Prolactin and wool growth in the Romney ewe

A thesis presented in partial
fulfilment of the requirements
for the degree
of

Doctor of Philosophy

in
Animal Science at
Massey University

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1999

Abstract

The effects of seasonal and experimental changes in plasma prolactin (PRL) concentration on wool growth in non-pregnant and breeding Romney ewes were assessed. Seasonal changes in plasma PRL concentration appeared to be primarily determined by photoperiod, rather than ambient temperature. The seasonal winter decline in wool production was prevented when circulating PRL levels were elevated during the winter by long day photoperiod. Endogenous PRL secretion was inhibited during pregnancy in breeding ewes, but was also influenced by photoperiod and season. A significant depression in wool growth was measured within the first 60 days of gestation, which was not associated with feed intake or changes in live weight. The reduction in wool growth was not associated with changes in circulating PRL concentration but is likely to be mediated by one or a combination of other maternal hormones. Clean wool growth rate, mean fibre diameter and fibre length growth rate all increased at or before parturition indicating that an inhibitory effect on wool growth was removed after the birth of the lamb. A consequence of higher wool growth rates during lactation was increased winter wool production in winter-lambing ewes. Photoperiod-induced increases in PRL concentration during pregnancy, at parturition, and during lactation were associated with significant medium- to long-term stimulatory effects on wool growth. The suppression of PRL concentration with bromocriptine, was associated with lower rates of long-term wool growth. Collectively these results suggest that plasma PRL has a stimulatory effect on wool growth in the Romney ewe.

Acknowledgements

I wish to acknowledge the assistance of colleagues, friends and family who have contributed in varying ways to the completion of these experiments. Firstly, I would like to thank AgResearch for the use of their facilities at the Ruakura Research Centre where I carried out all work relating to this project. I am extremely grateful to Wools of New Zealand who generously provided the funding for this project.

On a more personal note, I would like to thank my supervisors, Dr. Allan Pearson, Prof. Stuart McCutcheon, Dr. Tricia Harris and Dr. Sam Peterson for their advice and encouragement throughout.

I am grateful to past and present members of the Wool Follicle Growth Group for many helpful discussions and their practical assistance. In particular, a sincere thank you to Murray Ashby and Janet Wildermoth whose expert technical skills and overall contribution to the operation of these trials has been greatly appreciated.

Thank you to Dr. Roland Sumner for many helpful discussions relating to wool biology, to Dr. Harold Henderson for your guidance in the statistical analysis of all the experimental data, and to Pauline Hunt for the preparation of some of the diagrams presented in this thesis. Special thanks also to Trevor Watson, Ross Lasenby and Mat Holmes who assisted in the feeding and care of the experimental animals; and to John Parr and members of the Spermatology Group for your valuable expertise with the artificial insemination and ultrasonic pregnancy scanning.

Finally, a heartfelt thanks to my parents whose support, patience and understanding over the last 4½ years has made life a little easier. I've finished!!

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List of Abbreviations

A	allowance *(component of feed intake)*
ACTH	adrenocorticotrophic hormone
BSA	bovine serum albumin
CIDR	controlled internal drug release
DM	dry matter
EDTA	ethylenediaminetetraacetic acid
GH	growth hormone
h	hours
IGF(s)	insulin-like growth factor(s)
IRA	Innovative Research of America
LD	long days
min	minutes
M	maintenance *(component of feed intake)*
NaCl	sodium chloride
NaOH	sodium hydroxide
ND	natural days
OFDA	Optical Fibre Diameter Analyser
oPRL	ovine prolactin
PBS	phosphate-buffered saline
PEG	polyethylene glycol
PL	placental lactogen
PMSG	pregnant mare serum gonadotrophin
PRL	prolactin
s	seconds
SD	short days
T_3	tri-iodothyronine
T_4	thyroxine
$W^{0.75}$	initial wool-free metabolic live weight

INTRODUCTION

Long-woolled sheep breeds in New Zealand have a pronounced seasonal pattern of wool growth, with growth rates being highest in the summer and lowest in the winter. These seasonal changes in wool growth result from simultaneous changes in fibre diameter and fibre length growth rates, and are a function of both season and the physiological state of the ewe. Although breeding ewes play an important role in the New Zealand wool industry by providing replacement stock to the farmer, bearing and rearing a lamb reduces wool production by 10–15% compared to a non-breeding ewe. Furthermore, the period of minimal wool growth in the winter coincides with late pregnancy, adversely affecting commercially important wool properties such as fibre diameter and staple tensile strength. While restricted winter nutrient availability is likely to play a major role in the depression of wool production in breeding ewes under New Zealand pastoral conditions, hormonal mechanisms that alter nutrient flows to the skin and/or act directly on wool follicles could also be involved.

Previous experiments have implicated prolactin (PRL) in the control of wool growth cycles in primitive and shedding breeds of sheep (Lincoln & Ebling, 1985; Allain *et al.*, 1986; Lincoln, 1990). However, the effect of PRL on wool growth in seasonal but non-shedding breeds is not clearly defined (Dolling *et al.*, 1986; Foldes *et al.*, 1990; Lincoln, 1990; Curlewis *et al.*, 1991; McCloghry *et al.*, 1993). Recently, PRL receptors have been identified in the wool follicle (Choy *et al.*, 1995; 1997) suggesting that seasonal changes in circulating PRL concentration may mediate wool growth cycles. As an increase in plasma PRL concentration inhibits wool growth in the New Zealand Wiltshire (Pearson *et al.*, 1996) it seems possible that the rise in PRL concentration associated with parturition and lactation could be responsible for the depression in wool growth measured during late pregnancy.

In this research programme, a review of the literature was undertaken to characterise the profile of PRL and other important reproductive hormones during pregnancy, that could play a role in the regulation of wool growth.

Subsequently, experiments were designed to partition some of the physiological factors that underlie the wool growth depression in the New Zealand Romney ewe when she is pregnant or lactating.

The first and second experiments examined natural and experimental changes in plasma PRL concentration and their effect on wool production in spring- and winter-lambing Romney ewes. In the third experiment, the onset and duration of the wool growth depression in housed, pregnant Romney ewes was established, and its relationship to temporal changes in PRL concentration examined. Finally, in the fourth experiment, the diurnal profile of PRL concentration was characterised in ewes maintained under natural or experimentally-extended daylength during late pregnancy, at parturition, and in early lactation.

CHAPTER ONE

Review of the Literature

1.0 SEASONAL PATTERNS OF WOOL GROWTH IN SHEEP

1.0.1 Introduction

Most sheep breeds have an annual cycle of wool growth, with the rate being lowest in the winter and highest in summer. In primitive sheep breeds, such as the Mouflon and Soay, fibre growth stops during the winter. When fibre growth starts again in spring, the new fibre grows through the follicle canal alongside the old fibre with consequent fleece shedding. This cyclic pattern of wool growth, follicle inactivity and shedding was an obvious advantage to wild sheep because it gave them a thick coat in winter, without the burden of carrying heavier fleeces during each successive year (Ryder, 1971; 1973).

In modern breeds, however, the wool follicles have long periods of activity followed by short periods of inactivity. These rest phases occur largely at random and, for all practical purposes, fibre growth is continuous throughout the year. This pattern of wool growth appears to be an evolutionary remnant of the seasonal shedding that still persists in primitive breeds and the Wiltshire Horn (Hawker, 1986).

Wool growth rate is a function of mean fibre length growth rate, mean fibre cross-sectional area, density of active follicles within the skin, the surface area of the skin and specific gravity of the fibre produced (Sumner *et al.*, 1994). The changes with season in these and other wool characteristics will be outlined by comparing highly seasonal, shedding breeds (Mouflon, Soay and Wiltshire) with the main New Zealand crossbreds, and at the other extreme, the Merino, which displays a comparative lack of wool growth seasonality.

1.0.2 Seasonal Wool Growth Patterns in Shedding Sheep Breeds

(i) Mouflon

The European Mouflon *(Ovis musimon)* has a double outer coat, comprising coarse kemp fibres, and a dense undercoat of finer wool (Ryder, 1973). Of the three shedding breeds described here, the Mouflon is considered the wild ancestor of domesticated sheep. The most conspicuous feature of the Mouflon is the moult of the entire pelage over spring. Shedding of the fleece begins on the neck, belly and lower parts of the flank in late spring before moving dorsally towards the back of the sheep (Ryder, 1973). Wool growth resumes in late spring, producing a relatively short and sparse summer coat (Lincoln, 1990). The wool appears to grow most rapidly during late summer and autumn (Ryder, 1973) with the rapid development of the coarse outer fibres and of the fine under wool seen in the winter coat (Ryder, 1973; Lincoln, 1990). By early winter, the coat is complete and there is little or no fibre growth.

The highly seasonal pattern of wool growth seen in the Mouflon is reflected in the follicle activity. The activity of both primary and secondary follicles are highest from spring to early autumn and are largely inactive during the 6 months around the winter solstice (Ryder, 1973). This longer period of inactivity is thought to be a feature of primitive sheep breeds. Additionally, subsidiary peaks of follicle inactivity occur at 2–4 month intervals throughout the year, but are not reflected by observable changes in the coat (Ryder, 1973).

(ii) Soay

The Soay, which represents an early stage of domestication, has a similar seasonal moult to that of the wild Mouflon. Spring regrowth of the short, brown fleece of the Soay is associated with a partial or complete moult of the old coat (Lincoln, 1990), which sheds in a bilateral and symmetrical pattern (Ryder, 1971). In rams, new fibres begin to grow a month earlier in the spring than in ewes (Ryder, 1971), suggesting a differential response to seasonal

changes between sexes. In winter, the pelage consists of both coarse and fine fibres that form a dense coat (Lincoln, 1990). There is little or no wool growth in winter, although Ryder (1971) reported that the coat length in rams increased over this period while that in the ewe was unchanged.

Like the Mouflon, the shedding of fibres in the Soay is associated with an annual cycle of follicle activity in summer and inactivity in winter. Activation of primary follicles, which grow non-medullated fibres, and secondary follicles which produce only fine wool fibre, closely follows the spring equinox, and cessation follows the autumn equinox. Subsidiary fibre growth cycles have been identified in the summer (Ryder, 1971).

(iii) Wiltshire

Like the Mouflon and Soay breeds, the most characteristic feature of Wiltshire sheep is that the fleece undergoes regular shedding in the spring or early summer. Shedding progresses in a sequential, bilateral pattern commencing on the neck, chest and shoulders, and spreading across the abdomen, before moving towards the back and rump of the sheep (Slee, 1965; Ryder, 1969). In older sheep, this sequence becomes less consistent and the patterns more irregular (Slee, 1965).

In general, the Wiltshire has an unusually short fleece comprising kemp fibres and relatively coarse wool fibres. During the summer months there are subsidiary periods of moulting about every 2 months between the spring and autumn equinox (Ryder, 1969) before the longer and denser coat develops in the autumn (Lincoln, 1990). The wool fibres grown from the secondary follicles tend to become inactive later and to begin growing earlier than primary follicles. Primary follicles contain kemp fibres which develop brush ends during the 6-month autumn/winter period of inactivity (Ryder, 1969).

As wool growth in the Wiltshire is affected by the seasonal moult, which in turn is related to the wool follicle growth cycle, there is a marked seasonal

variation in the rate of wool growth. Wool growth is minimal near the end of winter and maximal in late summer. Fibre diameter is relatively high in early spring, coinciding with the resurgence of growth of kemp fibres, and lowest in the winter reflecting the predominance of fine secondary fibres (Slee & Carter, 1961).

The New Zealand Wiltshire is quite distinct from the English Wiltshire Horn. The Wiltshire Horn were first imported into New Zealand from Australia in 1974 and were subsequently crossed with Poll Dorsets to improve wool production and reduce the phenotypic expression of horns (Parry *et al.*, 1991). Although the New Zealand Wiltshire is not considered to be a pure breed, it also has a seasonal cycle in follicle growth with conspicuous shedding of the fleece similar to that of the Wiltshire Horn (Slee, 1965; Ryder, 1969). One feature of the New Zealand breed is that the extent and duration of wool follicle inactivity (telogen) is shorter than that reported in the Wiltshire Horn (Parry *et al.*, 1991). The New Zealand Wiltshire has been used extensively as a model in studies of seasonal variation in wool growth (e.g. Parry *et al.*, 1995; Pearson *et al.*, 1996).

1.0.3 The Major New Zealand Sheep Breeds

Total sheep numbers have declined in recent years with the June 1996 estimate at 47.4 million (The New Zealand Sheep and Beef Farm Survey, 1997). The New Zealand Romney dominates the New Zealand sheep industry and currently accounts for over 50% of the national flock. When the Perendale and Coopworth are included, a remarkable 75% of all the sheep in New Zealand are either Romney or Romney crosses (Figure 1.1). The Corriedale (including Halfbreds) and Merino are the other popular breeds.

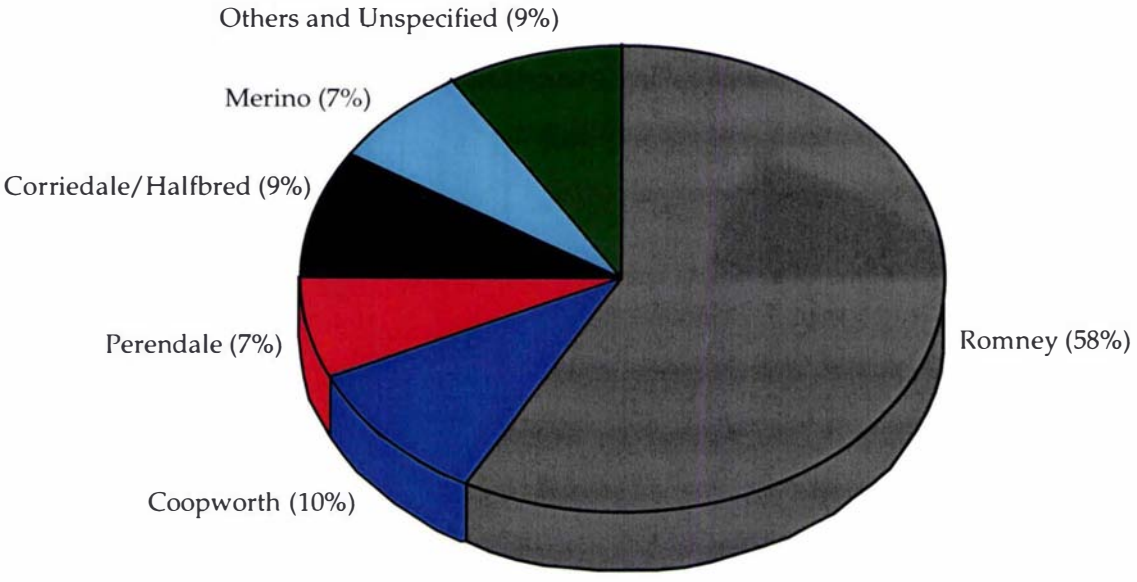

Figure 1.1: New Zealand's major sheep breeds (Source: NZ Meat & Wool Board's Economic Service, 1997).

1.0.4 Seasonal Wool Growth Patterns in New Zealand Sheep Breeds

The marked seasonal pattern of wool growth exhibited by the New Zealand Romney and other New Zealand crossbreds (Figure 1.2) has been well documented (Story & Ross, 1960; Ross, 1965; Bigham *et al.*, 1978a, 1978b; Geenty *et al.*, 1984; Woods *et al.*, 1995; Sumner *et al.*, 1998). In these sheep breeds, the seasonal wool growth was up to 4-fold higher for summer growth than for winter growth (Story & Ross, 1960; Sumner & Wickham, 1969; Bigham *et al.*, 1978b). However, it should be noted that in contrast to shedding sheep breeds, fibre growth continues through the winter.

Seasonal changes in wool growth result from simultaneous changes in fibre diameter and fibre length growth rates. Fibre diameter (Story & Ross, 1960; Bigham *et al.*, 1978b; Scobie *et al.*, 1993; Wuliji *et al.*, 1993; Woods *et al.*, 1995), length growth rate (Story & Ross, 1960) and fibre strength (Geenty *et al.*, 1984) are all at their greatest in summer and least during the period of low wool growth in winter. Annual changes of up to 40% in both fibre diameter and fibre length growth rates have been noted in Romney ewes (Story & Ross, 1960). Changes in these parameters are partly independent of each other as

fibre diameter changes occur about 1 month after the corresponding changes in fibre length (Story & Ross, 1960; Sumner & Wickham, 1969; Woods & Orwin, 1988). In the Romney, medullation also has a seasonal rhythm with the highest incidence in summer/early autumn with little or no expression in winter (Scobie *et al.*, 1993). While the level of medullation in the fleece is dependent on fibre diameter and season, the peak in medullation occurs 2 months after the peak in fibre diameter.

Numerous studies have detailed differences in wool production between the Romney, Coopworth, Perendale and Corriedale breeds in the same environment. Lambs (Dalton & Ackerley, 1974), yearlings (Dalton & Ackerley, 1974) and castrated males (Bigham *et al.*, 1978b) all have similar fleece weights between breeds, whereas Romney and Coopworth ewes have been shown to produce more wool than ewes of the other breeds (Bigham *et al.*, 1978a). Of the major breeds, the Coopworth exhibits the greatest seasonal variation in wool growth and the Corriedale the least (Sumner, 1979).

Breed differences in wool production are most likely to be present during periods of increasing wool growth and when wool production is highest. When wool growth rates are declining or at their minimum, breed differences are small (Bigham *et al.*, 1978b). This indicates that any difference in the fleece weight between these breeds results mainly from differences in early spring and summer wool growth rates. Variation in fibre diameter between breeds also appears to be greatest during periods of maximum wool growth rates (Bigham *et al.*, 1978b).

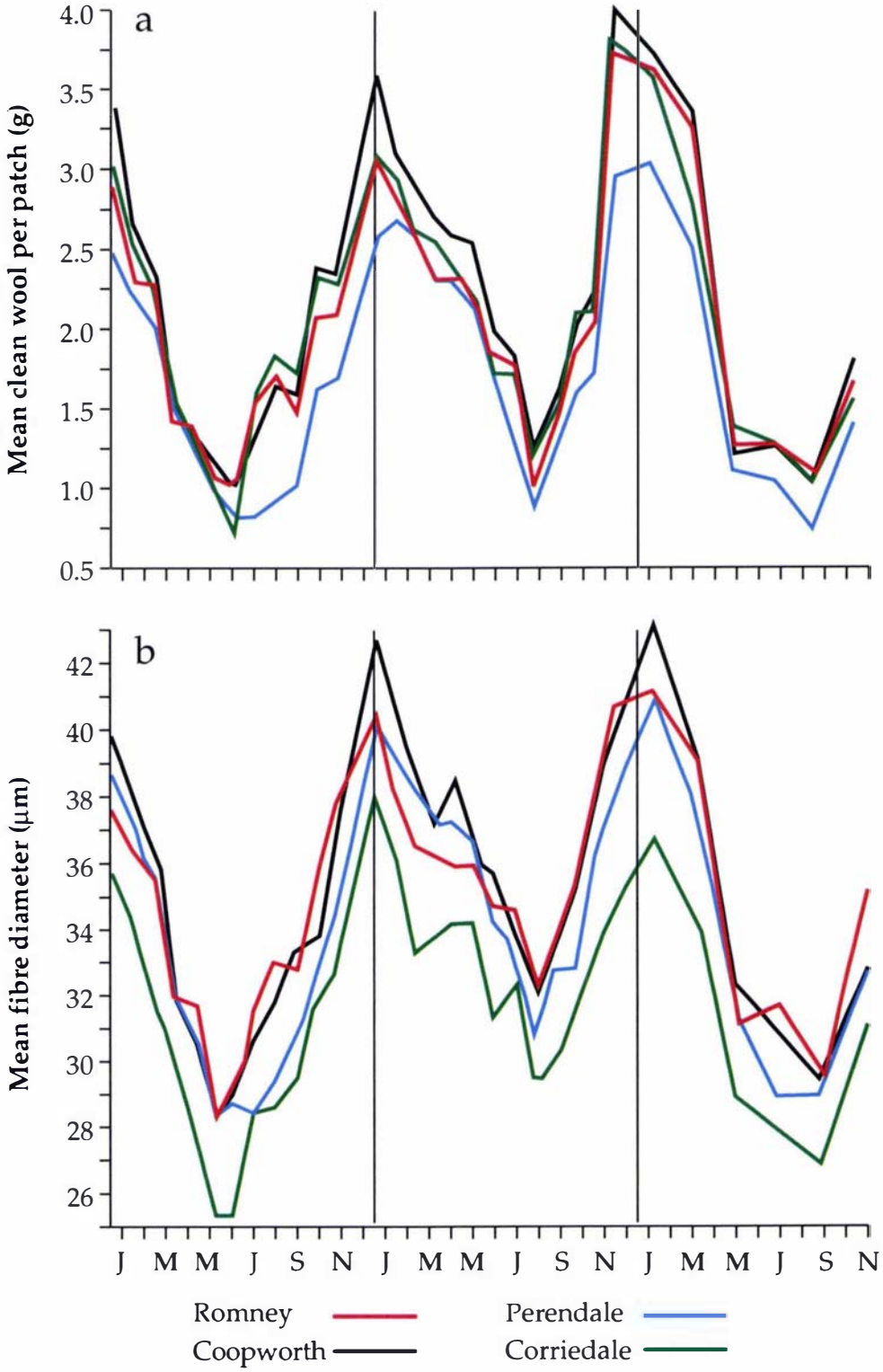

Figure 1.2: Seasonal changes in (a) clean wool growth and (b) fibre diameter of New Zealand crossbred sheep (adapted from Bigham *et al.*, 1978b).

In New Zealand conditions, the Merino exhibits a less-pronounced seasonal wool growth cycle than the principal New Zealand crossbreds (Wilkinson, 1986; Sumner *et al.*, 1994). The *rhythm*[1] of seasonal wool growth, which is a measure of wool growth seasonality enables direct comparisons between breeds. Bigham *et al.* (1978b) reported that the rhythm for both wool growth and fibre diameter in the Merino were approximately half the values derived from Romney, Coopworth, and Perendale sheep at the same location. The timing of the winter minimum of wool growth and mean fibre diameter (Sumner *et al.*, 1994) coincides with that previously reported for the Romney and other crossbreds (Bigham *et al.*, 1978b). However, in the same study (Sumner *et al.*, 1994), the period of maximum wool growth and fibre diameter in November was found to be approximately 4 weeks earlier than for the other breeds.

The seasonal cycle in wool growth rate can also affect annual wool production in breeding ewes. Winter-lambing ewes have been shown to consistently produce more greasy wool at shearing (0.2–0.8 kg) than their spring-lambing counterparts (Reid *et al.*, 1988; Reid & Sumner, 1991; Morris *et al.*, 1993; 1994). This is due to a reduced winter decline in wool growth in winter-lambing ewes, which are lactating, than in spring-lambing ewes, which are in late pregnancy at this time. The greater wool production in winter-lambing ewes compared to spring-lambing ewes is associated with an increase in staple tensile strength and fibre diameter of the fleece (Reid & Sumner, 1991; Morris *et al.*, 1994).

1.0.5 Summary

For sheep such as Mouflon, Soay and Wiltshire, the seasonal pattern of wool growth is very pronounced as the majority of the wool follicles become quiescent during the autumn and winter. In modern breeds such as the New Zealand Romney and the Merino, the follicles are rarely in telogen and fibres

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[1] Rhythm (%) = (Max.– Min.)/((Max. + Min.)/2) × 100 (see Sumner *et al.*, 1994)

are generally shed individually over an extended period so that a moult of the fleece does not occur. The wool growth rhythm in the Merino is almost totally absent while most of the crossbreds, like the Romney, lie in between these extremes. Seasonal changes in wool growth are caused by similar changes in a number of fibre characteristics, particularly fibre diameter and length growth rate. The timing of lambing can affect the pattern of wool growth and ultimately the quantity of the fleece produced.

1.1 THE EFFECTS OF PREGNANCY AND LACTATION ON WOOL GROWTH

1.1.1 Introduction

Seasonal patterns of wool growth represent the balance of the interactions between photoperiod, seasonal supply and quality of feed and physiological changes in the ewe associated with pregnancy and lactation. Pregnancy accentuates the winter decline in wool production so that during the second half of pregnancy, wool growth declines markedly and will have an impact on annual fleece production. In this section, the impact of pregnancy and lactation on wool production and wool characteristics will be discussed.

1.1.2 Annual Wool Production

The most direct measure of how pregnancy and lactation may affect wool production is the fleece weight obtained at shearing. Table 1.1 summarises studies which report a direct comparison of fleece weights from breeding ewes with those from non-pregnant ewes. Note that the period of fleece growth was 12 months in all experiments, with the exception of Parker *et al.* (1991), Reid (1978) and Masters *et al.* (1993), which were 7, 8 and 9 months respectively.

Table 1.1: Effect of pregnancy and lactation on fleece production.

Breed	Reduction in fleece (%) Greasy	Clean	Method of feeding	Reference
Romney	9	—	Grazing	Stevens & Wright (1952)
"	13*	—	Grazing	"
Border Leicester × Merino	7.1	—	Grazing	Seebeck & Tribe (1963)
"	14.6*	—	Grazing	"
Romney	3	—	Grazing	Ross (1965)
"	5*	—	Grazing	"
Merino	—	19.9	Grazing	Turner *et al.* (1968)
"	—	26.0*	Grazing	"
Merino	10.3	—	Grazing	Armstrong & O'Rourke (1976)
Polwarth	—	11.1	Grazing	Reid (1978)
Mixed NZ breeds	0–3	—	Grazing	Bigham *et al.* (1978a)
"	2-6*	—	Grazing	"
Romney and Coopworth	—	14	Grazing	Sumner & McCall (1989)
"	—	23*	Grazing	"
Merino	5–12	—	Grazing	Charlick & Arnold (1990)
"	15*	—	Grazing	"
Border Leicester × Romney	11	—	Grazing	Parker *et al.* (1991)
"	15*	—	Grazing	"
Romney	28	—	Grazing	Betteridge *et al.* (1992)
Merino	—	24	Pen-fed	Masters *et al.* (1993)

Percentage reduction in comparison with non-pregnant ewes. All values are for ewes with a single lamb unless marked with an asterisk which are for ewes with twins.

In quantitative terms, the average reduction in fleece production from these experiments equates to 0.35 kg or 12.5% as a result of bearing and rearing a single lamb. More specifically, grazing single-bearing Romney ewes or their crosses grew 0.14–0.45 kg less wool than non-pregnant controls (Stevens & Wright, 1952; Ross, 1965; Sumner & McCall, 1989; Parker *et al.*, 1991). In Merino ewes, the loss in fleece growth ranged from 0.29–0.56 kg (Seebeck & Tribe, 1963; Armstrong & O'Rourke, 1976). Significantly, the greatest reduction in clean fleece weight (0.61 kg) in breeding ewes at shearing was found in a pen-fed experiment in which diet was controlled (Masters *et al.*,

1993). This suggests that nutrition could be a major factor in many of the grazing studies, and that the decline in fleece production reported in breeding ewes could in fact be an overestimate. Under controlled feeding conditions, a reduction in fibre diameter of 1.6 μm (Masters *et al.*, 1993), in staple tensile strength of up to 30 N/Ktex (Hansford & Kennedy, 1988; Masters *et al.*, 1993) and in staple length of up to 9 mm (Masters *et al.*, 1993) can occur in the fleece wool of pregnant and lactating Merino ewes compared to non-pregnant ewes.

All studies agree that the reduction in annual fleece production is greatest in ewes bearing multiple lambs. On average, twin-bearing ewes grew 0.17 kg less fleece wool than single-bearing ewes. The loss in fleece growth ranged from 0.09–0.17 kg in Romney ewes (Stevens and Wright, 1952; Ross, 1965; Sumner & McCall, 1989; Parker *et al.*, 1991) and from 0.11–0.31 kg in Merino ewes (Seebeck & Tribe, 1963; Turner *et al.*, 1968; Oddy, 1985; Charlick & Arnold, 1990). In these data there is a suggestion that the fleece weight of New Zealand sheep breeds may be less affected by pregnancy and lactation, and increased fertility than is the case with the Australian Merino. Ewes giving birth to and rearing single lambs also tend to have stronger wool than twin-bearing ewes (Fitzgerald *et al.*, 1984; Morris *et al.*, 1994) with the staple tensile strength of the fleece being approximately 15% lower in the latter group. Likewise, fibre diameter (Fitzgerald *et al.*, 1984) and staple length (Dick & Sumner, 1997) of the fleece decrease as the number of lambs increase. When feed management conditions are similar, the decline in wool production with increasing number of lambs is remarkably consistent in most of the major New Zealand sheep breeds (Bigham *et al.*, 1978a).

Some studies have attempted to partition the effects of pregnancy and lactation on fleece production. The evidence is somewhat contradictory as one study (Reid, 1978) concluded that the reduction in fleece weight due to pregnancy in Polwarth ewes was 10% and lactation was only 1%, while another (Armstrong & O'Rourke, 1976), reported that the effect of lactation was nearly twice that of pregnancy in Merino ewes. In other experiments, the

removal of a lamb at birth in single-bearing ewes (Charlick & Arnold, 1990) and in twin-bearing ewes (Bigham *et al.,* 1978a) reduced the loss in fleece growth compared to their controls but this was not reflected in changes in the fibre diameter of the fleece (Reid, 1978). Clearly, these experiments have demonstrated that cumulative fleece weights are not a suitable measure of the respective effects of pregnancy and lactation on wool production. Therefore, a more in-depth investigation at changes in wool growth during each stage of pregnancy is warranted.

1.1.3 Early Pregnancy

In general, the effects of pregnancy on wool growth during the first 50 days of gestation are not well known. The little information that is available indicates that there is no difference in the wool production (Reid, 1978) or fibre diameter (Story & Ross, 1960; Oddy, 1985; Lee & Atkins, 1995) of pregnant ewes compared to non-pregnant ewes. The number of lambs the ewe is bearing also has no influence on wool growth at this stage of pregnancy (Story & Ross, 1960).

1.1.4 Mid-Pregnancy

Under pastoral grazing conditions, a reduction of 14% and 23% in clean wool growth rate was recorded in single- and twin-bearing Border Leicester × Romney ewes respectively between days 57 and 90 of gestation compared to non-pregnant ewes (Parker *et al.,* 1991). This is consistent with earlier findings of Henderson *et al.* (1970) who recorded a difference in wool growth rate between pen-fed pregnant and non-pregnant ewes in the third month of gestation. Lee and Atkins (1995) also reported that grazing pregnant Merino ewes grew 8% less wool than non-pregnant ewes in early pregnancy (given as 7 to 11 weeks after mating).

Other experiments have also shown that the wool growth in grazing (Reid, 1978; Hawker & Thompson, 1987) or pen-fed (Corbett, 1966) pregnant ewes is

depressed relative to non-pregnant ewes during this period, although Oddy (1985) found no difference in wool growth rate or fibre diameter in Merino ewes fed the same diet. As the clean wool growth rates between days 51–108 of gestation are higher in ewes that are aborted on day 24 of gestation than those of pregnant ewes, and similar to non-pregnant ewes (Reid, 1978), the potential to grow wool is not affected by the early loss of a lamb. During this stage of pregnancy, the greater impact of multiple lambs on wool growth first becomes evident (Story & Ross, 1960; Lee & Atkins, 1995).

1.1.5 Late Pregnancy

The changes in wool production during the last 50 days of gestation have been studied extensively. Over late pregnancy, wool growth is significantly lower in grazing pregnant ewes compared to non-pregnant ewes (Reid, 1978; Hawker & Thompson, 1987; Sumner & McCall, 1989; Betteridge *et al.*, 1992) as the wool growth rate continues to decline in pregnant ewes. Hawker and Thompson (1987) estimated that pregnant Romney ewes on pasture grew 26% less clean wool per day than non-pregnant ewes in the month prior to parturition. A comparable reduction in the wool growth rate of penned Merino ewes has been recorded during the last 2 months of pregnancy (Corbett, 1966; Oddy, 1985; Masters *et al.*, 1993). Masters *et al.* (1993) measured 30–43% lower rates of wool growth in ewes in late pregnancy than in non-pregnant ewes on the same diet.

Many other wool characteristics are also depressed during the last 50 days of gestation. Staples were 13% shorter in pregnant Romney ewes compared to non-pregnant ewes over late pregnancy (Hawker & Thompson, 1987). While Oddy (1985) found no difference in the fibre diameter in pen-fed pregnant Merino ewes compared to non-pregnant ewes, in general, the diameter of the wool progressively decreases as pregnancy advances (Hansford & Kennedy, 1988) leading to reduced tensile strength. The weakest point of the staple is often associated with the stress of parturition, giving rise to the so-called *lambing break*.

The decrease in wool production over this period is more pronounced in twin-bearing ewes than in ewes with a single lamb (Slen & Whiting, 1956; Story & Ross, 1960; Oddy, 1985; Hawker & Thompson, 1987). Lee and Atkins (1995) reported that the decline in wool growth by single- and twin-bearing Merino ewes compared to non-pregnant ewes was 11% and 18% respectively. A similar trend is observed with fibre diameter and fibre length (Slen & Whiting, 1956), with the reduction in fibre length occurring earlier in ewes with twin lambs.

1.1.6 Lactation

Wool growth rate remains significantly lower in lactating ewes compared to non-pregnant ewes (Story & Ross, 1960; Reid, 1978; Oddy, 1985; Betteridge *et al.*, 1992). Hawker (1979) showed that yearlings that reared lambs grew 20–30% less wool than non-pregnant yearlings from just before lambing to a month after weaning. In penned Merino ewes where diet was controlled, this reduction in wool growth rate during lactation could be as high as 40% (Masters *et al.*, 1993), although differences in fibre diameter were only measured in the first month of lactation compared to non-pregnant ewes (Oddy, 1985). Wool production generally increases over lactation (Betteridge *et al.*, 1992; Masters *et al.*, 1993), and often there is no difference in wool growth rate between lactating and non-pregnant ewes at weaning (Parker *et al.*, 1991).

Ewes rearing two lambs consistently grow less wool than ewes rearing one lamb until weaning (Sumner & McCall, 1989; Lee & Atkins, 1995). Lee and Atkins (1995) reported a further 7% reduction in wool growth in twin-bearing ewes than in single-bearing ewes. The length of lactation may also be important as there was an improvement in the wool growth rate of ewes whose lamb was removed at birth (Corbett, 1966; Reid, 1978). Similarly, in ewes that had twins but only raised a single lamb, wool growth, fibre diameter and fibre length all showed a small increase over lactation (Slen & Whiting, 1956).

1.1.7 Post-weaning

Lee and Atkins (1995) reported that grazing Merino ewes that reared a single lamb grew 6–8% more wool, which had a higher fibre diameter, than non-pregnant ewes in the 4-month period following weaning. Other experiments found no such difference between breeding and non-pregnant ewes (Corbett, 1966) or between single- and twin-bearing ewes (Sumner & McCall, 1989).

1.1.8 Summary

The research summarised here displays considerable variation in the magnitude of the wool growth depression during pregnancy and lactation which is due mainly to the variation in grazing environments and breeds both within and between different experiments. Few pen-fed experiments have been conducted where feed intake is known and has been controlled.

1.2 OTHER FACTORS CONTROLLING WOOL GROWTH

1.2.1 Introduction

Apart from the reproductive status of the ewe, the annual cycle of wool growth is also responsive to the level of feeding, the length of daylight and temperature. Breeds differ in their responsiveness, as the wool growth rate in British breeds like the Romney are strongly light dependent, while wool growth in the Merino was more dependent on changes in nutrition (Hawker, 1985). There are also significant differences in annual wool production between sexes and with the age of the sheep (see review by Sumner & Bigham, 1993). These factors will not be discussed in further detail in this thesis as only ewes of a similar age (3–5 years) were used.

1.2.2 Nutrition

(i) Nutritional requirements of the fetus

Numerous studies indicate that the energy requirements of the growing fetus are small during the first 100 days of gestation (Rattray, 1974; Rattray & Trigg, 1979; Robinson, 1983). As fetal growth is exponential, with 80% of growth taking place during the last third of pregnancy (Coop, 1950; Rattray, 1986), the nutritional cost of pregnancy rapidly accelerates. In lower temperatures, the partitioning of nutrients between mother and fetus may also be altered in favour of the fetus (Thompson *et al.*, 1982). The overall energy requirements of single- and twin-bearing ewes during pregnancy have been estimated to increase by 12% and 25% respectively compared to non-pregnant ewes (Lodge & Heaney, 1973).

(ii) Nutrient partitioning to the wool follicle

The reduction in wool growth during pregnancy arises from a decrease in the efficiency of the use of nutrients for wool growth relative to maternal live weight gain (Masters & Stewart, 1990). Over lactation, wool growth usually increases despite a decline in live weight, indicating a significant change in the partitioning of nutrients in these two periods. The wool follicle is influenced by the supply of amino acids, particularly the sulphur amino acids which are available for fibre production. It is accepted that the sulphur-containing amino acids, cystine and methionine, are the primary limiting nutrients in non-pregnant ewes (Reis, 1979) and fetal growth and milk production increase the requirement of the ewe for these amino acids. However, in experiments where ewes were fed to maintain a constant conceptus-free live weight, the plasma concentrations of total, free essential amino acids declined significantly during the last 3 weeks of pregnancy, but the concentration of cystine and methionine exhibited little change (Masters *et al.*, 1993; Stewart *et al.*, 1993). These findings, and the diminished response of wool growth to supplementary sulphur-rich

protein during pregnancy and lactation (Williams *et al.*, 1978), suggests that these amino acids are not limiting in the pregnant ewe.

(iii) Effects of the quality and quantity of feed on wool growth

Numerous researchers have reported that changes in feed intake will lead to changes in wool production. Feeding level affects wool growth through changes in both fibre length and fibre diameter (Sumner & Wickham, 1969; Hawker & Thompson, 1987) which result from an alteration in the rate of cell division in the follicle (Hawker, 1985). Under New Zealand pastoral conditions the responsiveness of wool growth to changes in the feed intake is greatest in the summer and lowest in the winter (Hawker & Crosbie, 1985).

There is a direct increase in wool growth as feed intake increases, although the proportion of nutrients allocated to wool growth declines (Hawker, 1985). In pen-fed experiments, feed intake was also positively correlated with wool growth (Daly & Carter, 1955; Sumner, 1979). When the plane of nutrition was high throughout pregnancy and lactation (Coop, 1950; Papadopoulos & Robinson, 1957) or during the latter stages of pregnancy (Fitzgerald *et al.*, 1984; Hawker & Thompson, 1987), wool production improved. Grazing management has also been shown to affect wool production with the depression in wool growth over winter being greatest in pregnant ewes grazed at the highest stocking rates (Sumner & Wickham, 1969; Betteridge *et al.*, 1992), although no difference between single- and twin-bearing ewes were found (Sumner & Wickham, 1969).

While most agree that the level of nutrition during lactation has a relatively small influence on wool growth compared to pregnancy, there are still conflicting reports on the merits of nutritional management in the pregnant ewe. Despite Williams and Butt (1989) reporting that the provision of additional feed can prevent the decline in wool growth as pregnancy advances, it is generally thought that the wool growth depression in pregnant

ewes occurs irrespective of feed intake (Lodge & Heaney, 1973; Oddy, 1985; Masters *et al.*, 1993). None of these experiments used Romney sheep.

Lowering the plane of nutrition at any time of the year will reduce wool growth rates and alter the inherent wool growth cycle. At very low intakes wool growth is an obligatory process and continues despite live weight loss until death. Many experiments have demonstrated that sub-maintenance feeding throughout pregnancy (Papadopoulos & Robinson, 1957; Alexander *et al.*, 1961) or during mid-pregnancy (Monteath, 1971) reduce the quantity and quality of wool produced. Furthermore, it appears that the decline in wool growth is accelerated in pregnant ewes on lower levels of nutrition (Slen & Whiting, 1956). When feed intake is held near maintenance levels in lactating ewes, wool growth is reduced by over 50% (Corbett, 1966).

The feeding of supplements at different stages of pregnancy is a common farming practice in Australian Merinos and there is ample evidence that wool growth rate is enhanced and the sulphur content of the wool increased if sheep on moderate dietary intakes are given supplements of sulphur-rich proteins (e.g. Ralph, 1984; Kelly & Ralph, 1988; Kelly *et al.*, 1992). The magnitude of this effect is greater when the supplements are protected and not degraded in the rumen (Masters *et al.*, 1996). In pregnant Romney ewes, however, the results obtained are more variable as both Coop (1950) and Henderson *et al.* (1970) found that wool growth in pregnant ewes did not respond to supplementary feeding.

1.2.3 Photoperiod

An annual rhythm of wool growth is now generally regarded as being induced by photoperiod (Hutchinson, 1965; Nagorcka, 1979). This rhythm can be altered by artificial photoperiod which has usually been demonstrated by reversing the photoperiodic rhythm or by changing its periodicity. In most of

these experiments the diet was controlled but a paucity of information on pregnant ewes remains.

Morris (1961) was the first to report that reversing the photoperiodic seasons reversed the rhythm of wool growth in Romney ewes, but this reversal was gradual, taking 2 years. In contrast, the effect of reversed photoperiodic seasons on wool growth was nearly immediate in another experiment (Hart *et al.,* 1963), although the ambient temperature and pattern of photoperiod treatment differed. Hutchinson (1965) demonstrated that the rhythm of wool production in Southdown ewes was entrained to an accelerated photoperiodic rhythm with a lag of 2–3 months.

Maxwell *et al.* (1988) found that the frequency of the seasonal rhythm was increased to a cycle of approximately 8 months when sheep were exposed to continuous LD, but declined in continuous SD. In the extreme case of zero daylength (Hart, 1961), the seasonal wool growth rhythm was suppressed and eventually disappeared. There is, however, one report of a short-term increase in winter and spring wool growth in response to SD (Hart, 1961). In shedding breeds such as the New Zealand Wiltshire, a SD to LD transition induces a follicle growth cycle (Pearson *et al.,* 1996) resulting in a short-term inhibition and a long-term stimulation of wool growth.

Despite some conflicting results, from the various experiments reviewed it seems that the annual rhythm of wool growth is entrained by photoperiod. The effects of photoperiod on wool growth appear to require a period of adaptation but if the photoperiodic stimulus is increased by bright lighting and rapid changes of daylength, entrainment of the rhythm is more successful. Daylength is influenced by latitude as well as season. Sumner *et al.* (1998) recently reported that the wool growth of Romney ewes exposed to a longer summer daylength and a shorter winter daylength had a greater degree of seasonality.

1.2.4 Temperature

Long-woolled sheep breeds show a marked depression in wool growth in winter, although raising the ambient temperature during winter does not necessarily affect the rate of wool growth of well-fleeced animals (Coop, 1953). This study, and others (Morris, 1961), has led to the conclusion that temperature plays a relatively small part in the wool growth cycle. However, the situation is not so clear in clipped areas of skin, as studies indicate that wool growth rate can differ from that of the fleece as a whole (Bennett *et al.*, 1962; Wodzicka-Tomaszewska & Bigham, 1968). Bennett *et al.* (1962), Doney and Griffiths (1967), and Downes and Hutchinson (1969) all observed a reduction in length growth rate on closely clipped skin patches after short-term exposure to cold. Similarly, Lyne *et al.* (1970) and Jolly and Lyne (1970) showed that lowered subdermal temperatures reduce length growth rate but not diameter. The depression in wool growth becomes more pronounced as the degree of skin cooling intensifies (Jolly & Lyne, 1970). Exposure to elevated temperatures produces the reverse effect on fibre diameter (Jolly & Lyne, 1970; Lyne *et al.*, 1970) and either an increase (Jolly & Lyne, 1970) or no effect on length growth rate (Lyne *et al.*, 1970).

It has also been demonstrated that wool growth rate was increased relative to uncovered patches when the midside patch was covered with a bag of wool to simulate fleece cover (Bennett *et al.*, 1962; Wodzicka-Tomaszewska & Bigham, 1968). When the entire sheep is exposed to cold by shearing, more wool is produced in the subsequent months (e.g. Sumner & Armstrong (1987)). The stimulation of wool growth rate following shearing is contrary to the generally observed depression in wool growth when a small area of skin is exposed to cold following clipping. Exposure of skin to cold reduces blood flow (Doney & Griffiths, 1967), which is correlated with wool production (Hocking Edwards & Hynd, 1994), and therefore nutrient supply to the exposed area. However, in the shorn sheep, the reduction in blood flow is likely to be compensated by an increase in feed intake (Elvidge & Coop, 1974).

1.2.5 Summary

Nutrition plays an important role in the control of wool growth, this effect being pronounced in the pregnant ewe. The need to meet the nutritional requirements for the growing fetus, mammary gland development, an increase in live weight during pregnancy and milk production during lactation, is partially at the expense of wool growth. Photoperiod and temperature both affect wool growth directly, and indirectly via their influence on pasture quantity and quality. Blood flow and skin temperature are also important physiological factors regulating local wool growth.

1.3 CHANGES IN HORMONE CONCENTRATIONS DURING PREGNANCY AND LACTATION

1.3.1 Introduction

Endocrine changes resulting from the presence of an embryo in the uterus are central to the continuation of luteal function, to the maintenance of pregnancy and fetal development, and to the triggering of parturition and the onset of lactation (Cox, 1975). Concentrations and balances of many hormones have been shown to change during gestation, and the establishment and maintenance of pregnancy requires interactions between the developing conceptus and the maternal system (Bazer & First, 1983). The importance of the conceptus, as well as the mother, as a source of gestational hormones has long been recognised. During the last month of gestation, in addition to the secretions of the maternal pituitary and corpus luteum, the conceptus may also secrete hormones into the maternal system.

The following is a detailed account of the changes in plasma hormone concentrations during pregnancy and lactation in sheep from the literature. A variety of external factors have been shown to influence the levels of these hormones in the maternal circulation and these will also be discussed.

1.3.2 Progesterone

Progesterone is synthesised and secreted by luteal cells (Niswender *et al.*, 1985). There is little evidence of any major increase in plasma progesterone concentration above approximately 2–5 ng/mL during the first 50 days of gestation (Bassett *et al.*, 1969; McNatty *et al.*, 1972; Stabenfeldt *et al.*, 1972; Tsang, 1978; Boulfekhar & Brudieux, 1980; Sawyer, 1995). This is consistent with the view that the corpus luteum is the main source of progesterone in the maternal circulation up to this time (Bassett *et al.*, 1969; Stabenfeldt *et al.*, 1972; Bassett & Thorburn, 1973).

From day 50 until day 120 of gestation, progesterone concentrations in the pregnant sheep increase steadily to about 8–13 ng/mL (Bassett *et al.*, 1969; Fylling, 1970; McNatty *et al.*, 1972; Tsang, 1978; Mukasa-Mugerwa & Viviani, 1992). At this time the progesterone concentration is about 2–6 times that during the first 50 days of gestation (Bassett *et al.*, 1969; McNatty *et al.*, 1972; Boulfekhar & Brudieux, 1980). This increase in the peripheral plasma progesterone concentration is due to an increase in the placental production and secretion of progesterone (Bedford *et al.*, 1973; Liggins *et al.*, 1973) which has been confirmed by the abrupt decline in maternal progesterone concentrations following total fetectomy and/or fetal death (Rueda *et al.*, 1995).

In the last 2 weeks of gestation there is a decrease in progesterone concentration from peak values of 10–22 ng/mL between days 125–140 (Stabenfeldt *et al.*, 1972; Tsang, 1978; Boulfekhar & Brudieux, 1980) and the decline accelerates 2–3 days prepartum (McNatty *et al.*, 1972; Tsang, 1978; Boulfekhar & Brudieux, 1980; Mukasa-Mugerwa & Viviani, 1992). Levels decrease from about 4 ng/mL to 2 ng/mL during the last 12 h before labour (Stabenfeldt *et al.*, 1972), although the changes in the peripheral progesterone concentration are variable at this time. On the day of parturition, progesterone levels can range from 3 ng/mL (Tsang, 1978; Mukasa-Mugerwa & Viviani, 1992) to below 1 ng/mL (Bassett *et al.*, 1969; McNatty *et al.*, 1972). The decrease

in circulating progesterone before parturition has been attributed to a reduction in the placental secretion of progesterone (Fylling, 1970) and reduced progesterone synthesis (Stabenfeldt *et al.*, 1972), and is associated with the initiation of labour.

Immediately after parturition, progesterone almost disappears from the plasma. This decline, from approximately 2 ng/mL to 0.8 ng/mL, usually occurs within 30–45 min after delivery, and levels remain low during the following 48 h period (Stabenfeldt *et al.*, 1972). Progesterone concentrations are less than 1 ng/mL for the first week postpartum (Boulfekhar & Brudieux, 1980; Mukasa-Mugerwa & Viviani, 1992) and throughout the first 6 weeks of lactation the values are similar to those of anoestrus non-pregnant ewes (Bassett *et al.*, 1969). The metabolic clearance rate of progesterone during lactation is greater than over pregnancy, but production rates are considerably less (Slotin *et al.*, 1971).

While progesterone levels in the peripheral plasma of single- and twin-bearing ewes are similar during the first 50 days of gestation, in general, concentrations are consistently higher in those with twins from this time until parturition (Bassett *et al.*, 1969; Stabenfeldt *et al.*, 1972; Butler *et al.*, 1981; Peterson *et al.*, 1997). Two studies (McNatty *et al.*, 1972; Mukasa-Mugerwa & Viviani, 1992) found no relationship between the number of fetuses and progesterone concentrations. However, progesterone levels during the latter stages of pregnancy may be confounded by the nutrition of the ewe (Shevah *et al.*, 1975). Continuous underfeeding can also delay the decrease in maternal progesterone concentration (Mellor *et al.*, 1987). Progesterone concentration may also vary with season (Rhind *et al.*, 1978), breed (Butler *et al.*, 1981) and age (McNatty *et al.*, 1972).

1.3.3 Oestrogens

Oestrogens are secreted by the *theca interna* cells of the ovarian follicles, by the corpus luteum and by the placenta (Baird *et al.*, 1973; Findlay & Seamark,

1973). The changes in peripheral plasma concentrations of unconjugated oestrogens (Challis, 1971; Carnegie & Robertson, 1978; Tsang, 1978; Butler *et al.*, 1981) and of oestrogen sulphates (Carnegie & Robertson, 1978; Tsang, 1978) in the pregnant ewe have been well documented.

The major oestrogens in the maternal circulation throughout gestation are oestrone, oestradiol-17β and oestradiol-17α although these biologically active oestrogens appear to be metabolised and excreted as less potent conjugated products (Challis *et al.*, 1973). Oestradiol-17β is secreted by the ovary and is the principal oestrogen in the non-pregnant ewe (Baird *et al.*, 1973). During the early stages of pregnancy, the concentrations of oestrogens in peripheral plasma are so low as to be barely detectable. The concentration of unconjugated oestrogens remain less than 5 pg/mL until about day 120 of gestation, after which time they increase slowly to 20–40 pg/mL 5 days before parturition (Challis, 1971). Oestrone levels are less than 10 pg/mL until day 110 of gestation before rising to nearer 20 pg/mL (Carnegie & Robertson, 1978). By day 140, oestrone concentrations reach 27–59 pg/mL (Carnegie & Robertson, 1978; Tsang, 1978). Oestradiols are first measurable on day 90 of gestation and are present at a concentration of 15 pg/mL (Carnegie & Robertson, 1978).

The most dramatic change in the concentration of oestrogens in maternal circulation is seen in the last 2 days of gestation when there can be up to a 10-fold increase in oestrogen concentration (Challis, 1971). Peak oestrone concentrations can be as high as 160--400 pg/mL on the day of parturition (Tsang, 1978). In the week preceding parturition oestradiol values range from 10–54 pg/mL (Carnegie & Robertson, 1978; Tsang, 1978; Mellor *et al.*, 1987) but may be as high as 110--400 pg/mL at term (Tsang, 1978). The oestrogen surge is also derived from the conceptus and there is therefore a sharp fall within a day or so after birth. Although the oestrogen surge plays an important role in the events of parturition (Cox, 1975), it appears that such an increase is not a prerequisite for successful parturition (Rueda *et al.*, 1995) Maternal oestrogen

concentrations 2–5 weeks prepartum are not related to breed or the number of lambs (Butler *et al.*, 1981), or to nutrition (Mellor *et al.*, 1987).

All studies confirm that oestrone sulphate is the predominant oestrogen present in the maternal circulation throughout gestation in the ewe, although there are conflicting reports about when the level of this hormone increases. Carnegie and Robertson (1978) reported a rise in the jugular venous concentration by day 35 of gestation, however, levels were still below 0.1 ng/mL at this time. In another study (Tsang, 1978), oestrone sulphate was detectable at around 70 days of gestation with values ranging from 0.1–0.3 ng/mL. While it appears that this discrepancy is most likely due to differences in assay sensitivity, there is little doubt that concentrations of oestrone sulphate are low at this stage of pregnancy. The levels steadily increase to 1–9 ng/mL a few days before lambing (Carnegie & Robertson, 1978; Tsang, 1978), before a dramatic increase to 15–50 ng/mL about 2 days before parturition (Tsang, 1978).

The pattern of oestradiol sulphate concentration in the maternal plasma was very similar to that of oestrone sulphate, although the observed concentrations were much lower. Oestradiol sulphate was first detectable on day 31 (56 pg/mL) and the concentration began to increase by day 80, rising to concentrations of around 500 pg/mL by day 120 of gestation. The levels of oestrone sulphate and oestradiol sulphate in fetal plasma are consistently higher than those measured in the maternal plasma, indicating that the mother is not necessarily the source of these hormones (Carnegie & Robertson, 1978).

1.3.4 Prolactin

PRL is a peptide hormone secreted from specialised cells of the anterior pituitary gland called lactotrophs. During pregnancy the maternal pituitary increases in size, primarily as a result of hyperplasia and hypertrophy of these lactotrophic cells (Djiane & Kelly, 1993). As is true for most pituitary hormones, PRL is secreted in a pulsatile manner (Lamming *et al.*, 1974).

Maternal plasma PRL levels remain low and relatively stable throughout most of pregnancy and increase rapidly in late term to reach maximal levels around the time of parturition. Most studies (e.g. Kelly *et al.,* 1974; Lamming *et al.,* 1974; Munro *et al.,* 1980; Fitzgerald *et al.,* 1981) agree that maternal PRL concentrations range between 10 ng/mL and 50 ng/mL during the first 100 days of gestation. These levels are comparable to basal PRL concentrations in non-pregnant ewes over the same period (Fitzgerald *et al.,* 1981). It is generally accepted that, while PRL concentrations may increase over the last 6 weeks of gestation, there is a wide variation in circulating PRL levels between individual animals at this time.

A rise in maternal PRL concentration was usually observed 1–3 days before parturition (Kelly *et al.,* 1974; Lamming *et al.,* 1974). Kelly *et al.* (1974) reported that plasma PRL concentrations began to rise about 36 h before parturition and reached 300–600 ng/mL approximately 3–6 h prepartum. During the final stages of labour and at parturition, rapid spurts of PRL are released, concentrations reaching between approximately 100 ng/mL and 700 ng/mL (Kelly *et al.,* 1974; Lamming *et al.,* 1974; Munro *et al.,* 1980; Koritnik *et al.,* 1981; Peterson *et al.,* 1990). The role played by stress during lambing, which is known to lead to an increase in plasma PRL concentration (Lamming *et al.,* 1974; Djiane & Kelly, 1993) may also be significant.

As during parturition, lactation is associated with raised plasma PRL. Basal PRL concentrations throughout early lactation range from 100–150 ng/mL, however suckling causes a rise in PRL levels to as high as 800 ng/mL (Lamming *et al.,* 1974). By mid-lactation, basal levels are 20–100 ng/mL and suckling causes a rise in PRL concentration from 20 ng/mL to up to 400 ng/mL. Basal PRL concentrations remain low (5–20 ng/mL) during late lactation, equivalent to levels found in non-lactating anoestrus ewes and continue to decline as lactation advances (Lamming *et al.,* 1974). There is a further decrease in PRL concentration after weaning (Rhind *et al.,* 1980). In general, PRL concentrations during lactation are lower than those found

during the summer months when the ewes are no longer lactating (Munro *et al.*, 1980).

A well known seasonal pattern of PRL secretion has been confirmed in non-pregnant sheep (Munro *et al.*, 1980; Fitzgerald *et al.*, 1981; Webster & Haresign, 1983; Buys *et al.*, 1990) which is primarily determined by photoperiod. PRL concentrations are low in autumn and winter before rising in spring to reach a peak in summer. There is also a seasonal effect on the PRL concentrations in pregnant (Munro *et al.*, 1980; Bosc *et al.*, 1982; Buys *et al.*, 1990; Peterson *et al.*, 1990) and lactating ewes (Rhind *et al.*, 1980). Furthermore, PRL concentrations through late gestation (Bocquier *et al.*, 1986; Bassett *et al.*, 1988; Ebling *et al.*, 1989; Bassett, 1992) and lactation (Bocquier *et al.*, 1990) are higher in ewes kept in artificial LD photoperiod. Further evidence of the influence of environmental effects on PRL secretion can be seen in the effect of shearing, when there is a marked decline in plasma PRL concentrations (Carr & Land, 1982; Webster & Haresign, 1983; Pijoan & Williams, 1984). This change in PRL levels was related to temperature, date of shearing and voluntary feed intake (Pijoan & Williams, 1984).

Feed restriction in pregnant ewes reduces PRL concentrations (Koritnik *et al.*, 1981). Conversely, the plane of nutrition has little effect on maternal PRL levels over lactation (Rhind *et al.*, 1980). Litter size does not influence maternal PRL concentrations prior to parturition (Buys *et al.*, 1990; Peterson *et al.*, 1997) but lactational PRL levels are higher in twin-bearing ewes compared to single-bearing ewes (Peterson *et al.*, 1997).

1.3.5 Placental Lactogen

Placental lactogen (PL), or chorionic somatomammotrophin, is structurally similar to PRL. PL is secreted by binucleate cells of the chorionic epithelium and sheep placentae during pregnancy (Carnegie *et al.*, 1982; Ogren & Talamantes, 1988; Kappes *et al.*, 1992) and is known to have both lactogenic and somatotropic activity (Martal & Djiane, 1975; Reddy & Watkins, 1978). A

live fetus is required to maintain PL synthesis and secretion (Rueda *et al.*, 1995). PL has been implicated in the prepartum preparation of the mammary gland for lactation, in the stimulation of steroidogenesis in both the ovary and the placenta, and regulating the metabolism and growth of the fetus (Talamantes *et al.*, 1980; Anthony *et al.*, 1995).

PL in the peripheral circulation can usually be detected as early as day 48 to day 60 of gestation (Kelly *et al.*, 1974; Chan *et al.*, 1978a; Bazer & First, 1983) when levels are greater than 2 ng/mL (Chan *et al.*, 1978a). Circulating PL concentrations increase as pregnancy advances, reaching maximal levels of 1000–2000 ng/mL by days 95–114 (Kelly *et al.*, 1974). In subsequent studies (Chan *et al.*, 1978a; Talamantes *et al.*, 1980; Butler *et al.*, 1981; Kappes *et al.*, 1992) PL concentrations reached a peak somewhat later (days 121–141) in gestation. In contrast, circulating PRL levels remain below 50 ng/mL at this time (Munro *et al.*, 1980; Fitzgerald *et al.*, 1981). Additionally, treatment of pregnant sheep with bromocriptine lowers circulating pituitary PRL levels, but does not change circulating levels of PL (Kann, 1976a, 1976b; Martal & Lacroix, 1978; Gow *et al.*, 1983) suggesting that PRL and PL secretion is controlled via different receptor pathways (Chan *et al.*, 1978b; Emane *et al.*, 1986).

In late gestation PL concentration declines, commencing approximately 5–15 days before parturition (Chan *et al.*, 1978a; Talamantes *et al.*, 1980; Taylor *et al.*, 1980). PL concentrations fall slowly from approximately 1000 ng/mL to 500–700 ng/mL, 12 h before parturition and then decrease quite rapidly postpartum (Kelly *et al.*, 1974; Taylor *et al.*, 1980; Mellor *et al.*, 1987; Kappes *et al.*, 1992). On the day of delivery PL levels are about 50% of the mean value recorded in late gestation (Taylor *et al.*, 1980). By way of contrast, fetal PL concentrations can reach peak values by mid-gestation, and plateau or gradually decline until parturition (Gluckman *et al.*, 1979; Kappes *et al.*, 1992).

PL levels are known to increase with increasing number of offspring (Martal & Djiane, 1977; Gluckman *et al.*, 1979; Taylor *et al.*, 1980; Butler *et al.*, 1981; Oddy

& Jenkin, 1981; Bosc *et al.*, 1982; Kappes *et al.*, 1992), particularly over the last third of pregnancy. Furthermore, maternal PL concentration is thought to be related to total placental mass (Taylor *et al.*, 1980) although it is unlikely that the rate of PL secretion is solely a function of placental mass (Talamantes *et al.*, 1980). Maternal PL concentrations are affected by diet over late pregnancy, as fasting will increase concentrations of PL (Brinsmead *et al.*, 1981) and underfeeding can delay the prepartum decrease in PL concentration (Mellor *et al.*, 1987). There are also highly significant differences among breeds in circulating PL levels (Butler *et al.*, 1981). The lack of either a seasonal effect (Bosc *et al.*, 1982), a marked circadian rhythm (Chan *et al.*, 1978a; Taylor *et al.*, 1980) or changes with photoperiod manipulation in the pregnant ewe (Bocquier *et al.*, 1986), indicates that, in contrast to PRL, PL secretion is not photoperiod-dependent.

1.3.6 Growth Hormone

As with reproductive hormones, concentrations of growth hormone (GH) present in the maternal circulation are dependent on the stage of pregnancy. Moore *et al.* (1984) noted that GH levels were 1–2 ng/mL at 57 days gestation and relatively stable (2–5 ng/mL) between days 70–120 of gestation (Bassett *et al.*, 1970; Blom *et al.*, 1976; Moore *et al.*, 1984; Mellor *et al.*, 1987; Jenkinson *et al.*, 1995). These lower levels are characteristic of those found in non-pregnant sheep (Trenkle, 1967). An increase in plasma GH only occurs approximately 5 weeks before parturition with GH concentrations averaging 6–18 ng/mL 5–10 days prepartum (Blom *et al.*, 1976; Gow *et al.*, 1983; McMillen *et al.*, 1987; Jenkinson *et al.*, 1995). A marked increase in GH concentrations has been shown to occur the day before parturition (Mellor *et al.*, 1987) when plasma GH levels have been reported to be as high as 65 ng/mL (Wróblewska & Domañski, 1981). This latter study reported GH concentrations considerably higher than those in other studies and probably reflects the variability in plasma hormone concentrations around parturition. Plasma GH concentrations remain elevated the day after parturition (Mellor *et al.*, 1987).

Plasma GH concentrations 8 weeks before parturition are similar in all sheep, regardless of the number of fetuses. However, 1 week before parturition, GH levels have been shown to markedly increase in ewes carrying multiple fetuses compared to those carrying a single fetus (Hove & Blom, 1976). Furthermore, it appears that the increase in GH concentrations towards term starts later in single compared to multiparous ewes (Blom *et al.*, 1976).

Ewes suckling twins have higher plasma GH concentrations than ewes suckling single lambs during the first week of lactation (Hoefler & Halford, 1987; Holcombe *et al.*, 1989). After this time, GH levels do not differ, although they tend to be higher in ewes with twins. In contrast to plasma PRL concentrations, which decline over lactation, GH levels remain relatively high (Wróblewska & Domañski, 1981; Gow *et al.*, 1983).

Nutrition is the single most important physiological regulator of the somatotrophic axis (Bauer *et al.*, 1995). Plasma GH concentration appears to be stimulated when nutrient availability is restricted in rams (Barenton *et al.*, 1987). Similarly, feeding pregnant sheep a restricted diet is associated with a rise in plasma GH concentration (Blom *et al.*, 1976; Koritnik *et al.*, 1981; Bauer *et al.*, 1995). This increase in GH concentration occurs earlier and reaches higher levels than in adequately fed pregnant controls (Blom *et al.*, 1976).

GH levels are increased in the pregnant ewe exposed to LD photoperiod (Bocquier *et al.*, 1986; Perier *et al.*, 1986). However, in a more recent study, there were no seasonal differences in GH concentration in pregnant sheep (Jenkinson *et al.*, 1995).

1.3.7 Insulin-like Growth Factors

Most studies have focused on the fetal insulin-like growth factors I and II (IGF-I and IGF-II) concentrations, and data available on maternal IGF concentrations during pregnancy are somewhat contradictory. Van Vliet *et al.* (1983) suggested that IGF-I levels between days 84–98 of gestation were

similar in pregnant ewes and adult non-pregnant ewes. However another study (Handwerger *et al.*, 1983), concluded that IGF-I concentrations in pregnant ewes were greater between day 51 of gestation and term than in non-pregnant ewes.

Serial measurement of maternal IGF-I concentrations have either shown a decline as pregnancy advances (Jenkinson *et al.*, 1995) or no change (Wallace *et al.*, 1997). In the former study, IGF-I levels increased from 90–105 ng/mL at day 70 of gestation to 115–145 ng/mL by day 100, while plasma IGF-I concentrations in the latter study were consistently above 200 ng/mL over this period. Nearer parturition, plasma IGF-I concentrations fell to 50–90 ng/mL (Jenkinson *et al.*, 1995) but were still elevated (> 170 ng/mL) in numerous other studies (Wylie & Chestnutt, 1990; Hall *et al.*, 1992; Iwamoto *et al.*, 1992; Liu *et al.*, 1994; Wallace *et al.*, 1997). This variation in IGF-I profile may be due to breed, or seasonal differences (Jenkinson *et al.*, 1995).

No data is available detailing plasma IGF-II concentrations throughout pregnancy in sheep although maternal IGF-II levels are considerably greater than those of IGF-I in late gestation (Gluckman & Barry, 1988; Iwamoto *et al.*, 1992; Oliver *et al.*, 1995) and at term (Gluckman & Barry, 1988).

Little is known about the effects of fetal number on maternal IGF concentration. Wylie and Chestnutt (1990) reported that IGF-I concentration on day 140 of gestation was not related to fetal number, whereas in another study (Hall *et al.*, 1992), IGF-I levels on the same day, and at parturition, were higher for single- than twin-bearing ewes. This was thought to be due to a lower nutritional status in multiparous ewes. Documentation of maternal IGF concentrations throughout lactation is also lacking.

Nutritional factors are important in the regulation of IGF-I secretion in the pregnant sheep, and changes in maternal plasma IGF-I concentrations during pregnancy are the inverse of those in GH (Jenkinson *et al.*, 1995). Maternal

undernutrition or starvation over late pregnancy causes a fall in both IGF-I and insulin concentrations (Gallaher *et al.*, 1992; Bauer *et al.*, 1995) whereas IGF-II plasma levels are unaffected by feed restriction. The fall in IGF-I levels is expected as glucose and insulin concentrations, which both decline, are significant determinants of IGF secretion (Bauer *et al.*, 1995).

1.3.8 Insulin

Maternal plasma insulin concentrations remain below 1 ng/mL for the first 100 days of gestation before decreasing nearer term (Blom *et al.*, 1976; Vernon *et al.*, 1981; Mellor *et al.*, 1987). It is thought that PL may be involved in the fall in insulin concentrations during late pregnancy (Tyson & Felig, 1971), the greatest decrease in insulin concentration during the last 5–6 weeks of gestation occurring in ewes with multiple lambs (Mellor *et al.*, 1987). Blom *et al.* (1976) suggests that the change in insulin concentration is a metabolic adaptation to pregnancy regulated by the maternal availability of glucose.

Plasma insulin concentrations remain low (< 1 ng/mL) following parturition but increase with advancing lactation (Vernon *et al.*, 1981; Shetaewi & Ross, 1991). While Holcombe *et al.* (1989) reported that ewes suckling a single lamb had similar insulin concentrations compared to ewes raising twin lambs over lactation, a more recent study (Peterson *et al.*, 1997) found plasma insulin concentrations to be higher in single-bearing ewes compared to twin-bearing ewes in the 3 weeks before and after parturition.

1.3.9 Corticosteroids

Corticosteroids are a class of steroid hormones which includes the glucocorticoids, such as cortisol. Corticotroph cells in the anterior pituitary gland secrete ACTH as their principal product (Ssewannyana *et al.*, 1990), which in turn stimulates the secretion of cortisol from the adrenal gland of the adult sheep.

In unstressed sheep circulating cortisol concentrations range from 1–10 ng/mL (Bassett & Hinks, 1969). Basal cortisol concentrations change little during the first 120 days of gestation, although this hormone responds readily to certain stresses including those associated with pregnancy (Cox, 1975). Maternal corticosteroid concentrations are generally less than 5 ng/mL before day 120 (Boulfekhar & Brudieux, 1980) and begin to increase about 15–20 days before term, increasing more rapidly over the last few days of gestation (Bassett & Thorburn, 1969; Mellor *et al.*, 1987). Plasma cortisol concentrations 3 days before parturition range from 9–25 ng/mL and remain at similar levels until the day before birth (Chamley *et al.*, 1973). During the 18 h preceding parturition, mean corticosteroid concentration begins to rise further, reaching a peak concentration of 70–200 ng/mL (Bassett & Thorburn, 1969; Chamley *et al.*, 1973; Strott *et al.*, 1974). Within 18 h after birth, concentrations decline to 4 ng/mL (Mellor *et al.*, 1987) and remain relatively stable for the first 4–8 days postpartum (Boulfekhar & Brudieux, 1980).

Plasma levels of corticosteroids are generally higher in twin-bearing ewes than in ewes which have a single lamb (Chamley *et al.*, 1973). There is no evidence in the literature to suggest that photoperiod can effect corticosteroid concentrations in the pregnant ewe, but as plasma concentrations of ACTH and cortisol display considerable seasonal variation (Ssewannyana *et al.*, 1990), it is possible. Plasma cortisol concentrations are relatively unaffected by plane of nutrition (Mellor *et al.*, 1987).

1.3.10 Thyroid Hormones

Thyroxine (T_4) is the major thyroid hormone in sheep (Annison & Lewis, 1959) and secretion from the thyroid gland varies markedly. Looking at the literature it is somewhat difficult to separate the effects of pregnancy and season on thyroid secretion, particularly as most previous studies involve grazing sheep and were confounded by variable feed intakes. Not surprisingly, there is considerable variation in T_4 concentrations reported in the pregnant ewe.

There is a minimal change in the maternal T_4 concentration during the first 120 days of gestation which range between 70–80 ng/mL (Dussault *et al.*, 1971; Ross *et al.*, 1985; Okab *et al.*, 1993; Wallace *et al.*, 1997). In general, plasma T_4 concentration declines as pregnancy advances (Annison & Lewis, 1959; Dussault *et al.*, 1971; Assane & Sere, 1990; Wallace *et al.*, 1997) although some studies (Sutherland *et al.*, 1974; Prasad, 1990; Okab *et al.*, 1993) have reported an increase followed by a marked decrease. T_4 levels fluctuate considerably over the last 6 weeks of pregnancy (Nathanielsz *et al.*, 1973; Mellor *et al.*, 1976) varying between 40–70 ng/mL closer to parturition (Dussault *et al.*, 1971; Nathanielsz *et al.*, 1973; Okab *et al.*, 1993; Wallace *et al.*, 1997). A possible cause for the reduction in maternal T_4 concentration is a negative feedback control caused by the active fetal thyroid (Nathanielsz *et al.*, 1973). Plasma tri-iodothyronine (T_3) (Okab *et al.*, 1993; Wallace *et al.*, 1997) and thyroid-stimulating hormone concentrations change little over pregnancy (Dussault *et al.*, 1971; Buys *et al.*, 1990).

During lactation, plasma T_4 concentrations are higher than in non-pregnant sheep (Sutherland *et al.*, 1974) but lower than throughout pregnancy (Okab *et al.*, 1993). T_4 levels also vary with the day of lactation and are lower (25 ng/mL) in the middle of lactation but similar (35 ng/mL) during early and late lactation (Shetaewi & Ross, 1991).

Measurements made at different times of the year in grazing sheep suggest that there are seasonal variations in plasma thyroid hormone concentrations (Henneman *et al.*, 1955; Sutherland *et al.*, 1974; Andrewartha *et al.*, 1980; Buys *et al.*, 1990; Okab *et al.*, 1993). In general, plasma T_4 and T_3 concentrations are minimal in early autumn and maximal in midwinter and spring, regardless of reproductive status (Sutherland *et al.*, 1974). In contrast, Buys *et al.* (1990) did not observe any seasonal influence on plasma thyroid-stimulating hormone concentrations. Most authors agree that environmental factors such as temperature, photoperiod and feeding level could be responsible for the circannual rhythm in thyroid hormones in sheep. Worth noting is that

seasonal changes in plasma T_4 levels are lessened when sheep are maintained indoors under natural lighting and consuming a constant amount of feed (Wallace, 1979b). T_4 concentrations are not influenced by litter size (Buys *et al.*, 1990) but have been shown to vary with breed (Henneman *et al.*, 1955; Ross *et al.*, 1985; Okab *et al.*, 1993) and age (Henneman *et al.*, 1955; Prasad, 1990).

1.3.11 Summary

The hormonal changes that take place over the first 120 days of gestation tend to be gradual in a temporal sense. However, particularly marked changes in endocrine systems are seen near parturition. These include, sequentially, an increase in fetal cortisol concentrations, followed by an increase in oestrogens; a decrease in plasma progesterone, and an increase in PRL in the maternal circulation. There is also a parallel decline in PL concentration. Thyroid hormones, GH, and IGF concentrations also change as pregnancy advances. A schematic diagram of the endocrine changes in the major reproductive hormones during pregnancy in sheep described in this section is shown in Figure 1.3. Clearly, other factors such as nutrition, season, photoperiod, temperature, breed, and interactions with other hormones are also important.

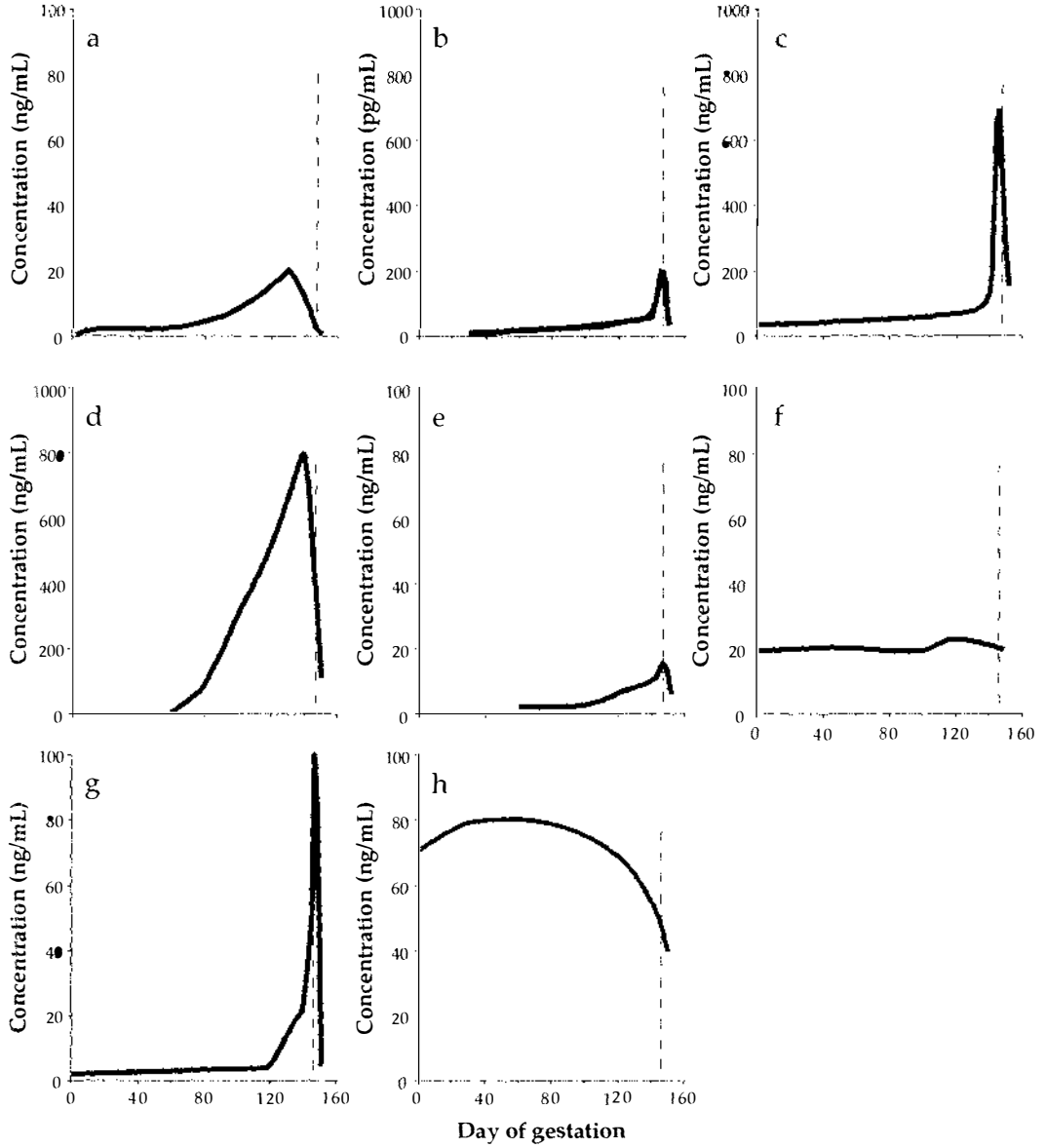

Figure 1.3: Endocrine changes during pregnancy in sheep. (a) progesterone, (b) oestrone, (c) prolactin, (d) placental lactogen, (e) growth hormone, (f) insulin-like growth factor-I, (g) cortisol and (h) thyroxine.

1.4 HORMONAL REGULATION OF WOOL GROWTH

1.4.1 Introduction

Hormonal regulation of wool growth was first reviewed by Ferguson *et al.* (1965) but studies were limited by the inability to accurately assay many plasma hormones at that time. In a later review, Wallace (1979a) noted that there had been few attempts to relate wool growth to concentrations of hormones in plasma in the intervening years. This was despite methods being

available for measuring plasma concentrations of hormones secreted from the pituitary, thyroid, adrenal gland and the ovaries. Both authors concluded that, although hormones have an important influence on wool growth, the existing endocrine knowledge did not account for many of the conflicting results in similar environmental conditions.

Changes in wool growth are readily observed after the removal of endocrine glands. Hypophysectomy removed the source of all pituitary hormones and eventually stopped wool growth (Ferguson *et al.*, 1965), which unequivocally proved that pituitary-derived hormones were required to maintain wool growth. Similarly the removal of the thyroid gland, adrenal glands or ovaries has been demonstrated to have an effect on wool growth in a variety of sheep breeds. Other common investigative methods have included the local and systemic administration of hormones and pharmacological manipulation of hormones.

1.4.2 Progesterone

The literature records only one study that has investigated the effects of progesterone on wool growth. Slen and Connell (1958) gave intramuscular injections of 125–250 mg progesterone every 14 days to non-pregnant Corriedale ewes and castrated males for a period of 7 months. At these doses progesterone had no significant effect on wool growth as measured by monthly clean wool weights compared to their controls. However, given that progesterone concentration was not measured in this study, and that levels do increase markedly in the pregnant ewe (Figure 1.3), it is premature to dismiss the possibility that progesterone could have an influence on wool growth based on this single experiment.

In other species such as the rat (Mohn, 1958) and mouse (Davis, 1963) progesterone was also found not to affect hair growth, although Farooq *et al.* (1963) reported that its administration to pregnant rabbits did decrease the hair shedding normally observed shortly before parturition. Conversely, in

the ferret, progesterone treatment during oestrus seemed to hasten both the shedding of hair and the growth of new hair (Harvey & MacFarlane, 1958). It is interesting to note that during pregnancy in the female ferret, there is a stimulation of hair growth and shedding takes place.

1.4.3 Oestrogens

There is some evidence that exogenous oestrogens may depress wool growth. The earliest account was by Slen and Connell (1958) who reported that a second 12 mg dose of the synthetic oestrogen diethylstilbestrol, 3 months after the first, resulted in a significant decrease in the average clean wool weight in Corriedale ewe lambs, a response which was independent of changes in body weight. In contrast, the administration of a single implant had no effect on wool growth. They also demonstrated that a reduction in clean wool weight was observed in Corriedale ewes treated orally with 4 mg oestradiol implants at 14-day intervals and 3 mg diethylstilbestrol every 3 days. This decline in wool growth was accompanied by a significant decrease in fibre length but not in fibre diameter. Oestradiol and diethylstilbestrol treatment resulted in a reduction in thyroid weight, but an increased pituitary and adrenal gland weight, suggesting that the reduction in wool production may have resulted from a lowered thyroid activity and/or adrenal and pituitary stimulation.

Harvey and MacFarlane (1958) suggested that oestrogens should inhibit hair growth and shedding in the ferret, though treatment with oestradiol had been ineffective as an inhibitor of hair growth during anoestrus. In a recent experiment oestrogen administered as a slow release implant was a powerful inhibitor of fibre growth initiation in male ferrets (Yu *et al.*, 1998). In the rat (Hale & Ebling, 1975) as well as reducing the rate of hair growth, oestradiol reduced the duration of the growing period of hairs.

In summary, high doses of exogenous oestrogens do have a depressant effect on wool growth in both non-pregnant ewes and castrated males. However, it is doubtful that this hormone has any effect on wool production during

pregnancy, as plasma oestrogen concentrations remain extremely low until the final stages of gestation when wool growth is already depressed.

1.4.4 Melatonin

Although the pineal hormone melatonin is thought to have a direct involvement in the development of secondary wool follicles in the fetus or neonate (Foldes & Maxwell, 1993) the effect on wool growth in adult sheep is not clear. Pinealectomy resulted in a short-term inhibitory effect on wool growth in young Merino lambs (Foldes & Maxwell, 1993) and was possibly mediated via a delayed development of secondary wool follicles (Foldes & Maxwell, 1993). In other experiments, however, acute or chronic melatonin treatment had no effect on wool growth (Harris *et al.*, 1989; Foldes *et al.*, 1990; McCloghry *et al.*, 1992). Furthermore, melatonin supplementation, at concentrations above normal physiological levels, had little effect on the fibre production rate of isolated wool follicles maintained in a control media (Winder *et al.*, 1995).

However, melatonin has been shown to influence wool production in primitive breeds, by regulating PRL secretion from the pituitary gland. Implantation with melatonin influenced PRL secretion and moulting of wool in Soay (Lincoln & Ebling, 1985) while, in the Limousine ram, the seasonal cycle of kemp follicles was abolished in pinealectomised animals (Allain *et al.*, 1986).

1.4.5 Prolactin

There is an abundance of evidence linking seasonal variation in plasma PRL concentrations with changes in wool growth rate in shedding breeds of sheep (Lincoln & Ebling, 1985; Allain *et al.*, 1986; Lincoln, 1990). The variation between breeds in their responsiveness to PRL is dependent on the degree of seasonality of wool growth. The highly seasonal Mouflon lies at one end of the spectrum, while the Merino is the least responsive to changes in circulating

PRL concentration (Lincoln, 1990). Recently, PRL receptors have been identified in the wool follicle (Choy *et al.*, 1995; 1997), providing further evidence that PRL may have a direct action on the wool follicle.

The earliest studies in which exogenous PRL (5 mg/day) was injected into hypophysectomised Merino sheep (Ferguson *et al.*, 1965), and into normal sheep, observed no effect on wool growth (Wallace, 1979a). In another study (Downes & Wallace, 1965), intradermal injections of PRL given at a range of doses gave an inconsistent response. At the highest dose of 625 μg a 20% increase in fibre length growth rate was measured, while intermediate dose rates ranging from 25 to 125 μg caused a reduction in length growth rate of individual fibres and a concurrent increase in fibre diameter. More recently, an experiment using New Zealand Wiltshire sheep showed that, while subcutaneous injections of PRL (5–50 mg/day) elevated circulating concentrations of PRL above those of controls, the decline in secondary follicle activity was variable and not dose-dependent (Pearson *et al.*, 1997). These studies demonstrate that although PRL generally does have a local influence on wool growth, the effect is inconclusive.

Of more interest, natural and experimental increases in daylength have been shown to have a short-term inhibitory effect on growing wool follicles in the Wiltshire, mediated through rising plasma concentrations of PRL (Pearson *et al.*, 1993b, 1996). The decreased follicle activity closely followed or was concurrent with increases in plasma PRL concentration. The follicles then rapidly resumed growth, accompanied by shedding of the fleece. Suppression of this PRL surge with bromocriptine prevents fleece shedding (Pearson *et al.*, 1993a). Significantly, the SD:LD induced PRL profile is similar to that observed near parturition (Figure 1.3), and allows for the possibility that PRL has a similar role in the regulation of the wool growth depression during pregnancy and lactation.

Suppression of the spring rise in PRL in Scottish Blackface ewes (Curlewis *et al.*, 1991), or induction of a premature decline in PRL secretion in Romney ewes during the summer (McCloghry *et al.*, 1993) by treatment with bromocriptine had no effect on wool growth. In the latter experiment, the sheep had already experienced the summer peak in PRL concentration, which may explain the lack of a wool growth response. Dolling *et al.* (1986) found that the wool growth rate in Merino sheep housed indoors and fed a constant diet was lower, but not significantly so, compared to their controls after a 6-week treatment with bromocriptine.

PRL may influence fibre growth by interacting with other hormones including melatonin. Farooq *et al.* (1963) found evidence that PRL, in conjunction with oestradiol and progesterone, was involved in control of hair shedding in the rabbit which normally occurs shortly before parturition. In the absence of PRL, treatment with oestradiol and progesterone failed to produce hair loosening.

1.4.6 Placental lactogen

Wallace (1979a) noted that, nothing was known of the effects of PL on wool growth. Since that time our understanding of the regulation of follicle growth by PL has been little advanced, as the role PL may play in this growth process has been largely overlooked. However, a more recent study (Wickham *et al.*, 1992), provided the first evidence that follicle development in newborn lambs may be regulated by PL as the infusion of 1.2 mg/day PL into chronically catheterised Dorset fetuses between days 122–136 of gestation resulted in a 17% reduction in the secondary/primary immature follicle ratio. The suppression of the initiation of new secondary follicles was attributed to the increase in the fetal arterial PL concentrations during the treatment period.

While specific PL receptors have been reported in ovine fetal liver (Freemark & Comer, 1989) they have not been identified in the skin or wool follicle of the adult sheep. However, there is increasing evidence that PL binds to the GH

receptor with higher affinity than GH in this species (Emane *et al.*, 1986; Breier *et al.*, 1994). GH receptor binding proteins are present in hair follicles of the rat, rabbit and human (Lobie *et al.*, 1990) and there is also a significant increase in plasma PL concentration from mid-gestation in the pregnant ewe (Figure 1.3). Therefore, the possibility exists that PL could exert a direct inhibitory influence on wool growth via the GH receptor, or potentiate the effects of GH (Ogawa *et al.*, 1995). Contrary to this evidence, is the fact that PL does not appear to have any GH-like effects in lactating ewes (Min *et al.*, 1997), nor does it alter plasma IGF-I concentration in pregnant and lactating ewes (Bassett *et al.*, 1993; Min *et al.*, 1997).

1.4.7 Growth Hormone

A GH receptor binding protein has been localised in the epidermis and surrounding tissues in other species (Lobie *et al.*, 1990). This suggests that GH has the potential to have a direct effect on the wool follicle, although intradermal injections of GH did not change length growth rate (Downes & Wallace, 1965; Wynn, 1982).

Daily injections of ovine GH generally depress wool growth. Wynn *et al.* (1988) reported that a 10 mg dose of GH for a 4-week period in Merino ewes decreased wool growth rate by 20% during GH treatment, largely because of reduced fibre diameter, which is consistent with the other experiments (Wallace, 1979a; Wynn, 1982). Following cessation of GH administration, wool growth was restored to normal growth levels within 14 days and then increased by up to 20% above control levels. This prolonged period of compensatory wool growth, occurring after the cessation of treatment can persist for up to 10 weeks and results from a proportional increase in fibre length and diameter (Wynn *et al.*, 1988). Wallace (1979a) found that only fibre diameter increased during the post-treatment period, while other researchers (Ferguson *et al.*, 1965; Wheatley *et al.*, 1966), noted an initial depression of wool growth during the treatment period.

GH does not appear to have a permissive role on wool growth as exogenous GH administration does not initiate wool growth in hypophysectomised sheep (Ferguson *et al.*, 1965). No increase in wool growth in hypophysectomised sheep maintained on T_4 has been observed either (Ferguson *et al.*, 1965), suggesting that the action of GH on wool production is dependent on another pituitary hormone.

It is intriguing to note that increased wool growth is usually observed in sheep treated with bovine GH. Sun *et al.* (1992) reported that daily administration of bovine somatotropin to Romney lambs for a 14-week period from birth, elevated plasma concentrations with a concurrent increase in clean wool growth during the treatment period. There was no effect of GH on wool growth during the post-treatment period, or on other wool measurements. Likewise, Dorset cross lambs given daily injections of 0.1 mg bovine GH grew one-third more clean wool (Johnsson *et al.*, 1987) and had a greater greasy fleece weight than control lambs (Johnsson *et al.*, 1985). This effect could be mediated by IGF production, which in addition to GH, is also modulated by circulating PRL (Schalch *et al.*, 1979) and insulin (Daughaday *et al.*, 1976). In the study by Johnsson *et al.* (1985), plasma concentrations of PRL and insulin were reduced by bromocriptine administration.

There is little doubt that administration of exogenous GH does affect wool growth, however, the size of the change and the duration of the response varies markedly between studies. It is possible that the increase in GH concentration during late gestation (Figure 1.3) has a direct inhibitory effect on wool growth or induces a response through the actions of other hormones in the circulation.

1.4.8 Insulin-like Growth Factors

IGF receptors are present in the ovine wool follicle (Nixon *et al.*, 1997) but their exact role in the control of wool growth remains uncertain. IGF receptors have also been shown to be responsive to PRL (Murphy *et al.*, 1988; Reiter *et al.*,

1992), therefore the PRL action on the wool follicle could be partially mediated via the IGFs. However, it would appear that IGFs have little or no direct effect on wool growth. Chronic treatment with IGF-I (Cottam *et al.*, 1992) or a variant of IGF-I (Hocking Edwards *et al.*, 1995) at physiological doses in well-fed castrated sheep had no effect on wool growth rate or any parameters associated with wool production, despite increased circulating plasma IGF-I concentrations. Furthermore, greasy fleece weights of Romney lambs obtained at shearing were not related to plasma IGF-I concentrations (Roberts *et al.*, 1990), nor was follicle bulb diameter affected by IGF-I treatment (Harris *et al.*, 1993; Hocking Edwards *et al.*, 1995).

Despite this weight of evidence, IGFs may be indirectly involved in hormone-influenced fibre growth via paracrine mechanisms. Hormones, including GH and PRL, may alter the synthesis and signalling capabilities of IGFs within the wool follicle, leading to changes in growth rate and/or differentiation of follicle cell lines.

1.4.9 Insulin

All studies (Wallace, 1979a; Wynn, 1982; Hocking Edwards *et al.*, 1995) have concluded that increases in plasma insulin concentration had no known quantitative effect on wool production.

1.4.10 Corticosteroids

There is an abundance of evidence that one class of corticosteroids, the glucocorticoids, depress wool growth when present in high concentrations (Ferguson *et al.*, 1965; Chapman & Bassett, 1970; Wallace, 1979a). Plasma cortisol concentrations are generally low (Bassett & Hinks, 1969), but can be elevated in sheep subjected to physical or emotional stress (Ferguson *et al.*, 1965). Depending upon the duration and severity of the stress, this could be sufficient to cause some degree of wool growth depression due to increased

adrenocortical activity (Wallace, 1979a) leading to fleece tenderness (Lindner & Ferguson, 1956).

Chapman and Bassett (1970) injected cortisol during a 12-week period, increasing the dose every 3 weeks, so that by the end of the 12 weeks, plasma concentrations of 50 ng/mL caused the complete cessation of wool growth. The effects of cortisol were variable because at low plasma concentrations, fibre diameter decreased and length growth rate was unchanged, but as the plasma concentration increased, the fibre length growth rate was progressively depressed. A similar administration of 1.8 mg/kg cortisone for 8 weeks also depressed wool growth (Lindner & Ferguson, 1956). Higher doses were associated with a reduction in fibre diameter and later the stimulation of fibre shedding and formation of brush ends (Thwaites, 1972). It appears that a prolonged elevation of plasma cortisol concentration is required before a significant reduction in wool growth rate is observed (Scobie & Hynd, 1995).

Cortisol applied topically to the skin can also produce local changes consistent with those induced by injected cortisol (Lindner & Ferguson, 1956; Chapman & Bassett, 1970). Similarly, fibre diameter and length growth were significantly decreased in sheep treated with intramuscular injections of ACTH for 10 weeks (Lindner & Ferguson, 1956) but rose sharply after cessation of treatment. In contrast, Thwaites (1972) reported that ACTH administration had little effect on the fibre diameter in Merino ewes.

Synthetic corticosteroids (including dexamethasone, flumethasone and betamethasone) are powerful inhibitors of wool growth and have been used as defleecing agents (McDonald *et al.*, 1982). Either a single intramuscular injection (Ferguson *et al.*, 1965) or daily injections (McDonald *et al.*, 1982) of dexamethasone produce a prolonged depression of wool growth.

In some situations, glucocorticoids may also stimulate wool growth. This was first reported by Downes and Wallace (1965), who noted that low intradermal

doses (0.6 and 3 μg) of cortisol stimulated fibre growth rate around the site of injection, but this response was not seen in all fibres. At higher dosages (320 and 1600 μg) the rate was decreased, as was the fibre diameter, which led to increased tendency for the fibres to break. Dexamethasone treatment (0.1–12.5 μg) also caused a similar, but less marked, pattern of response.

The mechanism by which glucocorticoids depress wool growth is uncertain but the evidence suggests that cortisol and its analogues appear to act directly on the wool follicle to suppress wool growth. Circulating levels of corticosteroids in the pregnant sheep are only elevated in the last few weeks of gestation (Figure 1.3), and Chapman and Bassett (1970) showed that plasma concentrations greater than 30 ng/mL were sufficient to reduce wool growth in sheep on a restricted diet. Although acute changes in cortisol concentration are unlikely to effect wool growth (Scobie & Hynd, 1995), the increased levels nearer term would be sufficient to produce an inhibitory response.

1.4.11 Thyroid Hormones

It is now widely recognised that the presence of thyroid hormones is necessary for normal wool growth and normal follicle development (Ferguson *et al.*, 1965; Maddocks *et al.*, 1985). Thyroidectomy decreases wool growth (Theriez & Rougeot, 1962; Ferguson *et al.*, 1965; Rougeot, 1965) by 30 to 60% of the normal rate in sheep maintained on a constant feed intake. The reduction in wool growth appears to be due entirely to a decrease in fibre length growth rate which occurred over a period of about 1 month as the thyroid hormones were gradually eliminated from the circulation (Theriez & Rougeot, 1962; Rougeot, 1965). Wool growth can be restored to, or increased above, pre-operative rates in thyroidectomised sheep by daily injections of T_4 (Ferguson, 1958; Ferguson *et al.*, 1965; Rougeot, 1965), but these treatments have no effect on fibre diameter (Theriez & Rougeot, 1962; Rougeot, 1965). These findings are supported by Downes and Wallace (1965), who reported that intradermal injections of 10 μg T_4 caused local increases in fibre length growth rate but had little effect on fibre diameter.

In a more recent experiment (Maddocks *et al.*, 1985), thyroidectomised Merino rams were given replacement therapy equivalent to 0, 30, 100 and 300% normal plasma T_4 concentrations. They noted that one of the major differences between their study and those by Ferguson and co-workers (1958; 1965) was that the sheep received T_4 doses equivalent to, or greater than, the normal T_4 secretion rate in the earlier studies. Similar results were produced using comparable T_4 concentrations, but the most significant result of their study was that T_4 concentrations only 30% of those found in normal plasma were sufficient to maintain wool growth at a normal rate. This supports the idea that T_4 plays a facilitatory role rather than a regulatory role in wool growth. Under normal conditions, circulating T_4 concentrations are rarely low enough to prevent wool growth (Maddocks *et al.*, 1985). An interesting outcome of these studies was the fact that high doses of T_4 elevate wool growth above normal in thyroidectomised, but not in hypophysectomised, sheep (Ferguson *et al.*, 1965), suggestive of the regulatory control exerted by thyroid-stimulating hormone.

In mature Romney ewes, injections of 0.75–5 mg/day of T_4 in the winter months can cause a marked increase in wool growth, but this effect was not replicated using T_4 implants (Kirton *et al.*, 1959). However, subcutaneous T_4 implants (90 mg) did increase the rate of wool growth in active follicles (Lambourne, 1964). Additionally, administration of T_4 to Merino lambs can re-establish the interrupted development of the secondary follicles following thyroidectomy at birth (Ferguson *et al.*, 1956).

There is a seasonal change in plasma T_4 concentration but this did not correlate with seasonal changes in the wool growth rate of Merino × Border Leicester castrated males maintained on a constant feed intake (Wallace, 1979b). As some seasonal changes in wool growth also occurred in thyroidectomised animals maintained with a constant amount of T_4 (Ferguson *et al.*, 1965), it is unlikely that seasonal fluctuations in T_4 were involved in seasonal wool growth modulation.

It is clear that the presence of thyroid hormones is necessary for the normal development of wool follicles and growth of fibres. However, there is little evidence to suggest that T_4 has any influence on the depression in wool growth during pregnancy as the low levels associated with reduced wool growth are unlikely to occur in the pregnant ewe (Figure 1.3).

1.4.12 Catecholamines

Catecholamines also play an important role in the regulation of wool growth. Adrenaline and noradrenaline act directly on wool follicles to reduce the rate of cell division (Scobie *et al.*, 1992; 1994), while the depression in wool growth observed from prolonged exposure to cold *(Section 1.2.4)* is associated with a reduction in blood flow caused by the secretion of catecholamines from sympathetic nerve terminals (Scobie *et al.*, 1994).

1.4.13 Summary

The studies reported here have demonstrated the importance of the endocrine system in influencing the rate of wool growth in sheep. There is little evidence to suggest that progesterone or oestrogen play an important role in the control of wool growth but there is the possibility that they mediate or potentiate the effect of other hormones. Increased PL concentration has been demonstrated to reduce the secondary/primary follicle ratio in neonates. Given the evidence that PL has a high affinity for the GH receptor, and the PRL receptor in some circumstances, this hormone remains a strong candidate. Despite the presence of IGF receptors in the wool follicle, there have been no reports of a direct effect of IGFs on wool production. Any such action is likely to be mediated via the paracrine mechanisms within the wool follicle that could be influenced by GH or PRL.

The more profound effect of hypophysectomy compared with that of thyroidectomy points to the involvement of pituitary hormones other than thyroid-stimulating hormone, which appears to be essential in the

maintenance of normal wool growth. The pituitary exerts both stimulatory and inhibitory influences through the secretion of thyroid-stimulating hormone and ACTH respectively, but variation in thyroid or adrenocortical activity does not seem to account fully for observed natural variations in wool growth or those associated with pregnancy.

Removal of the pituitary gland in sheep is followed by a reduction or total suppression of wool growth, which can be partly overcome by treatment with T_4, but not with GH or PRL. This indicates that pituitary hormones other than GH or PRL are required for normal wool growth. However GH or PRL, or both, still have a regulatory role on wool growth in the sheep. Both GH and PRL, which are elevated during late pregnancy, have been shown to inhibit, and on occasion stimulate, growth in a variety of sheep breeds. Any such rise could be the cause of the wool growth depression observed at this time.

1.5 CONCLUSIONS

From this review of the literature it is clear that many breeds of sheep exhibit seasonal variations in wool growth. The winter decline in wool production is superimposed on an additional depression in the pregnant ewe which cannot be prevented by nutritional management. This is suggestive of an endocrine signalling mechanism but our understanding of these biological processes and, even which hormones are involved, is rudimentary.

The relationship between changes in plasma PRL concentrations and fibre growth is well established in mustelids, goats and in shedding breeds of sheep. In highly developed sheep breeds, however, there is only circumstantial, and sometimes conflicting, evidence linking PRL to fibre growth. More importantly, there is little information available in breeding ewes. Nevertheless, PRL does display considerable seasonal variation in modern sheep breeds, and is also markedly influenced by reproductive status, with PRL levels increasing sharply in late gestation and fluctuating throughout

lactation. Furthermore, PRL receptors are present in the wool follicle and this hormone has been reported to have both stimulatory and inhibitory effects on wool growth. Given this wealth of evidence it seems possible that the depression in wool production in the breeding ewe could also be partially attributable to the changes in circulating PRL concentrations during pregnancy and lactation. Alternatively, as receptors for other hormones have also been identified in or near the hair follicle, fibre growth could be mediated by a number of other hormones. Another interpretation is that these hormones could interact with PRL to regulate fibre growth.

The project described in this thesis focused on the effects of seasonal and experimental changes in PRL concentration on wool growth in pregnant Romney ewes, the dominant sheep breed in the New Zealand wool industry. The aims of this project were to: *(i)* determine the period of minimal wool production in spring- and winter-lambing ewes without the confounding effects of differences in nutrient supply; *(ii)* relate changes in growth rates to changes in fibre characteristics; *(iii)* characterize the plasma PRL profile over pregnancy, parturition, and lactation; and *(iv)* determine the influence of changes in circulating PRL concentrations on wool growth in the pregnant and lactating Romney ewe.

CHAPTER TWO

General Materials and Methods

2.1 General Introduction

A series of 4 experiments was undertaken involving non-pregnant and breeding Romney ewes of a similar age. The sheep were held indoors at the Ruakura Agricultural Research Centre, Hamilton (latitude 37°46'S, longitude 175°19'E), under controlled conditions of diet and environment and weighed regularly. These experiments all involved the collection of jugular blood samples and midside patch wool samples, and treatment with bromocriptine, which will be described in detail in this chapter. Individual experimental designs will be detailed in their respective experimental chapters in addition to any methodologies specific to that experiment. Some sheep failed to adapt to a pelleted diet and only the number of experimental sheep that started each trial are reported. Additionally, any sheep excluded because of poor health during the experiment are not included in the results. All experimental procedures in this study were approved by the AgResearch Ruakura Animal Ethics Committee.

2.2 Animal Health

The sheep were treated for internal parasites on arrival at Ruakura, immunised against Salmonella with Salvexin (Pitman-Moore, Upper Hutt, NZ) and treated with Vetivax 5 plus selenium (Bomac Laboratories Ltd., Manukau City, NZ) to protect against a variety of clostridial (Pulpy Kidney, Tetanus, Malignant Oedema, Blackleg and Black Disease) and selenium-responsive diseases. Additionally all pregnant ewes were vaccinated with Campylovexin (Pitman-Moore, Upper Hutt, NZ) to prevent abortion due to campylobacteriosis and given a further treatment of Vetivax 2 weeks prior to lambing. An oral dose of 4 mL potassium iodide was given to all pregnant sheep on days 90 and 120 of gestation to prevent goitre in the lamb. The sheep were also dosed with 10 mL of a 10% zinc sulphate solution at weekly intervals to prevent copper toxicity associated with the pelleted diet.

2.3 Diet

The sheep were accustomed to a pelleted diet (Country Harvest Stock Feed, Cambridge, NZ) during their first 2–3 weeks at Ruakura.

Table 2.1: Composition of the diet.

Lucerne	60%	Dry Matter	95.1%
Barley	30%	Protein	14.6%
Linseed oil	5%	Ash	19.5%
Molasses	5%	Digestible fibre	33.6%
		Non-digestible fibre	58.9%
		Carbohydrate	15.9%

2.3.1 Rationale of feeding regimen

The aim of the feeding regimen was to feed the experimental sheep to a constant maternal live weight to remove or reduce the effect of variable nutrient availability on wool follicle growth.

The estimated total metabolisable energy (ME) requirements (MJ/day) for pregnancy in pen-fed ewes at different stages of gestation were determined using the data of Rattray and Trigg (1979). Additional references used to calculate the energy requirements in twin-bearing ewes included Lodge and Heaney (1973) and Russel *et al.* (1977).

Rattray and Trigg (1979) conducted 2 experiments to provide information on the effect of ewe live weight or body condition on fetal growth during late gestation. In the first experiment, 5 different feeding levels were offered to twin-bearing ewes penned indoors from day 98 of gestation to approximately day 140 when they were slaughtered. These feeding levels equated to maintenance (40 g DM/$W^{0.73}$ for non-pregnant sheep, where DM = dry matter and $W^{0.73}$ = metabolic live weight), 1.25, 1.50, 1.75 and 2.00 times maintenance (M). Feed was increased weekly from 85% to 90%, 95%, 100%, 110% and lastly to 120% of the nominal feeding level. The results showed that all groups

gained live weight, largely as the result of the weight of the conceptus, but ewes on the lower level of feeding lost a considerable amount of body weight while those on the highest level gained weight. Ewes receiving the 1.5 × M feeding level had only a –0.40 kg net empty weight change over this 6-week period.

The second experiment of Rattray and Trigg (1979) used both single- and twin-bearing ewes that were given 2 levels of feeding (low = M, high = 1.75 × M) from day 95 of gestation until slaughter on approximately day 135 of gestation. These ewes were also shorn on day 95 and were penned outdoors. Weight losses were greater at a similar level of feeding than those in the first experiment. This was thought to be due to the higher maintenance requirements of sheep housed outdoors and their shorn condition.

Based on this information, a daily feed regimen was developed for single-bearing ewes housed indoors to be used in the current trials. The standard feeding level of 1.5 × M was chosen and multiplied by the weekly increments (85%, 90%, 95%, 100%, 110% and 120%) used previously to produce an allowance (A, expressed in g/day/$W^{0.73}$) over the last 6 weeks of pregnancy. This allowance factor was fitted against the day of gestation to produce the exponential equation:

$$A = 6.074 \times 10^{-6}\ day^3 - 1.895 \times 10^{-3}\ day^2 + 2.070 \times 10^{-1}\ day - 6.542$$

This equation was used to adjust the daily feed ration of pregnant sheep as gestation advanced (e.g. the allowance changed between 1.00 on day 80 and 1.81 on day 135). For a non-pregnant sheep the allowance was set and remained at 1.00. Therefore, daily feed intake (g) = $A \times M \times W^{0.73}$.

The calculated daily feed intake of a standard 50 kg non-pregnant ewe, pregnant ewe with a single lamb, and ewe bearing twin lambs is shown in

Figure 2.1. The effect of sheep live weight in determining daily feed intake is illustrated in Figure 2.2.

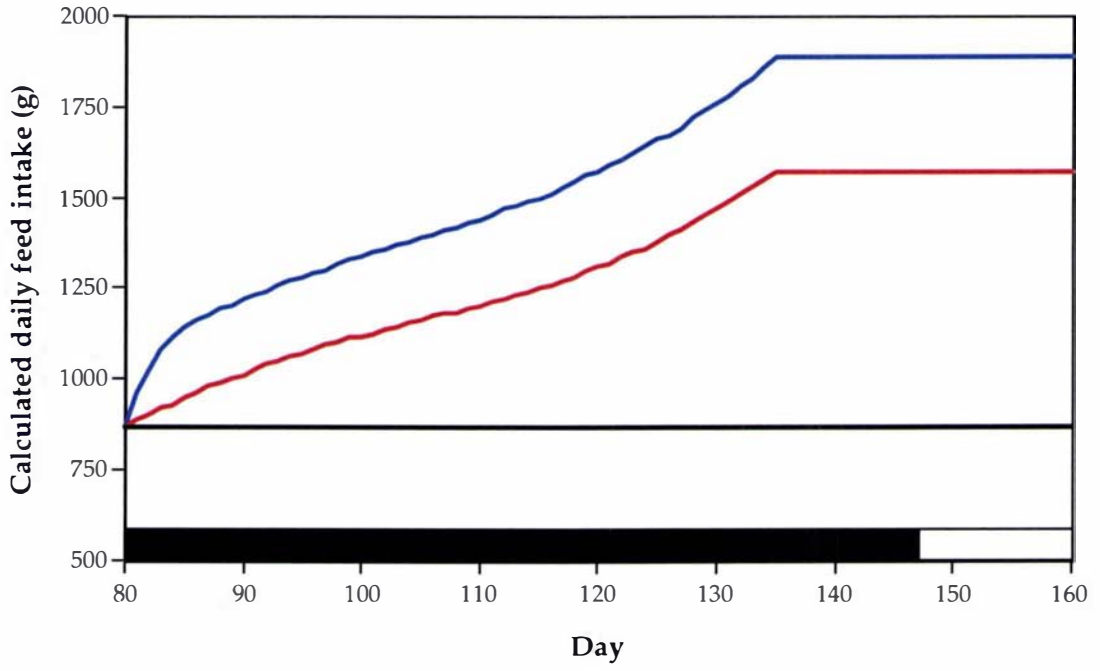

Figure 2.1: Calculated daily feed intakes for a 50 kg non-pregnant (–) and 50 kg pregnant ewes carrying either 1 (–) or 2 (–) lambs. Solid bar represents gestation; open bar represents lactation.

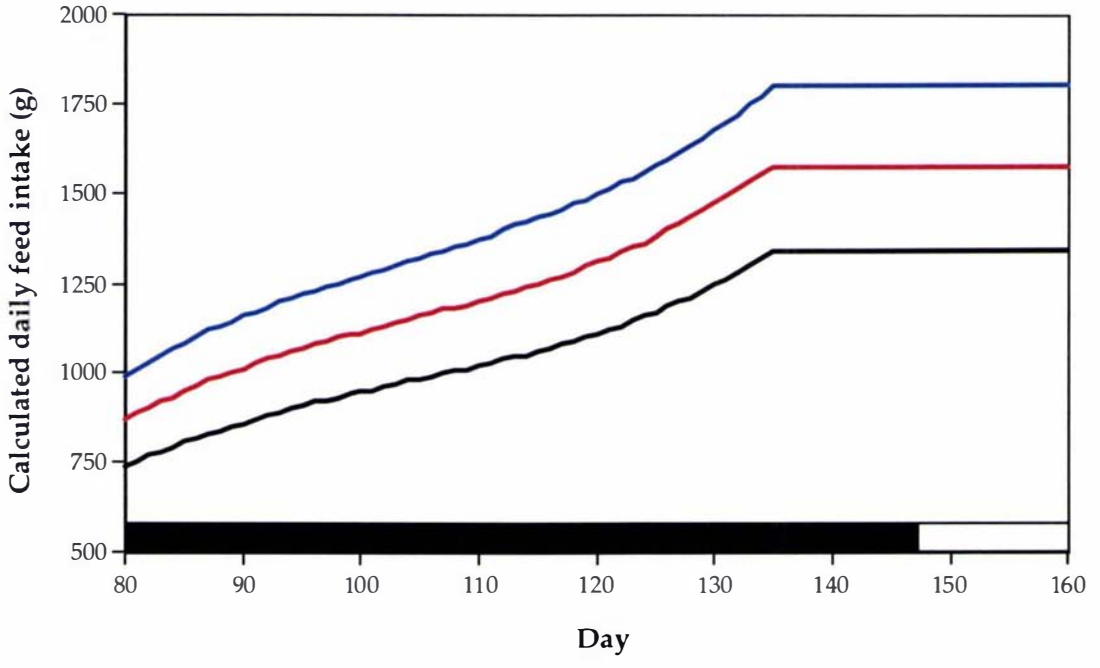

Figure 2.2: Calculated daily feed intake for pregnant ewes weighing 40 kg (–), 50 kg (–) or 60 kg (–) at day 80 of gestation. Solid bar represents gestation; open bar represents lactation.

In practice, pregnant ewes were fed at the same level as non-pregnant ewes until approximately day 90 of gestation (mean length is 147 ± 3 days), after which rations were increased daily until day 135 of gestation (Figure 2.1). After this time the daily feed ration remained constant until the lambs were weaned. From time to time the daily ration (approximately 800–1000 g/head/day) was adjusted on an individual basis to maintain live weight as constant as possible (for non-pregnant ewes or following parturition). All sheep were confined to individual pens and fed individual weighed rations between 0800 h and 1000 h daily. Daily feed intake was measured from weighing the dry weight refusals and deducting it from the food offered the previous day. Fresh water was available *ad libitum.*

2.4 Light Treatment

Three different light treatments were used:

Natural Days (ND)

The sheep were exposed to the normal seasonal photoperiod with no artificial manipulation of light regimen.

Short Days (SD)

The sheep were held under short day conditions of 8 h light and 16 h dark (8L:16D) through the use of window shutters opened at 0830 h and closed at 1630 h every day.

Long Days (LD)

The sheep were exposed to long day conditions of 16 h light and 8 h dark (16L:8D) through the use of a timer on fluorescent lighting activated from 0500 h–2100 h every day in addition to natural photoperiod.

Light levels, relative humidity and temperature were monitored at 15 min intervals using a Z88 data logger (Cambridge Computing, UK). Ambient air

temperature was not controlled but daily ambient outdoor air temperature and the hours of daylight at Ruakura were recorded at 0900 h for each experiment. The light intensity from the fluorescent lighting alone, determined 1 m above the floor at 5 locations, ranged between 245 and 275 lux in LD conditions (artificial fluorescent lighting only) while the light intensity in SD conditions once the shutters were closed was < 0.5 lux.

2.5 Live weight and Lamb measurements

All sheep and lambs were weighed on electronic scales (Howard's Weigh, Napier, NZ) regularly (weekly to monthly) during the trial to monitor changes in live weight. Lamb birth weight, crown-rump length, girth, hindleg length and head width were recorded within 24 h of birth. All lambs were identified by eartag and their tails removed by use of a rubber ring. Male lambs were also castrated at this time.

2.6 Wool Sampling

The sheep were shorn on entry to each experiment and a standardised 100 × 100 mm area was marked on the right side of each sheep with the aid of a template. To assess fleece growth rate, the wool within this area was clipped at monthly intervals using small animal clippers (Oster; Milwaukee, WI, USA) fitted with blade No. 70 (size 000). The sheep were shorn again at the conclusion of each experiment, the greasy fleece weighed, and a full length midside wool sample collected.

2.7 Wool Metrology

2.7.1 Scouring

The midside fleece samples and patch samples were weighed and then washed sequentially at a controlled temperature and time using non-ionic detergent (Teepol) in an adapted domestic washing machine. These steps are outlined below:

(i)	0.20 w/v detergent	75°C	4 min
(ii)	0.10 w/v detergent	60°C	4 min
(iii)	water	55°C	4 min

2.7.2 *Conditioning*

The scoured midside and patch wool samples were oven-dried at 50°C in a forced-draft oven for 10 min. Dried wool samples were conditioned for 48 h under standardised atmospheric conditions (20°C, 65% relative humidity) to attain 16% regain (Ryder & Stephenson, 1968) before reweighing to determine the washing yield.

2.7.3 *Measurement of fibre parameters*

Mean staple length, mean staple tensile strength and position of break were measured by WRONZ Developments Ltd., Christchurch. Staple length was determined by laying greasy staples that were not under tension against a ruler and measuring the length to ± 5 mm. Staple length was measured on 12 randomly selected staples per sheep. Staple tensile strength was measured on 7 randomly selected greasy staples per sheep using standard procedures for an Agritest system (Agritest Pty Ltd., Sydney, NSW, Australia). The tip and base of each staple broken in the strength test were weighed and the weight of the tip as a proportion of the total staple weight was used to indicate the position of break. Corebulk was measured according to the corebulk test method (Standards Association of New Zealand, 1994) by SGS New Zealand Ltd., Wellington.

Mean fibre diameter, fibre diameter variation and mean fibre curvature of the scoured fleece samples were measured using the automated Optical Fibre Diameter Analyser (OFDA). Similarly, mean fibre diameter, fibre diameter variation and mean fibre curvature of a sub-sample of each clipped patch were also measured. Aspects of the use of OFDA for the measurement of fibre diameter (Brims, 1993) and fibre curvature (Edmunds, 1995; 1997) have been

outlined previously. The mean rate of growth of clean wool (g/day) for an individual ewe during each collection period was estimated by proportioning each ewe's clean fleece weight according to the relative weight of clean wool clipped from her midside during that period.

2.8 Autoradiography of Wool Fibres

Wool growth rates of individual fibres were assessed according to a modified method based on that of Friend and Robards (1995). This procedure enabled an accurate determination of the length growth rates of individual wool fibres.

2.8.1 Preparation and radiolabelling

After shearing, a single spot was tattooed on the left flank of each sheep using a portable 12V electronic tattooing instrument (National Lightweight, Philadelphia, PA, USA). A 3 cc tuberculin syringe with a fine 30G needle was used to inject 300 µL of a saline solution containing 1 µCi of L-[^{35}S]cysteine (Amersham International, Australia) intradermally in the centre of each tattoo site to a depth of 1 mm. The injections were repeated into the same skin site at weekly intervals.

2.8.2 Removal of fibres and washing

At the end of the trial, the labelled wool staples were clipped off at skin level and tied with cotton thread close to the cut end of the wool fibres. Each wool sample was then washed sequentially in dichloromethane, absolute ethanol and distilled water for a minimum of 20 s in each solution. The wool samples were then allowed to air dry overnight.

2.8.3 Initial exposure

The wool samples were initially exposed to X-ray sensitive ^{3}H-Hyperfilm (Amersham International, Australia) by taping them to a cardboard sheet. Under darkroom conditions, the wool staples were placed in contact with X-

ray sensitive film in an Amersham Hypercassette for an exposure time of between 2 to 7 days at 4°C.

2.8.4 X-ray film development

The X-ray film was developed using the recommended protocol with Ilford (Australia) solutions:

Developer	Phenisol developer (diluted 1:4)	4 min
Stop	3% acetic acid	30 s
Fixer	Hypam fixer (dilution 1:4 containing	
	Hypam hardener diluted 1:40)	4 min

The film was then washed in running water for 30 min before drying.

2.8.5 Mounting of fibres to slides

Labelled clean 26 × 76 mm glass slides with a frosted end were coated in 10% gelatine solution containing 0.05% chrome alum (50 g gelatine, 0.25 g chrome alum/500 mL distilled water) and oven dried at 50°C. Individual wool fibres were plucked at random from the staple and adhered to the reverse side of the slide to maximise the full length of the slide. A moistened paint brush was used to brush along the length of the fibre moistening the dried gelatine. A total of 50 fibres (5 fibres per slide) were mounted for each sheep. After air-drying overnight, the fibres were re-brushed using a warm gelatine solution to prevent fibre lifting.

2.8.6 Coating slides with emulsion

All emulsion coating steps were performed under standard dark room conditions using an Ilford safelight 902 with a 15W light bulb. Twenty millilitre bottles of LM-1 Nuclear Emulsion (Amersham International, Australia) were melted in a 43°C water bath for at least 10 min. The required volume of melted emulsion was poured from the bottle into an Amersham

dipping chamber covered with aluminium foil. Both the bottle and dipping chamber remained in the water bath throughout the coating procedure.

In a predetermined order, each slide was dipped in the dipping chamber using plastic forceps and the undersurface was wiped with paper towels before the slide was placed on an ice-chilled metal tray for 10 min. The slides were placed in a light-proof slide box containing silica gel and dried overnight in the dark, at room temperature. The following day the silica gel was replaced before the box was wrapped in aluminium foil and a black plastic bag to ensure the slides were not exposed to light. The box was then stored in an isotope-free refrigerator at 4°C for as long as necessary. Test slides were placed in a separate box for routine developing at monthly intervals to determine optimum exposure time. Silica gel was checked regularly to ensure it remained active and replaced if necessary.

2.8.7 Development of slides

After the required exposure time the boxes containing the slides were removed from the refrigerator and allowed to equilibrate to room temperature (about 2 h) in a darkened room before processing. The developing protocol was similar to that previously described.

Developer	Phenisol developer (undiluted)	5 min
Stop	Distilled water	30 s
Fixer	Hypam fixer (dilution 1:4 containing Hypam hardener diluted 1:40)	10 min

The slides were then rinsed through 3 changes of distilled water over 10 min and allowed to air-dry before being cover-slipped using DPX mountant (BDH Laboratory Supplies, Palmerston North, NZ).

2.8.8 Quantification of fibre length growth

Length growth rate of the wool fibre was measured by observing the radiolabelled signals on the 50 fibres under an Olympus SZ40 zoom stereo dissecting microscope connected to a Macintosh IIfx computer by a COHU 4713-2000 CCD digital camera mount (COHU Inc. Electronics Division, San Diego, CA, USA). The distance between radiolabelled signals on individual wool fibres was measured from a captured image (magnification 10 ×) using the NIH Image V1.61 image processing and analysis program. The microscope was calibrated using a stage micrometer.

2.9 Blood Sampling for Radioimmunoassay

Jugular samples were collected between 0800 h and 1000 h using 10 mL evacuated blood collection tubes containing 0.34 M EDTA as the anticoagulant (Vacutainer; Becton Dickinson, Rutherford, NJ, USA). Five millilitre samples were taken from the lambs. The blood was then centrifuged at 4°C for 20 min. The plasma was separated and stored at –20°C until analysed.

2.10 Bromocriptine Administration

A 50 mg dose of bromocriptine (Parlodel® LA; Sandoz Pharma Ltd., Basle, Switzerland) was given intramuscularly in the hindleg of some groups of sheep at 2-week intervals (mean dose of 3.3 mg/day) throughout the duration of treatment. A standard dose of 100 mg (1.8 mg/kg) was given to each ewe on the first day of treatment in the initial trial to ensure effective PRL suppression over parturition and/or early lactation. A standard 50 mg dose was used in all subsequent trials after plasma PRL levels were shown to have been successfully suppressed. Pregnant ewes were first treated with bromocriptine approximately 10 days before parturition (day 137 of gestation) or approximately 3 days following parturition, to allow the periparturient PRL surge.

2.11 PRL Radioimmunoassay

Ovine PRL concentration was measured in duplicate by double-antibody radioimmunoassay using ovine PRL (NIDDK-oPRL-I-2) for standards and radioiodination, and rabbit anti-ovine PRL antiserum (NIDDK-anti-oPRL-2).

2.11.1 Materials in radioimmunoassay kit

(i) ovine prolactin (NIDDK-oPRL-I-2), 500 µg/ampoule

(ii) ovine PRL antiserum (rabbit) (NIDDK-anti-oPRL-2), 1 mL, 1:30 dilution of 2% normal rabbit serum in phosphate-buffered saline (titre = 1:800,000)

2.11.2 Preparation of oPRL antigen

Prior to iodination, 20–50 µg aliquots of oPRL standard were weighed and dissolved in 0.05 M sodium phosphate buffer (pH 7.5) to a final concentration of 5µg/10 µL (i.e. 40 µg of hormone in 80 µL buffer). These 11 µL aliquots were then stored at –20°C for up to 2 months.

2.11.3 Preparation of buffers

All laboratory chemicals used were from BDH (BDH Laboratory Supplies, Palmerston North, NZ) unless stated otherwise.

(i) 0.5 M sodium phosphate (pH 7.4)

A	0.5 M $Na_2HPO_4.12H_2O$	35.8 g/500 mL
B	0.5 M $NaH_2PO_4.2H_2O$	7.8 g/100 mL

Solution A (4.28 mL) and solution B (0.72 mL) were mixed and made up to final volume of 500 mL with distilled water.

(ii) 0.01 M phosphate buffered saline (PBS) was prepared using stock sodium phosphate solutions:

A	0.5 M $Na_2HPO_4.12H_2O$	35.8 g/200 mL
B	0.5 M $NaH_2PO_4.2H_2O$	7.8 g/100 mL

For pH 7.6 buffer, 42.8 mL solution A, 7.2 mL solution B and 22.5 g NaCl were added and diluted to 2.5 L with distilled water. PBS was stored at room temperature (~20°C) until used.

(iii) 0.05 M sodium phosphate (pH 7.5) was made by diluting 0.5 M sodium phosphate buffer 10 times.

(iv) 1% bovine serum albumin (BSA)

BSA (Sigma, St. Louis, MO, USA) weighing 1.5 g was dissolved in 150 mL 0.01 M PBS for use as a carrier buffer in the column.

2.11.4 Preparation of lactoperoxidase (Sigma L8257, EC 1.11.1.7)

A 20 µg sample of lactoperoxidase enzyme was dissolved in 400 µL 0.5 M sodium phosphate buffer (pH 7.4) and stored in 20 µL aliquots (0.025 nmole) at –20°C for up to 6 months.

2.11.5 Preparation of hydrogen peroxide

A 30% stock solution of hydrogen peroxide was diluted 600 times by adding 10 µL stock solution to 5.99 mL distilled water. This solution was then further diluted 100 times by adding 10 µL of this solution to 990 µL water to give a final dilution of 1:60,000 (6.2 nmole/20 µL). This working solution was made for each iodination.

2.11.6 Iodination of oPRL using lactoperoxidase method

oPRL was iodinated by the lactoperoxidase method (Thorell & Johansson, 1971) using [^{125}I]iodide (New England Nuclear, Wilmington, DE, USA). In a 1.5 mL microtube the following solutions were added:

20 µL lactoperoxidase

10 µL oPRL

5 µL ^{125}I-iodide (0.5 mCi)

20 µL hydrogen peroxide

The microtube was vortexed gently 3 times to ensure that all the liquid was at the bottom. The reaction was allowed to proceed for 3 min. The reaction was stopped by adding 300 µL 0.05 M sodium phosphate buffer (pH 7.5) and immediately transferred to a prepared Sephadex column (G 50) for separation of labelled peptide from excess ^{125}I-iodide.

2.11.7 Preparation of assay solutions

(i) Assay diluent (EDTA)

0.01M PBS

1% BSA (5 g/500 mL)

0.01% sodium azide (Riedel-de-Haën, Seelze, Germany)

(50 mg/500 mL)

0.05 M EDTA disodium salt (9.31 g/500 mL)

Adjusted to pH 7.6 with NaOH.

(ii) 4% polyethylene glycol (PEG)

A 4% working solution was made up from dissolving a 20% stock solution of PEG6000 in 0.01 M PBS.

2.11.8 Preparation of oPRL standards

A 20–50 µg aliquot of oPRL was weighed and dissolved in 0.01 M $NaHCO_3$ to give a concentration of 100 µg/mL. This solution was diluted 10 times in assay diluent, giving a final concentration of 10 µg/mL (or 1000 ng/100 µL). Aliquots of ~600 µL were stored at –20°C for up to 6 months. Later, a series of

working oPRL standards were prepared from the 10 µg/mL stock oPRL standard and diluted with assay diluent to give oPRL standards of the following concentrations (Table 2.2).

Table 2.2: Preparation of oPRL standards.

Working oPRL solution	**Concentrations of oPRL standards**	
(ng/100 µL)	**(pg/100 µL)**	**(ng/mL)**
1000	10000	100
1000	5000	50
5	2500	25
2.5	1250	12.5
5	500	5
0.50	250	2.5
0.25	125	1.25
0.125	62.5	0.625
assay diluent	0	0

All standards were aliquoted into 500 µL volumes in 1.5 mL microtubes and stored at –20°C until use.

2.11.9 Preparation of oPRL controls

oPRL controls were made by diluting pooled sheep plasma of a known concentration, with horse plasma containing no oPRL to give 3 controls of desired concentrations (low = 200 pg/tube, medium = 1000 pg/tube, high = 2500 pg/tube). The medium control was used as a quality control to determine the intra-assay coefficient of variation and the inter-assay coefficient of variation. These were stored at –20°C in 1 mL aliquots until use.

2.11.10 Preparation of antiserum

A 1:800,000 titre was used by diluting 30 µL of stock antisera with 200 mL assay diluent.

2.11.11 Preparation of second antibody

Normal rabbit serum and sheep anti-rabbit serum were combined in the following ratio in 4% PEG/PBS:

Watpa Normal rabbit serum	1:400
Sheep anti-rabbit serum (#A265)	1:90

2.11.12 oPRL radioimmunoassay protocol

Tubes for samples, controls, standards, blanks (B) and total counts (T) were labelled in duplicate using 10 × 75 polystyrene tubes (Galantai Manufacturing Ltd., Auckland, NZ). The following solutions were added in order:

(i) sample (100 µL or 10 µL if high concentration was expected) or 100 µL oPRL standard;
(ii) appropriate volume of assay diluent was added so that the final volume was 200 µL and the tubes were vortexed;
(iii) antiserum (100 µL);
(iv) oPRL tracer (100 µL = ~15000 cpm).

Tubes were vortexed and incubated for 40 h at room temperature. At the conclusion of this time, 200 µL of a second antibody was added and the tubes were vortexed again. The tubes were incubated for a further 2 h at room temperature to reach equilibrium before adding 1 mL 4% PEG to stop the reaction and vortexed once more. Assay tubes were centrifuged at 2300 *g* at 4°C for 20 min to separate bound label from free label with excess sheep anti-rabbit serum. The supernatant was decanted and the pellet counted using a 1261 Multigamma counter (LKB Wallac, Turku, Finland) and analysed using the RIA.CALC program. Sensitivity for the radioimmunoassay was 0.6 ng/mL. Inter-assay and intra-assay variations will be given in each experimental chapter.

2.12 Data Analysis and Statistical Methods

All patch wool data were plotted using the mid-point of the wool patch collection period. Plasma PRL concentrations were log transformed before analysis to allow assumption of homogeneous variance in all experimental groups. Data are expressed as means ± SEM and not the value corrected for any covariates. Otherwise, treatment means and the pooled standard error of the difference of those means are reported. Data relating to feed intake, live weight, all wool growth measurements and plasma PRL concentrations were subjected to an analysis of variance at each sampling time, to test the effects of treatment. For live weight and wool growth data, the initial value was used as a covariate for analysis within each reproductive class. Two-way analysis of variance was used to determine the effects of treatment and sex for all lamb data. Relationships between variables were also explored graphically and by multiple regression analysis. All statistical analyses were performed using the computer statistical packages Data Desk v. 6.0.2 (Ithaca, NY, USA, 1996) and Genstat v. 5.0 (Lawes Agricultural Trust, UK, 1997).

CHAPTER THREE

Effects of photoperiod and bromocriptine treatment on wool growth during pregnancy and lactation in September-lambing Romney ewes

3.1 ABSTRACT

Photoperiod control and treatment with long-acting bromocriptine were used to investigate potential wool growth effects of PRL surges associated with parturition and lactation in Romney ewes. Seventeen non-pregnant and 29 pregnant ewes were maintained indoors from early July 1994 until November under controlled photoperiod and dietary intake. Two groups (n = 7–9) were exposed to natural photoperiod (ND non-pregnant and ND-lambed) while two others (n = 8) were held under short day photoperiod (8L:16D; SD non-pregnant and SD-lambed). Two further groups (n = 7) of pregnant ewes housed in SD were treated with bromocriptine, either from 1 week before parturition (SD-BrB) or 1–3 days after parturition (SD-BrA), to suppress PRL secretion. Lambing occurred between 3 and 10 September. Photoperiod and treatment with bromocriptine did not affect the lamb birth weight or liveweight changes, but did influence their circulating PRL concentrations. In the ND non-pregnant ewes, plasma PRL concentrations increased ($P<0.001$) with natural daylength. PRL concentrations were suppressed ($P<0.001$) in the SD non-pregnant and SD-BrB groups throughout the trial, and in the SD-BrA group following the PRL peak associated with parturition. In all pregnant sheep, apart from the SD-BrB group, PRL increased rapidly a few days prior to parturition and subsequently remained elevated (ND-lambed) or declined (SD-lambed) during lactation. Wool growth rate ($P<0.001$) and mean fibre diameter ($P<0.01$) increased in all groups during spring and differed between treatment groups ($P<0.001$). More clean wool was grown by non-pregnant groups than pregnant groups ($P<0.001$), despite comparable maternal live weights. PRL surges during parturition and lactation were associated with higher ($P<0.05$) wool growth rate and mean fibre diameter in SD groups in October and November. These results indicate that, although the wool growth depression in late pregnancy may not be caused by changes in PRL levels, increased plasma PRL concentrations at parturition followed by a moderate decline over lactation may be linked to a long-term stimulatory effect on wool growth.

3.2 INTRODUCTION

Wool production of breeding ewes is depressed from 7 to 26% on an annual basis compared with that of non-breeding ewes, due to the demands of pregnancy and lactation (Corbett, 1979). The variability in reported wool growth responses to pregnancy and lactation arises partially from the diversity of grazing environments both within and between the different experiments (Corbett, 1979) as well as potential genotype effects. The reduction in wool growth is due to both a reduced fibre length growth rate and a reduced fibre diameter and can result in an increase in the susceptibility to tenderness. Ewes producing and rearing twin lambs tend to have a higher proportion of tender fleeces than those rearing a single lamb (Bigham *et al.*, 1983; Fitzgerald *et al.*, 1984). It has been suggested that the development of the tender region along the staple is associated with the stress of parturition, the so-called *lambing break.*

The rate of wool growth of mated Australian Merino ewes was similar to that of non-pregnant ewes during early pregnancy, but was significantly less during the fourth and fifth months of pregnancy and during lactation (Oddy, 1985). In the first month after lambing, fibre diameter was less than in non-pregnant ewes. The data of Fitzgerald *et al.* (1984) and Peterson *et al.* (unpublished) suggest that the minimum fibre diameter is temporally associated with late gestation and/or parturition and early lactation.

Definitive data are lacking on the stage of pregnancy or lactation associated with the depression in wool production in New Zealand sheep breeds. Constant environmental conditions and a known and controlled nutritional intake are necessary prerequisites if the contribution of these sources of wool growth variation are to be minimised.

The underlying endocrine mechanism controlling seasonal wool growth is thought to involve fluctuations in plasma PRL concentration in response to

photoperiod (Wallace, 1979a; Lincoln, 1990). A number of recent developments suggest that PRL could also be involved in wool growth depression during parturition and lactation. Firstly, the lambing season has a major impact on annual maternal wool growth in New Zealand sheep breeds that is difficult to attribute directly to differential nutrient intake (Morris *et al.*, 1993; 1994). This effect is likely to involve interactions with seasonally-modulated hormones such as PRL. Secondly, PRL receptors are present in the wool follicle (Choy *et al.*, 1995; 1997). Thirdly, PRL secretion in sheep is markedly influenced by reproductive status. Plasma PRL levels are known to increase sharply in late gestation and fluctuate throughout lactation (Peterson *et al.*, 1990; 1991). Given the link between rising PRL and inhibition of wool growth in New Zealand Wiltshire sheep (Pearson *et al.*, 1996) it seems possible that lambing break could also be attributable to the rise in circulating PRL levels associated with parturition and lactation.

The present experiment (FP003/01) was designed to examine whether PRL has an association with wool growth during pregnancy, parturition or lactation. The objectives were to characterise wool growth in Romney sheep during mid- to late pregnancy and early lactation (under controlled dietary intake and photoperiod) and to determine the short-term effects on wool growth of changes in circulating PRL concentration associated with parturition and lactation. The sheep were fed to a constant maternal live weight in an attempt to remove or reduce the effects of variable nutrient availability on fibre growth. Of the 46 sheep in the trial, 30 were held in short days in order to prevent the increase in PRL levels associated with increasing photoperiod in the spring (i.e. to remove a confounding effect on the parturition/lactation-induced rises in PRL levels).

3.3 MATERIALS AND METHODS

3.3.1 Experimental Animals

A flock of 320 Romney ewes at the Ballantrae Hill Country Research Station, Woodville, were synchronised with EAZI-breed CIDR™ type G devices (InterAg, Hamilton, NZ) for 14 days in April 1994. The ewes received 400 iu PMSG (Pregnecol; Heriot Developments Pty Ltd., Australia) at time of CIDR removal and were joined with harnessed vasectomised rams. Ewes were inseminated with Romney semen using an intra-uterine laparoscopic technique on 13 April 1994 (day 0 of gestation). Seventeen non-pregnant ewes were transported to Ruakura on 14 April and were grazed separately until required. Twenty-nine pregnant sheep carrying a single fetus were identified by ultrasonic scanning on 17 June (day 58) and were transported to Ruakura Agricultural Research Centre on 21 June (day 62).

3.3.2 Housing

Forty-six ewes were transferred indoors to individual pens in 2 separate rooms in the Ruakura Physiology Building on 19 July (day 89). Each sheep was maintained in an individual pen (1.4 × 1.3 m) from 19 July to 28 November.

3.3.3 Environmental Observations

The daily minimum and maximum air temperatures recorded at the Ruakura meteorological station over the experimental period are shown in Figure 3.1. The mean temperatures in July, August, September, October and November 1994 were 9.0, 9.7, 10.7, 12.7 and 14.6°C respectively. Over the same period, the hours of daylight (ND conditions) also increased (Figure 3.2).

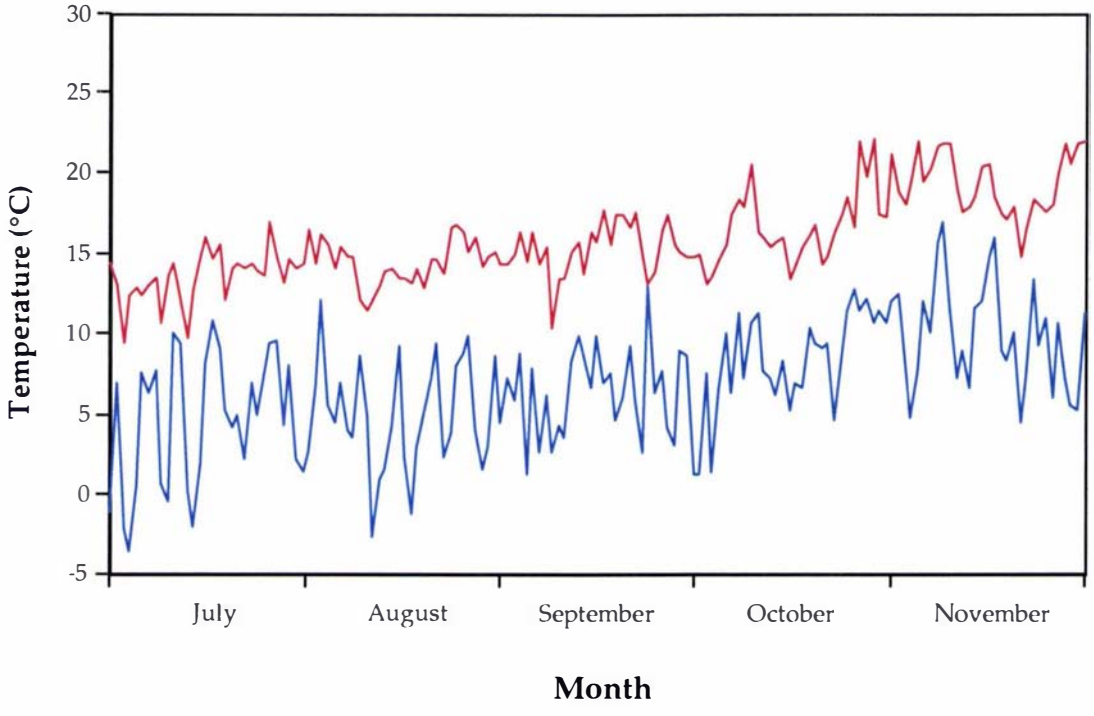

Figure 3.1: Daily minimum (–) and maximum (–) air temperatures during the 1994 experimental period.

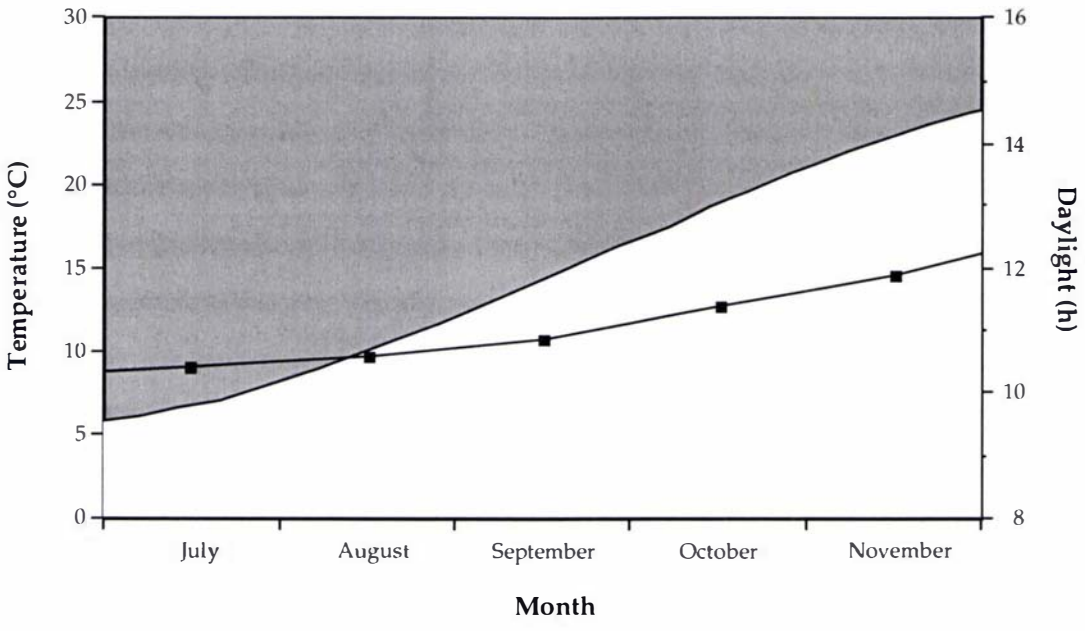

Figure 3.2: Monthly mean air temperature (■) and hours of daylight during the 1994 experimental period.

3.3.4 Experimental Groups

The experiment was a 2 × 2 factorial design incorporating 2 levels of reproductive status (non-pregnant versus pregnant) and 2 levels of

photoperiod (natural days versus short days) in Groups 1--4. There was also a comparison of 2 bromocriptine treatments with SD-lambed ewes (Groups 4–6, Table 3.1). The 46 sheep were allocated to one of 6 groups based on reproductive status, balanced for live weight and July greasy wool growth.

Lambs born to ewes from Groups 2, 4, 5 and 6 will be referred to as ND, SD, SD-BrB and SD-BrA lambs respectively.

Table 3.1: Experimental groups.

Group		n	Abbreviation
Natural Days			
1	Non-pregnant ewes	9	ND non-pregnant
2	Pregnant ewes	7	ND-lambed
Short Days			
3	Non-pregnant ewes	8	SD non-pregnant
4	Pregnant ewes	8	SD-lambed
5	Pregnant ewes treated with bromocriptine from day 135 of gestation	7	SD-BrB
6	Pregnant ewes treated with bromocriptine approximately 3 days after parturition	7	SD-BrA

3.3.5 Light Treatment

Groups 1–2 (Room 1) were exposed to natural photoperiod (Natural Days, ND) while groups 3–6 (Room 2) were held under short day (SD) conditions of 8 h light and 16 h dark (8L:16D) as described in Section 2.4.

3.3.6 Live weight

All sheep were weighed at 7 day intervals over the trial duration to monitor changes in live weight.

3.3.7 Feed Allowance

The daily feed allowance for pregnant and non-pregnant ewes was calculated and the daily feed intake measured as described in Section 2.3.

3.3.8 Lamb Measurements

All lambs were weighed within 24 h of birth and then at the same time as their dams until weaning. Crown-rump length, girth, maximum head width and hindleg length measurements were recorded along with the birth weight.

3.3.9 Wool Sampling

The sheep were shorn on entry to the experiment on 30 June and again at the conclusion of the experiment on 29 November 1994 when the fleece was weighed and a midside sample collected *(Section 2.7.3).* A midside patch was established on 6 July and re-clipped every month.

3.3.10 Blood Sampling

Blood samples were collected by venipuncture from all ewes from 13 July to 30 November. The intervals ranged from every 3 weeks at the beginning and end of the experiment to daily samples close to parturition. Following parturition, blood samples were also collected from the lambs, at the same times as their dams. Inter-assay and intra-assay coeffecients of variation for the PRL radioimmunoassay at 10 ng/mL were 12.5% and 14.3% respectively.

3.3.11 Bromocriptine Administration

Bromocriptine was given intramuscularly at 2-week intervals from 26 August (day 135 of gestation) in SD-BrB ewes and from 9 September (approximately 3 days postpartum) in SD-BrA ewes (Table 3.2). Gow *et al.* (1983) and Peterson *et al.* (1991) showed that suppression of PRL secretion using bromocriptine, immediately prior to parturition (and during lactation), reduced milk yield in sheep but did not prevent lactogenesis. However the administration of 100 mg

bromocriptine 10 days prior to parturition did temporarily affect lactogenesis. Consequently, lambs born to ewes in this trial group needed initial milk supplementation while some ewes were hand-milked to stimulate milk let-down.

Table 3.2: Bromocriptine treatment schedule administering 50 mg Parlodel LA* to SD-BrB and SD-BrA ewes except where specified.

Date		Day Post-conception	Group
26	August **	135	SD-BrB
~ 6	September (parturition)**	~ 146	SD-BrA
9	September	149	SD-BrB
16	September	156	SD-BrB, SD-BrA
30	September	170	SD-BrB, SD-BrA
14	October	184	SD-BrB, SD-BrA
28	October	198	SD-BrB, SD-BrA
11	November	212	SD-BrB, SD-BrA

*intramuscular 28-day formulation

**100 mg administered on first day of treatment

3.3.12 Statistical Methods

Data relating to feed intake, ewe and lamb live weight, plasma PRL concentration, clean wool growth rate and fleece characteristics were subjected to analysis of variance at each sampling time to test the effects of pregnancy, photoperiod and their interactions. For ewe wool data, the July value for each wool measure was used as a covariate for that parameter. Multiple regression analysis was used to analyse the change in PRL concentration over time in the lambs.

3.4 RESULTS

Lambing details

Thirteen male lambs and 16 female lambs were born between 3 and 10 September 1994 with the mean lambing date being 7 September.

Ewe data

3.4.1 Feed intake

Over the trial, total feed intake and mean daily feed intake (Figure 3.3) were higher in lambing ewes (236 ± 7 kg and 1824 ± 34 g/day) compared with non-pregnant ewes (115 ± 2 kg and 881 ± 16 g/day). There was no significant difference in daily or total feed intake between the 2 non-pregnant groups or between the 4 pregnant groups (analysed over prepartum and postpartum periods).

3.4.2 Live weight

On average, non-pregnant ewes were 7.6 kg heavier ($P<0.001$) than pregnant ewes at the start of the trial (Figure 3.4, Table 3.3). There was no difference in live weight between ewes of the same reproductive class. From July until late August (a week before parturition) pregnant ewes gained 6.4 kg on average as pregnancy advanced, compared with a loss of 3.2 kg in non-pregnant ewes ($P<0.001$), so that by this time there was no significant difference in live weight with reproductive status. Photoperiod had no effect on liveweight change (ND non-pregnant versus SD non-pregnant and ND-lambed versus SD-lambed comparisons) over this period.

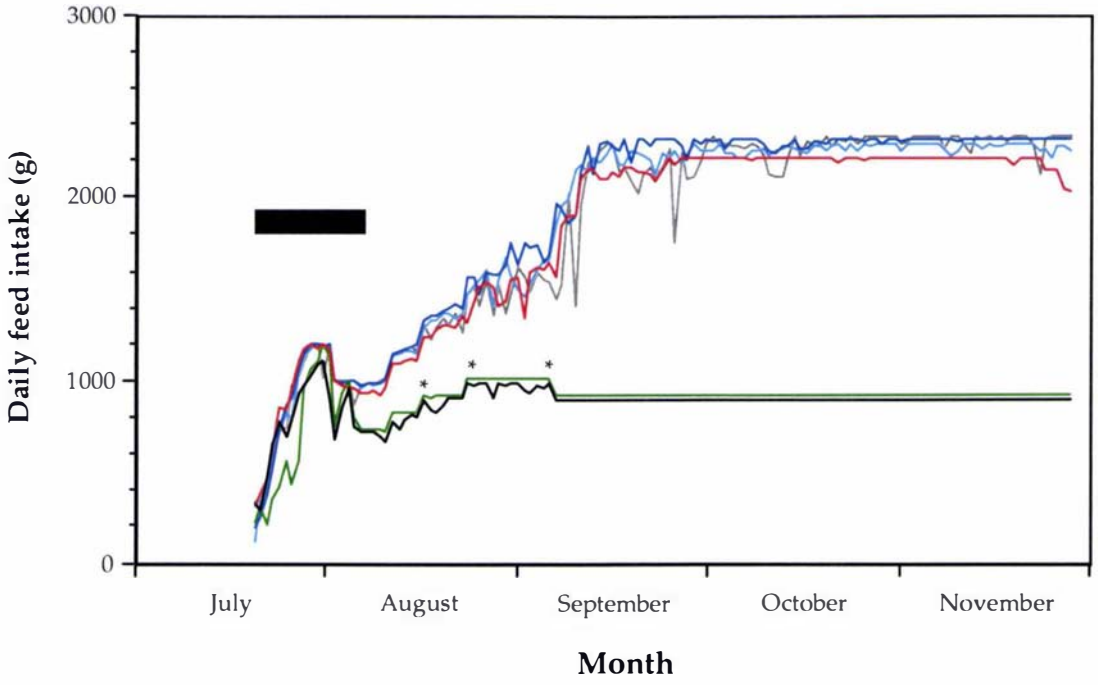

Figure 3.3: Mean daily feed intake of ND non-pregnant (–), ND-lambed (–), SD non-pregnant (–), SD-lambed (–) and SD-lambed ewes treated with bromocriptine before parturition (–) or after parturition (–). Solid bar represents period of feed adaptation; * represents when daily feed allowance was adjusted across some or all groups. NOTE: The variation in the SD-BrA group in September was due to the reduced feed intake of one animal.

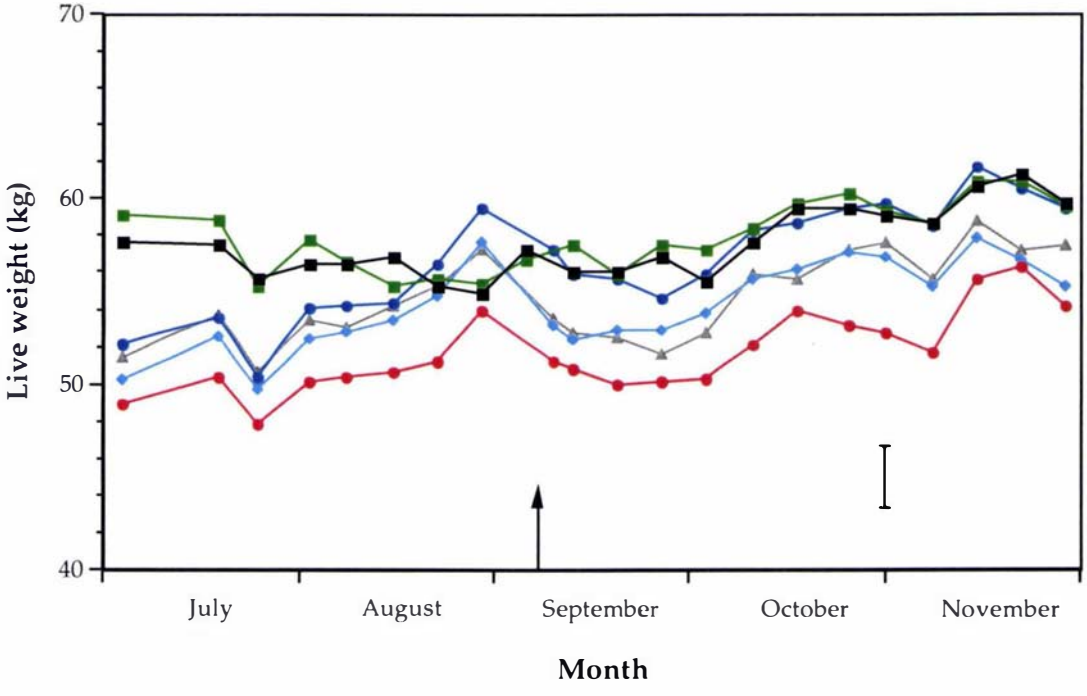

Figure 3.4: Mean live weight of ND non-pregnant (■), ND-lambed (●), SD non-pregnant (■), SD-lambed (●) and SD-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

There was no overall difference in live weight due to photoperiod immediately following parturition, but at this time non-pregnant ewes were significantly heavier ($P<0.05$) than ewes which had lambed. All treatment groups gained weight from September to November which was unaffected by photoperiod or bromocriptine treatment.

Table 3.3: Effects of pregnancy/lactation, photoperiod and bromocriptine treatment on the initial, prepartum, postpartum and fleece-free final live weights (LW), and the liveweight change of the experimental groups (Mean ± SEM).

Group	*n*	Initial LW (kg)	Prepart. LW (kg)	Postpart. LW (kg)	Final LW # (kg)	LW change (kg)
ND non-pregnant	9	57.6 ± 2.2^{b}	54.9 ± 2.4	56.0 ± 2.4^{ab}	57.6 ± 2.3	0.0. ± 0.8^{a}
ND-lambed	7	48.9 ± 0.5^{a}	53.9 ± 1.1	50.8 ± 0.6^{a}	52.5 ± 1.4	3.7 ± 1.4^{b}
SD non-pregnant	8	59.1 ± 2.4^{b}	55.4 ± 2.4	57.5 ± 2.4^{b}	57.3 ± 2.5	–1.7 ± 0.9^{a}
SD-lambed	8	52.1 ± 2.5^{a}	59.5 ± 2.5	55.9 ± 2.7^{ab}	57.5 ± 2.7	5.1 ± 0.8^{b}
SD-BrB	7	50.2 ± 2.4^{a}	57.6 ± 2.1	52.4 ± 1.8^{ab}	53.5 ± 1.7	3.2 ± 1.3^{b}
SD-BrA	7	51.4 ± 1.0^{a}	57.2 ± 1.2	52.8 ± 1.2^{ab}	55.5 ± 1.7	4.1 ± 1.7^{b}
Reprod. status						
Non-pregnant	17	58.3 ± 1.6^{b}	57.0 ± 1.7	56.7 ± 1.7^{b}	57.5 ± 1.6	–0.8 ± 0.6^{a}
Preg./Lact.	29	50.7 ± 0.9^{a}	57.1 ± 1.0	53.1 ± 0.9^{a}	54.9 ± 1.0	4.1 ± 0.6^{b}

Final live weight minus fleece weight at shearing.
^{ab} Within columns means within treatment groups and within reproductive status having superscripts with letters in common or no superscript are not significantly different ($P>0.05$).

The final live weights and post-shearing live weights were not significantly different between the 6 treatment groups although the trend for non-pregnant ewes to be heavier than lactating ewes in ND conditions continued. Differences in liveweight gain throughout the trial were also apparent. The fleece-free live weight change in ND and SD non-pregnant ewes was less than the weight change in ND-lambed ($P<0.05$) and SD-lambed ewes ($P<0.001$) respectively, and in SD-lambed ewes treated with bromocriptine ($P<0.01$). Weight gain in the latter group was no different to that in SD ewes not treated with bromocriptine.

3.4.3 Plasma PRL concentration

Markedly different plasma PRL profiles were achieved in the 6 treatment groups (Figure 3.5). One interesting feature was the difference between the mean plasma PRL concentrations in non-pregnant ewes compared with the pregnant groups in the period prior to 26 August when bromocriptine was first administered to the SD-BrB ewes (Figure 3.6). While non-pregnant groups averaged 39 ± 9 ng/mL, pregnant groups averaged 8 ± 1 ng/mL ($P<0.001$). Photoperiod had no effect on PRL concentrations within each reproductive class over the same period.

From 29 August until 2 September (the day before the first lambing), PRL levels continued to be significantly higher in non-pregnant ewes compared to pregnant ewes not under bromocriptine treatment (27 ± 5 versus 15 ± 4 ng/mL, $P<0.01$). The PRL concentration was suppressed in the SD-BrB group and was significantly lower compared to other SD pregnant groups (1.0 ± 0.4 versus 11 ± 2 ng/mL, $P<0.001$), and compared to ND-lambed ewes (24 ± 11 ng/mL, $P<0.001$).

The PRL profile in ND non-pregnant ewes followed the expected seasonal pattern (Figure 3.5a) where PRL concentrations remained low throughout winter and rose in spring. Photoperiod had no effect on plasma PRL concentrations in July and August. However, the SD conditions prevented the spring rise from September to November with mean PRL concentrations being higher in ND non-pregnant ewes than in equivalent SD ewes (77 ± 7 versus 36 ± 13 ng/mL, $P<0.001$) in these months.

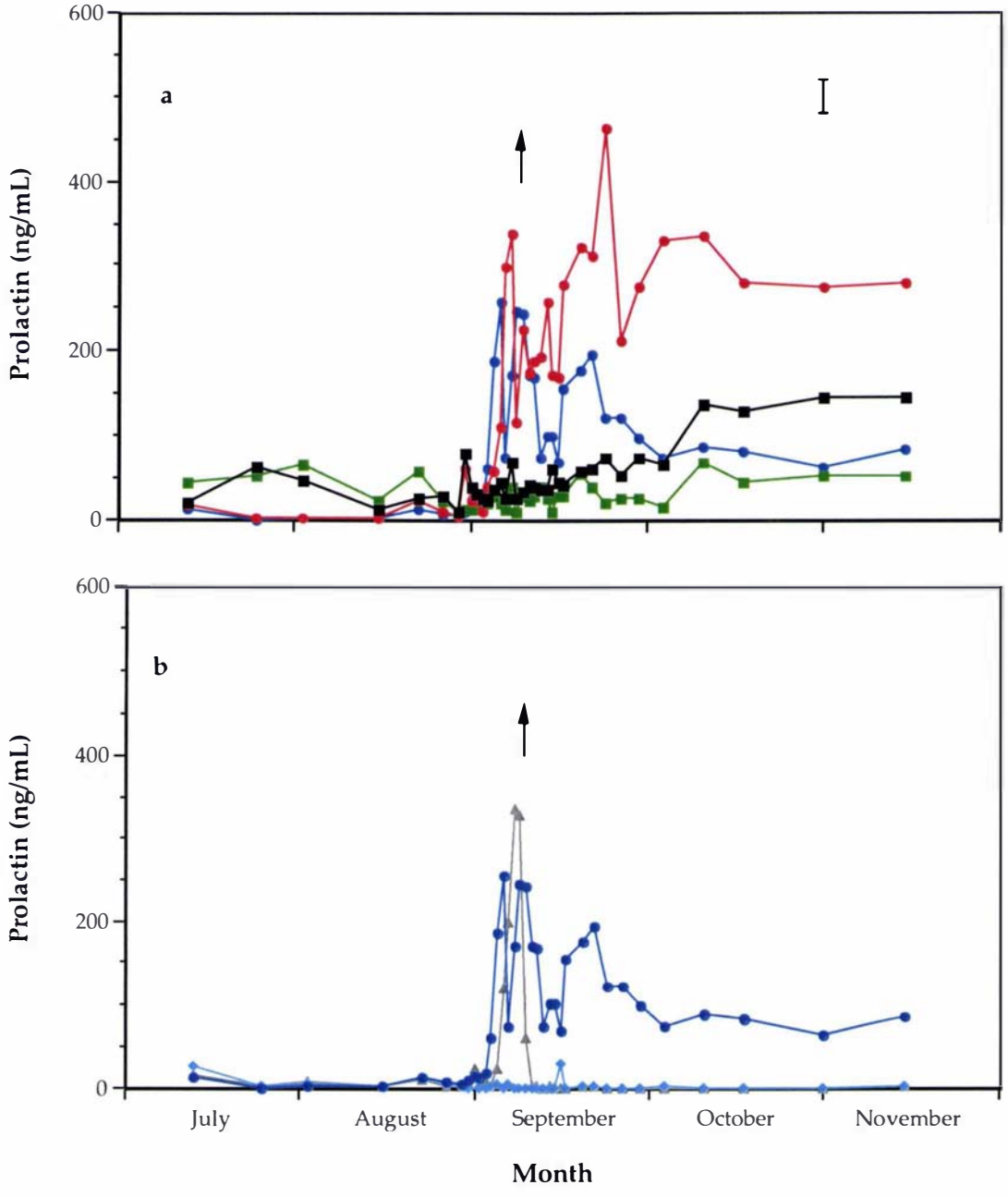

Figure 3.5: Mean plasma prolactin concentrations of (a) ND non-pregnant (■), ND-lambed (●), SD non-pregnant (■) and SD-lambed ewes (●); and (b) SD-lambed ewes (●) and SD-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

In ND-lambed ewes, PRL concentrations increased rapidly 1–2 days prior to parturition, reaching a peak of 340 ± 145 ng/mL (Figure 3.5a) and were significantly ($P<0.01$) higher than in ND non-pregnant ewes (38 ± 5 ng/mL) at this time. The PRL concentrations of SD-lambed ewes followed a similar trend to those of ND-lambed ewes and were not significantly different (245 ± 106

ng/mL) over this period, but were higher ($P<0.001$) than those of non-pregnant ewes in SD (24 ± 4 ng/mL). PRL concentrations of ND-lambed ewes remained elevated during lactation compared to those of SD-lambed ewes (277 ± 28 versus 107 ± 11 ng/mL, $P<0.001$) and non-pregnant ewes in ND ($P<0.001$). The mean PRL levels in SD-lambed ewes were also greater ($P<0.01$) than in SD non-pregnant ewes during this time.

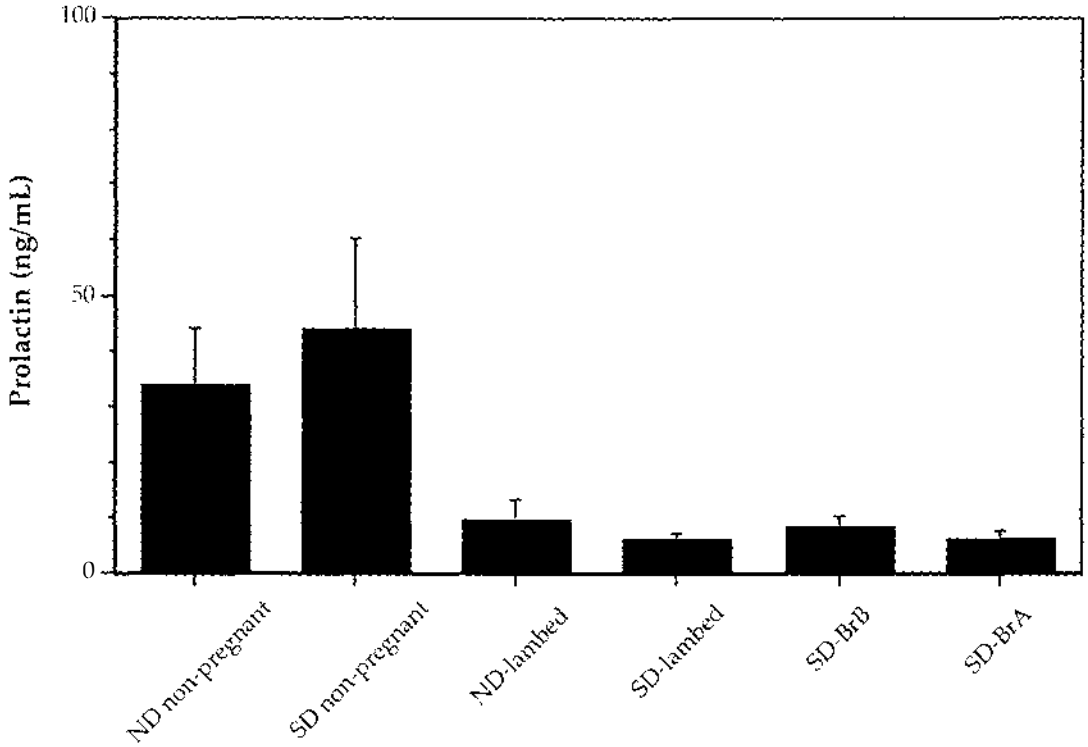

Figure 3.6: Plasma prolactin concentrations (Mean ± SEM) in all treatment groups from 13 July to 26 August inclusive when bromocriptine was first administered.

In the SD-BrB group, the PRL peaks associated with both parturition and lactation were completely abolished and PRL concentrations remained suppressed over the course of the trial (Figure 3.5b). The mean PRL concentration was significantly lower (0.8 ± 0.1 ng/mL, $P<0.001$) than in ND and SD ewes that produced lambs but were not treated with bromocriptine.

In the SD-BrA group, PRL concentrations were low during pregnancy but rose rapidly to 335 ± 99 ng/ml a few days before parturition. The administration of bromocriptine abolished PRL surges associated with lactation (Figure 3.5b). For the remainder of the trial, concentrations remained lower (0.7 ± 0.1

ng/mL, $P<0.001$) than in other untreated lactating and non-pregnant ewes but were not significantly different to plasma PRL concentrations in SD-BrB ewes.

3.4.4 Fleece weight and washing yield

Fleece weights at shearing indicated that non-pregnant ewes produced 13% more ($P<0.01$) greasy wool than ewes that had lambed (Table 3.4). These differences were not related to the initial live weight or weight change over the trial. SD-lambed ewes produced significantly ($P<0.001$) more greasy wool than lambing ewes maintained in ND, reflecting the wool growth rate on the midside patch *(Section 3.4.7)* but there was no difference between ND and SD non-pregnant ewes. In comparison to SD-lambed ewes, bromocriptine-treated SD ewes were unaffected by the drug treatment. Fleece washing yield was higher in ND ewes than in SD ewes (76.1 ± 0.8 versus 70.2 ± 0.8%, $P<0.001$) but was unaffected by reproductive status or bromocriptine treatment.

Table 3.4: Effects of pregnancy/lactation, photoperiod and bromocriptine treatment on greasy fleece weight (GFW), washing yield, clean fleece weight (CFW) and mean fibre diameter (MFD) of fleece wool at shearing of the experimental groups (Mean ± SEM).

Group	*n*	GFW (kg)	Yield (%)	CFW (kg)	MFD (μm)
ND non-pregnant	9	2.12 ± 0.10^{bc}	77.0 ± 1.1^{c}	1.61 ± 0.09^{bc}	35.1 ± 1.1
ND-lambed	7	1.64 ± 0.12^{a}	74.9 ± 1.2^{bc}	1.20 ± 0.11^{a}	35.0 ± 1.2
SD non-pregnant	8	2.22 ± 0.13^{b}	70.1 ± 1.2^{a}	1.69 ± 0.11^{c}	34.9 ± 1.6
SD-lambed	8	2.01 ± 0.07^{bc}	70.2 ± 1.2^{a}	1.44 ± 0.07^{ab}	31.8 ± 0.7
SD-BrB	7	1.85 ± 0.10^{ac}	73.0 ± 1.3^{ab}	1.28 ± 0.07^{a}	34.6± 1.0
SD-BrA	7	1.98 ± 0.07^{bc}	71.8 ± 1.4^{ab}	1.41 ± 0.06^{ab}	33.2 ± 1.6
Reprod. status					
Non-pregnant	17	2.17 ± 0.08^{b}	73.8 ± 1.2	1.65 ± 0.07^{b}	35.0 ± 0.9
Preg./Lact.	29	1.88 ± 0.05^{a}	72.4 ± 0.7	1.34 ± 0.04^{a}	33.6 ± 0.6

^{abc} Within columns means within treatment groups and within reproductive status having superscripts with letters in common or no superscript are not significantly different ($P>0.05$).

Significantly ($P<0.001$) more clean wool (19%) was grown by non-pregnant ewes compared with those that produced lambs (Table 3.4). While SD-lambed ewes grew significantly more wool ($P<0.05$) than ND-lambed ewes there was

no difference between SD and ND non-pregnant ewes. Clean fleece weights were unaffected by treatment with bromocriptine. Mean fibre diameter of fleece wool was unaffected by reproductive status, photoperiod or bromocriptine treatment.

3.4.5 Midside wool washing yield

Patch washing yield increased in all groups from July to August (Figure 3.7). While there was no difference with reproductive status over these months, patch yield was significantly higher ($P<0.05$) in non-pregnant ewes compared with pregnant and lactating ewes from September to November. This was reflected in higher 3-month average patch yields in non-pregnant ewes relative to ewes that produced a lamb (78.7 ± 1.0 versus 75.7 ± 0.7%, $P<0.05$). Photoperiod had little effect on washing yield in the first 3 months, but yield was significantly higher ($P<0.05$) in ND-lambed ewes (79.0 ± 1.6%) compared to SD-lambed ewes (73.7 ± 1.9%) in October and November, but similar in ND and SD non-pregnant ewes over this period. Bromocriptine treatment had no influence on washing yield in any month. Overall, there was a strong ($P<0.001$) positive relationship between patch washing yield and clean wool growth in July, August (Figure 3.8) and September but not in October or November.

3.4.6 Midside wool fibre diameter

Fibre diameter increased ($P<0.01$) with time in all groups (Figure 3.9) and generally reflected the rate of clean wool growth. Non-pregnant ewes had a significantly higher ($P<0.05$) mean fibre diameter in July, this difference ($P<0.001$) increasing over late pregnancy in August and September (the average fibre diameter was 11% higher in non-pregnant ewes over this period). Photoperiod did not influence fibre diameter at this time. No differences in fibre diameter with reproductive status or photoperiod occurred in October and November but the fibre diameter tended ($P<0.10$) to be higher in ewes maintained in SD. Mean fibre diameter was significantly lower

($P<0.05$) in bromocriptine-treated ewes than SD-lambed ewes in October and November.

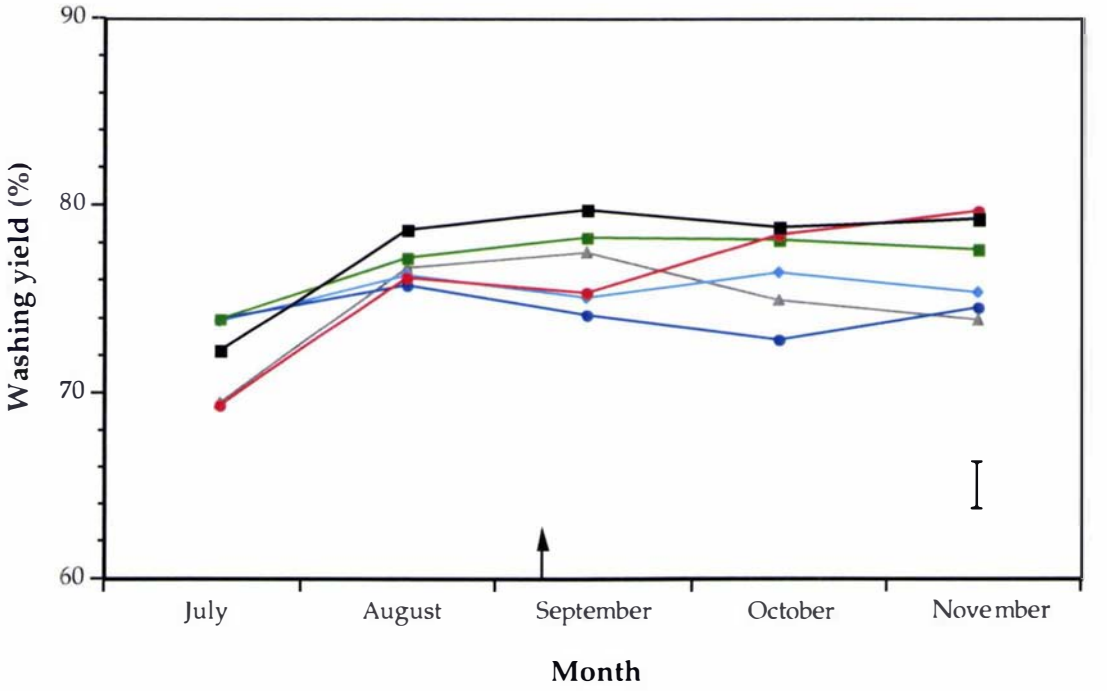

Figure 3.7: Midside wool washing yield of ND non-pregnant (■), ND-lambed (●), SD non-pregnant (■), SD-lambed (●) and SD-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

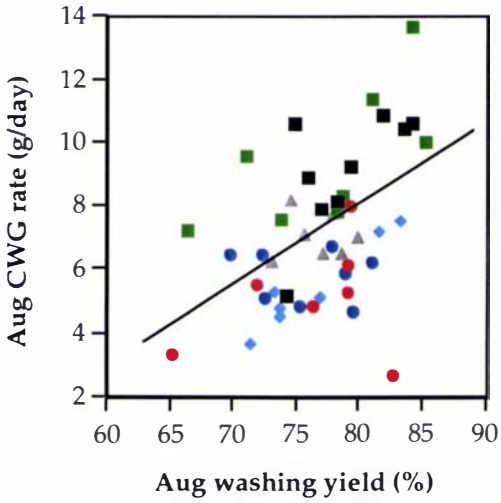

Figure 3.8: The relationship between August midside wool washing yield and clean wool growth rate. Legend: ND non-pregnant (■), ND-lambed (●), SD non-pregnant (■), SD-lambed (●), SD-BrB (◆) and SD-BrA ewes (▲).

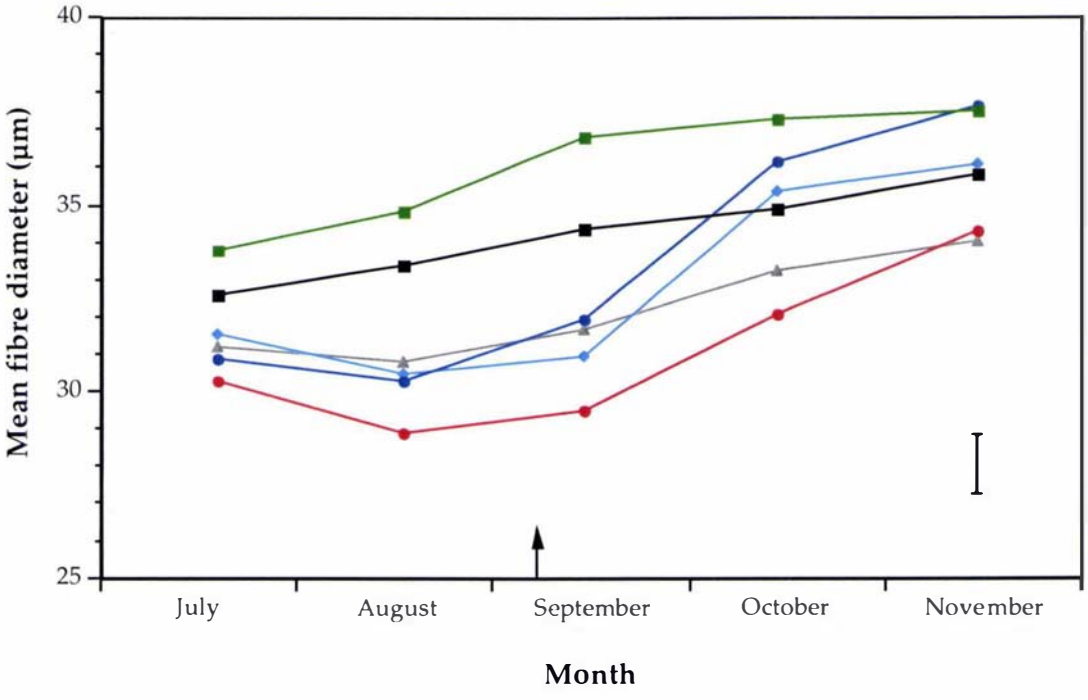

Figure 3.9: Midside mean fibre diameter of ND non-pregnant (■), ND-lambed (●), SD non-pregnant (■), SD-lambed (●) and SD-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

3.4.7 *Midside clean wool growth rate*

Monthly wool growth rate increased in all groups from July to November (Figure 3.10) and differed significantly among treatment groups ($P<0.001$). More clean wool was grown by both non-pregnant groups compared to those that produced lambs, despite comparable maternal live weights. Ewes that produced a lamb grew 33% less wool ($P<0.001$) over late pregnancy (July-September) but this difference was unrelated to photoperiod. Similarly, ewes suckling one lamb grew 14% less wool over lactation compared with non-pregnant ewes. The increase in wool growth rate from September to October was significantly greater ($P<0.001$) in lactating ewes so that by the month following parturition wool growth was similar in all groups regardless of reproductive status, photoperiod or bromocriptine treatment.

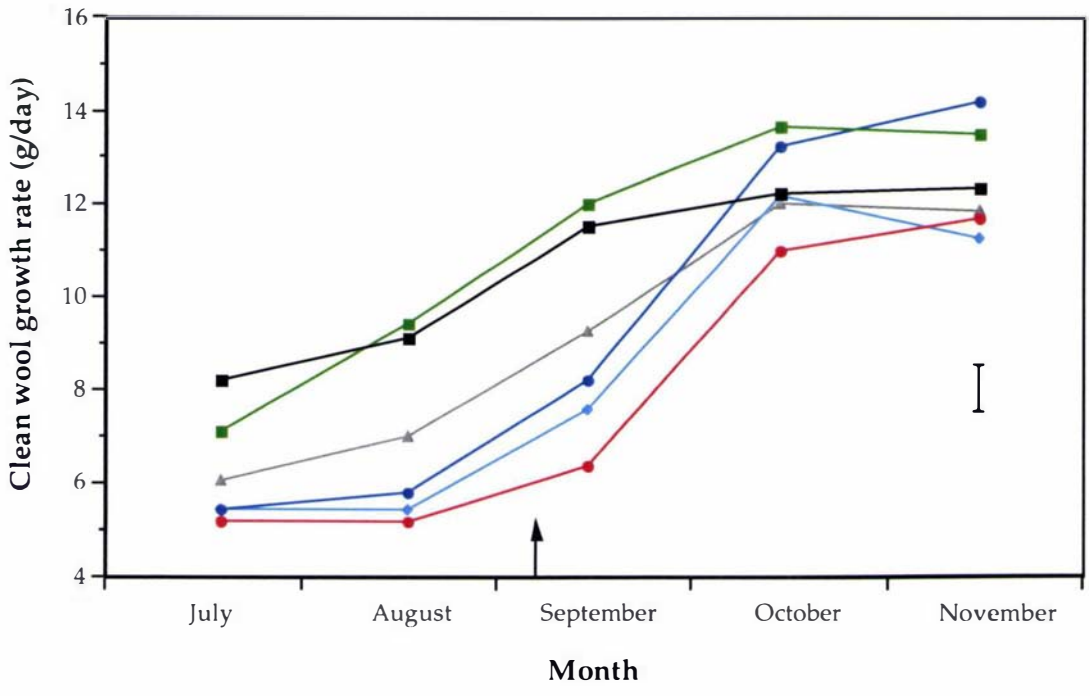

Figure 3.10: Midside clean wool growth rate of ND non-pregnant (■), ND-lambed (●), SD non-pregnant (■), SD-lambed (●) and SD-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

In SD-lambed ewes, the average wool growth from September to November was significantly higher (11.8 ± 0.6 g/day, $P<0.05$) than ND-lambed ewes (9.7

± 0.8 g/day). Wool growth in November was also significantly greater ($P<0.01$) in SD-lambed ewes (14.2 ± 0.6 g/day) compared to both SD-lambed groups treated with bromocriptine (11.6 ± 0.4 g/day). The increase in fleece growth rate throughout the experimental period was significantly greater ($P<0.05$) in ewes that produced lambs than in non-pregnant ewes and was greater ($P<0.01$) in SD ewes compared to ND ewes. Comparing SD and ND non-pregnant ewes, clean wool growth rates were similar in July and August, tended to be higher in SD ewes in September ($P=0.05$) and significantly higher ($P<0.05$) in October and November (Figure 3.10).

3.4.8 Wool growth and PRL profile interrelationships in non-pregnant ewes

Multiple regression analyses were used to determine whether changes in PRL concentration were related to the differences in wool growth rate between August and November.

While the monthly wool growth rate differed between the ND and SD groups in September, October and November (Figure 3.10) the change over these months was not significantly different between treatment groups (3.2 ± 0.4 versus 4.1 ± 0.4 g/day, $P>0.10$). Therefore, the non-pregnant ewes were analysed in the statistical model that ignored treatment. To eliminate differences in the inherent wool growth of individual sheep, the July wool growth rate was used as a covariate. It should be noted that although differing photoperiods had been imposed on the ND and SD ewes by July, there were no treatment effects in plasma PRL concentrations in that month (Figure 3.5a).

The PRL parameters used either singly or in various combinations for the regression analyses against the change in clean wool growth rate were:

(i) *Monthly PRL concentrations* – the mean PRL concentration in the months of July, August, September, October and November;

(ii) *Change in PRL concentration from July to September* – the difference between the mean July PRL concentration and the mean September PRL concentration;

(iii) *Mean September to November PRL concentration* – the average PRL concentration from September to November;

(iv) *Change in PRL concentration from September to November* – the difference between the mean September PRL and the mean November PRL concentration.

There was a strong negative correlation (r = –0.83) between the July PRL concentration and the change in PRL concentration from July to September and therefore these PRL parameters were not combined in the same model. Unlike July PRL concentration which was significantly ($P<0.05$) related to the increase in wool growth rate from August to November (Table, 3.5, Figure 3.11), the PRL concentrations in August, September, October and November were not ($P>0.10$). The change in PRL concentration from July to August, September, October or November all gave a significant ($P<0.05$) negative relationship with the wool growth change. However, the change between July and September provided the best fit to the model (Figure 3.11 and Table 3.5). Combining the July PRL levels ($P<0.05$) with the mean PRL concentration from September to November ($P>0.10$) improved the model fit to the wool growth data compared to July PRL levels alone.

Table 3.5: Percentage of variance in the change in wool growth from August to November accounted by various plasma prolactin parameters in non-pregnant ewes.

PRL parameter	Change in August to November wool growth rate
July PRL	39% *
Change in PRL from July to September	45% *
Mean September to November PRL	11% ns
Change in PRL from September to November	11% ns
July PRL + Mean September to November PRL	45% * ns
July PRL + Change in PRL from September to November	39% * ns

* $P<0.05$.

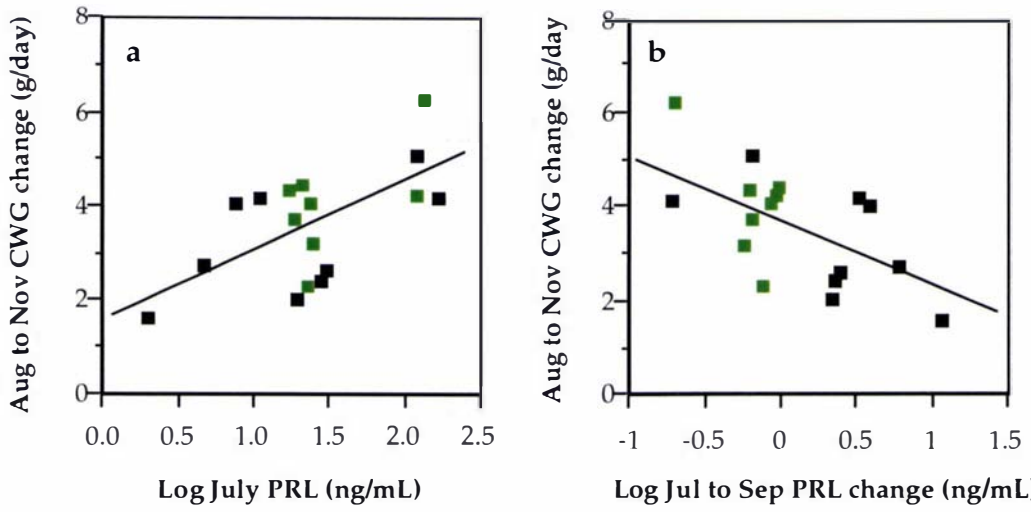

Figure 3.11: The relationship between the change in wool growth rate (CWG) from August to November and the (a) log July plasma prolactin concentrations and (b) the change in log prolactin concentration from July to September in ND (■) and SD (■) non-pregnant ewes.

3.4.9 *Wool growth and PRL profile interrelationships in the 4 pregnant groups*

A similar analysis was undertaken in the 4 pregnant groups to examine the relationships between the change in wool growth from September to November and plasma PRL concentrations at/and around parturition. The change in wool growth rate between September and November was significantly different ($P<0.01$) among the 4 treatment groups (Figure 3.10). Inspection of the PRL profiles from individual pregnant sheep revealed that PRL concentrations rose approximately 2 days before parturition and declined after a similar time postpartum. Four summary PRL parameters associated with parturition were used in this analysis (Figure 3.12):

(i) *Prepartum increase in PRL concentration* – the difference between the mean PRL concentration prior to parturition and the mean periparturient PRL concentration;

(ii) *Periparturient PRL concentration* – the mean PRL concentration for a 5-day period at parturition;

(iii) *Peak PRL concentration* – the highest PRL concentration during the 5-day periparturient period;

(iv) *Postpartum decline in PRL concentration* – the difference between the mean periparturient PRL concentration and the mean PRL concentration during lactation.

The mean periparturient PRL concentration was highly correlated (r = 0.90–0.92) to the peak PRL concentration. In general, the relationships between wool growth and the peak PRL concentration were weaker than those for the periparturient PRL concentration (alone or combined with other PRL parameters) (Table 3.6). Only the results using periparturient PRL concentration are presented.

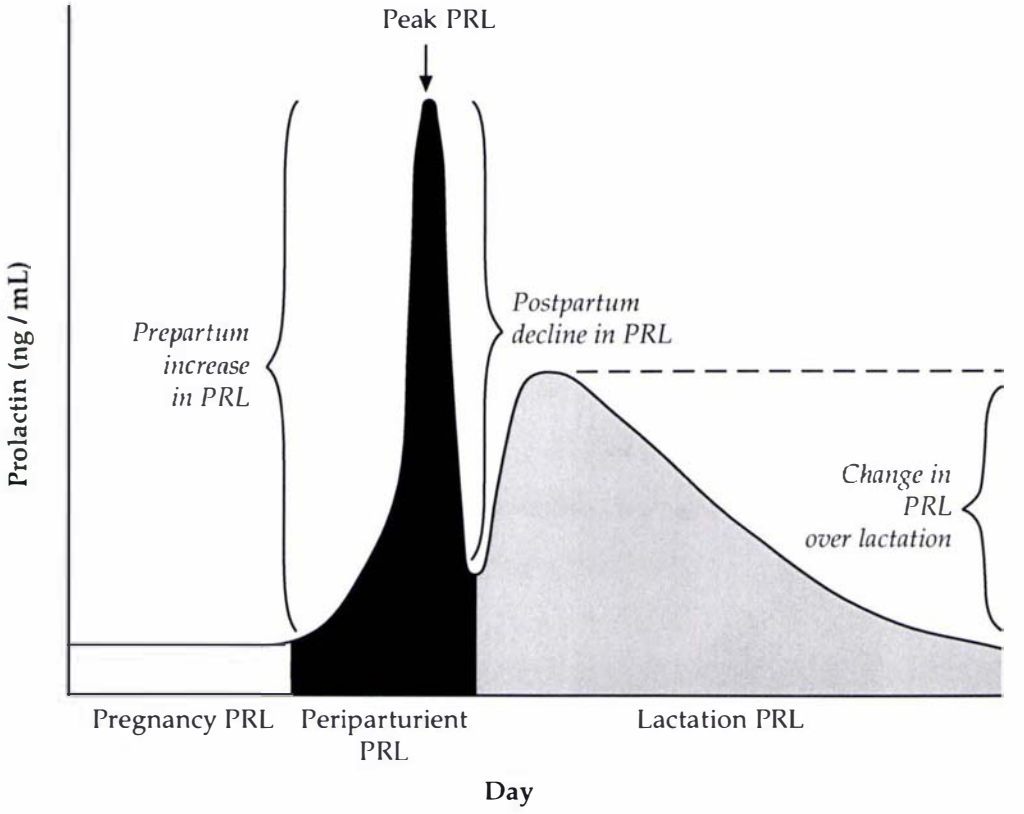

Figure 3.12: Diagrammatic view of the prolactin parameters used over pregnancy, parturition and lactation.

Two additional PRL parameters were derived from the lactation period (Figure 3.12):

(v) *Lactation PRL concentration* – the mean PRL concentration for the 2½ month period after the periparturient period;

(vi) *Change in PRL concentration over lactation* – the difference in PRL concentration from the start (a 5 sample day mean in September) to the end (a 5 sample day mean in October and November) of lactation.

The analyses for the pregnant treatment groups involved a systematic 3-step analysis of these PRL parameters. One of the treatment groups was excluded from the analysis after each step to avoid difficulties with confounding effects. Any inferences made using all the treatment groups could be driven by the fact that the SD-BrB group had a PRL profile quite different from the other 3 treatment groups (Figure 3.5b) and, in some instances, the PRL parameters

(e.g. the periparturient PRL concentration, Figure 3.13 a and c) were not applicable to the bromocriptine-treated groups. It is possible that the inclusion of the SD-BrB group was responsible for some of the effects observed in the 4 pregnant group models. The 3 remaining groups all had a PRL surge at parturition but differing PRL concentrations over lactation. In the final comparison both bromocriptine-treated groups were excluded from the analysis as PRL was suppressed during lactation in both these groups (Figure 3.5b). The ND-lambed and SD-lambed ewes had a similar PRL profile at parturition but subsequent levels were modified by photoperiod (Figure 3.5a).

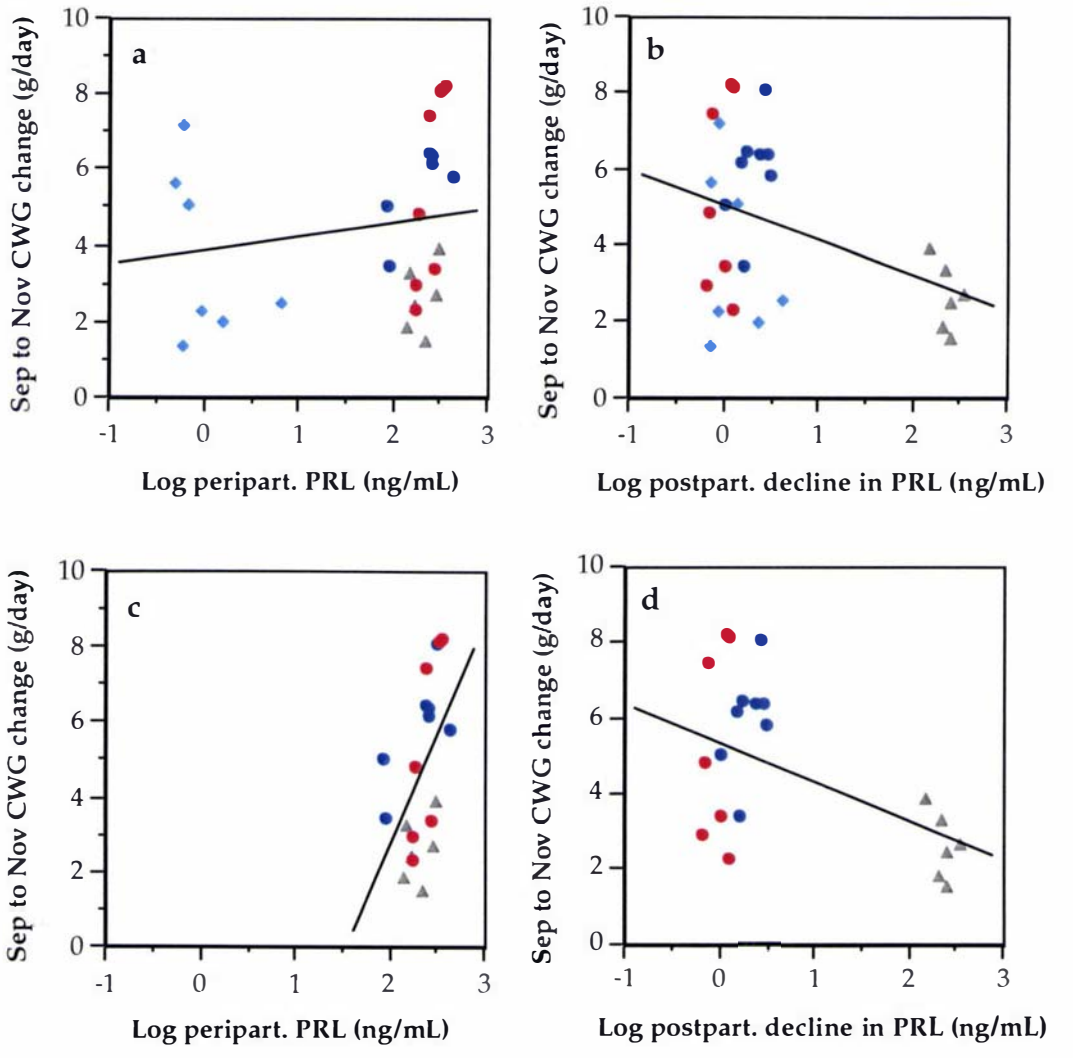

Figure 3.13: The relationship between the change in wool growth rate (CWG) from September to November and the mean log periparturient prolactin concentration and the log postpartum decline in prolactin concentration in (a, b) all pregnant ewes and (c, d) pregnant ewes excluding SD-BrB ewes. Legend: ND-lambed (●), SD-lambed (●), SD-BrB (◆) and SD-BrA (▲) ewes.

Table 3.6: Percentage of variance in the change in wool growth from September to November accounted by various plasma prolactin parameters in pregnant ewes.

PRL parameter	All Groups	Excluding SD-BrB ewes	ND and SD ewes only
Prepartum Increase in PRL	4% ns	0% ns	5% ns
Peak PRL	1% ns	11% ns	25% †
Periparturient PRL	5% ns	25% *	42% *
Postpartum decline in PRL	23% **	42% **	13% ns
Lactation PRL	35% ***	52% ***	11% ns
Change in PRL over lactation	3% ns	2% ns	5% ns
Prepartum increase in PRL + Lactation PRL	39% ns ***	52% ns ***	12% ns ns
Prepartum increase in PRL + Change in PRL over lactation	6% ns ns	2% ns ns	5% ns ns
Periparturient PRL + Postpartum decline in PRL	39% * ***	66% ** ***	42% * ns
Periparturient PRL + Lactation PRL	39% ns ***	66% * ***	42% * ns
Periparturient PRL + Change in PRL over lactation	7% ns ns	25% * ns	43% * ns

† $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

All pregnant groups: The periparturient PRL concentration was not related ($P>0.10$) to wool growth changes, either alone or in combination with mean PRL over lactation, or with the change in PRL over lactation (Table 3.6). However, when the periparturient PRL concentration ($P<0.05$) was combined

in a model with the postpartum decline in PRL concentration ($P<0.001$) the periparturient PRL concentration appeared to be implicated in the long-term wool growth changes observed (Figure 3.13). The mean PRL concentration over lactation was highly significant ($P<0.001$) alone, or when combined with other PRL parameters ($P<0.01$, Table 3.6).

Three pregnant groups excluding the SD-BrB group: Periparturient PRL concentrations were significantly related ($P<0.05$) to the wool growth change whether combined with PRL concentrations over lactation ($P<0.001$) or not. Similarly, periparturient PRL concentrations were significantly related ($P<0.05$) to wool growth change when combined with the change in PRL levels over lactation (Table 3.6). There were also highly significant effects with the periparturient PRL concentrations ($P<0.01$) combined with the postpartum decline in PRL concentration ($P<0.001$).

ND and SD-lambed groups: September to November wool growth rate was unaffected by PRL levels over lactation or the postpartum PRL concentration in ND-lambed and SD-lambed ewes (Table 3.6). However, the wool growth changes were still related ($P<0.05$) to periparturient PRL concentrations (Figure 3.14). The R^2 values were not improved when combined with PRL concentrations over lactation or the change in PRL profile over this period.

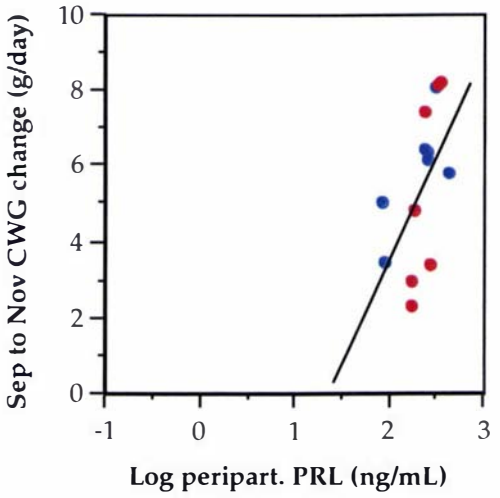

Figure 3.14: The relationship between the change in wool growth rate (CWG) from September to November and the log periparturient prolactin concentration in ND-lambed (●) and SD-lambed (●) ewes.

Lamb data

3.4.10 Lamb birth measurements

There was no significant treatment × sex interaction for lamb birth weight, crown-rump length, girth or head width. Therefore, means have been pooled across sex. Hindleg length in SD lambs was significantly greater ($P<0.01$) than in ND lambs (Table 3.7).

Table 3.7: Effects of photoperiod and maternal bromocriptine treatment on birth weight (BW), crown-rump length (CRL), girth, hindleg length and head width of the lamb experimental groups (Mean ± SEM).

Group	*n*	BW (kg)	CRL (mm)	Girth (mm)	Hindleg (mm)	Head (mm)
ND	7	5.1 ± 0.3	478 ± 14	386 ± 8	344 ± 7[a]	77 ± 3
SD/SD-BrA	15	5.1 ± 0.2	462 ± 10	393 ± 8	370 ± 5[b]	77 ± 2
SD-BrB	7	4.8 ± 0.2	475 ± 12	387 ± 7	356 ± 7[ab]	77 ± 3

[ab] Means within columns having superscripts with letters in common or no superscript are not significantly different ($P>0.05$).

3.4.11 Lamb live weight

Figure 3.15 shows the liveweight changes of the 4 lamb-treatment groups. Mean live weights increased from 5.0 ± 0.1 kg at birth in early September to 24.5 ± 0.4 kg at weaning in late November. There were no significant differences in lamb live weight between treatments or with sex, but ND lambs were consistently heavier on almost all occasions. The similarity in the birth and weaning weights for each treatment was reflected in the average daily weight gains which were 238.0 ± 9.1, 231.4 ± 4.6, 237.2 ± 11.7 and 244.1 ± 13.7 g/day for ND, SD, SD-BrB and SD-BrA lambs respectively.

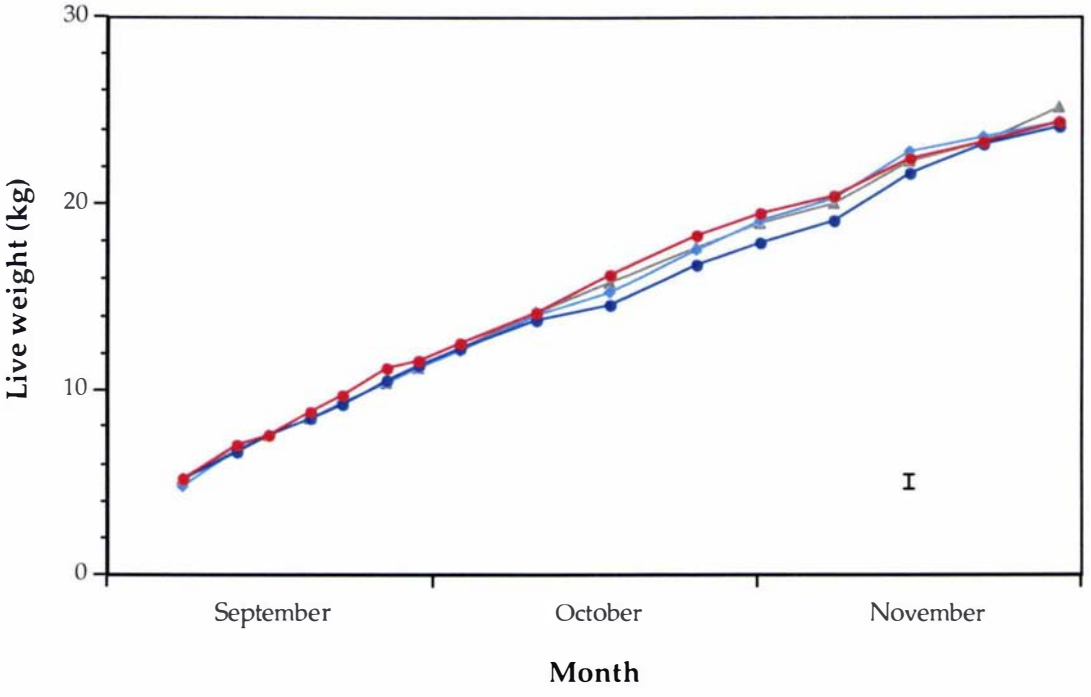

Figure 3.15: Mean live weight of lambs born to ND (●), SD (●), SD-BrB (◆) and SD-BrA ( ) ewes. Error bar represents the pooled SED.

3.4.12 Lamb plasma PRL concentration

At birth, PRL concentrations were significantly different ($P<0.05$) between groups. PRL concentrations were higher in ND and SD lambs than in SD-BrB lambs (21 ± 5 and 22 ± 7 ng/mL versus 7 ± 2 ng/mL, $P<0.05$) but were not different from those of SD-BrA lambs (13 ± 3 ng/mL, $P>0.10$) in both cases.

Plasma PRL concentrations followed the expected seasonal increase in ND lambs (Figure 3.16).

Trends in plasma PRL concentrations were significantly ($P<0.05$) influenced by photoperiod and drug treatment, and reflected in the slope of the regression line for each treatment. Both ND and SD lambs showed a significant increase ($P<0.001$) in PRL concentration with time (b = 0.0101 ± 0.0011 log ng/mL/day and b = 0.0045 ± 0.0013 log ng/mL/day for ND and SD lambs respectively). The rise in PRL concentration in ND lambs was over twice that in SD lambs from birth until weaning (Figure 3.16). There was no significant change in either bromocriptine-treated group (b = –0.0005 ± 0.0017 log ng/mL/day and b = 0.0014 ± 0.0012 log ng/mL/day for SD-BrB and SD-BrA lambs respectively).

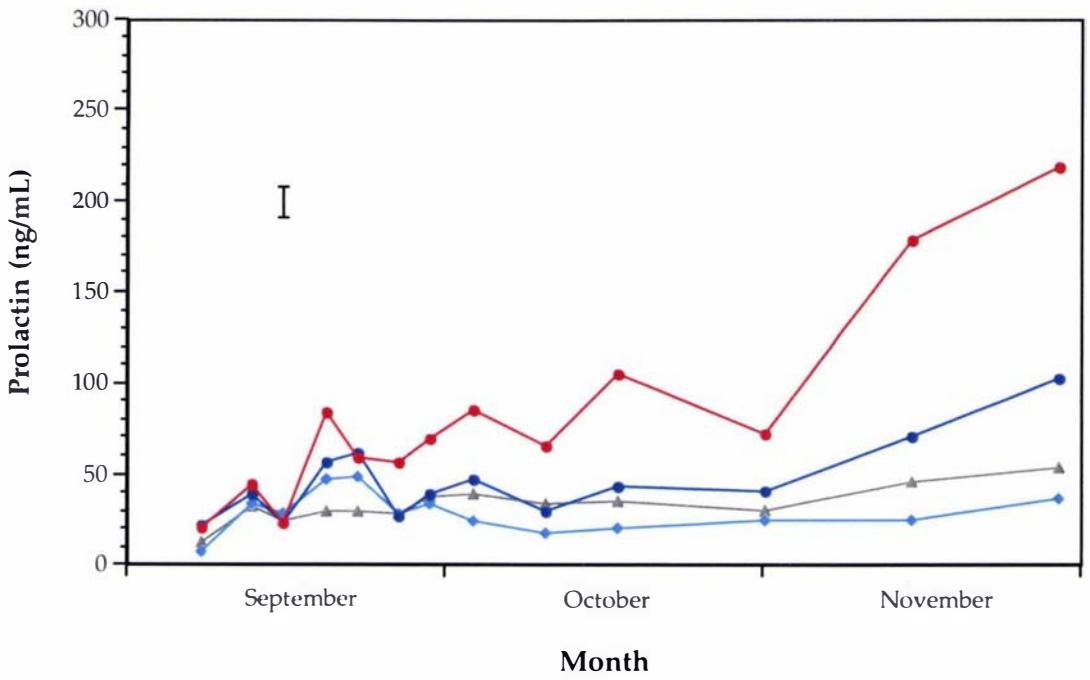

Figure 3.16: Mean plasma prolactin concentrations of lambs born to ND (●), SD (●), SD-BrB (◆) and SD-BrA (▲) ewes. Error bar represents the pooled SED.

The mean PRL concentration over the duration of the trial was determined by averaging each PRL concentration, excluding that found at birth. For the purpose of this analysis the PRL data from the SD-BrB and SD-BrA lambs

(referred to as SD-Br lambs) were pooled. Mean PRL concentrations in ND lambs (88 ± 6 ng/mL) were significantly higher than in SD lambs (54 ± 9 ng/mL, $P<0.01$) and SD-Br lambs (33 ± 3 ng/mL, $P<0.001$). In SD lambs, mean plasma PRL concentrations were also higher ($P<0.05$) than in SD-Br lambs.

3.5 DISCUSSION

The aims of this experiment were to characterise wool growth in Romney sheep during mid- to late pregnancy and early lactation under conditions of controlled dietary intake and photoperiod, and to determine the short- to medium-term effects on wool growth of changes in circulating levels of PRL concentration in pregnant and lactating Romney ewes.

A number of studies (Coop, 1950; Sumner, 1983; Hawker *et al.*, 1984; Hawker & Crosbie, 1985) have shown that the seasonal variation in the quality and quantity of feed play an important role in the control of wool growth. In the present study the daily feed intake and total feed intake were not significantly different within each reproductive class.

Liveweight changes are partially indicative of nutritional status and this was reflected in the differences in the live weight between non-pregnant and pregnant ewes at the beginning of the trial. Non-pregnant ewes weighed 58.3 ± 1.6 kg whereas the pregnant ewes, which were in mid-pregnancy at the time, weighed 50.7 ± 0.9 kg. Although these sheep were from the same parent flock, the non-pregnant ewes gained weight while grazing at Ruakura prior to the commencement of the trial. This was also reflected in the net liveweight change over the trial of the ewes depending on reproductive status. Non-pregnant ewes lost 0.8 ± 0.6 kg throughout the 5 months of the trial whereas ewes that produced and reared a single lamb gained 4.1 ± 0.6 kg. All groups had similar live weights by the completion of the trial suggesting that photoperiod and treatment with bromocriptine (Curlewis *et al.*, 1991) did not influence feed intake or liveweight changes.

PRL concentrations in both non-pregnant groups were significantly higher (39 ± 9 versus 8 ± 1 ng/mL) than in the 4 pregnant groups during late gestation. It is possible that the reduced PRL levels in pregnant sheep, as in pregnant rats, are due to a central inhibitory effect of high progesterone concentrations (Vermouth & Deis, 1974) or placental lactogen acting in a negative feedback fashion (Voogt *et al.*, 1982; Voogt, 1984). Plasma PRL concentrations in pregnant ewes not treated with bromocriptine increased rapidly 1–2 days prior to parturition. This pattern is similar to that described by previous authors (Lamming *et al.*, 1974; Munro *et al.*, 1980). While photoperiod did not influence PRL concentrations at parturition, ewes maintained in ND had higher PRL concentrations throughout lactation than ewes exposed to SD photoperiod. Experimental (Bocquier *et al.*, 1990) and seasonal (Rhind *et al.*, 1980) increases in photoperiod are known to affect PRL concentrations in the lactating ewe.

Treatment with bromocriptine was successful in reducing plasma PRL concentrations to low levels, consistent with previous results in pregnant ewes (Kann, 1976a; Peterson *et al.*, 1997). Bromocriptine treatment successfully eliminated the peripartum PRL surge and lactation PRL surge in SD-BrB ewes and the PRL surge associated with lactation in SD-BrA ewes. There is no evidence that bromocriptine can affect plasma concentrations of GH (Gow *et al.*, 1983), placental lactogen (Kann, 1976b; Martal & Lacroix, 1978; Gow *et al.*, 1983), oestrogens (Kann, 1976b), progesterone (Peterson *et al.*, 1997) or cortisol (Kann, 1976b). However, bromocriptine has been reported to reduce insulin concentrations and the GH-stimulated elevation of insulin concentrations (Johnsson *et al.*, 1985).

Suppression of PRL secretion during late pregnancy has been shown to delay the initiation of lactogenesis (Kann, 1976a, 1976b; Peterson *et al.*, 1997) and cause a reduction in milk yield in early lactation (Kann, 1976a; Gow *et al.*, 1983; Peterson *et al.*, 1991, 1997), although the milk yield is not affected if the bromocriptine treatment is terminated a few days prepartum (Kann, 1976a).

In the present experiment the initial dose of bromocriptine (100 mg/14 days) was considerably higher than those used by previous authors so it was not surprising that the onset of normal milk production was temporarily delayed and that some ewes required hand-milking to stimulate milk let-down. However, the similarity in SD-BrB lamb live weights to those of other treatment groups suggests that we were successful in overcoming any negative effect of bromocriptine on milk production.

Plasma PRL concentrations in the adult sheep are primarily determined by photoperiod (Munro *et al.*, 1980) with minimal levels in winter and spring rising to a peak in the summer, although some effect of temperature cannot be discounted (Schillo *et al.*, 1978). In the present experiment, increasing photoperiod was associated with a concomitant increase in PRL concentrations in ND non-pregnant ewes but not in non-pregnant ewes held under constant SD photoperiod. Although Mori *et al.* (1985) observed marked increases of plasma PRL concentrations in goats subjected to artificial SD when the ambient temperature exceeded 27°C, the monthly temperatures remained below 20°C over the experimental period in the present study, suggesting that the effect of temperature on PRL levels in SD non-pregnant ewes was likely to have been small.

A significant reduction in wool growth and mean fibre diameter in pregnant ewes had occurred at the start of the trial which was not attributable to maternal live weight or the nutritional demands of the conceptus at that time (Robinson, 1983; Rattray, 1986). Both groups had a winter minimum in wool growth rate in July, with pregnant sheep having a greater reduction than non-pregnant sheep. This result is consistent with Sumner and McCall (1989).

The effects of pregnancy and lactation on wool growth have been well documented in a variety of sheep breeds (see review by Corbett, 1979). A reduction in the annual fleece growth, due to both reduced length growth rate and reduced fibre diameter, is the cost normally associated with producing

lambs. In the present experiment, the average clean fleece weight obtained at shearing was 0.31 kg lower (a 19% reduction) compared with ewes that did not have lambs. Wool growth was reduced at all stages of pregnancy and early lactation irrespective of feed intake. In this study, wool growth rate was depressed by about 33% in mid- to late pregnancy compared with non-pregnant ewes, similar to that in pen-fed Merinos (Masters *et al.,* 1992). By comparison, the reduction in wool growth during lactation (14%) was considerably less than during pregnancy. Reid (1978) also reported that pregnancy had a greater impact on wool production than did lactation, while Story and Ross (1960), Armstrong and O'Rourke (1976), and Lee and Atkins (1995) recorded the opposite.

There are suggestions that the decline in wool production in late pregnancy is the inevitable result of a decline in the efficiency of the use of nutrients for wool growth (Oddy, 1985), and can be avoided by increasing the nutrient intake to allow for the increased requirements of the fetus (Williams & Butt, 1989). However, in the current experiment, like that of Masters *et al.* (1993), the sheep were fed to maintain the same conceptus-free live weight. This regime did not prevent the reduction in wool growth over pregnancy and suggests it is unlikely to be the result of simple competition for available nutrients between body tissues and conceptus, but could be due to a direct hormonal influence on wool growth *(see Chapter Five).*

The effects of pregnancy and lactation on wool growth were reflected by changes in fibre diameter. In relative terms, the reduction (–0.9 μm) in the fibre diameter of pregnant ewes from mid- to late pregnancy was similar to that reported by Masters and Stewart (1990). Minimum fibre diameter in pregnant ewes occurred in August, a month later than the minimum wool growth rate, but before parturition (Bigham *et al.,* 1983). Clearly, while wool growth rate and fibre diameter may, to some extent, change independently of each other, both contribute to the reduced fleece growth associated with producing a lamb.

Long-term exposure to SD photoperiod has previously been shown to increase spring wool growth rate in Corriedale ewes (Hart, 1961), although contrary to the current experiment, this was due to an increase in fibre length as opposed to fibre diameter. However, in another experiment (Pearson *et al.*, 1996) where non-pregnant Wiltshire ewes were exposed to SD over the same period, the fibre diameter also increased from October to December in SD ewes relative to ND ewes. If the present trial had been continued for a longer period, these differences in wool growth and fibre diameter may have been more pronounced given the trends observed.

Washing yield provides an estimate of the loss of grease, suint and dust during the scouring process, but the effects of climate and numerous technical difficulties have made the measurement of sebaceous and sudoriferous gland output difficult to quantify in the past. An added complication is that the proportions of grease and suint in the fleece vary independently of each other (Henderson, 1965). The experimental sheep were outdoors for the first 2 weeks of the July sampling period, and it is possible that the reduced patch washing yield in this month was partly influenced by the environmental conditions, including 52 mm of rainfall, over that period.

It has been reported that grease and suint production follows a similar seasonal pattern to that of wool growth (Batchelar, 1985; Butler & Head, 1993) and, in the present experiment, there was a strong positive ($P<0.001$) relationship between patch washing yield and wool growth rate in the first 3 months of the trial (Figure 3.8). This corresponds to the period of lowest wool growth. When wool growth rates increased in October and November this relationship disappeared. The differences noted between reproductive status and photoperiod are likely to be explained by this relationship with wool growth. Alternatively, the indoor environment may also have had an effect on washing yield with shutters being opened and closed for ND and SD ewes respectively.

The presence of PRL receptors in sweat and sebaceous glands (Choy *et al.*, 1995; 1997) provides indirect evidence that endogenous PRL concentrations could influence gland output. The monthly differences in fibre diameter and washing yield were not as apparent in midside wool collected from the fleece at shearing, but are consistent with an earlier report by Reid (1978), when both measurements tended to be lower in ewes that produced a lamb.

Photoperiod-induced increases in circulating PRL concentrations have been positively correlated with wool growth (Lincoln & Ebling, 1985; Lincoln, 1990). In non-pregnant ewes, the increase in clean wool growth from August to November was associated with the July PRL concentrations and the change in PRL concentration from the winter minimum. These observations are supported by Lincoln (1990) who reported that variation between breeds in the degree of seasonality in wool growth was positively correlated with the PRL concentration at the nadir of the cycle.

In pregnant ewes, differences in PRL concentrations at or around parturition and throughout lactation, and the decline in PRL concentrations from parturition to lactation, may play a role in the medium-term changes in wool growth observed in these experimental sheep. The almost complete absence of PRL (SD-BrB ewes) was associated with low rates of medium-term wool growth, while the suppression of PRL during lactation (SD-BrA ewes) was also associated with lowered rates of medium-term wool growth. Furthermore, PRL concentrations during lactation were associated with higher rates of wool growth, especially when concentrations gradually declined (SD-lambed ewes). Significantly, plasma PRL concentrations at parturition had no immediate effect on wool growth rate or fibre diameter, as both wool measures increased after the birth of the lamb, despite markedly different PRL profiles in the 4 treatment groups. The lag before changes in wool growth can be detected above the skin may highlight a limitation of using monthly midside patch clips for this purpose. The lack of an inhibitory wool growth response as a

consequence of rising PRL concentrations (Pearson *et al.*, 1996) eliminates any possibility of PRL being associated with the lambing break in the fleece.

In the present study, the birth weight of lambs born in the spring were similar to those reported in previous studies using the same breed (Dalton & Ackerley, 1974; Hawker & Thompson, 1987). Neither photoperiod nor treatment with the dopamine agonist bromocriptine had any effect on lamb weight, crown-rump length, girth or head width measurements recorded at birth, suggesting that the manipulation of maternal PRL concentrations has little influence on lamb growth over the last 6 weeks of gestation. However, photoperiod may affect long bone growth, as the hindleg length was greater in SD lambs compared to those whose dams were exposed to ND photoperiod.

There are numerous studies supporting an effect of changing daylength on lamb growth rate where lambs grow faster when exposed to increasing daylength (Forbes *et al.*, 1975, 1979a, 1979b; Schanbacher & Crouse, 1980). Observations in this study do not support this hypothesis as both the daily weight gain and weaning weight were similar in all treatment groups despite differing environmental conditions. One possible explanation is that the lambs were exposed to short or increasing photoperiod from birth in the present study but were at least 10 weeks old in the other studies.

The level of feeding has also been shown to influence lamb growth (Forbes *et al.*, 1975, 1979a) with higher growth rates observed in lambs fed *ad libitum*. While lamb feed intake was not recorded in the present experiment, the similarity in liveweight gain, weaning weight and lack of significant changes in dam live weights would suggest that feed intake was similar between treatment groups despite the fact that photoperiod (Bocquier *et al.*, 1986, 1997) and bromocriptine treatment (Kann, 1976a, 1976b; Gow *et al.*, 1983; Peterson *et al.*, 1997) are known to influence ewe milk yield. On the latter point, it should be noted that SD-BrB lambs were initially hand-fed to equalise milk intakes. Data from a subsequent experiment *(Section 4.4.14)* indicate that it is unlikely

that the lambs were under any nutritional constraint in the second and third months.

Plasma PRL concentrations in lambs were markedly different in the 4 treatment groups at birth. These observations are supported by previous authors (Bassett *et al.*, 1988; Ebling *et al.*, 1989; Helliwell *et al.*, 1997; Houghton *et al.*, 1997) who provided evidence that PRL concentrations in the ovine fetus are entrained to the photoperiod experienced by the mother during late gestation. A clear pattern emerges over time with PRL concentrations being elevated in ND lambs as a result of the influence of photoperiod (Ravault, 1976). PRL levels are known to increase in lambs exposed to longer daylength compared to shorter daylength (Forbes *et al.*, 1975; Brown & Forbes, 1980; Schanbacher & Crouse, 1980; Fitzgerald *et al.*, 1982; Brinklow & Forbes, 1984). Both the mean PRL concentration and rate of increase with time were significantly higher in ND lambs compared to SD and SD-Br lambs. Unlike SD ewes, the PRL concentration in SD lambs also increased from September to November despite a constant photoperiod, possibly reflecting the role of temperature in regulating circulating PRL concentrations in juvenile ruminants (Wettemann & Tucker, 1974; Peters *et al.*, 1980; Fitzgerald *et al.*, 1982; Eisemann *et al.*, 1984).

PRL concentrations in SD-Br lambs were significantly lower than in lambs exposed to ND and SD photoperiod throughout the experiment. This study provides the first evidence that treatment of ewes with bromocriptine before parturition also suppresses plasma PRL concentrations in their lambs (SD-BrB) from birth. The reduction in neonatal PRL concentrations supports the placental passage of bromocriptine previously reported in humans (Bigazzi *et al.*, 1979; Roti *et al.*, 1986) and in the rat (Reusens *et al.*, 1979). Furthermore, plasma PRL concentrations in SD-BrA lambs were lower than in SD lambs, raising the possibility that this effect may also be mediated via the milk.

3.6 CONCLUSIONS

Wool production was depressed during mid- to late pregnancy despite maintenance of maternal live weight. The treatment groups had markedly different plasma PRL profiles over the 5 months of the trial. In comparison with non-pregnant ewes, PRL concentrations were lower in pregnant ewes before parturition. This is likely to be mediated by changes in the concentrations of other hormones such as progesterone and placental lactogen. Wool growth increased in all pregnant groups around parturition, indicating that this increase was not dependent on PRL concentrations. In non-pregnant ewes, the increase in spring wool growth rate was positively correlated to the winter PRL concentration. In pregnant ewes, however, this increase in wool growth was linked to the PRL concentrations at parturition and the decline in PRL concentration following parturition. While the PRL surge at parturition may not be the cause of the lambing break in the fleece of breeding ewes, it could have a medium- to long-term stimulatory effect on wool growth.

CHAPTER FOUR

Effects of photoperiod and bromocriptine treatment on wool growth during pregnancy and lactation in June-lambing Romney ewes

4.1 ABSTRACT

Photoperiod control and treatment with long-acting bromocriptine were used to investigate potential effects of PRL surges on wool growth associated with parturition and lactation in Romney ewes bred out of season. Fourteen non-pregnant and 29 pregnant ewes were maintained indoors from early April 1995 for 12 months under controlled photoperiod and controlled dietary intake. Two groups (n = 8) were held under long day photoperiod (16L:8D; LD non-pregnant and LD-lambed) while 2 others (n = 6–7) were exposed to natural photoperiod (ND non-pregnant and ND-lambed). Two further groups (n = 7) of pregnant ewes housed in natural days were treated with bromocriptine, either from 1 week before parturition (ND-BrB) or 1–3 days after parturition (ND-BrA), to suppress PRL secretion. Lambing occurred between 12 and 18 June. Photoperiod and treatment with bromocriptine did not affect the birth weight or liveweight changes of lambs, but did influence their circulating PRL concentrations. LD lambs had higher ($P<0.001$) PRL concentrations than ND lambs and ND lambs whose dams were treated with bromocriptine. In ND non-pregnant ewes, changes in plasma PRL concentrations were associated with seasonal changes in daylength over the duration of the trial. In LD non-pregnant ewes, continued exposure to LD photoperiod from April caused a significant increase in PRL concentrations over the winter months. PRL concentrations were low in the ND-BrB group throughout the trial, and in the ND-BrA group following the PRL peak associated with parturition. Apart from the ND-BrB group, PRL concentrations in pregnant ewes increased rapidly a few days prior to parturition and subsequently remained elevated. PRL concentrations over parturition and lactation were highest in LD-lambed ewes.

Despite a comparable maternal live weight, lamb production was associated with a lower clean fleece weight, and a reduced mean fibre diameter, staple length and staple tensile strength compared to non-pregnant groups. LD ewes grew significantly more ($P<0.01$) clean wool than their ND counterparts as a

consequence of an increase in mean fibre diameter and length growth rate. PRL surges associated with parturition and lactation had a significant stimulatory ($P<0.001$) effect on both wool growth rate and fibre diameter in LD-lambed ewes from June to September relative to ND-lambed ewes, while the complete absence of the periparturient PRL surge was associated with longer-term inhibitory effects on wool growth rate. These results indicate that, although the depression in wool growth rate during late pregnancy is unlikely to be associated with changes in PRL concentrations in winter-lambing ewes, elevated plasma PRL concentrations during pregnancy, and at parturition, followed by a gradual decline in PRL concentrations over lactation are associated with increased wool growth rate associated equally with increases in mean diameter and length growth rate of the fibres.

4.2 INTRODUCTION

The seasonal nature of New Zealand's lamb production has been identified as a major limitation to the value of New Zealand's lamb exports (Johnson, 1989). In response to this, there has been an increasing trend for farmers to adopt a mix of winter- and spring-lambing policies. Research to evaluate ewe and lamb performance in such flocks has led to the finding that winter-lambing ewes produce more greasy wool (up to 1.1 kg) at annual shearing than spring-lambing ewes (Reid *et al.*, 1988; Reid & Sumner, 1991; Morris *et al.*, 1993). This difference in wool production was later shown to be a consequence of a significantly reduced winter decline in wool production by the June-lambing ewes, which was associated with an increase in mean fibre diameter and staple tensile strength (Morris *et al.*, 1994). An improvement in the annual wool production of the conventional spring-lambing national flock as seen in winter-lambing ewes (Morris *et al.*, 1993; 1994), would clearly have direct benefits to the sheep industry by improving production and wool processing efficiency.

While it has been well demonstrated that the winter decline in wool production seen in the British longwool sheep breeds results in a significant reduction in both wool production and tensile strength, the cause of the winter decline remains obscure. As outlined in Chapter Three, there appear to be interactions between nutrition, the extra metabolic demands of the fetus in pregnant ewes and photoperiod (possibly driven by plasma PRL concentrations). Separating these effects and determining their relative importance has not yet been achieved.

The present experiment (FP003/02) was designed to examine whether manipulation of maternal PRL concentrations in June-lambing ewes was associated with changes in wool growth rate and related characteristics. This study also provided the opportunity to make a comparison with wool production in September-lambing ewes *(Chapter Three)*. The sheep were fed to

a constant maternal live weight in an attempt to remove or reduce the effects of variable nutrient availability on fibre growth. Of the 41 sheep in the trial, 15 were held in long days in order to elevate circulating PRL levels to those normally associated with the photoperiod during the summer months and the remaining 26 were held in natural days.

4.3 MATERIALS AND METHODS

4.3.1 Experimental Animals

A flock of 250 Romney ewes from the Horotiu property of Mr. John Reeves, were induced to cycle using progesterone-impregnated EAZI-breed CIDR™ type G devices on 4 January 1995. After removal of the CIDRs 14 days later, ewes were administered 400 iu PMSG and joined with harnessed vasectomised rams (14:1 ratio). Approximately 48 h later, on 20 January (day 0 of gestation), 150 Romney ewes were artificially inseminated using the laparoscopic technique with frozen semen pooled from 6 Romney rams. Twenty-nine pregnant ewes carrying single fetuses were identified by ultrasonic scanning on 17 March (day 56). The 29 pregnant ewes and 14 non-pregnant ewes were transported to Ruakura Agricultural Research Centre, Hamilton, on 22 March.

4.3.2 Housing

All the ewes were transferred indoors to individual pens in 2 separate rooms in the Ruakura Physiology Building on 6 April (day 76). The trial commenced on 12 April. Each ewe was maintained in an individual pen from 6 April 1995 to 8 April 1996.

4.3.3 Environmental Observations

The daily minimum and maximum air temperatures recorded at the Ruakura meteorological station from April 1995 to April 1996 are shown in Figure 4.1.

Figure 4.2 shows the mean monthly temperatures and hours of daylight over the same period.

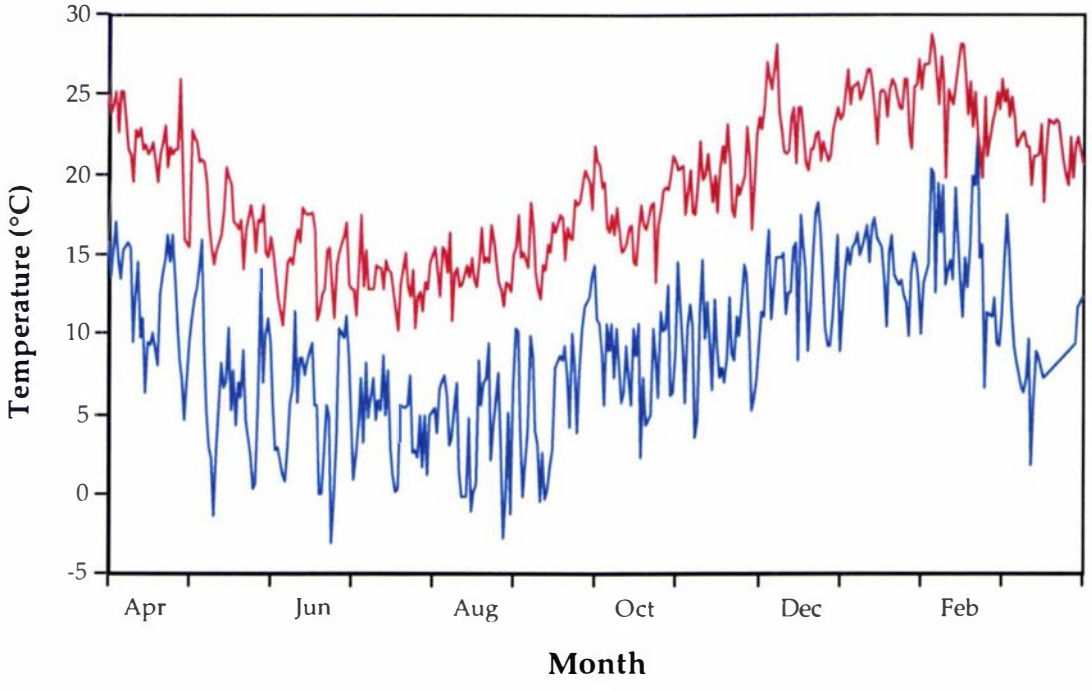

Figure 4.1: Daily minimum (–) and maximum (–) air temperatures during the 1995–96 experimental period.

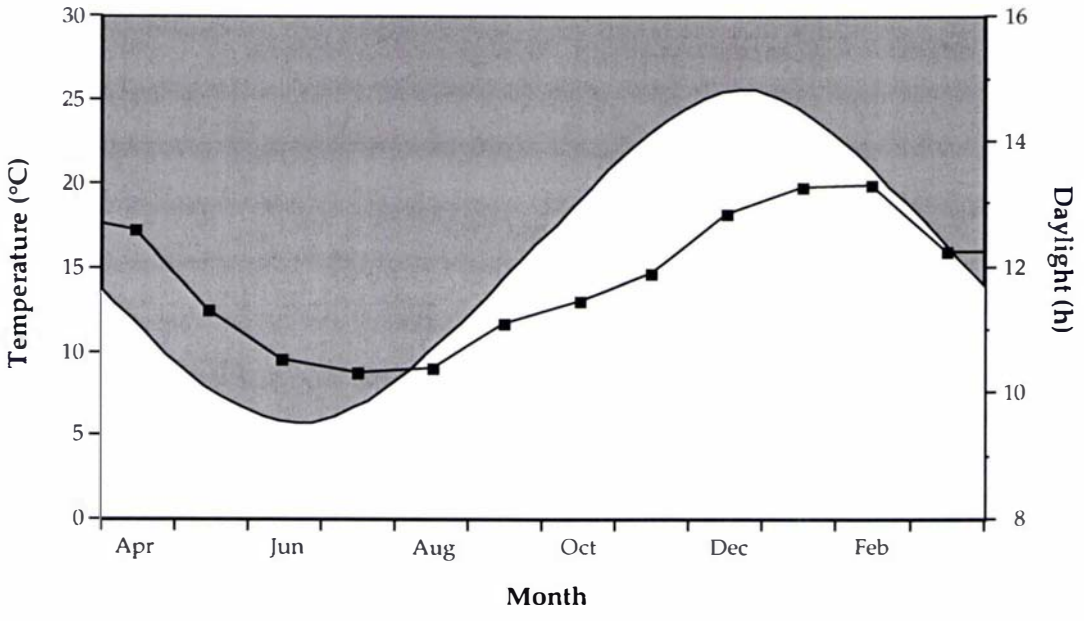

Figure 4.2: Monthly mean air temperature (■) and hours of daylight during the 1995–96 experimental period.

4.3.4 Experimental Groups

The experiment was a 2 × 2 factorial design incorporating 2 levels of reproductive status (non-pregnant versus pregnant) and 2 levels of photoperiod (natural days versus long days) in Groups 1--4. There was also a comparison of 2 bromocriptine treatments with ND-lambed ewes (Groups 4–6, Table 4.1). The 43 sheep were allocated to one of 6 groups based on reproductive status, balanced for live weight and April greasy wool growth.

Table 4.1: Experimental groups.

Group		n	Abbreviation
Long Days			
1	Non-pregnant ewes	8	LD non-pregnant
2	Pregnant ewes	8	LD-lambed
Natural Days			
3	Non-pregnant ewes	6	ND non-pregnant
4	Pregnant ewes	7	ND-lambed
5	Pregnant ewes treated with bromocriptine from day 135 of gestation	7	ND-BrB
6	Pregnant ewes treated with bromocriptine approximately 3 days after parturition	7	ND-BrA

Lambs born to ewes from Groups 2, 4, 5 and 6 will be referred to as LD, ND, ND-BrB and ND-BrA lambs respectively.

4.3.5 Light Treatment

Groups 1–2 (Room 1) were held under long day conditions (16L:8D) while groups 3–6 (Room 2) were exposed to natural photoperiod as described in Section 2.4.

4.3.6 Live weight

All sheep and lambs were weighed at 7 to 14 day intervals over the duration of the trial to monitor changes in live weight.

4.3.7 Feed Allowance

The daily feed allowance for pregnant and non-pregnant ewes was calculated and the daily feed intakes measured as described in Section 2.3.

4.3.8 Lamb Measurements

All lambs were weighed within 24 h of birth and subsequently, at the same time as their dams until they were weaned at 12 weeks of age on 12 September. Crown-rump length, girth, maximum head width and hindleg length measurements were recorded along with the birth weight.

4.3.9 Wool Sampling

The sheep were shorn on entry to the experiment on 31 March 1995 and again at the conclusion of the experiment on 9 April 1996 when the fleece was weighed and a midside sample collected *(Section 2.7.3).* A standardised midside patch clip was established on 3 April 1995 and re-clipped every month. Weekly intradermal injections of ^{35}S-cysteine at a tattooed site were used to determine the length growth rates of individual wool fibres in all sheep from 1 week prior to parturition (7 June) until 3 weeks after parturition (5 July). Wool staples were harvested on 26 July for autoradiographic determination of growth rates *(Section 2.8).*

4.3.10 Blood Sampling

Blood samples were collected from all ewes by venipuncture of the jugular vein at regular intervals between 13 April 1995 and 6 April 1996. The intervals ranged from every 3 weeks at the beginning and end of the experiment to daily sampling closer to parturition. Following parturition, blood samples were also collected from the lambs at the same times as their dams. Inter-assay and intra-assay coeffecients of variation for the PRL radioimmunoassay at 10 ng/mL were 7.7% and 12.5% respectively.

4.3.11 Bromocriptine Administration

Bromocriptine was given intramuscularly at 2-weekly intervals from 6 June (day 137) in ND-BrB ewes and from 19 June (day 150) in ND-BrA ewes. The treatment schedule is shown in Table 4.2.

Table 4.2: Bromocriptine treatment schedule administering 50 mg Parlodel LA* to ND-BrB and ND-BrA ewes.

Date		Day Post-conception	Group
6	June	137	ND-BrB
~ 15	June (parturition)	~ 146	
19	June	150	ND-BrA
3	July	164	ND-BrB, ND-BrA
17	July	178	ND-BrB, ND-BrA
31	July	192	ND-BrB, ND-BrA
14	August	206	ND-BrB, ND-BrA
28	August	220	ND-BrB, ND-BrA
11	September	234	ND-BrB, ND-BrA
25	September	248	ND-BrB, ND-BrA
9	October	262	ND-BrB, ND-BrA
24	October	277	ND-BrB, ND-BrA
7	November	291	ND-BrB, ND-BrA
21	November	305	ND-BrB, ND-BrA
5	December	319	ND-BrB, ND-BrA
3	January	334	ND-BrB, ND-BrA
17	January	348	ND-BrB, ND-BrA
31	January	362	ND-BrB, ND-BrA
29	February	377	ND-BrB, ND-BrA

*intramuscular 28-day formulation

4.3.12 Statistical Methods

Data relating to feed intake, ewe and lamb live weight, plasma PRL concentration, clean wool growth rate and other fleece measurements were

subjected to analysis of variance at each sampling time to test the effects of reproductive status, photoperiod and their interactions. For ewe wool data, the April value for each parameter was used as a covariate for that parameter. Data from one ND-BrB ewe (#1308; whose lamb died), and one LD-lambed ewe (#1341; who had respiratory problems) were excluded from analyses. Multiple regression analysis was used to analyse the change in PRL concentration over time in the lambs.

4.4 RESULTS

Lambing details

Nineteen male and 8 female lambs were born between 12 and 18 June 1995 with the mean lambing date being 15 June.

Ewe data

4.4.1 Feed intake

Total feed intake and mean daily feed intake (Figure 4.3) were higher in lambing ewes (488 ± 5 kg and 1330 ± 13 g/day) compared with non-pregnant ewes (313 ± 2 kg and 853 ± 7 g/day). In non-pregnant ewes, there was no difference in the daily feed intake or the total feed intake due to photoperiod. Likewise, the daily feed intake and the total feed intake were similar in the 4 pregnant treatment groups. Ewes in LD conditions had a higher daily feed intake (1439 ± 26 versus 1336 ± 21 g/day, $P<0.05$) than did ewes in ND over pregnancy but these differences were not significant ($P>0.10$) following parturition. Although some ewes took longer to adapt to indoor feeding and therefore had lower total feed intakes in April, this was not reflected in April wool growth rates.

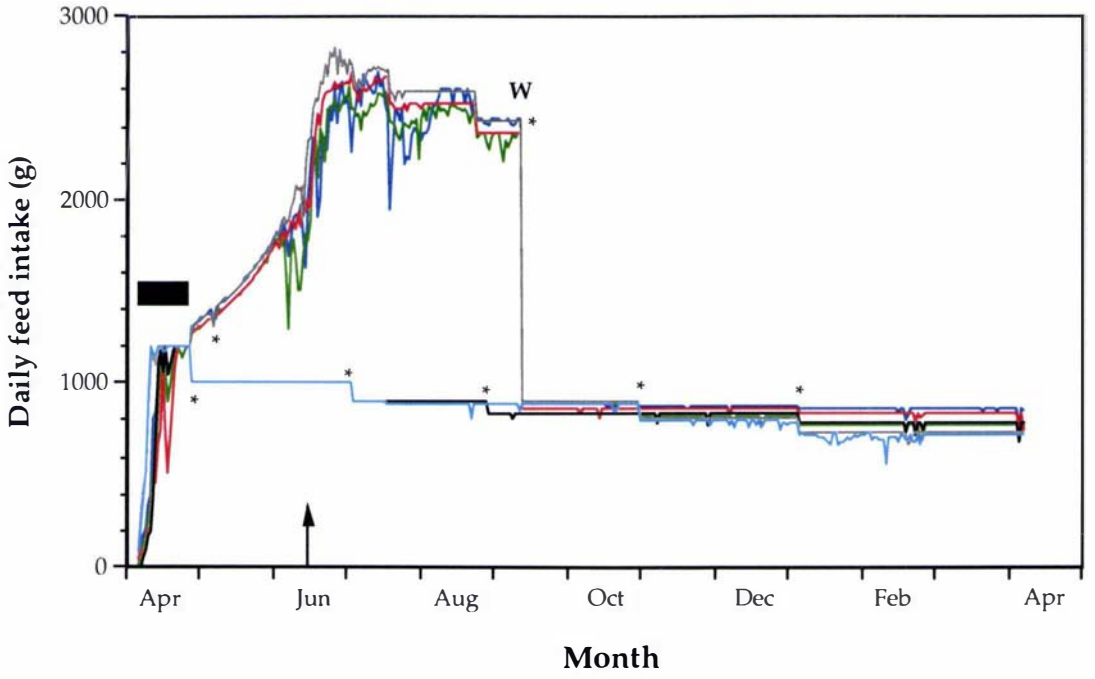

Figure 4.3: Mean daily feed intake of LD non-pregnant (–), LD-lambed (–), ND non-pregnant (–), ND-lambed (–) and ND-lambed ewes treated with bromocriptine before parturition (–) or after parturition (–). Solid bar represents the period of feed adaptation; arrow represents mean date of parturition; W represents weaning; * represents when daily feed allowance was adjusted across some or all groups.

4.4.2 Live weight

Non-pregnant and pregnant ewes were not significantly different in live weight at the start of the experiment and during April (Figure 4.4, Table 4.3). Pregnant ewes gained significantly more weight than non-pregnant ewes (11.3 ± 0.6 versus 1.4 ± 0.8 kg, $P<0.001$) from April until early June, as pregnancy advanced. This weight change did not differ by treatment within each reproductive class. One week before parturition, all pregnant groups were significantly heavier ($P<0.001$) than non-pregnant groups. Following parturition, ewes that produced a lamb were still significantly heavier ($P<0.05$) than non-pregnant ewes. Treatment with bromocriptine had no effect on live weight (Table 4.3).

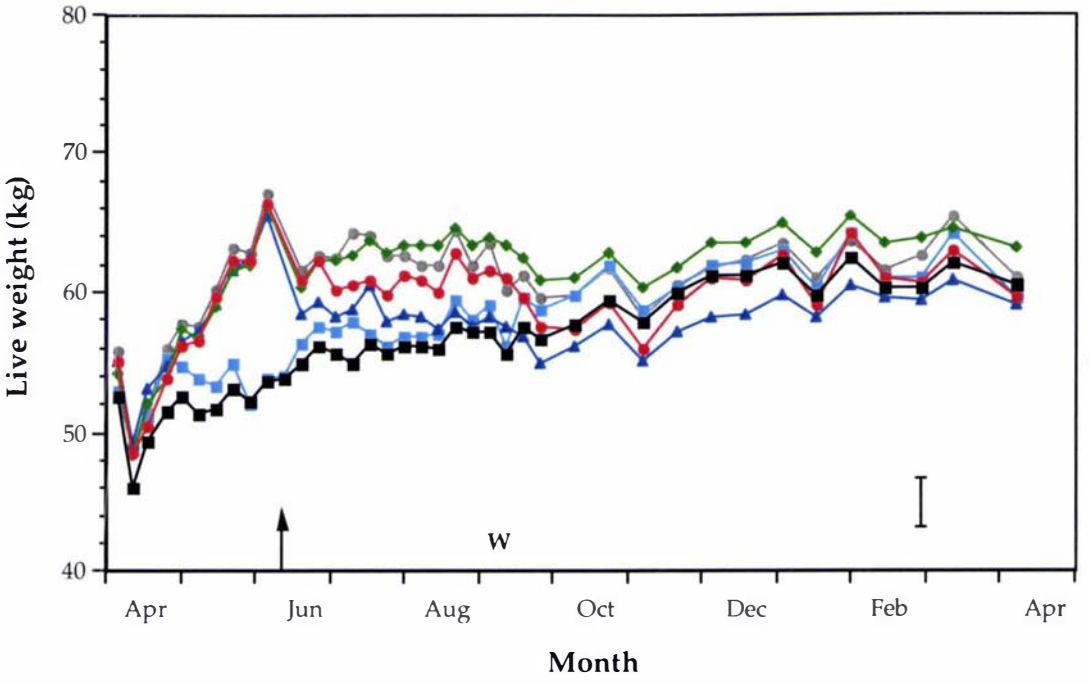

Figure 4.4: Mean live weight of LD non-pregnant (■), LD-lambed (●), ND non-pregnant (■), ND-lambed (●) and ND-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition; W represents weaning. Error bar represents pooled SED.

Table 4.3: Effects of pregnancy/lactation, photoperiod and bromocriptine treatment on the initial, prepartum, postpartum, weaning and fleece-free final live weights (LW) of the experimental groups (Mean ± SEM).

Group	*n*	Initial LW (kg)	Prepart. LW (kg)	Postpart. LW (kg)	Weaning LW (kg)	Final LW # (kg)
LD non-pregnant	8	52.9 ± 1.7	54.0 ± 1.9^{a}	56.3 ± 1.7	56.1 ± 1.4^{a}	53.8 ± 1.1
LD-lambed	7	55.7 ± 2.4	67.0 ± 2.1^{b}	61.5 ± 2.1	60.1 ± 2.6^{ab}	55.2 ± 2.5
ND non-pregnant	6	52.6 ± 2.4	54.5 ± 2.2^{a}	54.8 ± 2.1	55.6 ± 1.7^{a}	55.3 ± 1.2
ND-lambed	7	55.0 ± 2.6	66.3 ± 2.9^{b}	60.8 ± 3.0	61.1 ± 2.7^{ab}	54.7 ± 2.0
ND-BrB	6	54.1 ± 2.2	66.2 ± 3.0^{b}	60.4 ± 2.2	63.3 ± 3.5^{b}	57.8 ± 3.0
ND-BrA	7	55.0 ± 2.2	65.5 ± 2.2^{b}	58.4 ± 1.9	57.5 ± 2.5^{ab}	54.0 ± 2.4
Reprod. status						
Non-pregnant	14	52.7 ± 1.3	54.2 ± 1.4^{a}	55.7 ± 1.3^{a}	55.9 ± 1.0^{a}	54.4 ± 0.8
Preg./Lact.	27	55.0 ± 1.1	66.3 ± 1.2^{b}	60.3 ± 1.1^{b}	60.4 ± 1.4^{b}	55.3 ± 1.2

Final live weight minus fleece weight at shearing
^{ab} Within columns means within treatment groups and within reproductive status having superscripts with letters in common, or no superscript, are not significantly different (P>0.05).

Lactating ewes were consistently heavier than non-pregnant ewes (61.2 ± 1.3 versus 56.8 ± 1.2 kg, P<0.05) but the weight gain over lactation was not

influenced by reproductive status. These differences were reflected in the mean live weight at weaning (Table 4.3) when lactating ewes were still heavier ($P<0.05$) than non-pregnant ewes. ND-BrB ewes were heavier than other lactating ewes at weaning but this difference was not significant ($P>0.10$).

From weaning in September 1995 until the completion of the trial in April 1996 non-pregnant ewes gained significantly more weight than ewes which had lambed (4.1 ± 0.5 versus 0.3 ± 0.6 kg, $P<0.001$) so that the final mean live weight was similar for all treatment groups (Figure 4.4 and Table 4.3). ND and LD photoperiod had no effect on live weight in either reproductive class throughout the experiment.

On average, non-pregnant ewes gained 1.7 kg compared to a gain of 0.3 kg for ewes that reared a single lamb. This difference was not significant ($P>0.10$). Photoperiod did not influence liveweight change (0.3 ± 1.0 versus 1.1 ± 0.8 kg for LD and ND ewes respectively, $P>0.10$) but ND-BrB ewes gained significantly more weight than the other ewes which lambed (3.7 ± 1.5 versus −0.6 ± 0.8 kg, $P<0.05$).

4.4.3 Plasma PRL concentration

Markedly different plasma PRL profiles were achieved in the 6 treatment groups over the experimental period (Figure 4.5). PRL concentrations in non-pregnant ewes were significantly higher than in pregnant ewes (80 ± 18 versus 17 ± 4 ng/mL, $P<0.001$) in the period prior to 2 June when bromocriptine was first administered (Figure 4.6). PRL concentrations over the same period were also influenced by photoperiod, with levels being significantly higher in LD non-pregnant ewes compared to ND non-pregnant ewes (115 ± 21 versus 33 ± 9 ng/mL, $P<0.01$) and LD-lambed ewes compared to ND-lambed ewes (36 ± 12 versus 12 ± 2 ng/mL, $P<0.01$).

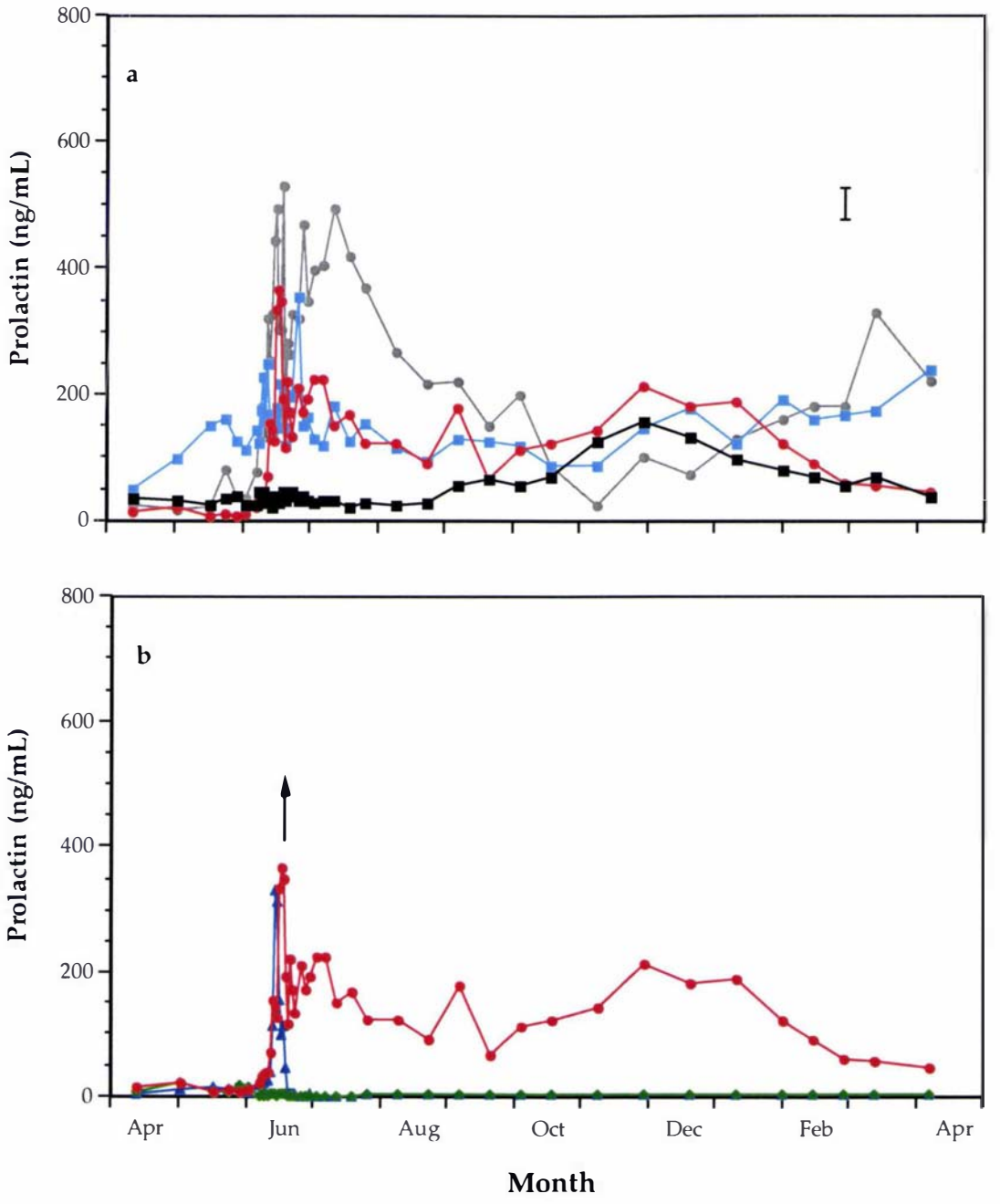

Figure 4.5: Mean plasma prolactin concentrations of (a) LD non-pregnant (■), LD-lambed (●), ND non-pregnant (■) and ND-lambed ewes (●); and (b) ND-lambed ewes (●) and ND-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

The PRL profile in ND non-pregnant ewes followed the expected seasonal pattern with PRL concentrations remaining low throughout autumn and winter (April-August). In contrast, the PRL concentrations of non-pregnant ewes maintained in LD photoperiod increased over this period reaching a peak in late June/early July (Figure 4.5a). The mean PRL concentrations from

April to August were significantly higher in LD non-pregnant ewes compared to non-pregnant ewes held in ND conditions (159 ± 18 versus 32 ± 5 ng/mL, $P<0.001$). From September, PRL concentrations in ND non-pregnant ewes increased ($P<0.001$), peaking in late November. PRL concentrations in LD non-pregnant ewes remained elevated over this period and there was no difference between ND and LD non-pregnant ewes from mid-October to early January inclusive. PRL concentrations in LD non-pregnant ewes had increased above those of ND non-pregnant ewes by 31 January and continued to increase over late summer/early autumn as levels in ND non-pregnant ewes declined.

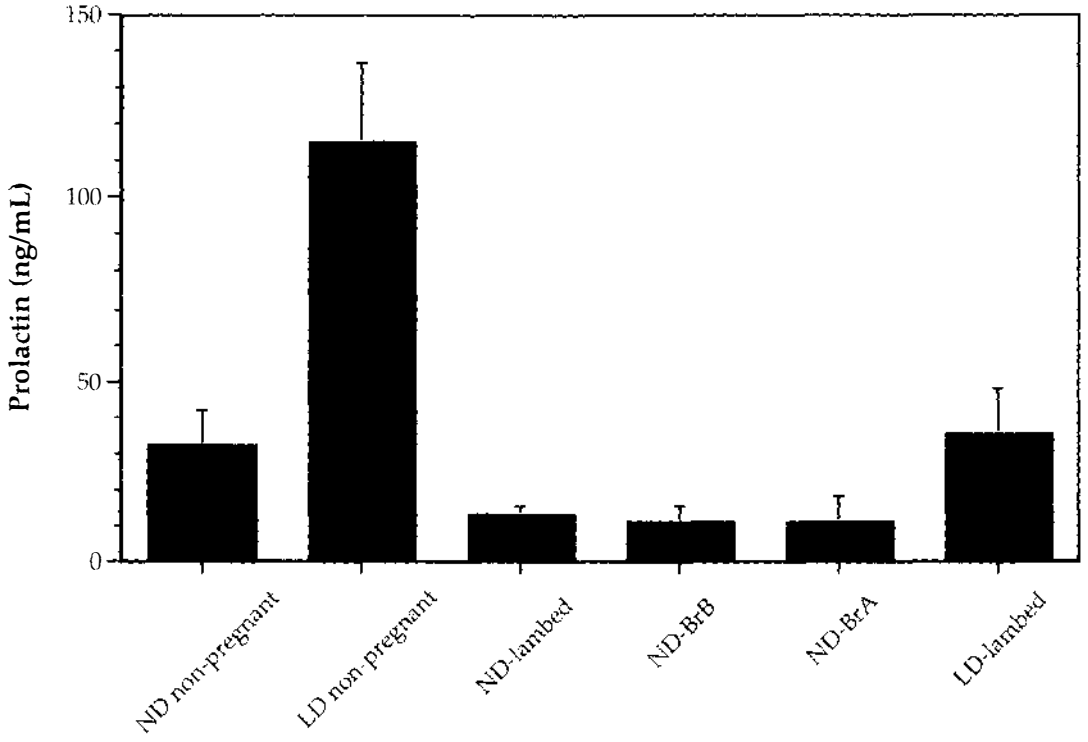

Figure 4.6: Plasma prolactin concentration (Mean ± SEM) in all treatment groups from 13 April to 2 June inclusive when bromocriptine was first administered.

In the ND-lambed group, PRL concentrations increased rapidly 1–2 days prior to parturition, reaching a peak of 366 ± 124 ng/mL (Figure 4.5a). Plasma PRL concentrations in ND non-pregnant ewes remained low (30 ± 5 ng/mL, $P<0.001$) by comparison. Similarly, PRL concentrations were elevated over lactation in ND-lambed ewes compared to non-pregnant ewes in ND (162 ± 27

versus 35 ± 5 ng/mL, $P<0.001$) until weaning on 12 September. Following weaning, PRL levels in ND ewes which had lambed were generally no different to those of non-pregnant ewes (Figure 4.5a).

The PRL profile of LD-lambed ewes followed a similar trend to that of ND-lambed ewes except that concentrations increased earlier and were significantly higher ($P<0.001$) in the week before parturition (Figure 4.5a). Peak PRL concentrations averaged 492 ± 83 ng/mL and the mean PRL levels around parturition were significantly higher (348 ± 42 versus 197 ± 32 ng/mL, $P<0.05$) in LD-lambed ewes compared to ND-lambed ewes. Exposure to LD photoperiod also resulted in significantly higher mean PRL concentrations during lactation (341 ± 33 versus 170 ± 31 ng/mL, $P<0.01$). By mid-October, plasma PRL concentrations were similar in both lambing groups regardless of photoperiod, but levels continued to decline in LD-lambed ewes (Figure 4.5a) and remained significantly lower over summer (98 ± 19 versus 170 ± 23 ng/mL, $P<0.05$). While PRL concentrations did not differ between ND-lambed and LD-lambed ewes on 31 January, PRL concentrations in LD ewes had increased above those of ND ewes by 14 February (184 ± 38 versus 92 ± 15 ng/mL, $P<0.05$) and increased ($P<0.001$) further by March/April.

In comparison to LD non-pregnant ewes, plasma PRL concentrations in LD-lambed ewes were significantly lower ($P<0.01$) until 8 June (1 week before parturition) when PRL levels started to increase and, by parturition, were significantly higher (379 ± 62 versus 163 ± 22 ng/mL, $P<0.01$, Figure 4.5a). PRL concentrations throughout lactation remained elevated (341 ± 33 versus 160 ± 19 ng/mL, $P<0.001$) in LD-lambed ewes compared to LD non-pregnant ewes. By 20 September (one week after weaning) PRL concentrations were not significantly different and the PRL profile was similar in both LD groups until the end of the trial in April (Figure 4.5a).

In ND-BrB ewes, the PRL peaks associated with parturition, lactation, and summer photoperiod were completely abolished and PRL concentrations

remained suppressed over the course of the trial (Figure 4.5b). The mean PRL concentration from 6 June (when bromocriptine was first administered) was significantly lower (1.3 ± 0.2 versus 144 ± 18 ng/mL, $P<0.001$) in ND-BrB ewes than in ND-lambed ewes. In ND-BrA ewes, PRL concentrations had a similar profile to those of ND-lambed ewes and rose rapidly to 330 ± 59 ng/mL a few days before parturition. The administration of bromocriptine completely abolished PRL surges associated with lactation and the expected summer rise (Figure 4.5b). For the balance of the trial concentrations remained lower (1.4 ± 0.1 ng/mL, $P<0.001$) than in ND-lambed and ND non-pregnant ewes. Plasma PRL concentrations in ND-BrA ewes were no different to those found in ND-BrB ewes following bromocriptine administration.

4.4.4 Fleece weight

Greasy fleece weights recorded at shearing in April 1996 indicated that non-pregnant ewes produced more greasy wool than ewes that had lambed (5.50 ± 0.22 versus 5.33 ± 0.13 kg). The difference was not significant ($P>0.10$, Table 4.4). LD-lambed ewes produced significantly ($P<0.05$) more greasy wool than ND-lambed ewes. This photoperiod effect was not seen in non-pregnant ewes. Greasy fleece weights were unaffected by bromocriptine treatment. Sheep maintained in LD photoperiod produced significantly more ($P<0.01$) clean fleece wool at shearing than those in ND (Table 4.4). LD-lambed ewes grew 26% more clean fleece wool than ND-lambed ewes (4.46 ± 0.24 versus 3.54 ± 0.14 kg, $P<0.01$). There was a 10% increase in the clean fleece weight of non-pregnant ewes held in LD compared to ND ewes (4.20 ± 0.27 versus 3.81 ± 0.30 kg, $P>0.10$). Clean fleece weights were unaffected by reproductive status (4.03 ± 0.20 versus 3.78 ± 0.12 kg for non-pregnant and lambed ewes) or bromocriptine treatment.

Table 4.4: Effects of pregnancy/lactation, photoperiod and bromocriptine treatment on greasy fleece weights (GFW), washing yield and clean fleece weights (CFW) at shearing of the experimental groups in April 1996 (Mean ± SEM).

Group	*n*	GFW (kg)	Washing Yield (%)	CFW (kg)
LD non-pregnant	8	5.75 ± 0.31^{ab}	72.8 ± 1.2^{bc}	4.20 ± 0.27^{a}
LD-lambed	7	5.95 ± 0.29^{b}	74.9 ± 1.1^{c}	4.46 ± 0.24^{b}
ND non-pregnant	6	5.18 ± 0.30^{a}	73.0 ± 2.1^{bc}	3.81 ± 0.30^{a}
ND-lambed	7	4.98 ± 0.19^{a}	71.2 ± 1.6^{bc}	3.54 ± 0.14^{a}
ND-BrB	6	5.25 ± 0.28^{ab}	65.9 ± 1.7^{a}	3.46 ± 0.21^{a}
ND-BrA	7	5.13 ± 0.18^{a}	70.7 ± 1.0^{b}	3.62 ± 0.12^{a}
Reproductive status				
Non-pregnant	14	5.50 ± 0.22	72.9 ± 1.1	4.03 ± 0.20
Preg./Lact.	27	5.33 ± 0.13	70.9 ± 0.9	3.78 ± 0.12

^{abc}Within columns means within treatment groups and within reproductive status having superscripts with letters in common, or no superscript, are not significantly different ($P>0.05$).

4.4.5 Fleece wool measurements

Reproductive status and photoperiod had no effect on washing yield of the midside sample, although this tended to be lower in ewes that produced lambs, and in ND ewes respectively. The washing yield of fleece wool was significantly lower ($P<0.05$) in ND-BrB ewes compared to other ND ewes which produced a lamb (Table 4.4).

Mean fibre diameter of the midside fleece sample was unaffected by reproductive status (Table 4.5) or bromocriptine treatment but was influenced by photoperiod (36.0 ± 0.5 versus 38.7 ± 0.7 μm for ND and LD ewes, $P<0.01$). Fibre curvature was significantly greater ($P<0.05$) in ewes that had lambed compared to non-pregnant ewes (Table 4.5) but was not affected by treatment with bromocriptine or photoperiod.

Non-pregnant ewes had a significantly longer ($P<0.01$) staple than ewes which produced a lamb (Table 4.6). Photoperiod also influenced staple length, the fleece wool of LD non-pregnant ewes being significantly longer ($P<0.01$)

compared with that of ND non-pregnant ewes. There was no difference in the staple length between ND and LD ewes which lambed. Bromocriptine-treated ewes had wool with significantly shorter staples than ND-lambed ewes (130 ± 2 versus 140 ± 4 mm, $P<0.01$, Table 4.6).

Table 4.5: Effects of pregnancy/lactation, photoperiod and bromocriptine treatment on mean fibre diameter (MFD) and fibre curvature (FC) in fleece samples at shearing of the experimental groups (Mean ± SEM).

Group	*n*	MFD (μm)	FC (deg/mm)
LD non-pregnant	8	38.4 ± 0.9^{ab}	27.2 ± 1.3^{a}
LD-lambed	7	39.0 ± 1.0^{b}	28.2 ± 1.2^{ab}
ND non-pregnant	6	36.3 ± 0.9^{ab}	29.4 ± 0.9^{abc}
ND-lambed	7	35.8 ± 1.2^{a}	29.7 ± 1.4^{abc}
ND-BrB	6	35.9 ± 0.9^{a}	32.8 ± 1.3^{c}
ND-BrA	7	36.1 ± 0.9^{a}	31.6 ± 1.2^{bc}
Reproductive status			
Non-pregnant	14	37.5 ± 0.7	28.1 ± 0.9^{a}
Preg./Lact.	27	36.7 ± 0.5	30.5 ± 0.7^{b}

^{abc} Within columns means within treatment groups and within reproductive status having superscripts with letters in common, or no superscript, are not significantly different ($P>0.05$).

Non-pregnant ewes also had a significantly greater ($P<0.05$) staple tensile strength than ewes which had lambed (Table 4.6). Tensile strength was unaffected by photoperiod (although it tended to be greater in LD ewes, 50.0 ± 1.1 versus 46.7 ± 1.1 N/Ktex, $P<0.10$) or bromocriptine treatment. Position of break was not influenced by reproductive status or photoperiod (Table 4.6). The position of break in the fleece staples of ewes treated with bromocriptine tended to be further from the tip compared to ND-lambed ewes (59.8 ± 1.4 versus 54.6 ± 3.0%, $P<0.10$). Reproductive status, photoperiod (22.4 ± 0.3 versus 22.8 ± 0.3 g/cm^3 for LD and ND ewes respectively) and bromocriptine treatment had no effect on corebulk (Table 4.6).

Table 4.6: Effects of pregnancy/lactation, photoperiod and bromocriptine treatment on staple length (SL), staple tensile strength (SS), position of break (POB) and corebulk in wool samples taken at shearing of the experimental groups (Mean ± SEM).

Group	*n*	SL (mm)	SS (N/Ktex)	POB (%)	Corebulk (cm^3/g)
LD non-pregnant	8	163 ± 2^{c}	51.4 ± 1.6^{b}	58.3 ± 1.9^{abc}	22.1 ± 0.6
LD-lambed	7	144 ± 5^{b}	48.5 ± 1.5^{ab}	62.9 ± 1.2^{c}	22.7 ± 0.3
ND non-pregnant	6	146 ± 5^{b}	50.1 ± 2.5^{ab}	56.5 ± 1.5^{ab}	22.4 ± 0.4
ND-lambed	7	140 ± 4^{b}	44.4 ± 2.4^{a}	54.6 ± 3.0^{a}	22.6 ± 0.5
ND-BrB	6	132 ± 3^{ab}	46.4 ± 2.0^{ab}	62.1 ± 2.3^{bc}	22.9 ± 0.6
ND-BrA	7	128 ± 3^{a}	46.4 ± 2.2^{ab}	57.9 ± 1.6^{abc}	23.1 ± 0.6
Reprod. status					
Non-pregnant	14	156 ± 3^{b}	50.8 ± 1.4^{b}	57.5 ± 1.2	22.2 ± 0.4
Preg./Lact.	27	136 ± 2^{a}	46.4 ± 1.0^{a}	59.3 ± 1.2	22.8 ± 0.3

[abc] Within columns means within treatment groups and within reproductive status having superscripts with letters in common, or no superscript, are not significantly different ($P>0.05$).

4.4.6 Midside wool washing yield

In general, the patch washing yield was lower ($P<0.001$) at the end of the experiment than at the start in all treatment groups (Figure 4.7). There was a strong ($P<0.001$) positive relationship between wool growth and washing yield in April and May (mid- to late pregnancy) as the average washing yield was significantly lower ($P<0.01$) in pregnant ewes compared to non-pregnant ewes (73.8 ± 0.7 versus 77.0 ± 0.9%). In June and July, when wool growth was similar in all groups, this relationship was not apparent. The washing yield increased ($P<0.001$) in all pregnant groups postpartum, the change being most marked in LD-lambed ewes. Over the final 6 months, long-term bromocriptine treatment resulted in lower patch washing yields compared to ND-lambed ewes (64.5 ± 1.0 versus 67.3 ± 1.6%, $P<0.10$).

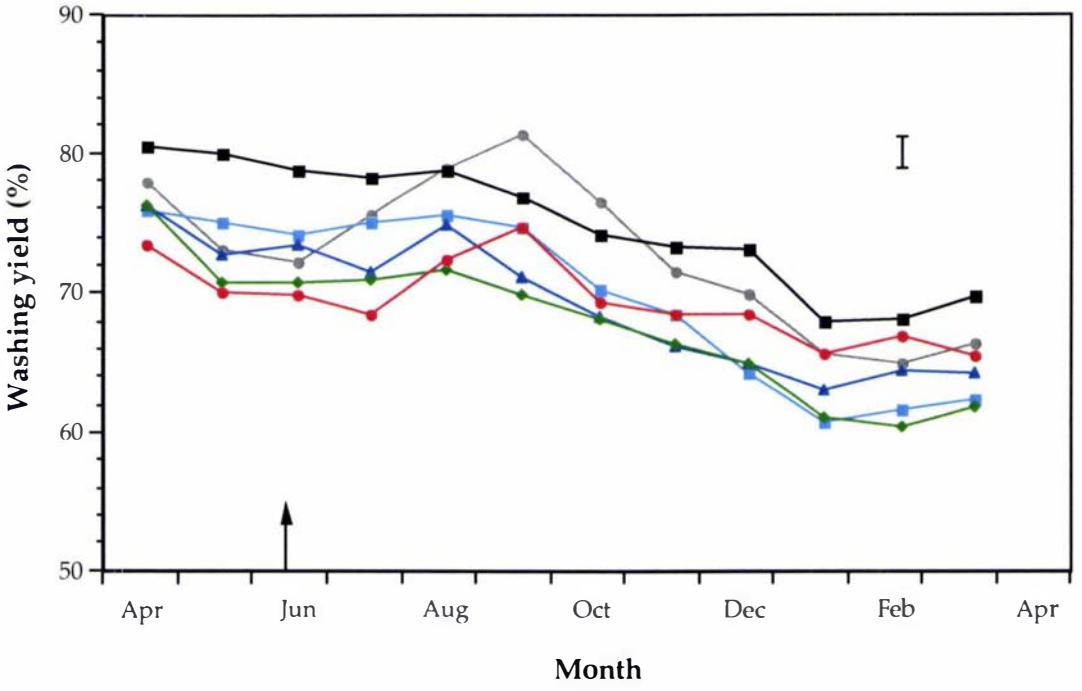

Figure 4.7: Midside washing yield of LD non-pregnant (■), LD-lambed (●), ND non-pregnant (■), ND-lambed (●) and ND-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

4.4.7 Midside wool fibre diameter

There were no differences in the mean fibre diameter of the patch samples from April to June (mid- to late pregnancy) as a result of reproductive status (Figure 4.8). Mean fibre diameter was not significantly different in ND and LD non-pregnant ewes during the same period. A similar trend was apparent in pregnant ewes, with the exception of April, when mean fibre diameter was significantly greater ($P<0.05$) in LD-lambed ewes compared to ND-lambed ewes. All groups exhibited a decrease in fibre diameter from April to June, at which time there were no treatment differences.

Postpartum, the change in diameter in ND and LD-lambed ewes was significantly greater ($P<0.001$) than that observed in both non-pregnant groups (4.1 ± 0.9 versus −1.7 ± 0.7 µm, Figure 4.8). Fibre diameter increased following parturition in all ewes that produced a lamb, and the change in fibre diameter between June and September differed significantly between groups

(P<0.001). The fibre diameter in LD-lambed ewes showed a marked increase while that in ND-lambed ewes increased only slightly (6.4 ± 1.1 versus 1.8 ± 0.7 µm, P<0.001). ND-BrB ewes (0.5 ± 0.6 µm) and ND-BrA ewes (–0.1 ± 0.8 µm) had a similar change in diameter to ND-lambed ewes (Figure 4.8). The decline in diameter from July to September was less in LD non-pregnant ewes compared to ND non-pregnant ewes (–0.2 ± 0.8 versus –3.6 ± 0.7 µm, P<0.05).

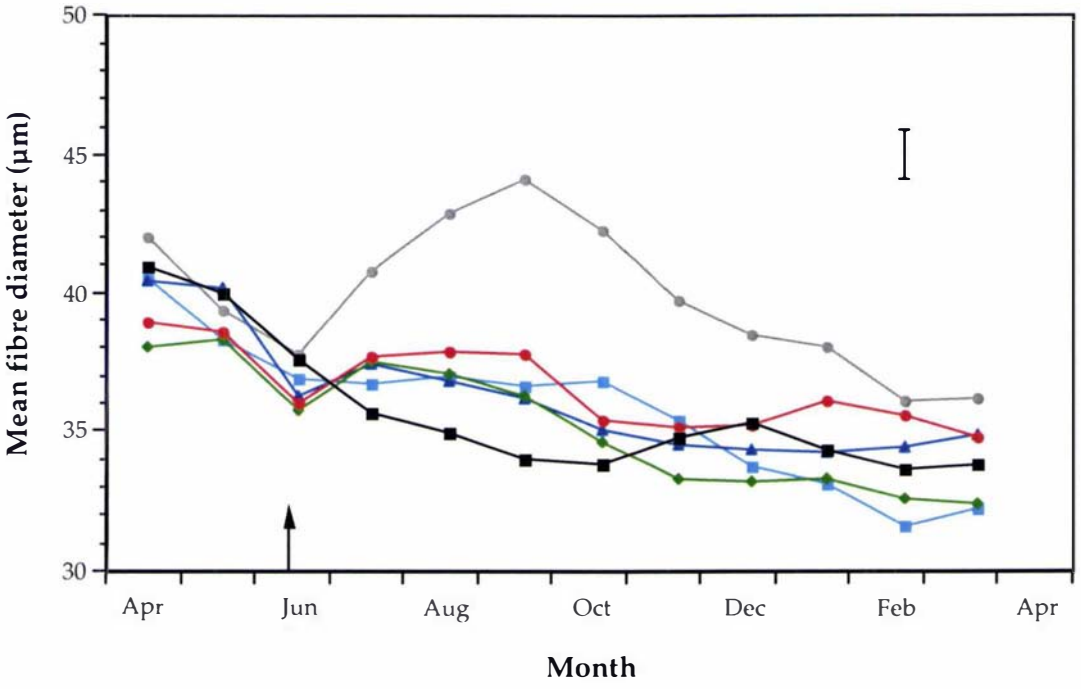

Figure 4.8: Midside mean fibre diameter of LD non-pregnant (■), LD-lambed (●), ND non-pregnant (■), ND-lambed (●) and ND-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

The mean fibre diameter in LD-lambed ewes fell from a peak in September but continued to be significantly greater (P<0.001) than in ND-lambed ewes until November (Figure 4.8). There were no differences in diameter due to bromocriptine treatment compared with ND-lambed ewes from October until the end of the experiment in April.

In non-pregnant ewes, the change in diameter between October and March was influenced by photoperiod, although this was not always reflected in monthly midside patches (Figure 4.8). In October, the diameter remained

higher ($P<0.01$) in LD non-pregnant ewes, but exhibited a significantly greater decline to March compared to ND non-pregnant ewes (–6.1 ± 0.7 versus –0.6 ± 0.5 μm, $P<0.001$). While the fibre diameter decreased in both LD groups from October to March, the diameter averaged over the last 6 months was greater in LD-lambed ewes compared to the LD non-pregnant group (38.5 ± 0.6 versus 33.8 ± 1.3 μm, $P<0.01$, Figure 4.8). The fibre diameter in ND-lambed ewes also tended to be higher by comparison to ND non-pregnant ewes over this period (35.4 ± 0.9 versus 34.3 ± 0.9 μm, $P<0.10$).

4.4.8 Midside wool fibre curvature

Midside fibre curvature exhibited a significant increase ($P<0.001$) over the trial in all groups and differed ($P<0.01$) between treatment groups (Figure 4.9). Monthly fibre curvature increased ($P<0.001$) from April to June with no differences due to reproductive status over this period. However, curvature was significantly greater in LD ewes in May compared to ND ewes (30.3 ± 0.9 versus 27.6 ± 0.8 deg/mm, $P<0.001$), but not in June, when the fibre curvature was similar in all groups. Although fibre curvature did not differ with treatment in each month, there were significant changes ($P<0.01$) with treatment between June and September (Figure 4.9). While fibre curvature increased in ND non-pregnant ewes, that in LD ewes declined (3.9 ± 1.7 versus –1.4 ± 1.4 deg/mm, $P<0.05$). Similarly, the decrease in fibre curvature in LD-lambed ewes was significantly greater compared to ND-lambed ewes (–6.5 ± 1.6 versus –2.8 ± 1.4 deg/mm, $P<0.05$). By comparison to non-pregnant ewes, the decline was greater ($P<0.05$) in ewes that produced a lamb. Bromocriptine treatment had no effect on the change in fibre curvature over this period (Figure 4.9).

Following weaning in September, the change in fibre curvature over the last 8 months differed ($P<0.001$) between the 6 treatment groups (Figure 4.9). In LD non-pregnant ewes the monthly fibre curvature increased significantly compared to ND non-pregnant ewes (6.9 ± 1.8 versus –0.2 ± 1.7 deg/mm,

$P<0.05$), while the trend was similar between the LD-lambed and ND-lambed groups (9.7 ± 1.6 versus 2.1 ± 1.5 deg/mm, $P<0.01$). Prior reproductive status did not influence the change in fibre curvature over this period but there were significant differences ($P<0.01$) between the groups treated with bromocriptine. The increase in ND-lambed ewes was less than that in ND-BrB ewes (2.1 ± 1.5 versus 13.4 ± 2.5 deg/mm, $P<0.001$) but not in ND-BrA ewes (5.9 ± 2.1 deg/mm, $P>0.10$). There was a difference in fibre curvature change ($P<0.05$) between the two bromocriptine-treated groups (Figure 4.9).

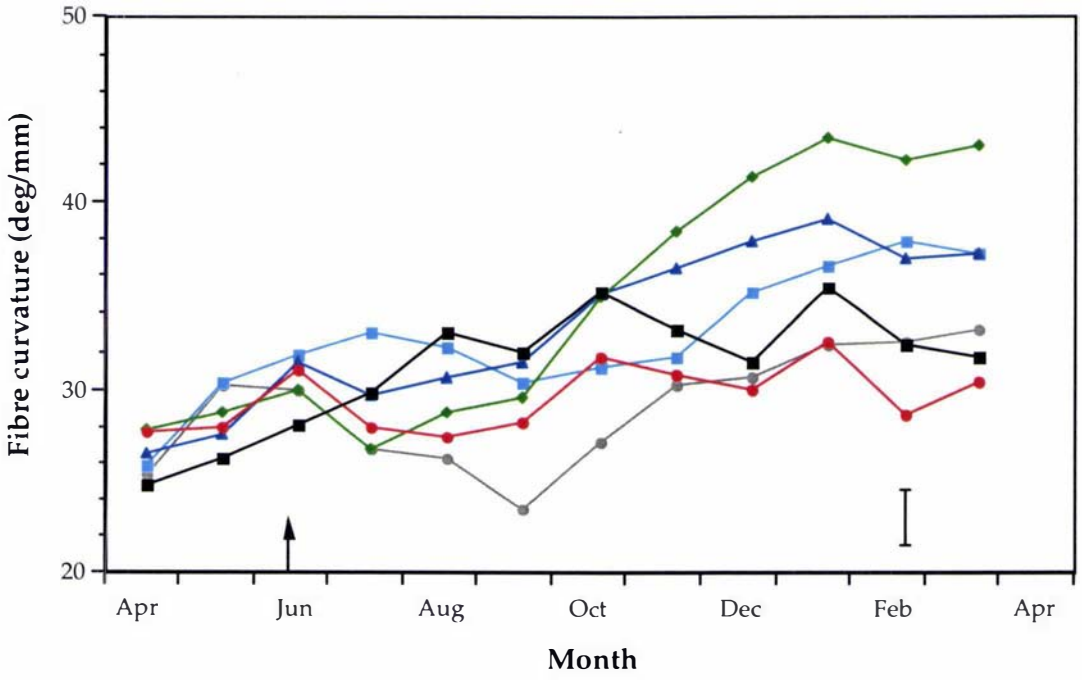

Figure 4.9: Midside fibre curvature of LD non-pregnant (■), LD-lambed (●), ND non-pregnant (■), ND-lambed (●) and ND-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

4.4.9 Midside clean wool growth rate

In general, wool growth rates followed the expected seasonal patterns (Figure 4.10) and the changes were similar to those observed for fibre diameter (Figure 4.8). Clean wool growth rate was significantly lower ($P<0.01$) in pregnant ewes in April and May (mid- to late pregnancy) compared to non-pregnant ewes irrespective of photoperiod. Wool growth declined over this period to

reach a winter minimum in June (the month of parturition), at which time there was no difference between treatment groups.

Following photoperiod treatment there were no differences in wool growth rates in April and May in ND and LD non-pregnant ewes (Figure 4.10). However, wool production fell to minimal levels between July and October in ND non-pregnant ewes compared to LD non-pregnant ewes, which grew significantly more wool over these months (11.6 ± 0.3 versus 8.2 ± 0.3 g/day, $P<0.001$). Wool growth rate increased from October in ND ewes and by December wool growth was similar in both non-pregnant groups and remained so until the completion of the trial in April. By comparison, both ND-lambed and LD-lambed ewes grew significantly more ($P<0.01$) wool than ND and LD non-pregnant ewes respectively from July to September, but there were no differences in monthly wool production after that point.

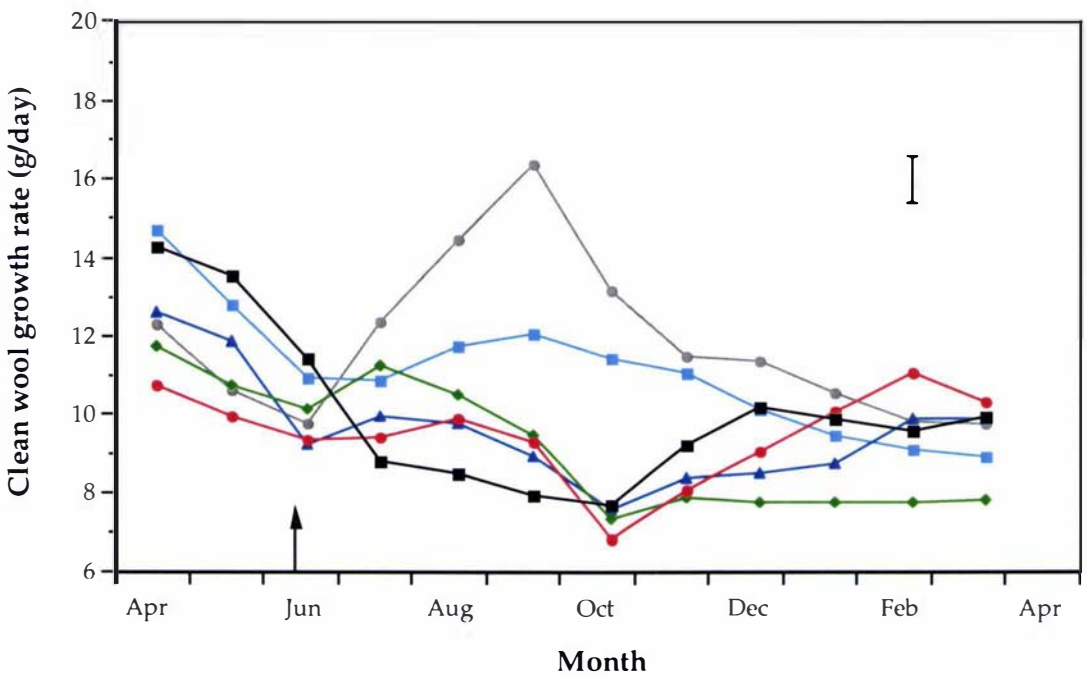

Figure 4.10: Midside clean wool growth rate of LD non-pregnant (■), LD-lambed (●), ND non-pregnant (■), ND-lambed (●) and ND-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲). Arrow represents mean date of parturition. Error bar represents the pooled SED.

Following parturition, wool growth increased in all ewes that produced a lamb, although this increase was dependent on the treatment imposed (Figure 4.10). In LD-lambed ewes, there was a significant increase in wool production from June to September compared to ND-lambed ewes (6.6 ± 1.2 versus 0.0 ± 0.7 g/day, $P<0.001$) reflecting higher average wool growth rates over lactation (14.4 ± 0.9 versus 9.6 ± 0.4 g/day, $P<0.001$). While wool growth declined from September to March in LD-lambed ewes (compared to an increase in ND-lambed ewes), significantly more ($P<0.01$) wool was grown by LD ewes until December, after which wool growth rates did not differ between ND and LD ewes.

Prior to parturition, there were no differences in the monthly wool growth among the 3 groups of ND pregnant ewes. Following bromocriptine administration in June, there were no treatment effects on wool production and all ND-lambed ewes (whether given bromocriptine or not) displayed a marked decline in wool growth in October (Figure 4.10). However, the change in wool growth rate from October to March varied significantly ($P<0.001$) with treatment. ND-lambed ewes grew significantly more wool than ND-BrB ewes over this period (9.2 ± 0.3 versus 7.7 ± 0.3 g/day, $P<0.05$) but did not differ from ND-BrA ewes (8.8 ± 0.6 g/day, $P>0.10$), while ND-BrA ewes also tended to grow more ($P<0.10$) wool than ND-BrB ewes (Figure 4.10).

4.4.10 Fibre length growth rate

The fibre length growth rate was significantly lower in pregnant ewes compared to non-pregnant ewes (0.44 ± 0.01 versus 0.48 ± 0.01 mm/day, $P<0.01$) at the start of the radiolabelling period (one week before parturition). Fibre length increased at a greater rate (0.07 ± 0.01 versus 0.00 ± 0.01 mm/day, $P<0.001$) over the next 4 weeks, so that by 1 July length growth rate was higher (0.50 ± 0.01 versus 0.48 ± 0.01 mm/day, $P<0.05$) in ewes that had lambed (Figure 4.11). While the fibre length growth rate in ND non-pregnant ewes was similar to that in LD non-pregnant ewes at each sampling point the

change in growth rate differed (–0.03 ± 0.01 versus 0.01 ± 0.01 mm/day, $P<0.01$) over the sampling period. In LD-lambed ewes, the increase in length growth rate was also greater (0.08 ± 0.01 versus 0.05 ± 0.01 mm/day, $P<0.05$) compared to ND-lambed ewes (Figure 4.11). Treatment with bromocriptine did not affect the length growth rate at each date, but the increase was significantly greater in ND-BrB ewes compared to ND-lambed ewes (0.12 ± 0.03 versus 0.05 ± 0.01 mm/day, $P<0.05$), but not when compared to ND-BrA ewes (0.07 ± 0.01 mm/day, $P>0.10$).

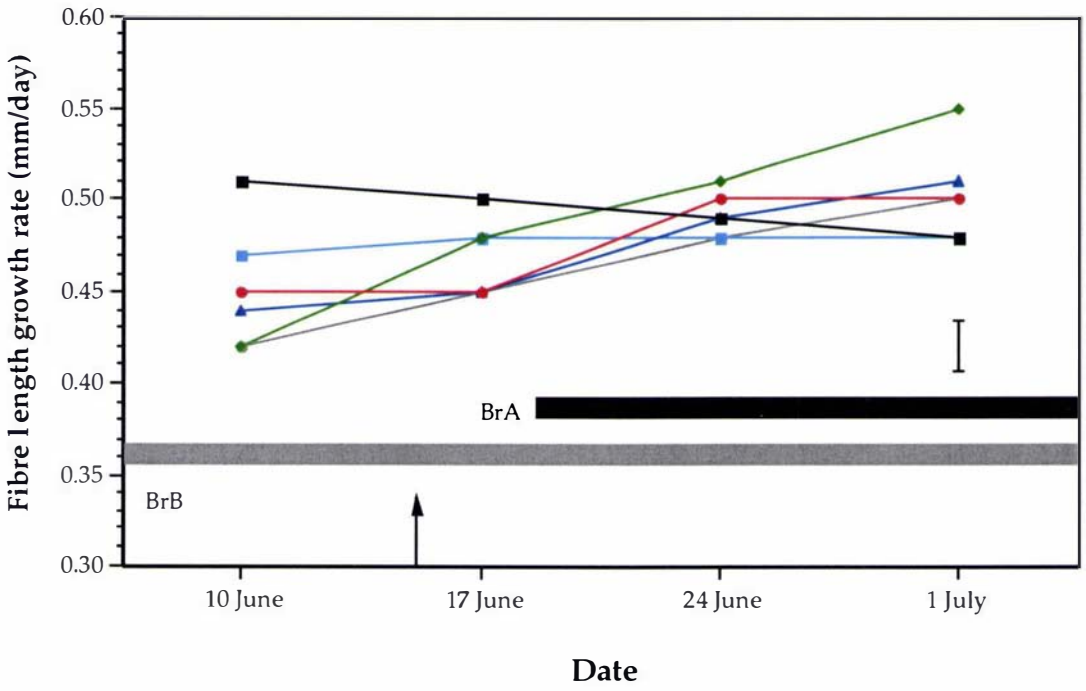

Figure 4.11: Fibre length growth rate of LD non-pregnant (■), LD-lambed (●), ND non-pregnant (■), ND-lambed (●) and ND-lambed ewes treated with bromocriptine before parturition (◆) or after parturition (▲) in a 5-week period around parturition. Arrow represents mean date of parturition. Shaded areas refer to respective periods of bromocriptine treatment. Error bar represents the pooled SED.

4.4.11 Wool growth and PRL profile interrelationships in non-pregnant ewes

The comparison between the 2 groups of non-pregnant ewes focused on the change in wool growth rate from June to October (Figure 4.10) which was significantly different between ND and LD ewes (–3.0 ± 0.7 versus 0.5 ± 0.7 g/day, $P<0.01$). As described in the previous chapter *(Section 3.4.8)*, multiple regression analysis was used to determine whether monthly PRL

concentrations or changes in PRL concentration over time were related to the differences in wool growth rate in individual sheep over this period. April wool growth rate was used as a covariate in all models. Data from one ND ewe was excluded from the analysis due to an error in ultrasound scanning. This ewe was mistakenly identified as pregnant and was given a higher daily feed intake from April to June, which resulted in a higher wool growth rate over these months, compared to other non-pregnant ewes.

The main PRL parameters used for the regression analyses were:

(i) *Monthly PRL concentrations* – the mean PRL concentration in the months of April to October inclusive;

(ii) *Mean May to June PRL concentration* – the average PRL concentration from May to June;

(iii) *Change in PRL concentration from June to October* – the difference between the mean June PRL and the mean October PRL concentrations.

April PRL concentration was not related ($P>0.10$) to the difference in wool growth rate from June to October, but the mean PRL concentration in May was a significant factor ($P<0.05$) in the model (Figure 4.12). This positive relationship increased in significance when the June mean PRL concentrations were included in the model (Table 4.7). As the monthly PRL concentrations were highly correlated (r = 0.80 to 0.92), the PRL concentrations in July, August, September and the mean PRL concentrations between May and October also gave significant effects ($P<0.05$).

While the change in PRL concentrations from April to June, May to June and June to July were not significant ($P>0.10$), the change from June to October gave a significant negative relationship ($P<0.01$) with the June to October wool growth change over the same period (Figure 4.12). Alone, the change in PRL concentration from June to August was not significant ($P>0.10$), but combining

this parameter with the mean June PRL concentration ($P<0.05$) provided the best fit ($R^2 = 59\%$) to the model (Table 4.7).

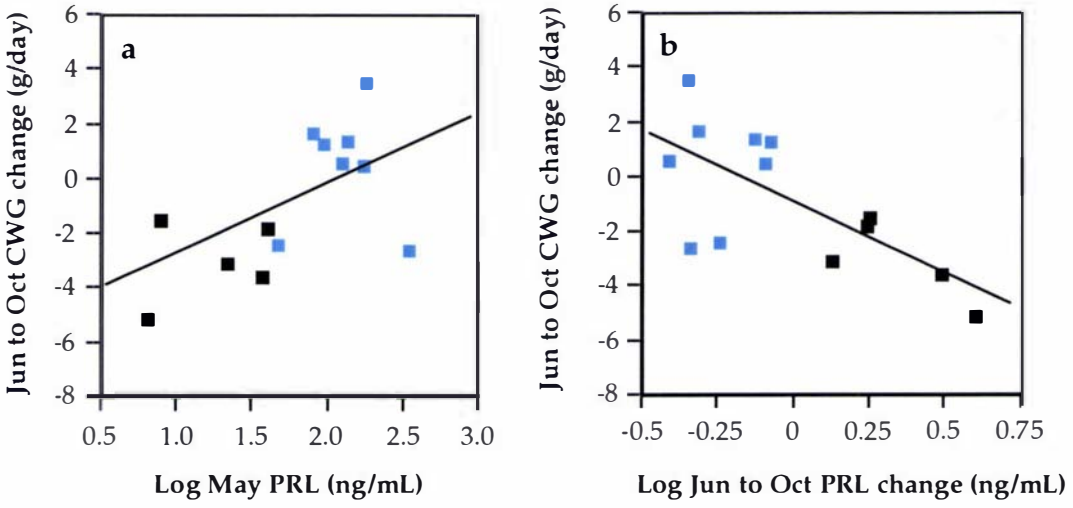

Figure 4.12: The relationship between the change in clean wool growth rate (CWG) from June to October and (a) the mean log prolactin concentrations in May and (b) the change in log prolactin concentrations from June to October in ND (■) and LD (■) non-pregnant ewes.

Table 4.7: Percentage of variance in the change in wool growth from June to October accounted by various plasma prolactin parameters in non-pregnant ewes.

PRL parameter	Change in June to October wool growth rate
April PRL	11% ns
May PRL	40% *
June PRL	48% *
Mean May to June PRL	47% *
Change in PRL from June to October	50% **
Mean June PRL + Change in PRL from June to August	59% ** ns

* $P<0.05$; ** $P<0.01$.

4.4.12 Wool growth and PRL profile interrelationships in the 4 pregnant groups

A sequential regression analysis *(previously described in Section 3.4.9)* was undertaken in the 4 pregnant groups to examine the relationships between the change in wool growth from June to September (Figure 4.10) and plasma PRL concentrations through gestation and lactation.

All pregnant groups: The mean PRL concentration over pregnancy was significant (P=0.001, Table 4.8 and Figure 4.13) in the model. The mean periparturient PRL concentration was also significant (P<0.05). Combining the PRL concentration over pregnancy with the periparturient PRL concentration did not improve the fit of the model. There was a suggestion of a relationship between the postpartum decline in PRL concentration and the wool growth change (P<0.10) and this relationship was strengthened (R^2 = 41%) when combined with the periparturient PRL concentration in a multiple regression analysis. The PRL concentration over lactation was also a significant (P<0.001) predictor (Figure 4.14) but the change in PRL concentration over lactation was not (P>0.10). However, combining the periparturient PRL concentration with the change in PRL concentration over lactation gave an improved fit (Table 4.8).

Three pregnant groups excluding the ND-BrB group: PRL concentrations over pregnancy were significantly related (P<0.001) to the June to September wool growth change (Figure 4.13) as was the increase in PRL concentration prior to parturition (P<0.05). The postpartum decline in PRL concentration became significant (P<0.05), while the relationship between the periparturient PRL concentration (P<0.05) and the wool growth change still held (Figure 4.13). The PRL concentration throughout lactation (Figure 4.14) remained a highly significant (P<0.01) factor and the change in PRL concentration over lactation tended towards significance (Table 4.8).

Table 4.8: Percentage of variance in the change in wool growth from June to September accounted by various plasma prolactin parameters in pregnant ewes.

PRL parameter	All Groups	Excluding ND-BrB ewes	ND and LD ewes only
Mean Pregnancy PRL	37%	48%	39%
	***	***	*
Prepartum increase in PRL	1%	28%	24%
	ns	*	ns
Peak PRL	12%	4%	6%
	†	ns	ns
Periparturient PRL	16%	24%	12%
	*	*	ns
Postpartum decline in PRL	13%	30%	21%
	†	*	ns
Lactation PRL	38%	37%	42%
	***	**	*
Change in PRL over lactation	4%	18%	8%
	ns	†	ns
	33%	36%	27%
Preg. PRL +	*	†	ns
Periparturient PRL	ns	ns	ns
	41%	39%	24%
Periparturient PRL +	**	ns	†
Postpartum decline in PRL	**	*	*
	41%	39%	42%
Periparturient PRL +	ns	ns	ns
Lactation PRL	**	*	*
	27%	29%	13%
Periparturient PRL +	*	ns	ns
Change in PRL over lactation	†	ns	ns

† $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

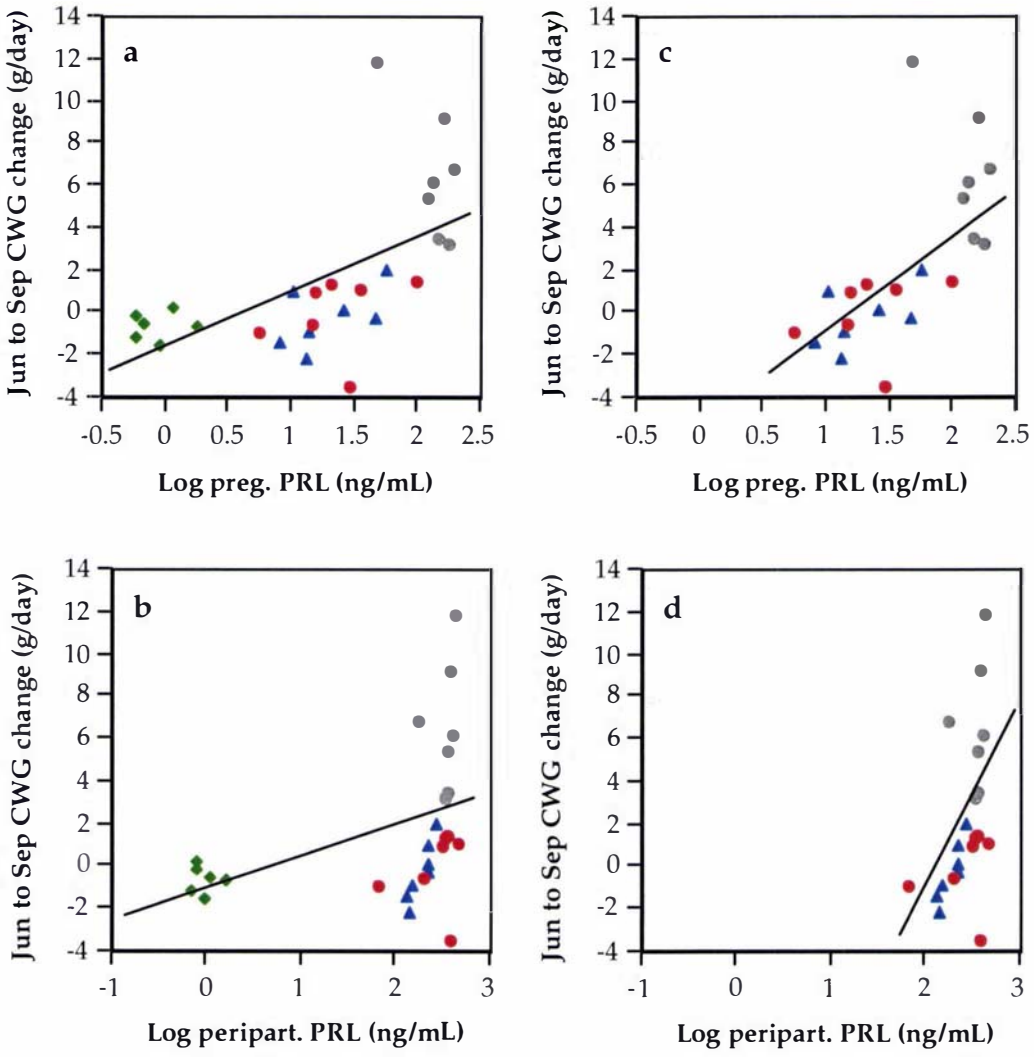

Figure 4.13: The relationship between the change in clean wool growth rate (CWG) from June to September and the mean log prolactin concentration during pregnancy and the log periparturient prolactin concentration in (a, b) all pregnant ewes and (c, d) pregnant ewes excluding ND-BrB ewes. Legend: LD-lambed (●), ND-lambed (●), ND-BrB (◆) and ND-BrA (▲) ewes.

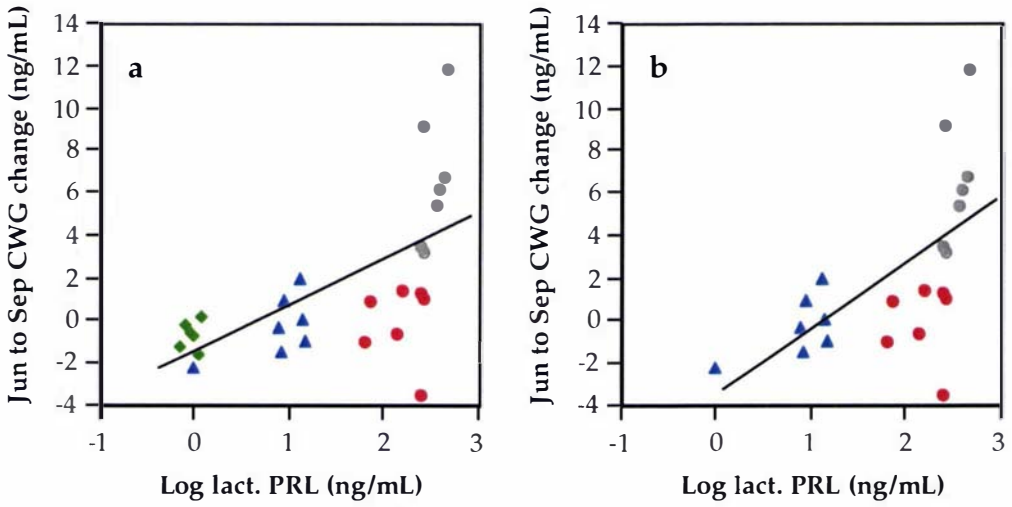

Figure 4.14: The relationship between the change in clean wool growth rate (CWG) from June to September and the mean log prolactin concentration during lactation in (a) all pregnant ewes and (b) pregnant ewes excluding ND-BrB ewes. Legend: LD-lambed (●), ND-lambed (●), ND-BrB (◆) and ND-BrA (▲) ewes.

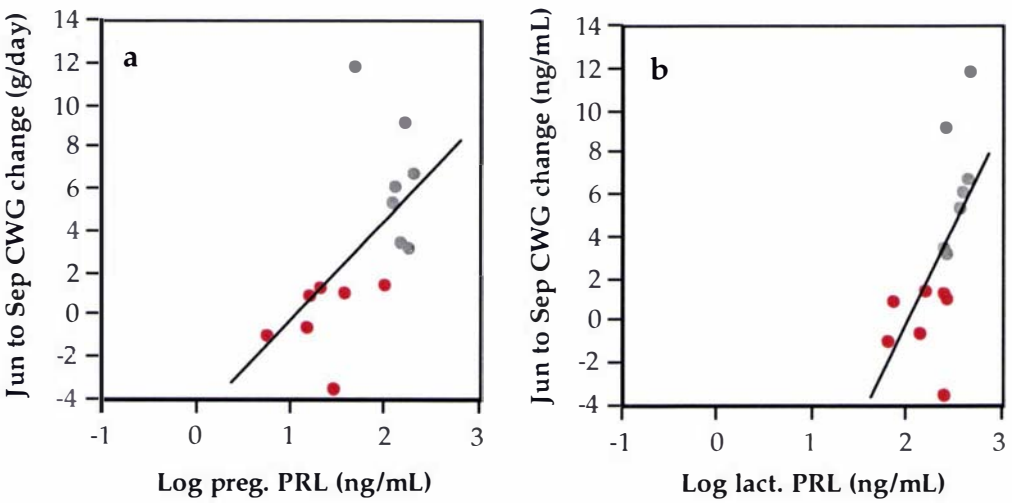

Figure 4.15: The relationship between the change in clean wool growth rate (CWG) from June to September and (a) the mean log prolactin concentration over pregnancy, and (b) the mean log prolactin concentration during lactation in LD-lambed (●) and ND-lambed (●) ewes.

ND and LD-lambed ewes: Again, there was an indication that PRL concentrations up to parturition (Figure 4.15) were important ($P<0.05$) but the periparturient PRL concentration became non-significant. PRL concentrations during lactation gave the strongest relationship ($P<0.05$) with the wool growth

change (Figure 4.15) and the fit of this model was not improved when including the periparturient PRL concentration (Table 4.8).

Lamb data

4.4.13 Lamb birth measurements

There was no significant treatment effect on lamb birth weight, crown-rump length, girth or head width (Table 4.9). The interaction between treatment and sex was also not significant for any measurement except for girth ($P<0.05$). While there was no significant difference in the hindleg length measurements of ND (ND and ND-BrA lambs combined) and ND-BrB lambs, hindleg length was significantly greater ($P<0.05$) in LD lambs compared to both ND groups.

Table 4.9: Effects of photoperiod and maternal bromocriptine treatment on birth weight (BW), crown-rump length (CRL), girth, hindleg length and head width of the lamb experimental groups (Mean ± SEM).

Group	*n*	BW (kg)	CRL (mm)	Girth (mm)	Hindleg (mm)	Head (mm)
LD	7	5.7 ± 0.2	431 ± 12	409 ± 5	372 ± 6[b]	89 ± 2
ND/ND-BrA	15	5.5 ± 0.2	422 ± 7	405 ± 4	355 ± 5[a]	85 ± 2
ND-BrB	7	5.3 ± 0.4	424 ± 10	409 ± 10	355 ± 4[a]	88 ± 2

[ab] Means within columns having superscripts with letters in common or no superscript are not significantly different ($P>0.05$).

4.4.14 Lamb live weight

Mean lamb live weight increased from 5.5 ± 0.1 kg at birth in mid-June to 29.7 ± 0.6 kg at weaning in mid-September (Figure 4.16). Except at birth, ND-BrB lambs were significantly lighter than either LD lambs ($P<0.01$) or ND-BrA lambs ($P<0.05$) throughout the experiment. ND lambs produced from ewes not given bromocriptine treatment, were heavier ($P<0.05$) than ND-BrB lambs until late August. There was no difference in live weight between LD, ND and ND-BrA lambs. At weaning, ND-BrB lambs remained significantly lighter ($P<0.01$) than LD and ND-BrA lambs but not ND lambs. This difference was

reflected in average daily weight gains for each treatment group which were 285 ± 12, 271 ± 15, 244 ± 10 and 287 ± 7 g/day for LD, ND, ND-BrB and ND-BrA lambs respectively.

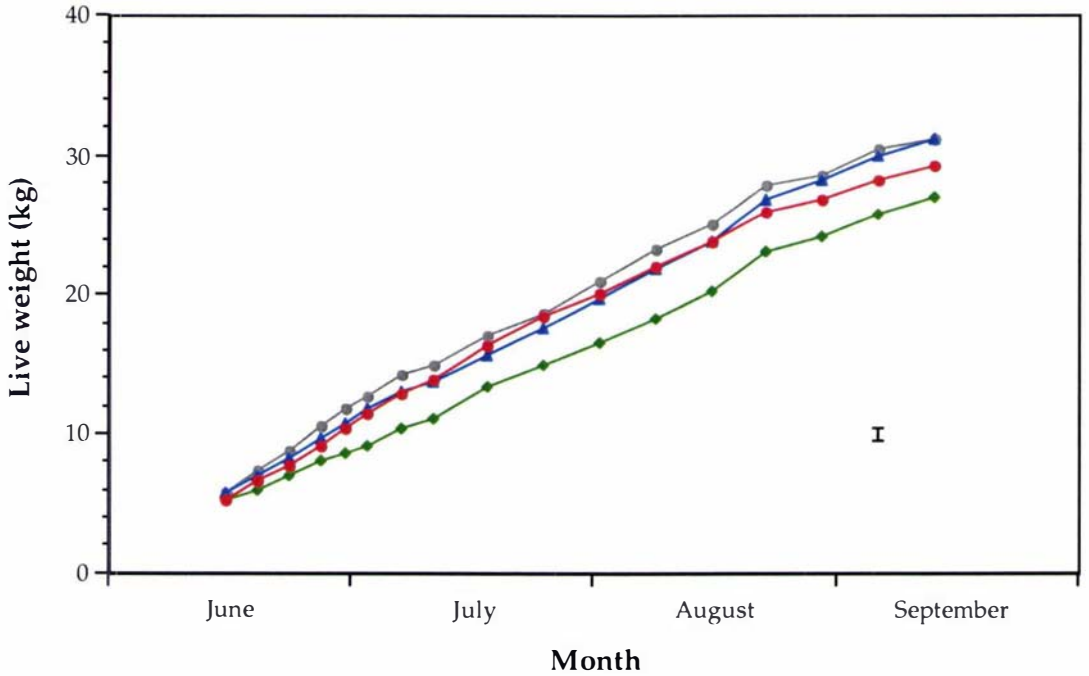

Figure 4.16: Mean live weight of lambs born to LD (●), ND (●), ND-BrB (◆) and ND-BrA (▲) ewes. Error bar represents the pooled SED.

4.4.15 Lamb plasma PRL concentration

Plasma PRL concentrations differed significantly ($P<0.001$) between treatment groups at birth (Figure 4.17). PRL concentrations in LD lambs fell after birth, but remained significantly higher ($P<0.001$) than all ND groups until 26 July. The mean PRL concentrations were not different in ND, ND-BrB or ND-BrA lambs over this period. PRL concentrations increased in all groups from 26 July, and although these were generally lower in ND-BrB lambs, PRL concentrations were not significantly different to other groups. The mean plasma PRL concentrations from birth to weaning did reflect significant ($P<0.001$) treatment effects. The mean PRL concentration in LD lambs (67 ± 7 ng/mL) was significantly higher ($P<0.001$) than in ND lambs (40 ± 4 ng/mL), ND-BrA lambs (35 ± 4 ng/mL) and in ND-BrB lambs (27 ± 2 ng/mL). In ND lambs, mean PRL concentrations were also higher ($P<0.05$) than in ND-BrB

lambs but not ND-BrA lambs. There was no difference in the mean PRL concentrations between groups whose dams were treated with bromocriptine.

Changes in PRL concentration with time within each treatment group were tested by linear regression analysis. The slopes of the regression lines in bromocriptine treatment groups were positive (b = 0.0079 ± 0.0017 and b = 0.0052 ± 0.0012 log ng/mL/day for ND-BrB and ND-BrA lambs respectively, $P<0.001$). Plasma PRL concentration also increased during the spring in ND lambs (b = 0.0027 ± 0.0013 log ng/mL/day, $P=0.05$), and although it did not change significantly in LD lambs (b = −0.0003 ± 0.0009 log ng/mL/day, $P>0.10$), the intercept was higher compared to other groups (1.8 versus 1.2 log ng/mL).

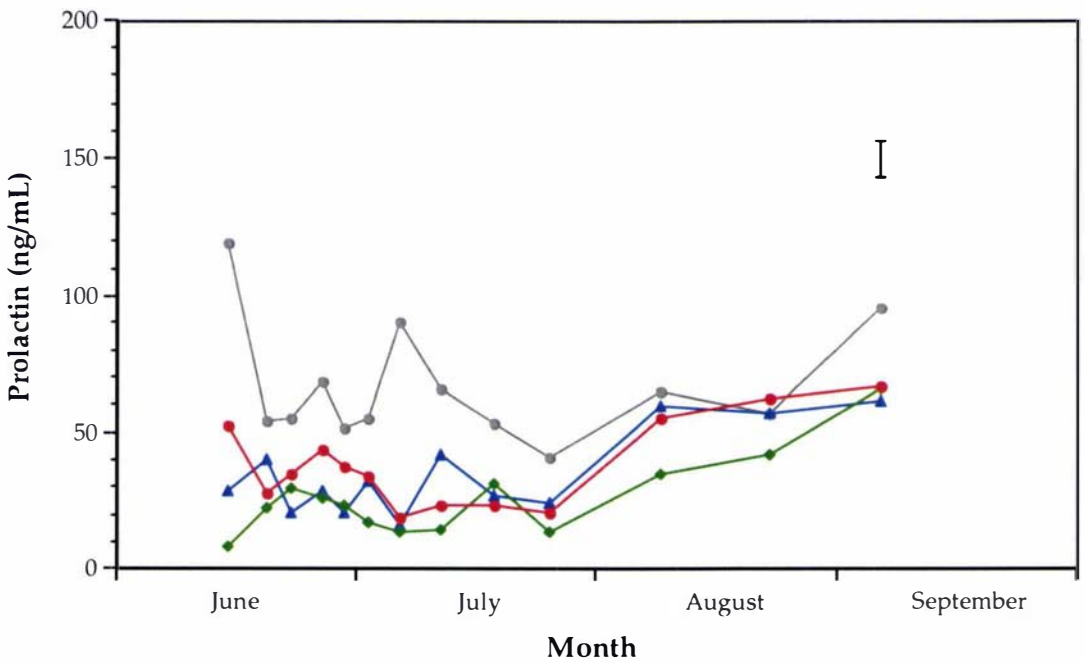

Figure 4.17: Mean plasma prolactin concentrations of lambs born to LD (●), ND (●), ND-BrB (◆) and ND-BrA (▲) ewes. Error bar represents the pooled SED.

4.5 DISCUSSION

The aims of this experiment were to characterise wool growth in Romney sheep during mid- to late pregnancy and during lactation under conditions of controlled dietary intake and controlled photoperiod, and to determine

whether modified maternal PRL concentrations in June-lambing ewes were associated with changes in wool growth.

As in all experiments described in this thesis, feed intake in pregnant and non-pregnant ewes was controlled to minimise the nutritional effect on wool growth rate. While the daily feed intake was not affected by photoperiod in either reproductive class, treatment of ND-BrB ewes with bromocriptine did result in a temporary loss of appetite in some ewes prior to parturition. However, this was not reflected in differences in June wool growth or ewe liveweight change.

Both reproductive classes had a similar live weight at the beginning of the trial (Table 4.3). There were no differences in mean live weight after parturition, at weaning, or at the completion of the trial, suggesting that photoperiod treatment did not influence ewe live weight. Long-term treatment with bromocriptine did, however, cause an increase in the live weight of ND-BrB ewes during lactation relative to the other lactating ewes. Despite reductions in their daily feed allowance, the live weights of their lambs were consistently lower than those of other lambs. Together this evidence suggests that bromocriptine may have reduced milk production (Kann, 1976a; Gow *et al.*, 1983; Peterson *et al.*, 1997). Non-pregnant ewes exposed to LD and ND photoperiod also had similar live weights. Overall, a relatively constant maternal live weight was achieved within each reproductive class and, therefore, any changes in wool growth rate were unlikely to be attributable to differences in nutrient availability.

The higher plasma PRL concentrations in non-pregnant ewes compared to pregnant ewes (80 ± 18 versus 17 ± 4 ng/mL) over late pregnancy in the latter group is consistent with data reported in September-lambing ewes *(Chapter Three)*. In addition to the effects of pregnancy on prepartum PRL concentrations, a pronounced seasonal response not seen in the previous experiment was observed in these experimental sheep. Higher PRL

concentrations during late gestation in ewes kept in artificial LD compared to ewes subjected to a nocturnal mid-winter photoperiod, have been reported previously (Bocquier *et al.*, 1986; Bassett *et al.*, 1988; Ebling *et al.*, 1989; Bassett, 1992).

Plasma PRL concentrations in LD non-pregnant ewes were also higher compared to ND non-pregnant ewes (Figure 4.5a). There was an immediate rise in PRL concentrations (reaching a peak in June) in non-pregnant ewes upon exposure to LD photoperiod in April against a background of falling ambient temperature. In the present experiment, there was also evidence to suggest that there was still a degree of responsiveness among LD ewes to longer summer days and/or higher temperatures, as PRL concentrations began to rise again in December (Figure 4.5).

In ND non-pregnant ewes, however, PRL concentrations remained low throughout winter and increased gradually during the period from winter to summer (June to December). The seasonal variation in PRL concentration is influenced by the duration of daylight (Munro *et al.*, 1980) but also, to some extent, by the rise in ambient temperature (Schillo *et al.*, 1978) over these months. The evidence from the ND and LD groups in this study suggests that photoperiod effects on PRL concentrations override the effects of temperature.

The change in plasma PRL concentrations in pregnant ewes followed the same pattern as in spring-lambing ewes *(Chapter Three)* and as described by other authors (Lamming *et al.*, 1974; Munro *et al.*, 1980; Peterson *et al.*, 1997). However, contrary to Peterson *et al.* (1990) there was no indication of a seasonal difference in the mean PRL levels at parturition in spring-lambing *(*ND-lambed, *Chapter Three)* ewes and in winter-lambing (ND-lambed) ewes in the present study. The most obvious difference in the PRL profile in ND-lambed and LD-lambed ewes in this study was that PRL concentrations in ewes maintained in LD (summer photoperiod) increased to higher levels before the onset of the periparturient rise. Although PRL concentrations

remained high in both groups throughout lactation, levels were higher in LD ewes (341 ± 33 versus 170 ± 31 ng/mL). These findings are consistent with those reported by Bassett (1992). PRL concentrations declined post-weaning and both groups exhibited a summer increase in PRL concentration, which corresponded with their respective non-pregnant controls.

Treatment with bromocriptine successfully suppressed plasma PRL concentrations in pregnant ewes, as described previously (Kann, 1976a; Peterson *et al.*, 1997), and eliminated the peripartum PRL surge in ND-BrB ewes and the hyperprolactinaemia throughout lactation in ND-BrB and ND-BrA ewes. Following weaning, PRL levels remained below 5 ng/mL in both bromocriptine-treated groups until the completion of the experiment in April and no spring rise was observed.

The marked inherent seasonal pattern of wool growth in Romney sheep (Story & Ross, 1960; Bigham *et al.*, 1978b; Hawker, 1985) was modified in the present trial, possibly as a result of the constant plane of nutrition and an indoor environment (Figure 4.10). All groups exhibited the expected winter decline in wool growth and fibre diameter (Hawker & Thompson, 1987) with higher wool growth rates in summer compared to winter (Story & Ross, 1960; Bigham *et al.*, 1978b), but the wool growth minimum in ND ewes, at least, was shifted to October. This corresponded to the minimal tensile strength within the fleece, which usually occurs around parturition in ewes fed to maintain a constant live weight (Masters *et al.*, 1993). It is possible that a reduced feed intake after weaning in September may have accounted for this shift, although there was no corresponding change in ewe live weight. Seasonal trends of midside fibre curvature amongst groups from June were the opposite to those observed in wool growth and fibre diameter reported by Dick and Sumner (1997).

A depression in wool production occurred during mid- to late pregnancy (April to June) in all pregnant groups compared to non-pregnant groups and

was due to a 20% reduction in clean wool growth rate and a 4% reduction in fibre diameter. These findings are similar to those in September-lambing ewes of the same breed *(Chapter Three)* and studies reviewed by Corbett (1979), and could not be attributed to the extra nutritional requirements of the fetus (Rattray, 1986). The physiological costs of pregnancy and lactation on wool growth in pen-fed ewes observed in this and earlier experiments (Masters & Stewart, 1990), included not only lower annual fleece weight at shearing, but also a reduction in fibre diameter, staple length and staple tensile strength.

In the present experiment, the magnitude of this depression in wool growth was significantly diminished compared to breeding ewes that lambed in spring *(Chapter Three).* The average fibre diameter from July to September was 28% higher (37.8 ± 1.0 versus 29.5 ± 0.9 μm) in winter-lambing ewes, compared to spring-lambing ewes, equating to a 75% increase in clean wool growth rate. This is consistent with Morris *et al.* (1994), who also reported an increase in mean fibre diameter over this period, leading to higher overall fleece production. It is possible that this difference is merely a reflection of the physiological state of the ewe (lactating and pregnant respectively), but is unlikely to be explained by maternal feed intake.

Although Hutchinson (1962) reported substantial variation in wax and suint production throughout the year, evidence for a rhythm was not established. The limited published information available (Batchelar, 1985; Butler & Head, 1993) does suggest, however, that wool yield is associated with the cyclic pattern of wool growth as seen in the present experiment (Figures 4.7 and 4.10). Washing yield was lower in pregnant ewes throughout pregnancy, when wool growth was also depressed. Perhaps the best illustration of this relationship was seen in LD-lambed ewes. In this group, midside patch washing yield exhibited the same significant increase from June to September as observed for clean wool growth rate and mean fibre diameter. Another feature was the progressive decline in yield over the trial, this being in general agreement with outdoor studies in the same breed (Ross, 1965; Sumner, 1969;

Wuliji *et al.*, 1993). Without exposure to rain the washing yield of sheep housed indoors can be considered a measure of secondary gland output. It was likely that suint production increased with fleece growth, which resulted in a decline in washing yield.

Artificial increases in PRL concentrations in both breeding and non-pregnant ewes exposed to LD photoperiod were associated with significant increases in wool production compared to their ND counterparts. In LD-lambed ewes, which had PRL concentrations elevated above those normally observed around parturition and lactation, clean wool growth rate increased by over 50% (Figure 4.10). An 18% increase in mean fibre diameter from June to September and an equivalent increase in washing yield was also measured over these same months (Figures 4.7 and 4.8). However, the increased wool growth rates in LD-lambed ewes postpartum were not detected using autoradiographic techniques. In this instance, the period covered by the radiolabelling data finished before any significant increase was likely to be observed in the patch clips. The higher wool growth in LD-lambed ewes from June to December resulted in a 0.9 kg (26%) increase in clean fleece weight relative to ND-lambed ewes which was associated with coarser fleece wool. Staple length and staple tensile strength also tended to be higher in LD ewes, which accounted for the shift in the region of minimum tensile strength of the staple. Subsequently, it is possible that the wool follicles entered a refractory phase following the period of high wool growth, which may have explained the absence of the expected seasonal rise in wool production.

A similar pattern was observed in LD non-pregnant ewes as increased plasma PRL concentrations from May to July were associated with a 36% increase in wool growth rate from July to November, thus preventing the winter decline in wool production seen in ND non-pregnant ewes (Figure 4.10). The overall increase in wool production in non-pregnant ewes maintained in LD photoperiod led to a 10% increase in clean fleece weight which could be

explained by increased length growth rate (confirmed by autoradiography and a 12% higher staple length), and a moderate 6% increase in fibre diameter.

Contrary to Curlewis *et al.* (1991), who recorded no significant effects on wool growth rate from chronic treatment with bromocriptine in Scottish Blackface ewes, wool growth patterns and some fleece characteristics were influenced by bromocriptine treatment in the present experiment. Dolling *et al.* (1986) also reported that the increase in wool growth rate over a 6-week period following bromocriptine treatment was lower than in control sheep, although this difference was not significant. In the current study, long-term treatment with bromocriptine modified the wool growth cycle so that by November there were 3 distinct groupings in terms of relative wool growth rate (Figure 4.10). Wool growth rate was lowest in bromocriptine-treated groups compared to ND-lambed and LD-lambed ewes. These treatment differences were not reflected in differences in fleece weight or mean fibre diameter and staple tensile strength, although staple length tended to be lower in bromocriptine-treated ewes. The region of minimum tensile strength was also altered.

In summary, this study provides evidence for a positive association between wool growth and photoperiod-induced increases in plasma PRL concentrations (Lincoln & Ebling, 1985; Lincoln, 1990) in the New Zealand Romney. Furthermore, the differences in wool growth rate from July to October in ND and LD non-pregnant ewes lend further support to the hypothesis of Lincoln (1990), that elevated PRL concentrations in winter ameliorate the seasonal decrease in wool growth rate. In the present trial, the change in PRL concentrations from winter to spring also gave a strong negative relationship with wool growth rate.

Experimentally-induced increases in PRL concentrations during pregnancy, around parturition, and throughout lactation were associated with higher rates of wool growth over lactation. Moreover, it was possible that the enhanced wool production in LD-lambed ewes was associated with an initial

large increase in PRL concentrations followed by a gradual decline, as opposed to a more rapid decline in ND-lambed ewes. Lastly, the almost complete absence of PRL (ND-BrB ewes) was associated with low rates of long-term wool growth, while the suppression of PRL during lactation and prevention of the summer rise in PRL (ND-BrA ewes) was also associated with a reduction in wool growth rate.

In agreement with the data reported in the previous chapter, photoperiod and maternal bromocriptine treatment (and PRL concentrations in the ewe) had no effect on the fetal growth estimates measured. LD lambs did have longer hindlegs compared with ND lambs, suggesting that summer photoperiod and elevated maternal concentrations of PRL or other hormones may have promoted fetal long bone growth.

The season of birth has a pronounced influence on lamb performance (Reid *et al.*, 1988; Peterson *et al.*, 1990; Morris *et al.*, 1993, 1994) with lambs born in autumn or winter having substantially lower birth weights (up to 25–30%) than those born in spring. A comparison of the average birth weights of spring lambs *(Chapter Three)* and winter-born lambs in the present study gave the opposite result. In September-born lambs, the average birth weight was 5.0 ± 0.1 kg, while the June-born lambs averaged 5.5 ± 0.1 kg. This difference was most likely a reflection of the heavier ewes used in the present experiment, and therefore greater placental size (Jenkinson *et al.*, 1995), but the genetic variation of the ewes and their lambs may also account for the conflicting results.

Previous studies (Forbes *et al.*, 1975, 1979a, 1979b; Schanbacher & Crouse, 1980; Schanbacher *et al.*, 1982) have shown that the growth of lambs could be altered by exposure to LD photoperiod through a more efficient conversion of feed into weight gain and possibly increased secretion of PRL. In the present study however, no evidence was found to support this hypothesis. Lamb feed intake was not measured directly, but it was restricted by the competition of

lambs with their dams for food which could explain the discrepancy with earlier reports. However, the lack of a significant difference between the average daily weight gain and in the weaning weights between LD and ND lambs suggests that changes in photoperiod (Fitzgerald *et al.,* 1982; Ebling *et al.,* 1989; Bassett, 1992), PRL (Eisemann *et al.,* 1984) or in other photoperiod-responsive hormones (Bocquier *et al.,* 1986) had a minimal effect on postnatal growth. Maternal prepartum treatment with bromocriptine did have an adverse effect on the weight gain of ND-BrB lambs which were consistently lighter than the other groups (Figure 4.16). This may be related to lower milk yields (Kann, 1976a; Gow *et al.,* 1983; Peterson *et al.,* 1991, 1997) in their dams despite efforts to equalise milk intake between treatments, and this was reflected in the slightly slower weight gain of the lambs from birth.

In lambs born to ewes exposed to LD photoperiod, the elevated PRL concentrations at birth are consistent with previous researchers (Ebling *et al.,* 1989; Bassett, 1992; Helliwell *et al.,* 1997). Houghton *et al.* (1997) suggested that the duration of the maternal melatonin signal provides the fetal lamb with information about the length of the prevailing photoperiod. PRL concentrations at birth were also significantly lower in ND-BrB lambs, allowing the possibility of placental transfer of bromocriptine (Bigazzi *et al.,* 1979; Reusens *et al.,* 1979; Roti *et al.,* 1986) in the sheep.

The reason for the unanticipated fall in PRL concentrations after birth in LD lambs is not known, but could be related to a decline in the body temperature of the neonate postpartum. Significantly, Bassett (1992) and Helliwell *et al.* (1997) also reported that PRL levels in lambs decreased early in postnatal life under continued exposure to LD photoperiod. The former author suggested that this may have been due to a disruption in the photoperiodic mechanism regulating PRL secretion during perinatal life. Nevertheless, plasma PRL concentrations remained higher in LD lambs compared to all ND lambs until late July, similar to other work (Forbes *et al.,* 1975; Schanbacher & Crouse, 1980; Brinklow & Forbes, 1984; Bassett, 1992). After this date, however, all

groups exhibited a spring rise in PRL concentrations irrespective of photoperiod, suggesting that ambient temperature (Fitzgerald *et al.*, 1982; Schanbacher *et al.*, 1982; Eisemann *et al.*, 1984) and not photoperiod, could have been an overriding factor regulating plasma PRL secretion at this time.

4.6 CONCLUSIONS

In breeding ewes, wool production was depressed during mid- to late pregnancy compared to non-pregnant ewes despite a controlled dietary intake. The decline in wool production was associated with a reduction in length growth rate and reduced mean fibre diameter. Other costs of producing a lamb were reduced staple length, staple tensile strength and increased secondary gland output. The inherent seasonal wool growth cycle was modified as a consequence of a constant maternal feed intake, photoperiod and long-term treatment with bromocriptine. All treatment groups had markedly different plasma PRL profiles over the 12-month duration of the trial. The main features included lower PRL concentrations in pregnant ewes prior to parturition compared to non-pregnant ewes, which may be mediated by other reproductive hormones. Wool growth as measured by autoradiography increased in all pregnant groups around parturition, indicating that the peripartum increase in wool growth rate was not dependent on PRL concentrations. In non-pregnant ewes, photoperiod-induced increases in PRL concentration above those normally associated with winter appeared to reset the wool growth cycle and were positively correlated with an increase in wool growth, primarily due to increased length growth rate in the subsequent months. In pregnant ewes, however, elevated PRL concentrations over pregnancy, around parturition and throughout lactation were associated with a more rapid increase in wool growth through a corresponding increase in fibre diameter. The suppression of plasma PRL was linked with long-term suppression of wool growth rate.

CHAPTER FIVE

The timing of the depression in wool growth during pregnancy in Romney ewes

5.1 ABSTRACT

Clean wool growth rate and plasma PRL concentration were measured in order to define the onset and duration of the wool growth depression in pregnant and lactating Romney ewes. Twelve breeding and 12 non-pregnant ewes were maintained indoors from early February 1996 until February 1997 under controlled dietary intake. Both groups were exposed to natural photoperiod. Lambing occurred between 11 and 15 September. A significant ($P<0.01$) depression in wool growth and fibre diameter occurred within the first 60 days of gestation which was not associated with differences in feed intake or changes in maternal live weight. PRL concentrations did not differ with reproductive status until day 47 of gestation, at which time levels were significantly lower ($P<0.001$) in pregnant ewes compared to non-pregnant ewes, before rising immediately prior to parturition. These results suggest that pregnancy is associated with an early hormonally-mediated depression in wool follicle output which is superimposed on the decline associated with winter photoperiod. PRL does not appear to be involved in the gestational depression in wool growth as plasma concentrations did not change significantly until the last month of pregnancy.

5.2 INTRODUCTION

Results from Chapter Three and Four demonstrated that the decline in wool growth and fibre diameter in pregnant ewes occurred before day 75 of gestation (mid-pregnancy) when both these trials commenced. PRL status did not alter wool growth during late pregnancy, and while wool growth was depressed during late pregnancy, it increased sharply at parturition regardless of PRL profile. These findings suggested that maternal reproductive hormones other than PRL could be involved in the wool growth depression observed over pregnancy and lactation.

The present experiment (FP003/03) was designed to establish the onset and duration of depressed wool growth in the pregnant ewe, and its relationship with changes in plasma PRL concentration. Wool growth was measured in the period before mating, and throughout pregnancy. As in all experiments, the sheep were fed to a constant maternal live weight to reduce the effects of variable nutrient availability on fibre growth. The 24 sheep, including 12 pregnant ewes, were exposed to natural photoperiod.

5.3 MATERIALS AND METHODS

5.3.1 Experimental Animals

Twenty-four Romney ewes from the Hight and Woodland fleece growth flocks at Tokanui were used in the trial. The Hight flock was established in 1967 from a foundation flock of commercial Romney ewes, while the Woodlands flock was set up in 1973 from a base population of Romney ewes that were established in 1969-71. Ewes selected for yearling greasy fleece weight and their control lines were used (see Clarke & Johnson (1993) for a further description of selection lines). Fourteen ewes were chosen randomly and balanced for February greasy wool growth and for selection line. These ewes were synchronised using CIDR devices on 2 April 1996 and mated with 3 Romney rams between 17 and 19 April before being returned to their pens.

Scanning on 17 June (day 60 of gestation) indicated that 9 ewes were carrying single fetuses and 3 ewes twin fetuses. The remaining 2 ewes were not pregnant and were returned to the control group (Table 5.1).

Table 5.1: Experimental groups.

Group		n	
Natural Days			
1	Non-pregnant ewes	12	non-pregnant
2	Pregnant ewes	12	pregnant (n = 9 for single and n = 3 for twin-bearing ewes)

This experiment was part of a larger 12-month trial performed by the Wool Follicle Growth Group at Ruakura, although this chapter only focuses on the period until October and therefore all data collected after 5 November will not be discussed.

5.3.2 Housing

On 8 February 1996, the sheep were transferred indoors and were housed together in the Ruakura Physiology Building until 12 February 1997.

5.3.3 Environmental Observations

The daily minimum and maximum air temperatures recorded at the Ruakura meteorological station from February 1996 to September 1996 are shown in Figure 5.1. Figure 5.2 shows the mean monthly temperatures and hours of daylight over the same period.

5.3.4 Light Treatment

All sheep were exposed to natural photoperiod.

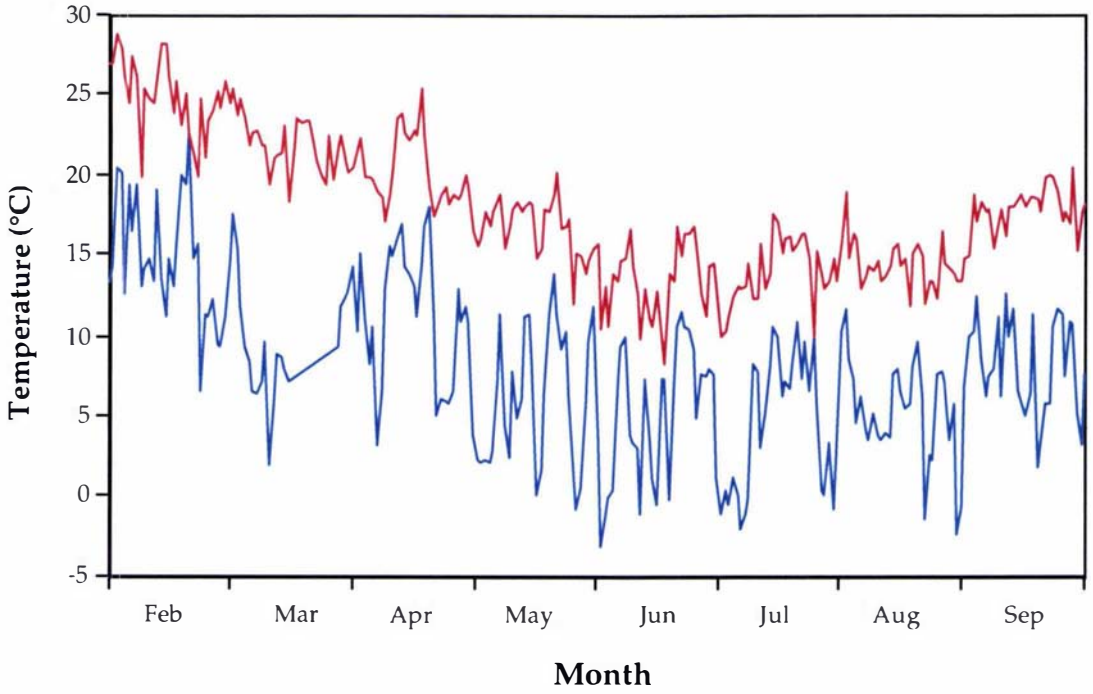

Figure 5.1: Daily minimum (–) and maximum (–) air temperatures during the 1996 experimental period.

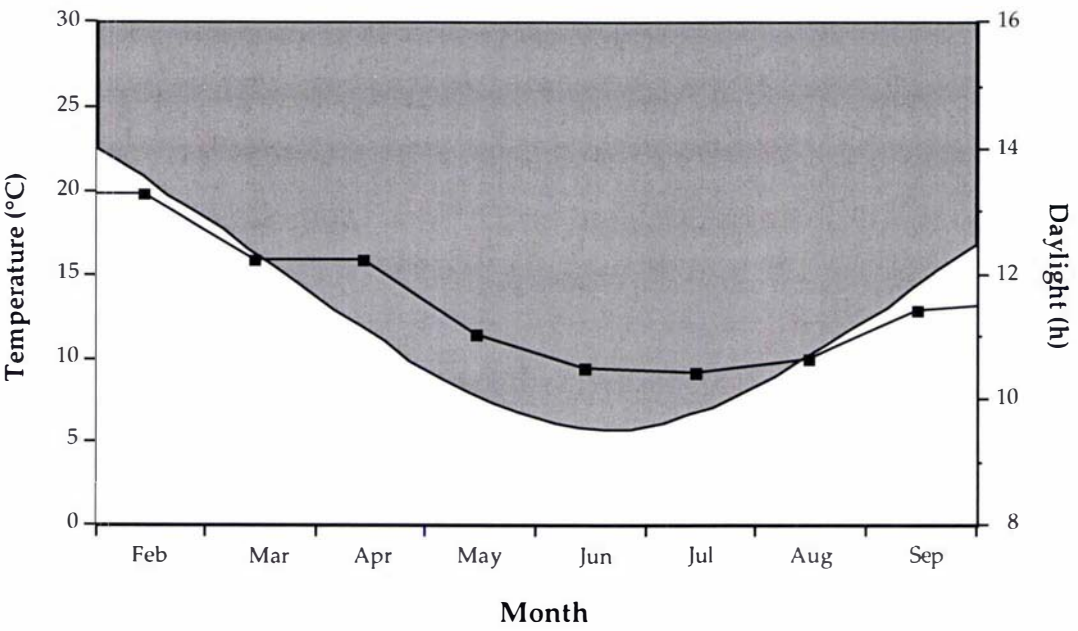

Figure 5.2: Monthly mean air temperature (■) and hours of daylight during the 1996 experimental period

5.3.5 Live weight

All sheep were weighed at 2-weekly intervals for the first 2 months and weekly around parturition.

5.3.6 Feed Allowance

The daily feed allowances for pregnant and non-pregnant ewes were calculated and the daily feed intake measured as described in Section 2.3. For twin-bearing ewes, the feed allowance was adjusted to 1.2 times that of single-bearing ewes from day 87 of gestation to allow for the metabolic demands of an additional fetus.

5.3.7 Wool Sampling

The sheep were shorn on entry to the experiment on 14 February 1996. A midside patch was established on 22 February and re-clipped every month. The sheep were shorn at the conclusion of the experiment on 12 February 1997 when the fleece was weighed and a midside sample collected *(Section 2.7.3)*.

5.3.8 Blood Sampling

Blood samples were collected from all ewes by venipuncture of the jugular vein at weekly intervals between 14 February 1996 and 24 September 1996. Inter-assay and intra-assay coeffecients of variation for the PRL radioimmunoassay at 10 ng/mL were 10.5% and 11.7% respectively.

5.3.9 Lamb Sampling

No samples were collected from the lambs (although the lambs were weighed at weekly intervals to monitor health status). The lambs remained with their dams until weaning at approximately 12 weeks of age on 11 December 1996.

5.3.10 Statistical Methods

Data relating to feed intake, ewe live weight, plasma PRL concentration and wool growth were subjected to analysis of variance at each sampling time to test the effects of pregnancy. For wool growth and fibre diameter data, the pre-treatment (March) value was used as a covariate to adjust for inherent

wool growth capability. Two pregnant ewes and one non-pregnant ewe died in the course of the trial and their data were excluded from all analyses.

5.4 RESULTS

Lambing details

All ewes lambed between 11 and 15 September 1996 with the mean lambing date being 13 September. Of the 15 lambs born, which included 3 sets of twins, 9 were males and 6 females.

Ewe data

5.4.1 Feed intake

Total feed intake and mean daily feed intake (Figure 5.3) were higher in ewes that lambed (239 ± 6 kg and 1027 ± 26 g/day) compared to non-pregnant ewes (203 ± 1 kg and 871 ± 4 g/day). The daily feed intake during pregnancy tended to be higher in twin-bearing ewes compared to single-bearing ewes (1166 ± 6 versus 1034 ± 30 g/day, $P<0.10$).

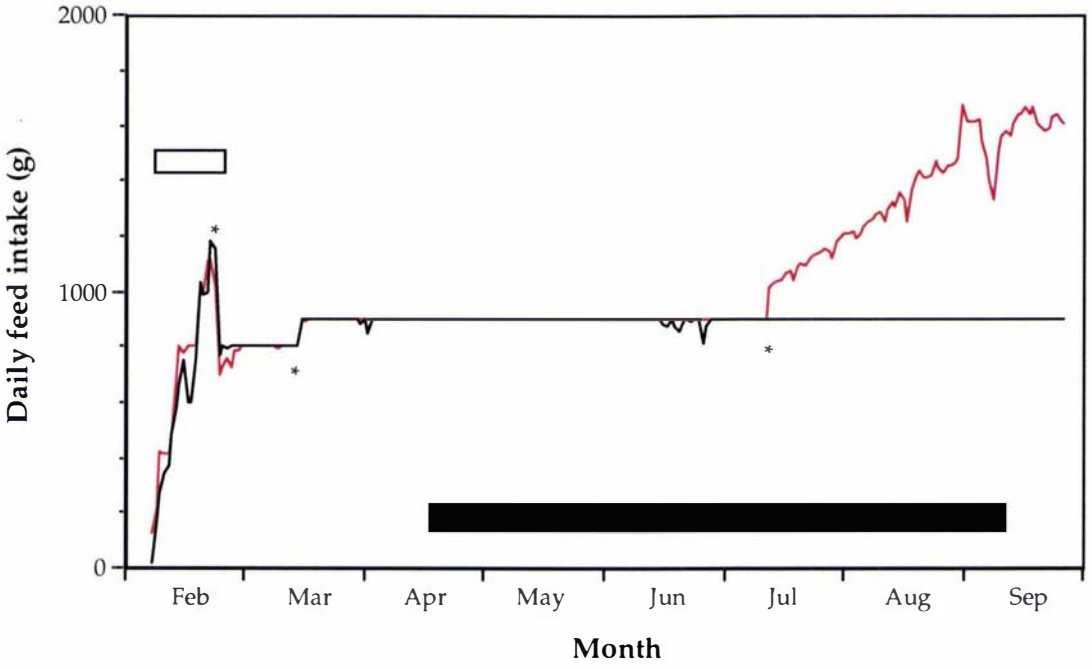

Figure 5.3: Mean daily feed intake of non-pregnant (–) and pregnant (–) ewes. Open bar represents the period of feed adaptation; solid bar represents period of gestation; * represents when daily feed allowance was adjusted across some or all groups.

5.4.2 Live weight

Both treatment groups had similar live weights on 8 February (49.2 ± 2.6 versus 50.1 ± 1.4 kg, $P>0.10$, Figure 5.4). Although breeding ewes were heavier ($P<0.01$) at joining, there was no difference in live weight with reproductive status until 19 June (day 62 of gestation) when pregnant ewes were significantly heavier than non-pregnant ewes (52.3 ± 2.1 versus 50.8 ± 1.1 kg, $P<0.05$). Pregnant ewes gained more weight from 20 May until 30 August (15.6 ± 1.2 versus 5.3 ± 0.3 kg, $P<0.001$). The prepartum increase in weight was greater in twin-bearing ewes compared to single-bearing ewes (18.1 ± 0.3 versus 11.3 ± 1.0 kg, $P<0.01$). Live weight declined in pregnant ewes postpartum relative to non-pregnant ewes, and although pregnant ewes were heavier ($P<0.01$) on 16 September, there were no subsequent differences.

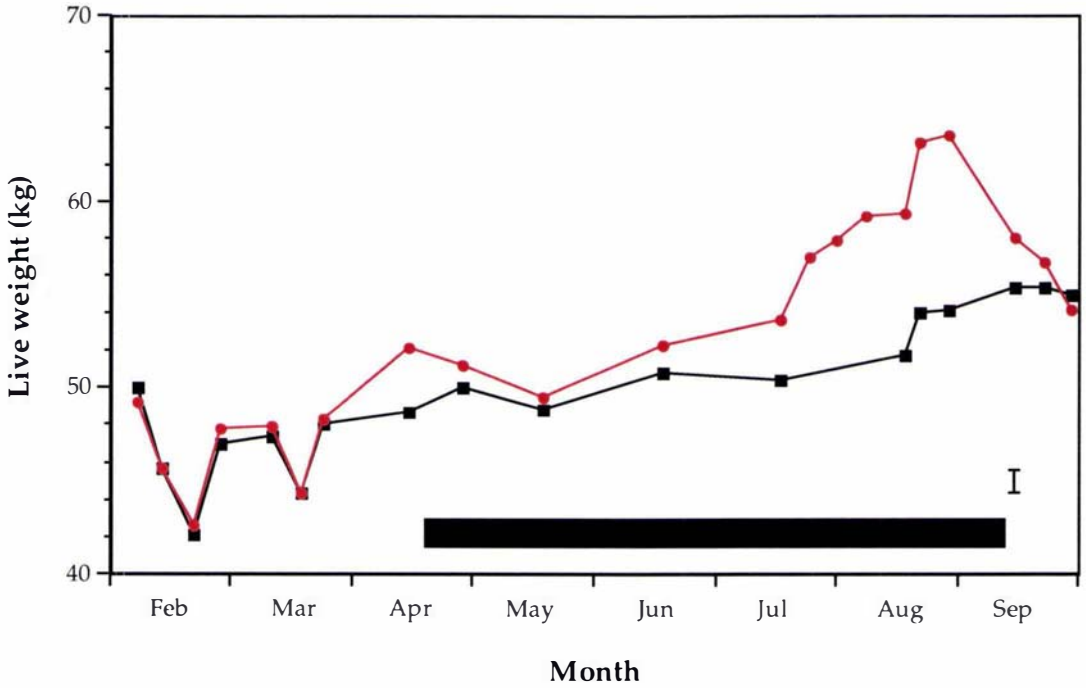

Figure 5.4: Mean live weight of non-pregnant (■) and pregnant (●) ewes. Solid bar represents period of gestation. Error bar represents the pooled SED.

5.4.3 Plasma PRL concentration

PRL concentrations declined from February, but the mean plasma PRL concentration in the period until 16 April (the day before joining) was similar in non-pregnant ewes (28 ± 5 ng/mL) and breeding ewes (26 ± 4 ng/mL, Figure 5.5). While PRL levels remained low (11 ± 2 ng/mL) in both groups until 4 June, the average PRL concentration was significantly higher in non-pregnant ewes compared to pregnant ewes (36 ± 4 versus 12 ± 2 ng/mL, $P<0.001$) in the subsequent period until 27 August. Although PRL concentrations continued to rise during September in non-pregnant ewes, the elevated PRL concentrations associated with parturition, meant that PRL concentrations were significantly higher ($P<0.01$) in pregnant ewes on all sampling dates after 3 September. In general, maternal plasma PRL concentrations throughout pregnancy were not affected by the number of fetuses, but in one of the twin-bearing ewes (#260), the prepartum rise in PRL concentration occurred a month before a rise was observed in other pregnant ewes.

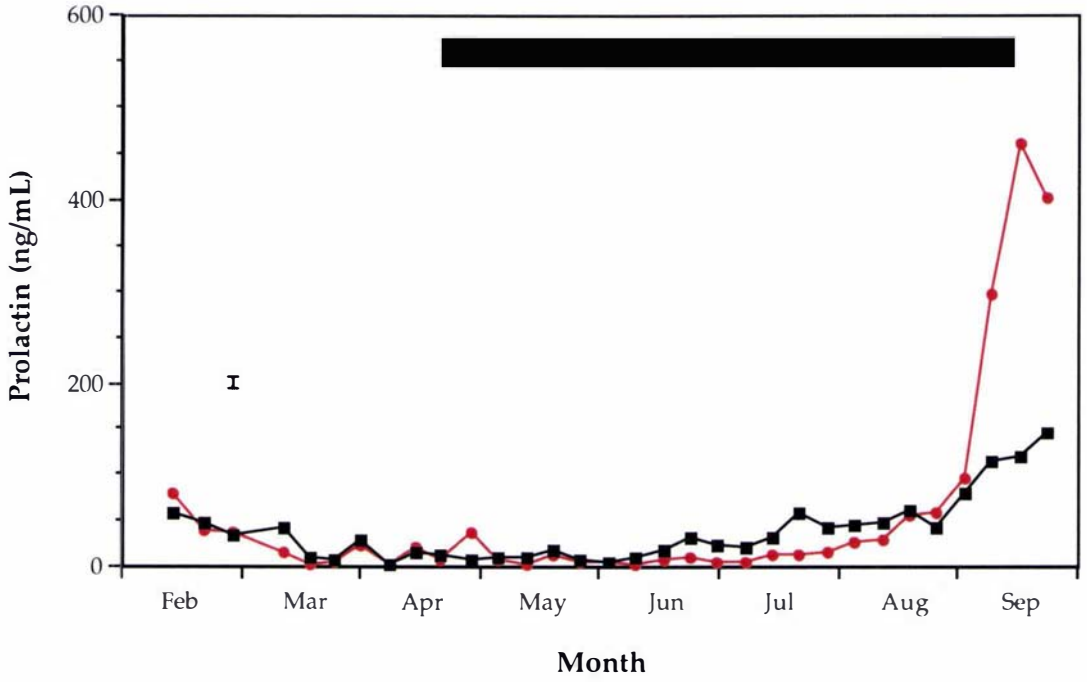

Figure 5.5: Mean plasma prolactin concentration of non-pregnant (■) and pregnant (●) ewes. Solid bar represents period of gestation. Error bar represents the pooled SED.

5.4.4 Midside clean wool growth rate and fibre diameter

Preliminary analyses indicated that the monthly wool growth rate and mean fibre diameter in single- and twin-bearing ewes were not significantly different during the course of the trial. These data therefore were pooled in the comparisons with the non-pregnant group.

Clean wool growth rate declined ($P<0.01$) in both groups from March but there were no measurable effects of pregnancy until June (Figure 5.6). From June to September, the average clean wool growth rate was 27% lower in pregnant ewes relative to non-pregnant ewes (6.3 ± 0.4 versus 8.4 ± 0.4 g/day, $P<0.05$). In October, 1 month after lambing, the wool growth rate in lactating ewes had increased and was similar to that in non-pregnant ewes (7.8 ± 0.7 versus 8.9 ± 0.4 g/day, $P>0.10$).

Monthly changes in mean fibre diameter generally reflected wool growth changes (Figure 5.7). While mean fibre diameter was not significantly different from March to May, diameter in the pregnant group showed a tendency to fall below non-pregnant controls in June (34.5 ± 0.7 versus 36.3 ± 1.3 μm, $P<0.10$). In subsequent months, up to and including October, mean fibre diameter remained significantly less ($P<0.05$) in pregnant and lactating ewes than in their non-pregnant controls (33.0 ± 0.7 versus 35.6 ± 1.0 μm).

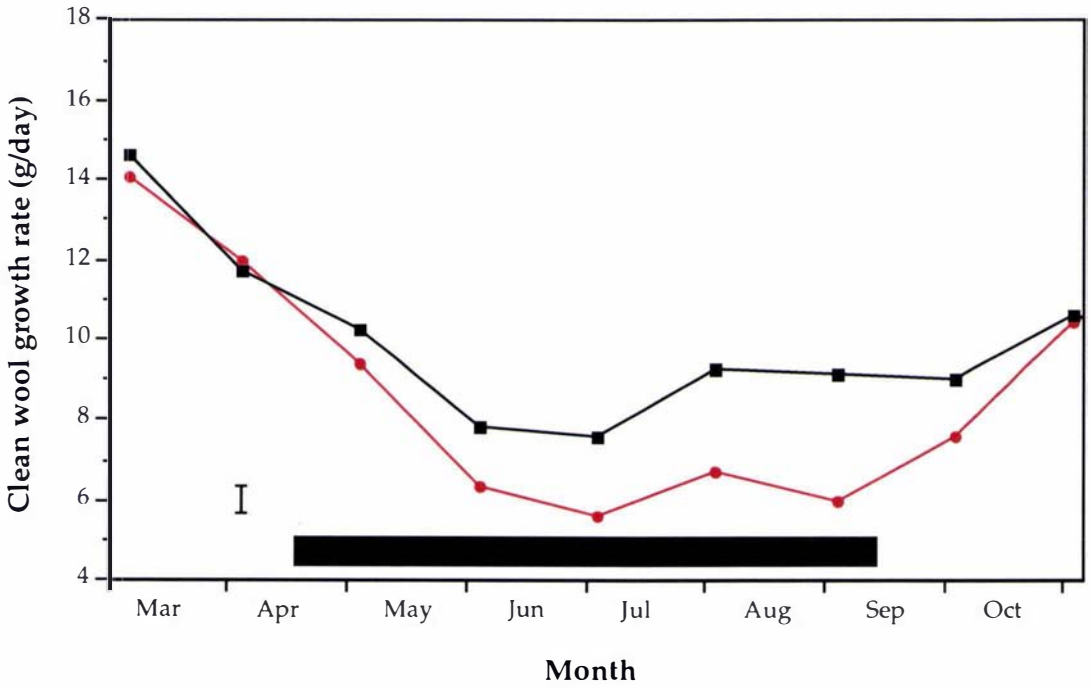

Figure 5.6: Midside clean wool growth rate of non-pregnant (■) and pregnant (●) ewes. Solid bar represents period of gestation. Error bar represents the pooled SED.

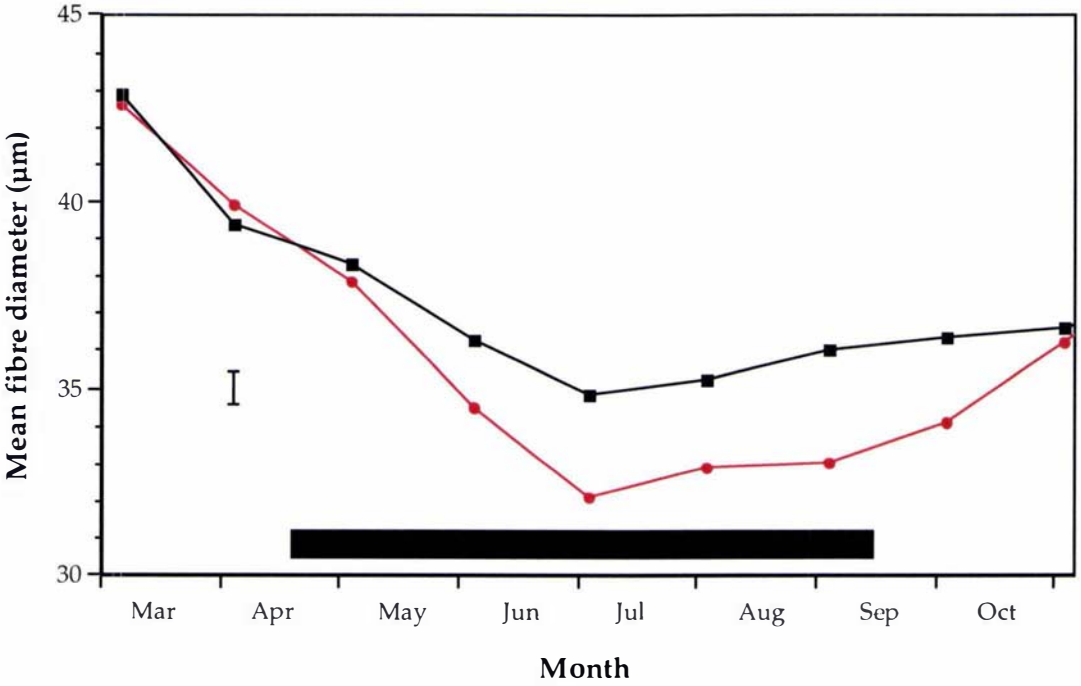

Figure 5.7: Midside mean fibre diameter of non-pregnant (■) and pregnant (●) ewes. Solid bar represents period of gestation. Error bar represents the pooled SED.

5.5 DISCUSSION

The aim of this experiment was to determine the timing of the wool growth depression in pregnant Romney ewes under conditions of controlled dietary intake, and to relate this reduction in wool growth with changes in the concentration of plasma PRL in the maternal circulation.

A limitation in previous studies has been the use of grazing sheep which have had access to diets of varying quantity and composition. Under these circumstances the effects of pregnancy have potentially been confounded with changes in feed intake and live weight. When both these factors are controlled, a reduction in Merino wool growth has been reported by the third and fourth months of pregnancy (Corbett, 1966; Oddy, 1985) relative to non-pregnant ewes. Parker *et al.* (1991) recorded a reduction of 14% and 23% clean wool growth in single- and twin-bearing Border Leicester × Romney ewes respectively between days 57 and 90 of pregnancy under pastoral grazing

conditions. There are no comparable data on wool production of the New Zealand Romney held under controlled indoor conditions.

It is generally accepted that the energy requirements of the growing fetus during the first 100 days of gestation are relatively small. Thus, the recommended feed requirements for pregnant ewes and non-pregnant ewes are the same over this period (Robinson, 1983; Rattray, 1986). In the present experiment therefore, all sheep were fed at maintenance levels until mid-July (day 87 of gestation in pregnant ewes). The constant feed intake over the first 150 days of the trial was reflected in the similarity in ewe live weight which only began to diverge between June and July in response to differential feeding and the growth of the conceptus.

The higher live weight in mid-April in breeding ewes (Figure 5.4) was the result of flushing prior to joining in an effort to increase the ovulation rate in mated ewes, although this had no influence on subsequent wool growth. The postpartum live weight of lactating ewes increased by 8.8 ± 0.8 kg from the February weight while, over the same period, the live weight of non-pregnant ewes increased by 5.2 ± 0.7 kg. The disparity in weight gain would be largely accounted by mammary gland development.

In the present experiment, differences in clean wool growth were observed by June (within the first 60 days of gestation). Furthermore, other data indicate that the influence of pregnancy on wool growth is not restricted to mid- and late pregnancy and may occur as early as 21-35 days after joining when differences in individual fibre length growth, determined by autoradiography, were first detected in this experiment (Pearson *et al.*, 1999). These differences were not associated with either liveweight changes or differences in feed intake. This is consistent with earlier findings by Henderson *et al.* (1970), who recorded a difference in wool growth rate between pen-fed non-pregnant and pregnant ewes by day 56 of gestation in the latter group. Lee and Atkins (1995) also reported that grazing pregnant Merino ewes grew 8% less wool

than non-pregnant ewes in early pregnancy (7–11 weeks after joining) without any differences in feed intake or live weight. Contrary to Oddy (1985), who found no differences in fibre diameter between non-pregnant and pregnant ewes on a similar intake, changes in fibre diameter over pregnancy followed the same trend as wool growth in pregnant sheep in the current experiment. However, fibre diameter was not significantly lower in pregnant ewes until July, a month after differences in wool growth were measured, which suggests that there may be a differential effect of pregnancy on fibre length and diameter.

It was reported in previous experiments *(Chapters Three and Four)* that plasma PRL concentrations were suppressed during late pregnancy. Results obtained in this study are consistent with those, but more importantly they have allowed the determination of the exact timing of this suppression in circulating PRL concentrations in pregnant ewes housed indoors. While there is evidence to suggest that reduced PRL concentrations in pregnant rats could be caused by progesterone (Vermouth & Deis, 1974) or placental lactogen (Voogt *et al.*, 1982; Voogt, 1984), it is not known whether a similar endocrine mechanism exists in the pregnant ewe. Plasma PRL concentrations in mated ewes were similar to those in non-pregnant ewes at joining, and in the early stages of pregnancy, but from 4 June (day 47 of gestation) were significantly lower, and the subsequent spring rise in PRL concentration was prevented. Interestingly, this corresponds to the time of increased secretion of both progesterone (Bassett *et al.*, 1969; McNatty *et al.*, 1972) and placental lactogen (Kelly *et al.*, 1974; Chan *et al.*, 1978a) in the maternal circulation. It also coincides with an increase in plasma progesterone concentrations in these same experimental sheep (Pearson *et al.*, unpublished data). While no firm conclusions can be made given the limited evidence available on the effects of these hormones on wool growth, further investigation seems warranted.

The concentrations of many hormones in the maternal circulation, including PRL, change during the course of pregnancy (Figure 1.3). However, it is

unlikely that PRL mediates the reduction in wool growth in the pregnant ewe. Firstly, differences in wool growth were apparent prior to any measurable change in plasma PRL concentration, and secondly, there was a lack of supporting evidence to suggest that a small reduction in plasma PRL concentrations have any immediate effect on wool production (Pearson *et al.*, 1996). Alternative candidates include GH (Wynn *et al.*, 1988) and cortisol, (Chapman & Bassett, 1970), which both have a strong inhibitory effect on wool growth. The plasma concentrations of these hormones are relatively low during early pregnancy (Figure 1.3), but it is possible that the inhibition of wool growth could be induced by a combination of these or other reproductive hormones.

5.6 CONCLUSIONS

A significant reduction in wool growth rate and fibre diameter was observed within the first 60 days of gestation in sheep despite a controlled dietary intake. This was not associated with changes in maternal live weight. Plasma PRL concentrations were also lower in pregnant ewes during early pregnancy (from day 47 of gestation) relative to non-pregnant ewes, an effect which could be mediated by increased secretion of progesterone or placental lactogen. These data suggest a direct hormonal influence on wool growth at an early stage of pregnancy which does not appear to involve PRL. In late pregnancy, other influences could be important, but the inhibitory effect ceases at the birth of the lamb. Reduced responsiveness of the wool follicles to circulating levels of nutrients and/or control of partitioning of nutrients are possible causes of the wool growth depression observed in the pregnant ewe.

CHAPTER SIX

Characterisation of the plasma prolactin profile during pregnancy, at parturition, and during lactation in Romney ewes under different photoperiods

6.1 ABSTRACT

The effect of photoperiod on plasma PRL concentrations in pregnant and lactating ewes, and the role of PRL in mediating the stimulatory effect on wool growth in sheep housed in long daylength (16L:8D) were investigated. Twenty-one pregnant ewes were maintained indoors from 23 June to 2 December 1997 under controlled photoperiod and dietary intake. One group (n = 8) was held under natural photoperiod (ND) while 2 others (n = 6 and 7) were exposed to long day photoperiod (LD). One of these groups was treated with bromocriptine (LD-Br) 1 week from parturition to suppress PRL secretion. Maternal plasma concentrations of PRL were measured in hourly samples collected during a 24-h period in 6 ND and 6 LD ewes on day 118 of gestation (late pregnancy) and mid-lactation (day 27 postpartum). Maternal PRL concentrations were also determined over a 120-h period during parturition (8–13 September). Photoperiod and bromocriptine treatment had no effect on feed intake or live weight. In LD ewes, PRL concentrations were significantly higher ($P<0.001$) over pregnancy than in ND ewes and throughout the 24-h sampling period in late pregnancy. There was a significant diurnal rhythm in PRL concentrations in LD ewes with maximal levels occurring before the hours of darkness. Under LD conditions, prepartum plasma PRL concentrations rose earlier and reached a peak 16 h before that of ND ewes. PRL concentrations remained higher ($P<0.05$) during lactation in LD ewes than in ND ewes although no significant diurnal rhythm was detected. Suckling had no effect on maternal PRL concentrations in ND and LD ewes at mid-lactation. There was an increase in clean wool growth rate, mean fibre diameter and the rate of growth of fibre length postpartum. No short-term wool growth response was obtained in any treatment group despite markedly different PRL profiles. Higher PRL concentrations at parturition were associated with medium-term stimulatory effects on wool growth as November wool production tended to be greater in LD ewes.

6.2 INTRODUCTION

The series of experiments conducted previously involved the manipulation of wool growth in pregnant and lactating Romney ewes using photoperiod or bromocriptine. The results suggest that changes in PRL profile are associated with both medium- and long-term effects on wool growth patterns. In particular, large increases in wool production were observed in sheep held in LD photoperiod throughout pregnancy and lactation *(Chapter Four)*. The increased wool production was associated with higher-than-normal PRL concentrations at parturition.

PRL concentrations in pregnant ewes rise sharply a few days prior to parturition (Lamming *et al.*, 1974), however the exact timing of this periparturient rise and the pattern of change over lactation remains unclear. The present experiment (FP003/04) was designed to *(i)* characterise the normal 24 h plasma PRL profile of pregnant ewes exposed to ND and LD photoperiod in late gestation, at parturition, and in early lactation; *(ii)* establish whether PRL was responsible for the increase in monthly wool production observed previously in LD-lambed ewes; and *(iii)* examine whether the increased wool production observed in winter-lambing ewes exposed to extended photoperiod could be replicated in spring-lambing ewes. All sheep were fed to a constant maternal live weight to remove the effects of variable nutrient availability on fibre growth. Of the 21 sheep, 8 were held in ND photoperiod and 13 in LD photoperiod.

6.3 MATERIALS AND METHODS

6.3.1 Experimental Animals

Thirty pregnant Romney ewes from the commercial breeding flock at the Whatawhata Research Station were identified to be carrying single fetuses using ultrasonic scanning on 5 June (day 50 of gestation) and transported to Ruakura Agricultural Research Centre on 19 June 1997 (day 64).

6.3.2 Housing

Twenty-one ewes were transferred indoors to individual pens in 2 separate rooms in the Ruakura Physiology Building on 23 June (day 68) and maintained indoors until 2 December 1997.

6.3.3 Environmental Observations

The daily minimum and maximum air temperatures recorded at the Ruakura meteorological station from June 1997 to December 1997 are shown in Figure 6.1. Figure 6.2 shows the mean monthly temperatures and hours of daylight over the same period.

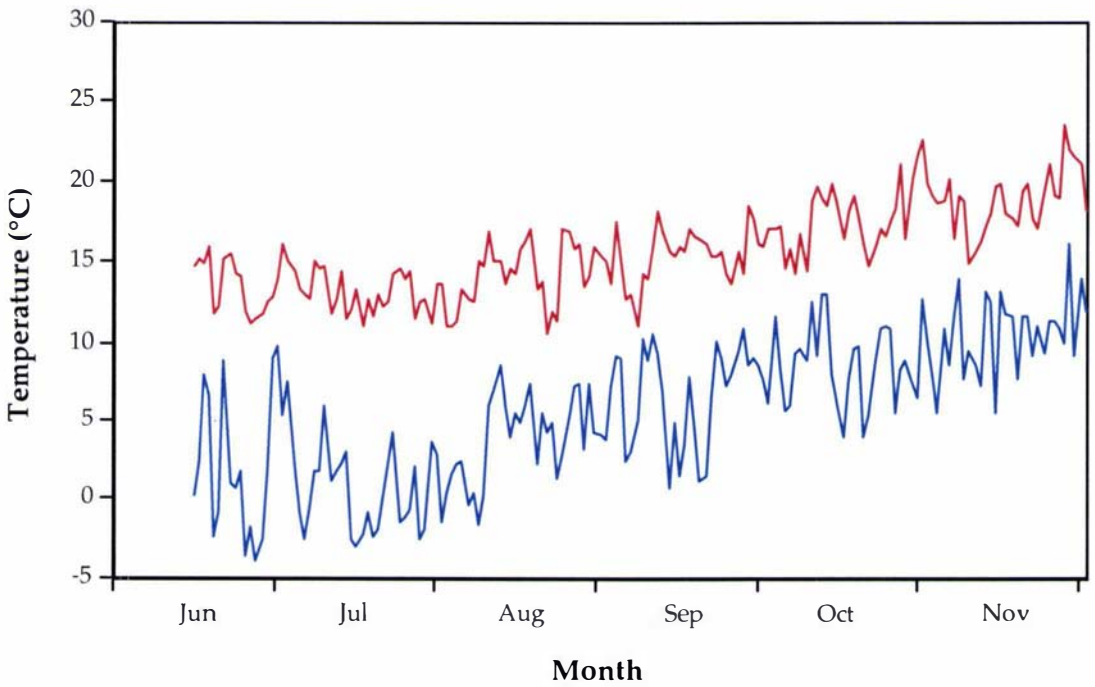

Figure 6.1: Daily minimum (–) and maximum (–) air temperatures during the 1997 experimental period.

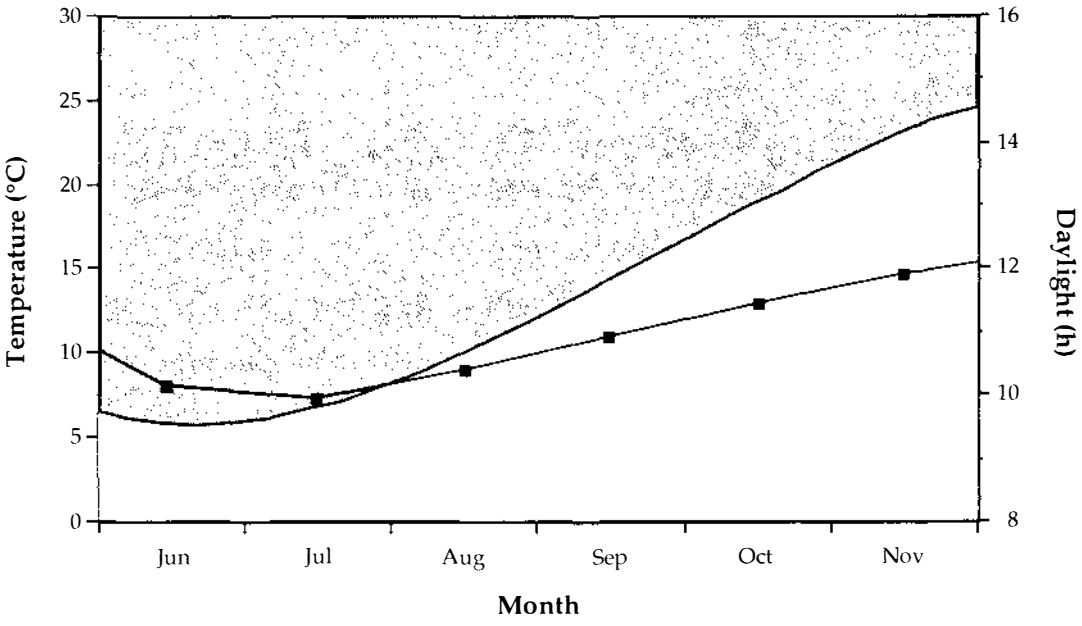

Figure 6.2: Monthly mean air temperature (■) and hours of daylight during the 1997 experimental period.

6.3.4 Experimental Groups

The experiment incorporated 2 levels of photoperiod (natural days versus long days) in pregnant ewes (Groups 1 and 2). There was also a comparison of bromocriptine treatment (Groups 2 and 3). The 21 sheep were divided into 3 groups balanced for the initial midside patch weights (Table 6.1).

Table 6.1: Experimental groups.

Group		n	Abbreviation
Natural Days			
1	Pregnant ewes	8	ND
Long Days			
2	Pregnant ewes	7	LD
3	Pregnant ewes treated with bromocriptine from day 137 of gestation	6	LD-Br

6.3.5 Light Treatment

Group 1 was exposed to natural photoperiod while groups 2 and 3 were held under long day conditions (16L:8D) as described in Section 2.4.

6.3.6 Live weight

All sheep were weighed at 7 to 14 day intervals over the trial duration to monitor changes in live weight.

6.3.7 Feed Allowance

The daily feed allowance for pregnant ewes was calculated and the daily feed intake measured as described in Section 2.3.

6.3.8 Wool Sampling

The sheep were shorn on entry to the experiment on 17 June and again at the conclusion of the experiment on 3 December 1997 when the fleece was weighed and a midside sample collected *(Section 2.7.3).* A standardised midside patch clip was established on 2 July and re-clipped monthly. Length growth rates of individual fibres were determined using repeated intradermal injections of ^{35}S-cysteine into a tattooed skin site at weekly intervals from 15 August until 10 October. Wool staples were harvested on 31 October for autoradiographic determination *(Section 2.8).*

6.3.9 Blood Sampling

Blood samples were collected from all ewes from 9 July until 18 November at intervals ranging from every 2 weeks to daily close to parturition. Additionally, a series of blood samples were taken at hourly intervals for a minimum of 24 h at 3 different stages of the trial: late pregnancy (12–13 August), parturition (8–13 September) and mid-lactation (7–8 October) in 6 ND and 6 LD ewes. For each blood sampling session, a cannula (Cavafix, Braun Melsungen AG, Melsungen, Germany) was inserted into a jugular vein under

local anaesthetic (Lopaine; Ethical Agents Ltd., NZ) at least 12 h prior to sampling. Cannulas were held in place by Leukoplast adhesive tape (BGF, Beiersdorf AG, Hamburg, Germany) and No. 6 elastic netting (Seton Healthcare Group, UK). A long-acting broad-spectrum antibiotic (Propen LA; Pitman-Moore, Upper Hutt, NZ) was injected intramuscularly at the conclusion of each cannulation. Cannulas were replaced as necessary to maintain patency. The cannulas were connected via a network of overhead 1.5 mm polyethylene tubing (Critchley Electrical Products Pty. Ltd., Auburn, NSW, Australia) which allowed the blood to be collected in a central room with minimal stress and disturbance to the ewes. Adjustments to cannula lines or assistance provided to lambing ewes during the hours of darkness was performed under dim red light. Each room had 2 infra-red cameras which were connected to a video recorder located in the central room. These were used to determine the exact time of parturition, to monitor any problems associated with labour and to observe suckling activity.

Blood collection commenced at 0400 h and continued at hourly intervals until 0400 h the following day (late pregnancy) or from 1000 h at hourly intervals until 1000 h the following day (mid-lactation). The collection of blood over parturition commenced at 1000 h and continued at hourly intervals until 1000 h 5 days later. All sheep were unrestrained at this time to allow normal labour to proceed. Throughout the 24-h lactation sampling period, a total of 60 samples from 7 ewes were taken at 15 min intervals until the next hour once suckling behaviour (n = 19) had been observed. Three millilitre blood samples were collected and processed as described previously. All collected blood was replaced with an equal volume of 0.9% saline (Baxter Healthcare Ltd., Toongagabbie, NSW, Australia) containing 25000 units/mL heparin (Leo Pharmaceuticals, Ballerup, Denmark). Inter-assay and intra-assay coeffecients of variation for the PRL radioimmunoassay at 10 ng/mL were 8.7% and 10.5% respectively.

6.3.10 Bromocriptine Administration

Bromocriptine treatment was administered by 90-day time-release pellets (Innovative Research of America (IRA), Sarasota, FL, USA) due to the unavailability of Parlodel. Three pellets (total release rate = 3.33 mg/day) were inserted subcutaneously in aseptic conditions. The skin between the scapulae was trimmed of excess wool, locally anaesthetised and a small incision was made of sufficient size to insert the pellets. The incision was then sutured.

6.3.11 Lamb Sampling

No samples were collected from the lambs. The lambs remained with their dams until weaning at the conclusion of the experiment.

6.3.12 Statistical Methods

Data relating to feed intake, ewe live weight, clean wool growth rate and wool characteristics were subjected to analysis of variance at each sampling time to test the effects of photoperiod and bromocriptine treatment. For ewe wool data, the July value for each parameter was used as a covariate. The mean 24-h PRL concentration was determined for each ewe during late pregnancy and mid-lactation by averaging the 25 samples collected during each sampling period and differences between photoperiod groups tested by analysis of variance. Data from one ND ewe which died during the experiment were excluded from the analyses.

6.4 RESULTS

Lambing details

Nine male and 12 female lambs were born between 8 and 15 September 1997 with the mean lambing date being 10 September.

Ewe data

6.4.1 Feed intake

Total feed intake was 222 ± 5 kg, 224 ± 4 kg and 220 ± 5 kg for ND, LD and LD-Br ewes respectively. The mean daily feed intake (Figure 6.3) over the 6 months of the experiment was 1379 ± 23 g/day, 1384 ± 24 g/day and 1360 ± 29 g/day. These values, and the average daily feed consumption over pregnancy and lactation, did not differ between treatments.

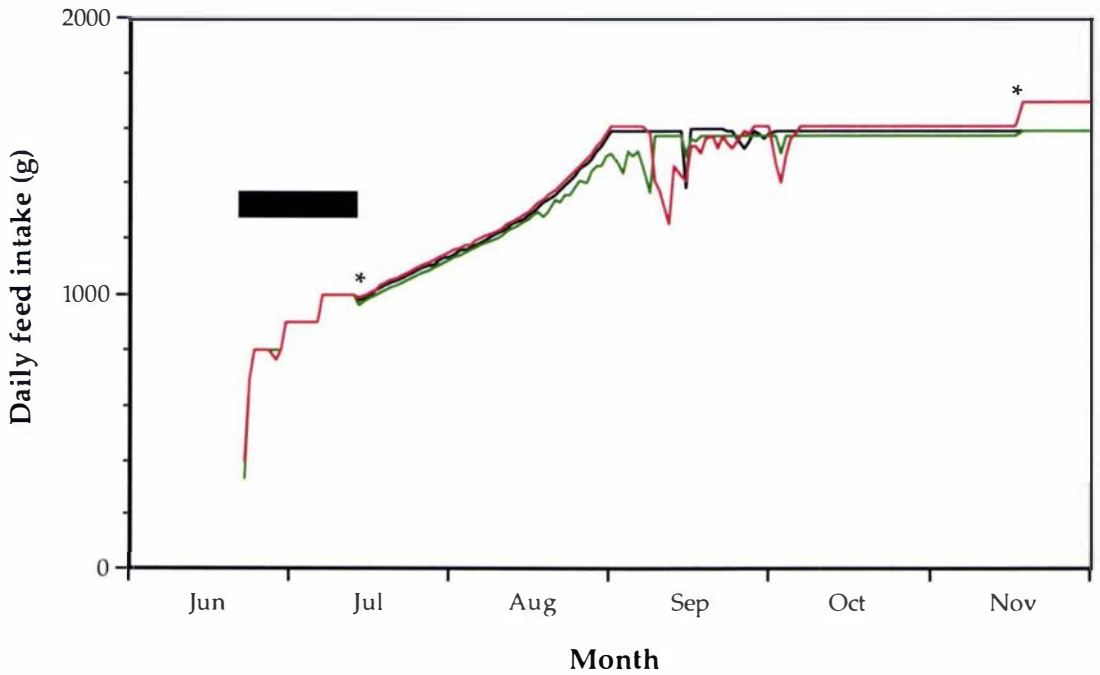

Figure 6.3: Mean daily feed intake of ND (–), LD (–) and LD-Br (–) ewes. Solid bar represents the period of feed adaptation; * represents when daily feed allowance was adjusted in some or all groups.

6.4.2 Live weight

Mean live weights increased ($P<0.001$) from 47.5 ± 0.7 kg in mid-June to 59.9 ± 0.9 kg, a week prior to parturition (Figure 6.4). A significant ($P<0.001$) weight loss occurred postpartum, so that by the end of the trial in December, the average live weight was 46.1 ± 0.9 kg. There were no treatment effects at any sampling date.

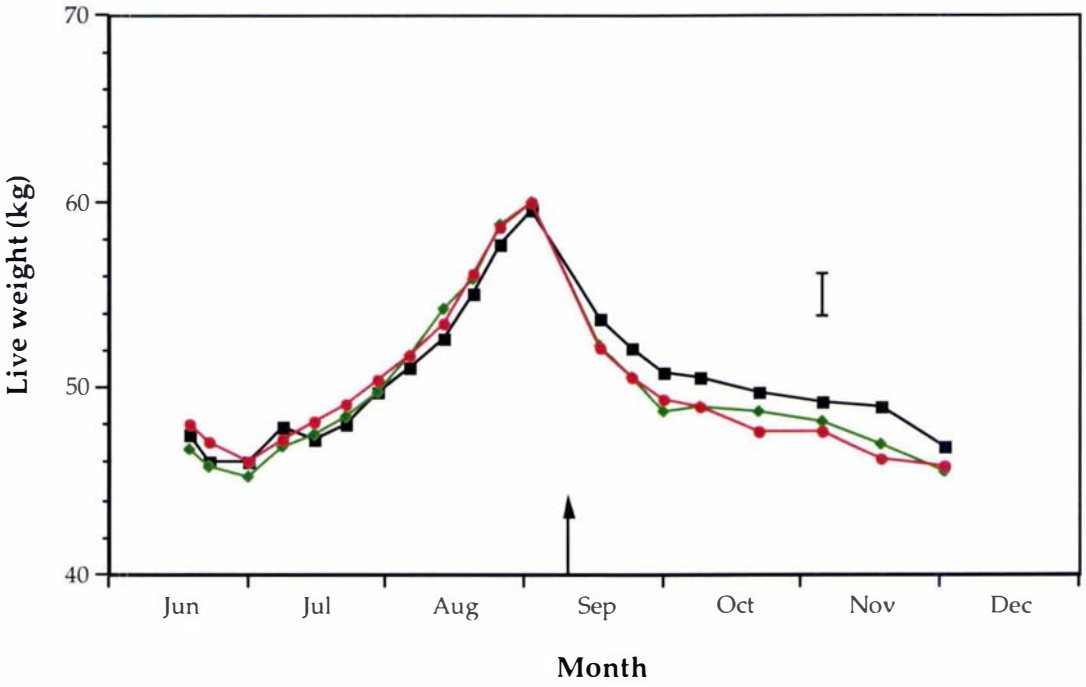

Figure 6.4: Mean live weight of ND (■), LD (●) and LD-Br (◆) ewes. Arrow represents mean date of parturition. Error bar represents the pooled SED.

6.4.3 *Plasma PRL concentration*

Different plasma PRL profiles were observed in the 3 treatment groups (Figure 6.5). While there was no difference between groups in PRL concentrations at the start of the trial in July, levels were significantly higher ($P<0.001$) in ewes maintained in LD compared to ND photoperiod over the rest of pregnancy (62 ± 9 versus 12 ± 5 ng/mL). Plasma PRL concentrations in samples taken every hour for 24 h on day 118 of pregnancy for ewes under the 2 photoperiods are shown in Figure 6.6. There were significantly higher ($P<0.001$) concentrations of PRL in ewes exposed to LD with the mean PRL concentration over this 24-h period being 120 ± 26 and 12 ± 6 ng/mL for LD and ND ewes respectively. Diurnal fluctuations in PRL concentrations were apparent in LD ewes, with PRL concentrations highest between 1400 and 2400 h. In ND ewes, PRL concentrations also changed with time but displayed considerable variability (Figure 6.6).

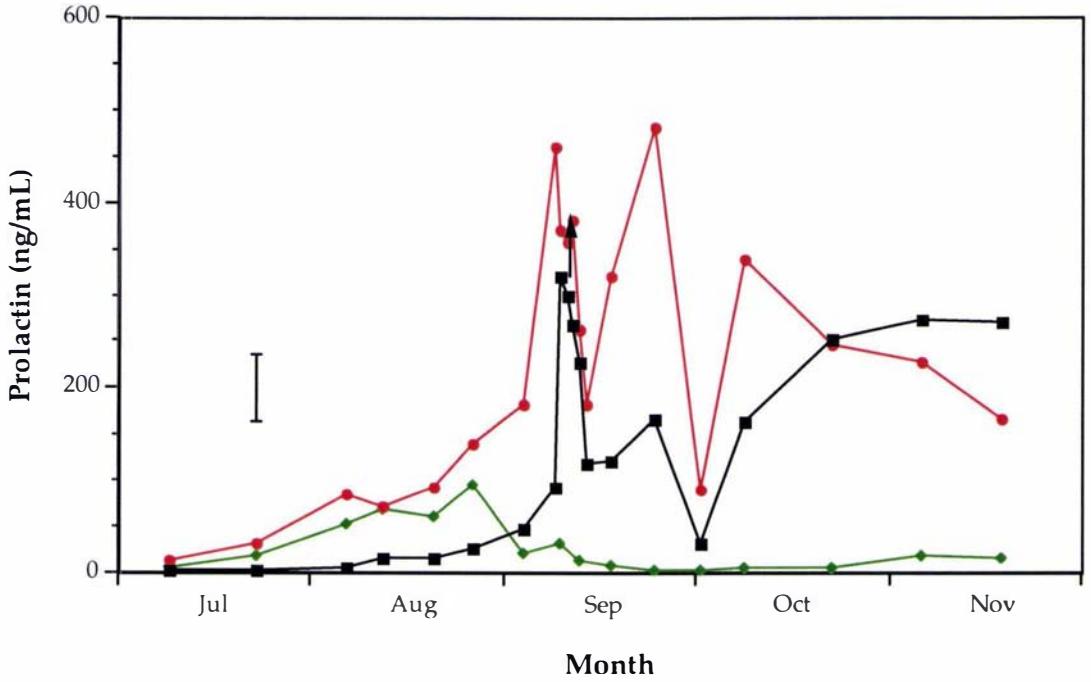

Figure 6.5: Mean plasma prolactin concentrations of ND (■), LD (●) and LD-Br (◆) ewes. Arrow represents mean date of parturition. Error bar represent the pooled SED.

In comparison to those in ND ewes, PRL concentrations remained higher ($P<0.001$) in LD ewes on 3 September but were not significantly different around parturition or early lactation (9–17 September, Figure 6.5). By 24 September, PRL concentrations were again higher ($P<0.05$) in LD ewes compared to ND ewes but were not different after 22 October. Bromocriptine treatment on 2 September suppressed the PRL surges associated with parturition and lactation (September–November) in LD-Br ewes and PRL concentrations were significantly lower ($P<0.001$) compared to those in LD and ND ewes over these months.

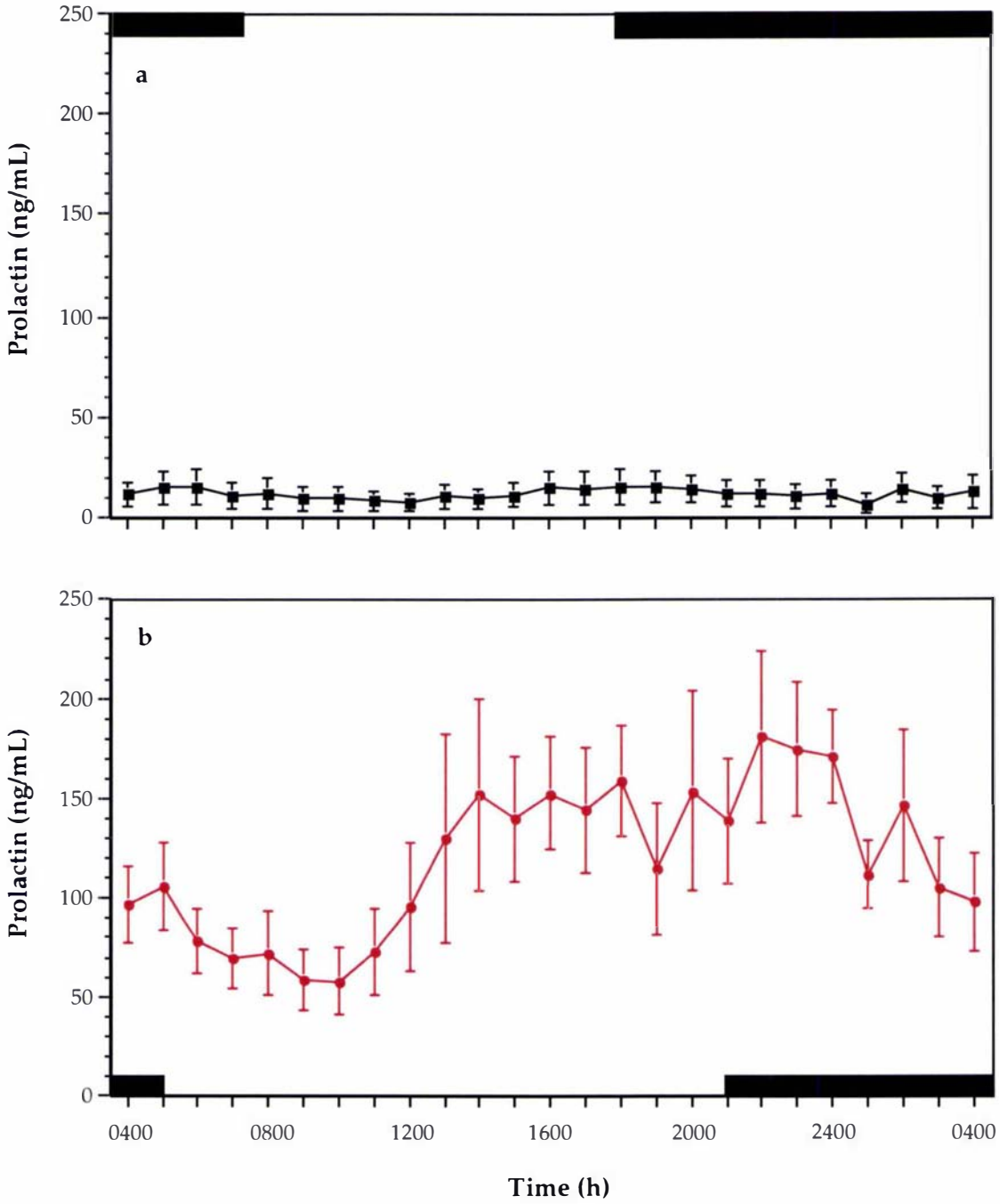

Figure 6.6: Mean plasma prolactin concentrations (±SEM) of (a) ND (■) and (b) LD (●) ewes collected over a 24-h period on day 118 of gestation. Solid bar represents period of darkness for each group (corresponds to the interval between dusk and dawn in the ND group).

Figure 6.7 shows the mean PRL profiles of ND and LD ewes in samples taken every hour for a 120-h period in the week of parturition. To allow for the variation in the time of lambing for each ewe, individual PRL levels were adjusted relative to parturition (0 h). There was a marked difference in the mean plasma PRL concentrations in ND and LD ewes at certain stages of this

120-h period, which is highlighted in the individual PRL profiles (Figure 6.8). One of the ND ewes (#406) lambed early in the 5-day sampling period, 3 others (#213, #449 and #711) displayed a complete periparturient PRL profile, while in another ND ewe (#206), only the prepartum PRL rise was observed. The last of the ND ewes (#285) lambed after the 5-day sampling period and had an incomplete PRL profile. As the LD ewes generally lambed earlier than their ND counterparts, only the postpartum PRL decline was observed in 4 of these ewes (#283, #427, #620 and # 931). In the other 2 LD ewes (#33 and #314), only the final stages of the prepartum PRL rise and the postpartum decline in PRL concentration was apparent.

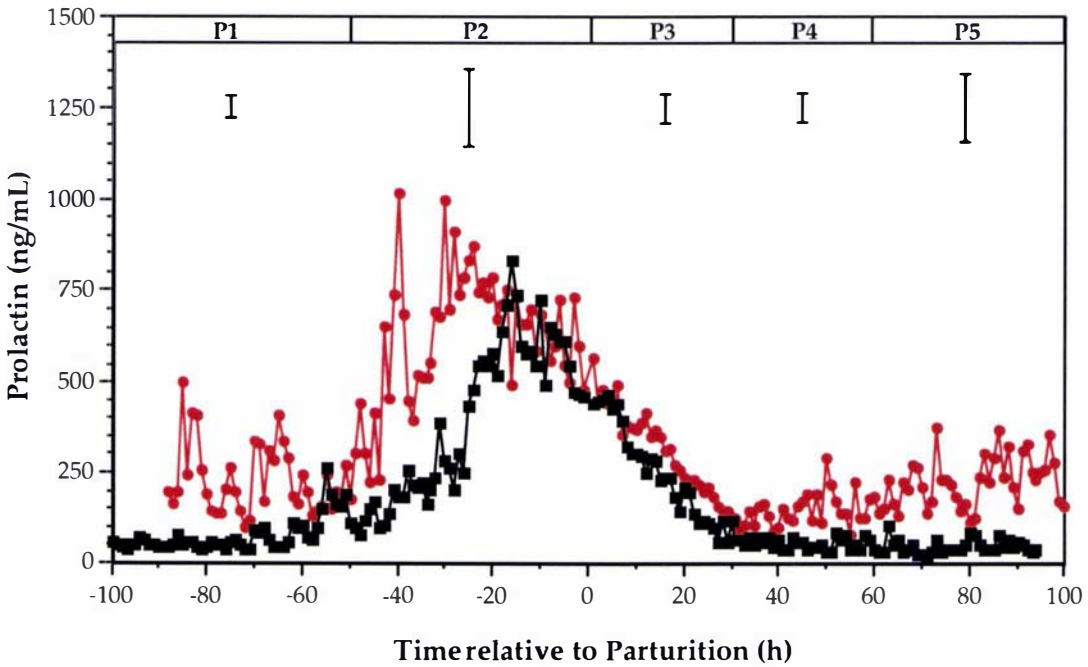

Figure 6.7: Mean plasma prolactin concentration of ND (■) and LD (●) ewes collected continuously for a 120-h period from approximately day –2 prepartum to day 2 postpartum. Values have been adjusted relative to the time of parturition (0). The 5 periods of interest are shown at the top of the graph and the error bars represent the pooled SED.

For analysis purposes, 5 periods of interest were considered based on the PRL profile from each group and the number of sheep sampled within each period. From 100 to 50 h before parturition, mean PRL concentrations were significantly higher ($P<0.05$) in LD ewes compared to ND ewes (223 ± 11 versus 71 ± 18 ng/mL). PRL concentrations increased earlier in LD ewes

relative to ND ewes and, while generally remaining higher from 49 to 1 h before parturition, were not significantly different (618 ± 74 versus 483 ± 185 ng/mL, $P>0.10$). In LD ewes, PRL levels reached a peak of 997 ± 399 ng/mL approximately 30 h before parturition while in ND ewes a peak PRL concentration of 833 ± 369 ng/mL was reached 16 h prepartum. There was no difference in PRL concentrations between LD and ND ewes from 0 to 30 h (319 ± 35 versus 275 ± 43 ng/mL, $P>0.10$) and 31 to 58 h (123 ± 41 versus 59 ± 4 ng/mL, $P>0.10$) after parturition. However, PRL levels tended to be higher in LD ewes compared to ND ewes from 59 to 100 h postpartum (211 ± 57 versus 44 ± 7 ng/mL, $P<0.10$).

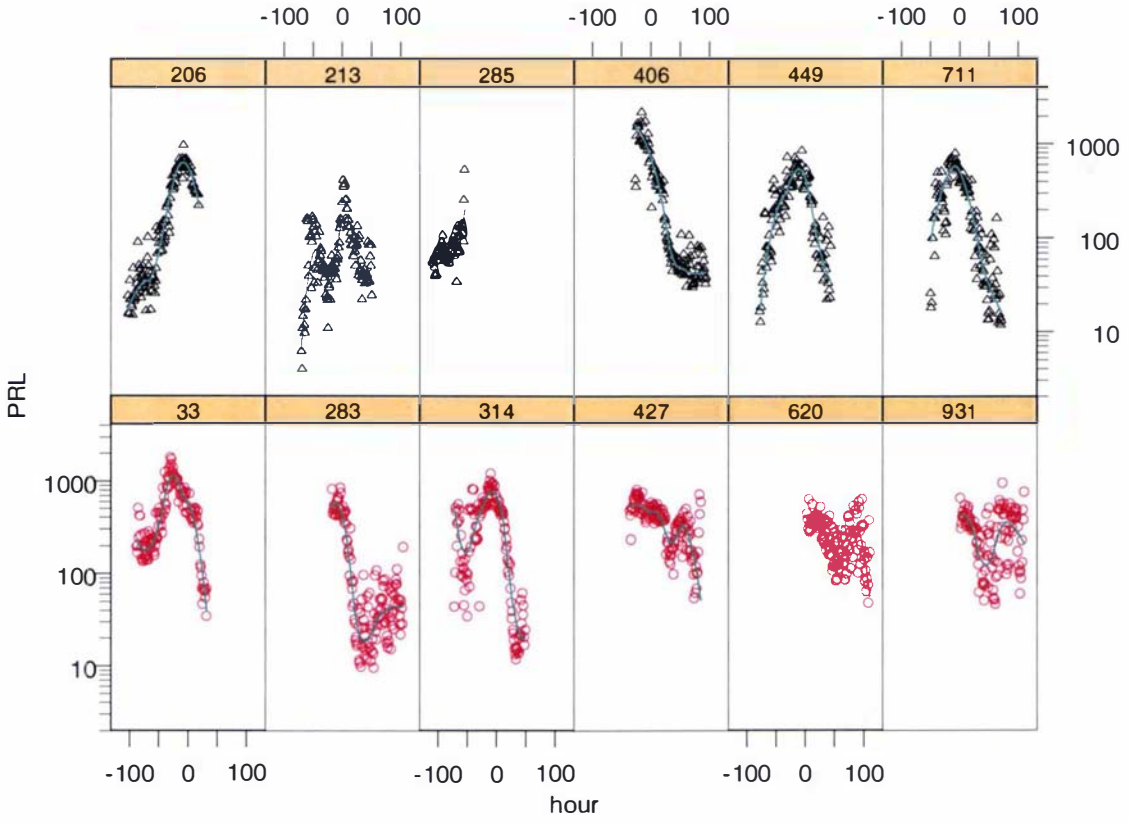

Figure 6.8: Individual log plasma prolactin profiles of ND (Δ) and LD (O) ewes collected continuously for a 120-h period from approximately day –2 prepartum to day 2 postpartum. Values have been adjusted relative to the time of parturition (0).

Mean PRL concentrations in samples taken every hour for 24 h on day 27 of lactation (Figure 6.9) were also significantly higher in LD ewes compared to

ND ewes (280 ± 36 versus 149 ± 36 ng/mL, $P<0.05$). PRL concentrations fluctuated in both groups and there was a diurnal rhythm in LD ewes but not ND ewes. Individual maternal PRL concentrations exhibited a large degree of variability in response to suckling, but in general concentrations declined by up to 30% from initial levels (Figure 6.10).

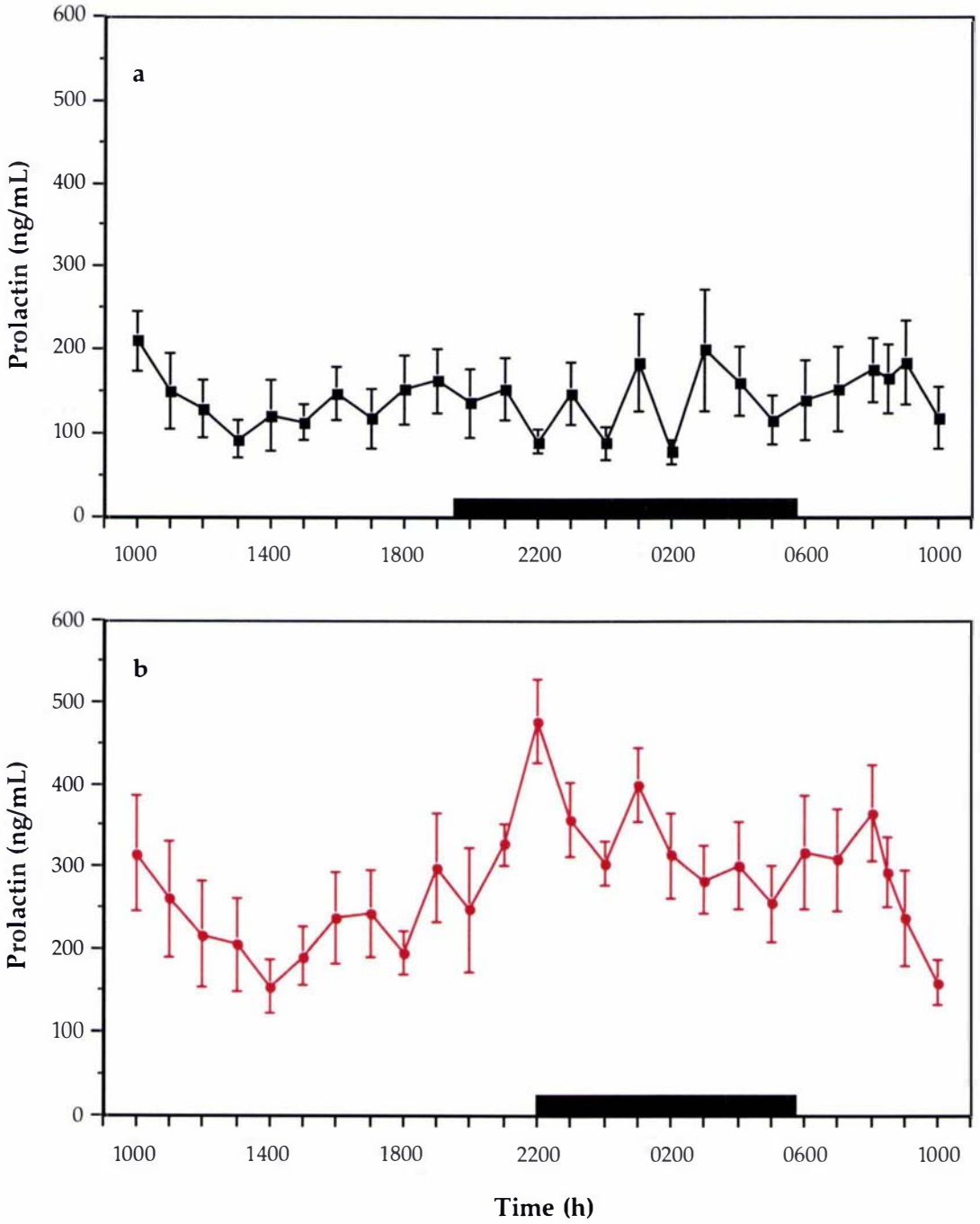

Figure 6.9: Mean plasma prolactin concentrations (±SEM) of (a) ND (■) and (b) LD (●) ewes collected continuously for a 24-h period on day 27 of lactation. Solid bar represents period of darkness for each group (corresponds to the interval between dusk and dawn in the ND group).

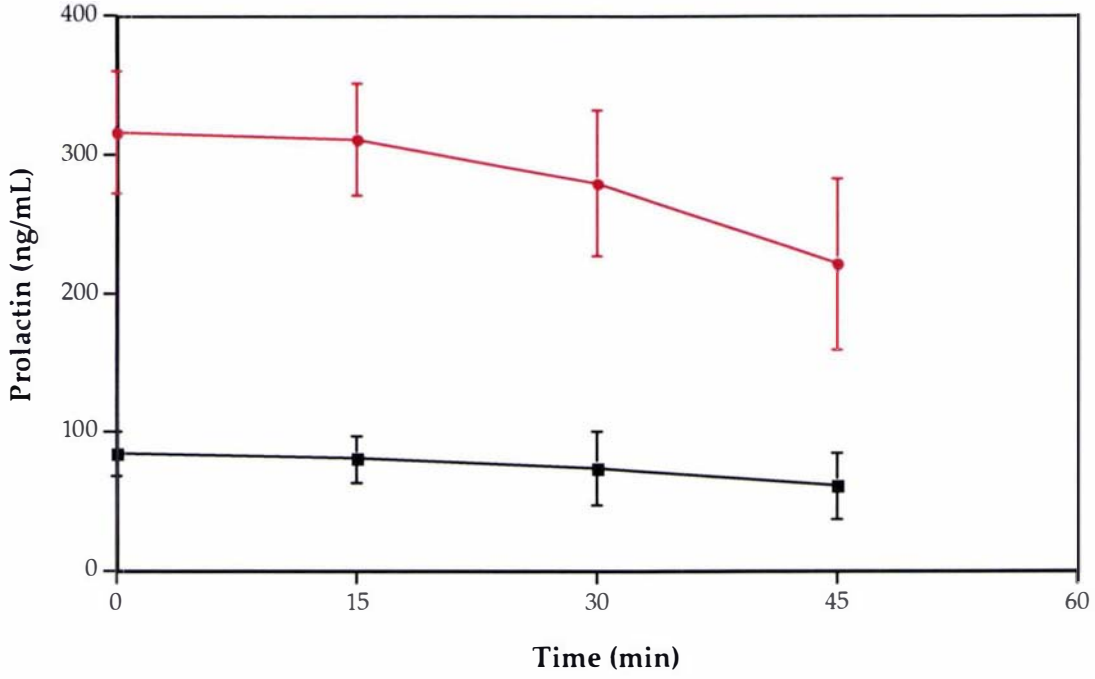

Figure 6.10: Change in plasma prolactin concentration (Mean ± SEM) of ND (■) and LD (●) ewes with time after lamb suckling (n = 60 observations).

6.4.4 *Fleece weight*

There were no treatment effects on 5-month greasy fleece weight, fleece washing yield, clean fleece weight or mean fibre diameter of the fleece wool sample at shearing (Table 6.2).

Table 6.2: Mean greasy fleece weight (GFW), washing yield, clean fleece weight (CFW) and mean fibre diameter (MFD) of fleece wool at shearing of the experimental groups (Mean ± SEM).

Group	*n*	GFW (kg)	Yield (%)	CFW (kg)	MFD (µm)
ND	7	1.85 ± 0.09	69.2 ± 2.5	1.28 ± 0.06	34.1 ± 0.5
LD	7	1.84 ± 0.11	73.4 ± 0.9	1.35 ± 0.08	36.1 ± 1.1
LD-Br	6	1.86 ± 0.12	68.9 ± 1.5	1.28 ± 0.08	34.3 ± 0.7

6.4.5 *Midside wool washing yield*

There were no treatment effects on midside patch washing yield in any month (Figure 6.11).

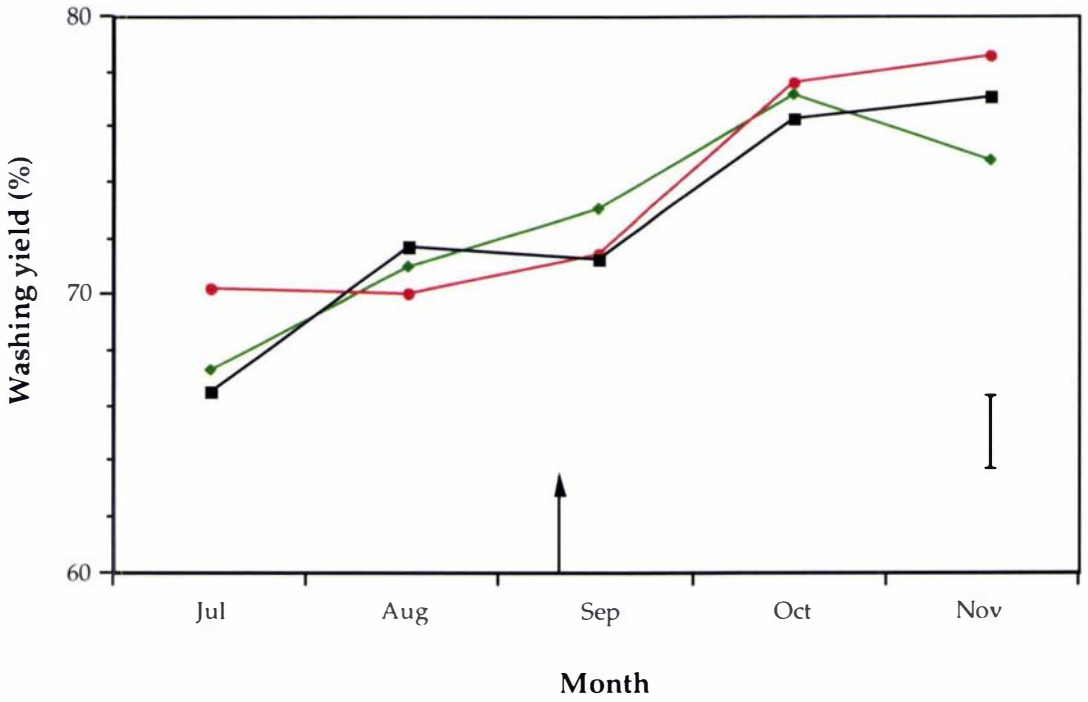

Figure 6.11: Midside washing yield of ND (■), LD (●) and LD-Br (◆) ewes. Arrow represents mean date of parturition. Error bar represents the pooled SED.

6.4.6 *Midside clean wool growth rate*

The change in clean wool growth rate increased ($P<0.001$) in all groups from July (mid-pregnancy) to November (Figure 6.12). There were no differences in monthly wool growth rate in any of the groups except in August when wool growth was significantly higher ($P<0.05$) in ND ewes compared to LD ewes with LD-Br ewes intermediate. Relative to ND and LD-Br ewes, clean wool growth rate tended to be higher ($P<0.10$) in LD ewes in October and November (11.2 ± 0.7, 11.3 ± 1.2 and 13.3 ± 1.0 g/day for ND, LD-Br and LD ewes respectively).

6.4.7 *Midside wool fibre diameter*

Changes in midside patch mean fibre diameter (Figure 6.13) reflected changes in wool growth rate. There were no treatment effects in any month, although November mean fibre diameter tended to be greater in LD ewes compared to ND ewes (38.3 ± 1.3 versus 35.2 ± 0.6 µm, $P<0.10$).

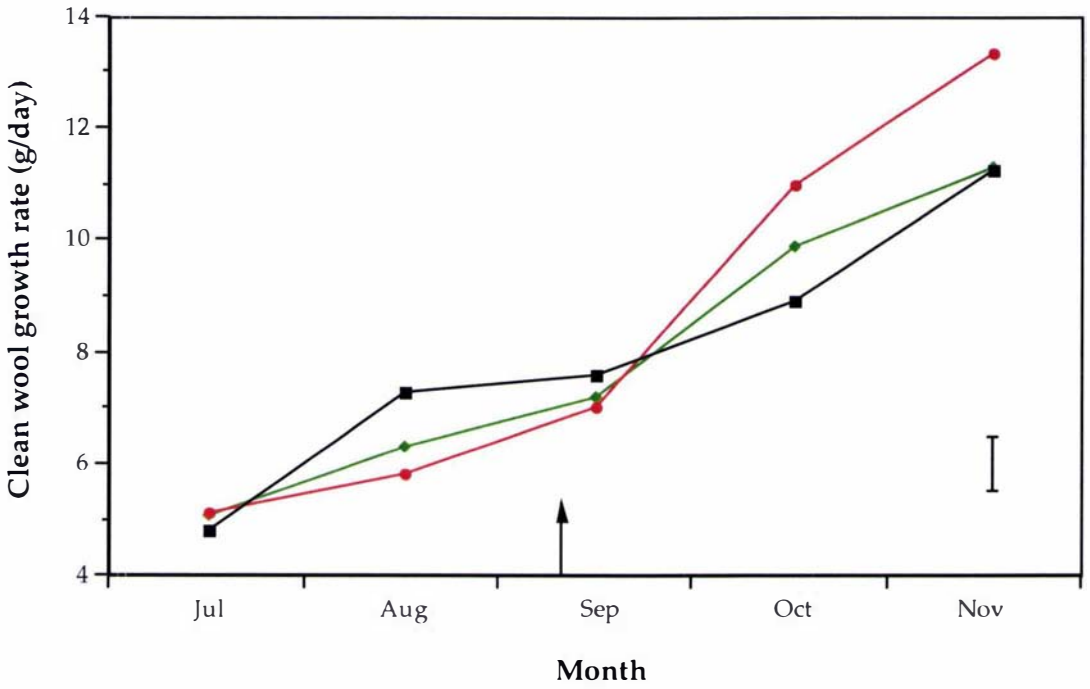

Figure 6.12: Midside clean wool growth rate of ND (■), LD (●) and LD-Br (◆) ewes. Arrow represents mean date of parturition. Error bar represents the pooled SED.

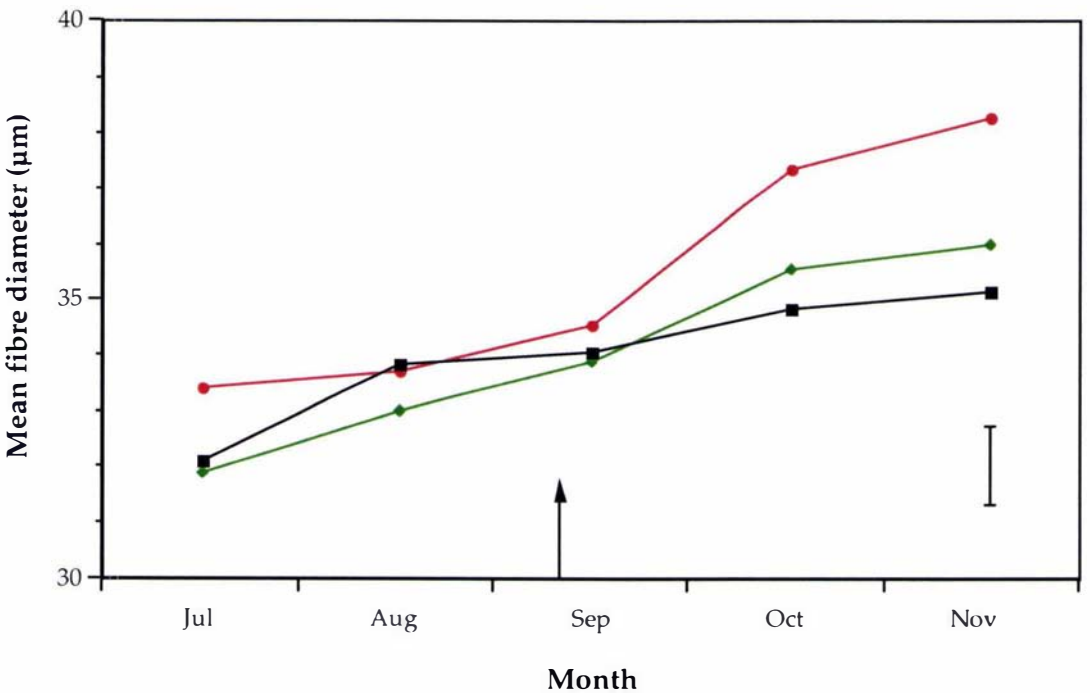

Figure 6.13: Midside mean fibre diameter of ND (■), LD (●) and LD-Br (◆) ewes. Arrow represents mean date of parturition. Error bar represents the pooled SED.

6.4.8 *Fibre length growth rate*

There was no difference in the fibre length growth rate among ND, LD or LD-Br ewes at any sampling date (Figure 6.14). Fibre length growth rate remained relatively constant from 21 to 7 days before parturition in all treatment groups, but increased ($P<0.05$) in LD-Br ewes compared to LD and ND ewes in the week immediately before parturition. There was a uniform postpartum increase ($P<0.001$) in fibre length in all groups.

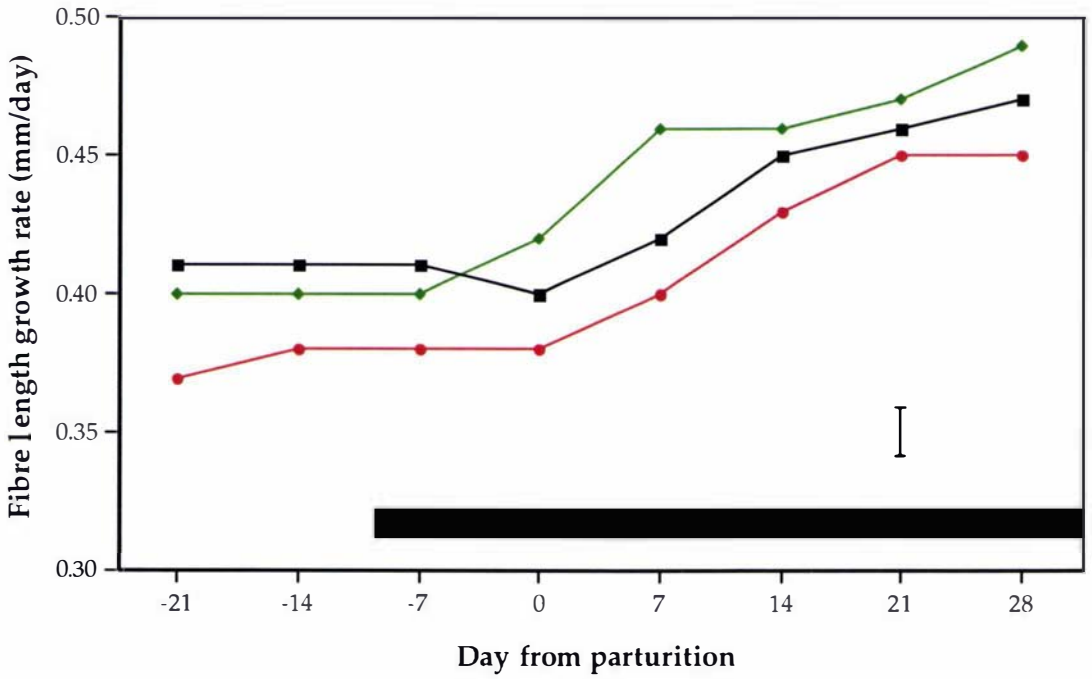

Figure 6.14: Fibre length growth rate of ND (■), LD (●) and LD-Br (◆) ewes. Solid bar represents the period of bromocriptine administration. Error bar represents the pooled SED.

6.4.9 *Wool growth and PRL profile interrelationships*

A sequential analysis *(previously described in Section 3.4.9)* was undertaken in the 3 groups to examine the potential relationships between the change in wool growth from September to November, and plasma PRL concentrations through gestation and lactation.

All groups: There was an indication that the mean PRL concentration during pregnancy was related ($P<0.10$) to the wool growth change from September to

November (Figure 6.15), but the prepartum increase in PRL concentration, the mean periparturient PRL concentration, the postpartum decline in PRL concentration and the PRL concentration during lactation (Figure 6.16) were not significant factors in the model (Table 6.3). Combining the PRL concentration over pregnancy ($P<0.05$) with the periparturient PRL concentration ($P<0.10$) improved the fit (R^2 = 41%) of the multiple regression analysis.

Table 6.3: Percentage of variance in the change in wool growth from September to November accounted by various plasma prolactin parameters in pregnant ewes.

PRL parameter	All Groups	Excluding LD-Br ewes
Mean Pregnancy PRL	34%	41%
	†	†
Prepartum increase in PRL	15%	23%
	ns	ns
Periparturient PRL	23%	28%
	ns	ns
Postpartum decline in PRL	15%	24%
	ns	ns
Lactation PRL	27%	51%
	ns	*
	41%	39%
Preg. PRL +	*	ns
Periparturient PRL	†	ns
	23%	55%
Periparturient PRL +	ns	*
Postpartum decline in PRL	ns	*
	23%	55%
Periparturient PRL +	ns	ns
Lactation PRL	ns	*

† $P<0.10$; * $P<0.05$.

Two groups excluding LD-Br group: The significance and the fit of the model using the mean PRL concentration over pregnancy strengthened in the absence of the LD-Br group (Table 6.3). The relationship between the wool

growth change and other PRL parameters improved but remained non-significant, except for the PRL concentration over lactation, which became significant ($P<0.05$, Figure 6.16). While the combination of the mean PRL concentration over pregnancy and the periparturient PRL concentration became non-significant in this multiple regression analysis, the periparturient PRL concentration ($P<0.05$) and postpartum decline in PRL concentration ($P<0.05$) gave the strongest relationship (R^2 = 55%) with the wool growth change.

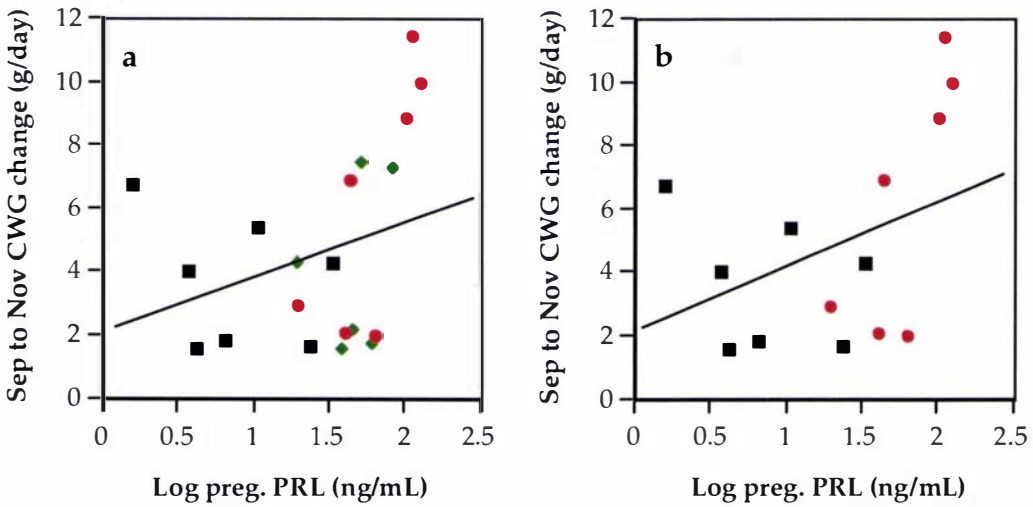

Figure 6.15: The relationship between the change in wool growth rate from September to November and the mean log prolactin concentration during pregnancy in (a) all pregnant ewes and (b) pregnant ewes excluding LD-Br ewes. Legend: ND (■), LD (●) and LD-Br (◆) ewes.

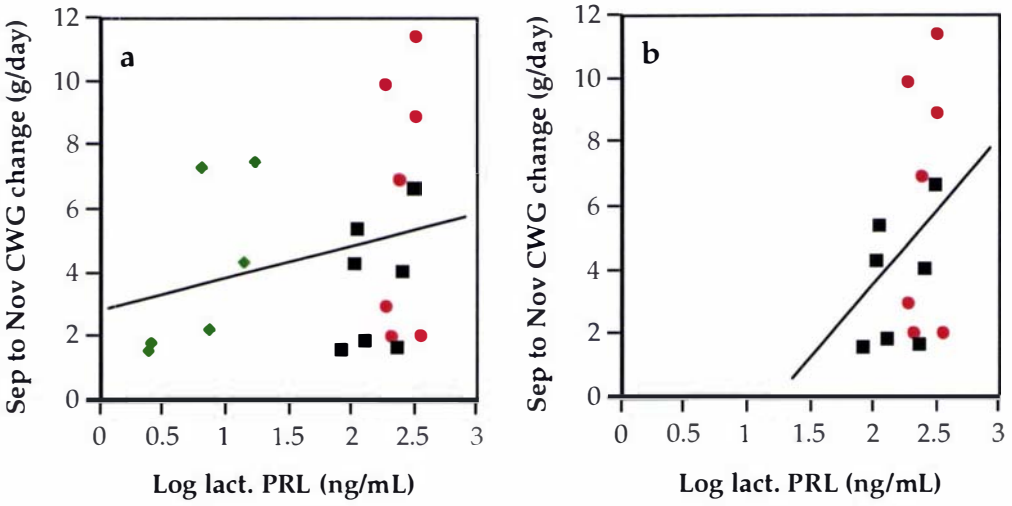

Figure 6.16: The relationship between the change in wool growth rate from September to November and the mean log prolactin concentration during lactation in (a) all pregnant ewes and (b) pregnant ewes excluding LD-Br ewes. Legend: ND (■), LD (●) and LD-Br (◆) ewes.

6.5 DISCUSSION

The aims of this experiment were: to characterise the normal 24-h plasma PRL profile of ND and LD pregnant ewes in late pregnancy, at parturition, and in early lactation; to establish whether PRL was responsible for the medium-term increase in monthly wool production observed previously in LD-lambed ewes; and to examine whether the increased wool production observed in winter-lambing ewes could be replicated in spring-lambing ewes.

Several studies in pregnant ewes have indicated that plasma PRL concentrations are elevated through late gestation when ewes are kept in artificial LD (Bocquier *et al.*, 1986; Bassett *et al.*, 1988; Ebling *et al.*, 1989; Bassett, 1992). This photoperiodic effect also exists in winter-lambing ewes *(Chapter Four)* and, in the present study, PRL concentrations were similarly higher in spring-lambing LD ewes than in ND ewes over pregnancy. PRL concentrations were higher during pregnancy (62 ± 9 versus 36 ± 12 ng/mL) and rose earlier prepartum in spring-lambing LD ewes in the present experiment compared to winter-lambing ewes exposed to LD photoperiod *(Chapter Four).* Together, these findings suggest that a strong seasonal

influence on PRL secretion persists in the pregnant ewe which modifies the effect of artificial LD and possibly the inhibitory actions of placental lactogen (Voogt *et al.*, 1982; Voogt, 1984) and progesterone (Vermouth & Deis, 1974).

Another finding of the current study was that, despite a similar daily dose, IRA bromocriptine pellets were not as effective in suppressing plasma PRL concentrations as Parlodel® LA intramuscular injections had been in previous trials. Under Parlodel treatment, PRL levels remained consistently below 2 ng/mL, but they were generally 10–15 ng/mL in the LD-Br group in this study. It is possible that the mode of delivery may account for these differences, but the small rise in plasma PRL concentration near parturition indicates that a combination of LD photoperiod, season and parturition provides a significant challenge to the central dopaminergic effects of bromocriptine.

While previous studies have measured PRL levels over a 24-h period in pregnant ewes (McMillen *et al.*, 1987; Piraux *et al.*, 1987) and non-pregnant ewes (Kennaway *et al.*, 1983), there are few comparable data on the diurnal rhythm of PRL concentrations at various stages of pregnancy and lactation in ewes maintained in different light regimes. The higher mean PRL concentrations in LD ewes relative to ND ewes during late pregnancy (day 118 of gestation) confirm that PRL concentrations are increased in LD conditions.

A peak in plasma PRL concentration in sheep has been previously associated with the time of 'lights out' or dusk in all photoperiods (Lincoln *et al.*, 1978; Wilson & Lapwood, 1978; Brinklow & Forbes, 1984; Poulton *et al.*, 1986; McMillen *et al.*, 1987) and in pinealectomised sheep (Brown & Forbes, 1980). However unlike these previous experiments, LD ewes in the present study had rising PRL concentrations during the early afternoon, some time before darkness. This was also a feature in earlier studies with pregnant ewes housed in ND photoperiod (McMillen *et al.*, 1987; Piraux *et al.*, 1987) and suggests that

a combination of pregnancy or LD may have modified the diurnal rhythm in PRL concentrations in these experimental sheep.

Melatonin secretion increases during the hours of darkness (Lincoln *et al.*, 1982; Kennaway *et al.*, 1983) and is higher in LD conditions (Lincoln *et al.*, 1982). However, it appears to have a relatively minor role in the regulation of the diurnal PRL rhythm as the daily rhythm in maternal PRL concentrations persists after pinealectomy in pregnant ewes (McMillen *et al.*, 1991).

This experiment provides the first comprehensive account of the changes in plasma PRL concentrations relative to parturition in the pregnant ewe and the influence of photoperiod on these changes. Although previous studies (Lamming *et al.*, 1974; Munro *et al.*, 1980; Peterson *et al.*, 1990) and other experiments described in this thesis have demonstrated that maternal PRL concentrations usually rise 1–3 days before parturition, all made use of single samples obtained by jugular venipuncture which are subject to stress-induced increases in PRL concentrations. PRL concentrations are not only higher over pregnancy, but increase to higher levels prior to parturition in pregnant ewes exposed to LD photoperiod (Figure 6.7). The timing of the prepartum PRL rise and the peak PRL concentration is estimated to be about 16 h earlier in LD ewes than in ND ewes. PRL concentrations then commenced a decline immediately before parturition and were similar in both ND and LD ewes in the 24-h period immediately following parturition. The stimulatory action of long daylength was again apparent 48 h after the birth of the lamb.

PRL concentrations remained higher in LD ewes compared to ND ewes over a 24-h period during early lactation in the present experiment. In lactating LD ewes, there was also a suggestion of a duirnal rhythm in circulating PRL concentration ewes as levels were elevated during the period of darkness. This is in contrast to earlier work by Piraux *et al.* (1987), who observed higher PRL concentrations during the day as a consequence of more frequent suckling by the lamb. Previous studies have reported marked increases in maternal

PRL concentrations in response to suckling (McNeilly *et al.*, 1972; Lamming *et al.*, 1974; Dingwall *et al.*, 1982), but there was no evidence in individual ewes or treatment groups to support this finding in the present study. In fact, PRL concentrations declined by up to 30% with time from their initial concentration. It is possible that 15-min intervals were too infrequent to measure any pulsatile change in PRL concentration as Lamming *et al.* (1974) measured a PRL peak within a 5-min period. Alternatively, this discrepancy may be a reflection of sampling procedures because the sampling interval in this study was shorter than the 19–21 min half-life for PRL in the circulation of sheep (Davis & Borger, 1973; Gow *et al.*, 1983).

Wool growth rate, as measured by clean wool growth, fibre diameter and fibre length, increased in all experimental groups following the birth of the lamb which supports the conclusion reported previously in this thesis. Monthly wool growth values were positively correlated to washing yield in every month except July, in agreement with other authors (Batchelar, 1985; Butler & Head, 1993) and earlier data in this thesis *(Chapter Three and Four).*

In contrast to the situation with winter-lambing ewes *(Chapter Four),* LD photoperiod and bromocriptine treatment had no effect on the 5-month fleece weight in these spring-lambing ewes. There was, however, a suggestion of an increase in other wool measurements that were not associated with increases in live weight or feed intake. Wool growth rate was higher in October and November in LD ewes relative to ND and LD-Br ewes, which was due to a corresponding increase in mean fibre diameter. A concurrent increase in fibre length was also conceivable, but the autoradiography measurements did not cover this period.

Although the magnitude of these increases was similar to that reported previously in LD ewes *(Chapter Four),* a non-significant divergence was apparent. While it is possible that the trial was terminated before any significant wool growth effect was measured, the most likely explanation is

that the wool growth response in ND ewes from the present experiment was higher than that measured in ND winter-lambing ewes *(Chapter Four)* which could be attributed to the higher circulating PRL concentration in spring. Plasma PRL concentrations were also generally higher at all stages of pregnancy in spring-lambing LD ewes compared to their winter-lambing counterparts. Nevertheless, inferences are limited by comparing different experimental sheep and trials. The periparturient PRL surge may be linked to the medium-term changes in wool production as observed in Chapter Four, but in the present experiment these relationships were not as strong, and in some cases were not significant (Table 6.3).

Another important objective of this experiment was to clarify the role PRL may play in stimulating short-term wool production. Although wool production was not significantly different, the almost complete absence of PRL at parturition and throughout lactation in LD-Br ewes was associated with levels of monthly wool growth and mean fibre diameter that resembled those of ND ewes rather than LD ewes. It should also be noted that, while the suppression of PRL was associated with lower levels of wool growth in a previous experiment, the timing of this inhibitory effect did not fall within the experimental period in the present study, preventing a direct comparison. Nevertheless, the divergence in wool growth rates in LD and LD-Br ewes lends support to a causal role of PRL in the control of wool growth in the pregnant Romney ewe.

6.6 CONCLUSIONS

This trial showed that there was a consistent positive effect of LD on plasma PRL concentrations during pregnancy and lactation. The clear diurnal rhythm in PRL concentrations in LD pregnant ewes indicates that the control mechanism for this rhythm persists in pregnancy. Maximal PRL concentrations were obtained in ND and LD photoperiod during the dark phase, while exposure to LD photoperiod altered the timing of the daytime

rise in PRL concentration. The prepartum PRL rise was shifted forward 16 h under LD conditions, although peak PRL concentrations occurred before parturition in both ND and LD ewes. Plasma PRL concentrations were higher over lactation in LD ewes than in ND ewes, although neither a diurnal rhythm, nor a positive response to suckling stimuli, was detected. Significantly, different PRL profiles over late pregnancy, at parturition and throughout lactation were associated with medium-term differences in clean wool growth rate in these experimental sheep. The higher November wool parameters in LD ewes may be indirect evidence to support the hypothesis that PRL is positively linked to stimulatory effects on wool growth.

GENERAL DISCUSSION AND CONCLUSIONS

Introduction

Significant depressions in annual wool production during winter and during pregnancy and lactation in the New Zealand Romney and similar breeds cannot be prevented by nutritional management (Hawker *et al.*, 1984; Oddy, 1985; Masters *et al.*, 1993), suggesting an endocrine signalling mechanism. Fluctuations in plasma PRL concentrations in response to photoperiod are thought to be an underlying endocrine mechanism controlling seasonal wool growth. Furthermore, rising PRL concentration has been linked to the inhibition of wool growth in the New Zealand Wiltshire sheep (Pearson *et al.*, 1996). As PRL receptors have recently been identified in the ovine wool follicle (Choy *et al.*, 1995; 1997) and PRL concentrations also increase markedly near parturition and during lactation (Peterson *et al.*, 1990; 1991), this study was focused on the role of PRL in modulating wool growth in Romney ewes.

The specific objectives were to: *(i)* determine the period of minimal wool production in spring- and winter-lambing Romney ewes fed to a constant maternal live weight; *(ii)* relate changes in growth rates to changes in fibre characteristics; *(iii)* characterise the plasma PRL profile over pregnancy, at parturition, and during lactation; and *(iv)* determine the influence of changes in circulating PRL concentration on wool growth. To achieve these objectives 4 experiments were conducted *(Chapters Three, Four, Five and Six)* involving the manipulation of PRL in pregnant and lactating ewes using different photoperiods (natural days, ND; short days, SD; and long days, LD) and bromocriptine, a dopamine agonist which suppresses pituitary PRL secretion.

Control of nutrition and live weight

A key requirement in this study was to minimise the confounding effects of feed intake in the experimental sheep. To this end, all sheep were fed to a constant maternal live weight. While daily feed intake provided a direct measure of feed consumption, the inherent ability of an individual ewe to convert food to wool could also be measured indirectly by monitoring live

weight (Turner & Young, 1969). In this respect, the feeding regimen employed in these experiments was successful in preventing significant changes in live weight allowing for the different reproductive states. The final live weight was held to within an average of ± 2% and ± 6% of the initial live weight for non-pregnant and pregnant ewes, respectively, in these experiments.

The importance of controlling nutrition was highlighted when the non-pregnant ewes were grazed separately from pregnant ewes and as a consequence were significantly heavier when the trial commenced (*Chapter Three),* and when ewes were flushed prior to mating *(Chapter Five).* Another practical problem was the adaptation of the experimental sheep from pasture to a pelleted diet *(Chapters Three and Four).* While some sheep had little problem with the transition, the majority took up to 2 weeks to fully adapt, often resulting in a temporary loss of live weight *(Chapter Four).* The feeding protocol was reviewed and an improved procedure was used in the final 2 experiments *(Chapters Five and Six).* This entailed feeding the pelleted diet to the experimental sheep while they were still grazing. In retrospect, it would have been advisable to have the sheep fully adapted to a pelleted diet before any wool measurements commenced.

Overall, the nutritional influence on live weight, and by inference on wool growth, was small in these experiments. Therefore treatment effects reported are unlikely to be attributable to differences in feed intake. Further, photoperiod treatments did not affect live weight. However, the prepartum administration of bromocriptine did cause a small increase in live weight over lactation (*Chapter Four).* This was presumably due to a reduction in lactational output, as maternal live weight increased at the expense of lamb growth rate.

Plasma PRL concentrations in non-pregnant ewes

Plasma PRL concentrations were successfully manipulated using photoperiod and treatment with bromocriptine. In non-pregnant ewes, PRL concentrations were closely aligned to natural daylength *(Chapters Three, Four and Five)* with a

consistent rise in late August or early September following the low levels over winter. The seasonal pattern in PRL concentration could be modified using artificial SD *(Chapter Three)* or LD *(Chapter Four)* photoperiod. It appears that photoperiod is the primary determinant of PRL concentration in the non-pregnant ewe. Thus levels remained low under constant SD photoperiod against a background of rising ambient temperatures *(Chapter Three),* and exhibited an immediate increase when ewes were exposed to LD photoperiod while the temperature was declining *(Chapter Four).* The PRL profile in non-pregnant ewes held under constant LD photoperiod *(Chapter Four)* also deviated from the annual temperature rhythm. However, the ambient temperatures experienced by SD and LD ewes held indoors were not entirely representative of winter and summer respectively, and this may have had some influence on PRL secretion (Schillo *et al.,* 1978). The use of temperature-controlled rooms to simulate winter and summer temperatures would be advantageous in any future indoor trials in order to improve control of PRL secretion.

Plasma PRL concentrations in breeding ewes

The higher PRL concentrations in non-pregnant ewes compared to pregnant ewes over the autumn *(Chapter Four)* and winter *(Chapter Three)* suggest that pregnancy inhibits endogenous PRL secretion and prevents the seasonal increase in circulating levels. These differences in PRL concentration were first measured from about day 50 of gestation *(Chapter Five)* and coincided with the time when placental secretion of progesterone and placental lactogen are reported to increase *(Section 1.3).* Photoperiod also played a significant role in control of PRL concentrations in the pregnant ewe as levels were consistently higher in LD ewes compared to ND ewes *(Chapters Four and Six).* During the winter the difference between ND and SD pregnant ewes was not significant *(Chapter Three).* Furthermore, the time of the year appeared to influence the differences in PRL concentrations, as levels were higher during pregnancy in LD September-lambing ewes *(Chapter Six)* than in LD June-lambing ewes *(Chapter Four).* Additionally, a diurnal rhythm of PRL persists in pregnant

ewes in both ND and LD, although the rhythm was more pronounced in LD ewes *(Chapter Six).*

Another feature of artificial LD photoperiod was that the prepartum increase in plasma PRL concentrations preceded the corresponding rise in pregnant ewes in ND and periparturient concentrations were generally higher *(Chapters Four and Six).* Intensive sampling around parturition revealed that the peak PRL concentration was approximately 16 h earlier in LD ewes than that measured in ND ewes, and that PRL concentrations declined prior to parturition irrespective of photoperiod *(Chapter Six).* The photoperiodic effect on PRL concentrations continued throughout lactation as levels were elevated in LD ewes compared to ND ewes *(Chapters Four and Six)* and lower in SD ewes relative to ND ewes *(Chapter Three).*

Bromocriptine has proven to be a valuable tool in the manipulation of maternal PRL secretion. Plasma PRL concentrations were reduced to very low levels, and the PRL surges associated with parturition and lactation. Also, the spring PRL rise in ewes held in natural *(Chapter Four)* and long *(Chapter Six)* daylengths were abolished by bromocriptine. However, there were minor problems associated with the prepartum administration of this drug to pregnant ewes. In particular, lactogenesis was delayed by 1–2 days *(Chapters Three and Four)* and there was sometimes a temporary loss of appetite *(Chapter Four)* following bromocriptine administration.

This study also offered a useful comparison of the mode of delivery of bromocriptine to the pregnant ewe, as a result of the unavailability of Parlodel® LA in a later experiment. These experiments indicate that intramuscular Parlodel injections *(Chapters Three and Four)* were more effective in suppressing PRL concentrations than subcutaneous IRA implants *(Chapter Six),* despite very similar dose levels. The mean plasma PRL concentration in sheep treated with bromocriptine was less than 2 ng/mL and 10–15 ng/mL using injections and implants respectively. It is possible that the greater

stimulatory effect of extended photoperiod in Chapter Six may be one reason for this difference. The inclusion of a LD bromocriptine-treated group in Chapter Four as a negative control would have given a definitive answer in this regard.

Wool growth in non-pregnant ewes

The seasonal cycle of clean wool growth in non-pregnant ewes was modified by photoperiod-induced changes in PRL concentrations *(Chapters Three and Four).* Winter PRL concentrations were positively associated with higher wool growth rates over the spring months in SD ewes *(Chapter Three)* and prevented the winter wool growth decline in LD ewes *(Chapter Four)* compared to their ND controls. This change in wool growth rate in response to increased plasma PRL concentration was not immediate. Hutchinson (1965) also reported a wool growth lag of 2–3 months following changes in photoperiod, suggesting that this effect may be mediated by PRL.

The higher clean wool production was predominantly due to an increase in fibre length growth combined with a moderate increase in fibre diameter. This increase in clean wool growth was also negatively correlated with the change in plasma PRL concentrations from winter to spring. This relationship suggests that the magnitude of the increase in PRL concentration from the nadir of the cycle may also be important in the regulation of spring wool growth in the Romney, as has been reported in other breeds (Lincoln, 1990).

The period of minimal wool growth in breeding ewes

A significant reduction in wool growth rate was measured within the first 60 days of pregnancy, which was not associated with feed intake or live weight. The gestational wool growth depression does not involve PRL, as there was no change in circulating PRL concentration at that time *(Chapter Five).* However, the wool growth depression could be due to a direct effect of other hormones. Alternatively, the responsiveness of the wool follicle to circulating levels of

nutrients or the control of partitioning of nutrients may be altered via an endocrine mechanism.

Of the likely candidate hormones, only GH concentrations are increased due to long daylength in pregnant and lactating ewes (Bocquier *et al.*, 1986; Perier *et al.*, 1986). GH does have a short-term depressant effect on wool growth (Wynn *et al.*, 1988), but it is doubtful that the circulating concentrations are sufficiently high during late pregnancy (Figure 1.3) to produce this inhibitory effect, which usually occurs much earlier in pregnancy. Cortisol can inhibit wool growth under some circumstances (Chapman & Bassett, 1970), although the concentration is relatively low during early pregnancy (Figure 1.3) making it an unlikely candidate. However, ACTH concentrations in the circulation have been shown to decrease when sheep are exposed to long daylength (Lincoln & Richardson, 1998), raising the possibility that the decline in maternal cortisol concentrations after the birth of the lamb could remove an inhibitory block on wool growth in LD ewes.

It remains possible that inhibition of wool growth could be induced by a combination of these or other reproductive hormones including placental lactogen and progesterone, which increase in concentration from day 60 of gestation (Figure 1.3). Progesterone has no known effect on wool growth (Slen & Connell, 1958), but placental lactogen has been linked to follicle development in newborn lambs (Wickham *et al.*, 1992). Apart from progesterone and placental lactogen, it is doubtful that the other hormones reviewed are directly associated with the depression in wool growth during pregnancy. Firstly, T_4 concentrations change little during pregnancy (Figure 1.3) and T_4 is generally thought to play a facilitatory role in wool growth (Maddocks *et al.*, 1985). Secondly, there is no change in plasma insulin or IGF-I concentrations (Figure 1.3) at the time when the reduction in wool growth rate was first measured, and neither hormone has any known effect on wool production (Hocking Edwards *et al.*, 1995). Although, oestrogens are barely detectable in the maternal circulation until the later stages of pregnancy

(Figure 1.3), they cannot be completely excluded as a candidate hormone as they have been reported to have an inhibitory action on fibre growth (Slen & Connell, 1958; Yu *et al.*, 1998).

It is likely that any endocrine inhibition of wool growth ceases following the birth of the lamb, accounting for the increase in wool production during lactation. Consequently, the magnitude of the decline in wool production during winter is diminished when the ewe is in a lactating state. In comparison to spring-lambing ewes *(Chapters Three and Six),* clean wool growth rate and mean fibre diameter were 58% and 20% higher respectively in winter-lambing ewes *(Chapter Four)* from July to September. These findings concur with those by Morris *et al.* (1994) in grazing ewes and demonstrate that this effect is not solely a consequence of the seasonal nature of pasture growth.

The influence of the prepartum rise in PRL concentration on wool growth

The lack of an inhibitory wool growth response at parturition or in early lactation suggests that the lambing break in the fleece of breeding ewes cannot be attributable to the PRL surge at parturition. In these studies, clean wool growth and fibre diameter increased in all pregnant groups at or before parturition *(Chapters Three, Four, Five and Six)* while the use of autoradiography confirmed that there was also a uniform prepartum increase in fibre length *(Chapters Four and Six).* Increases in PRL secretion associated with parturition had no immediate effect on wool growth in the Romney as opposed to the inhibitory response seen in non-pregnant Wiltshire sheep (Pearson *et al.*, 1996). This suggests that there may have been a loss of sensitivity to PRL as a consequence of selection for increased fleece weight in modern breeds. Alternatively, other endocrine changes associated with parturition may alter follicle responses to PRL.

Nevertheless, this study has identified that prepartum changes in PRL concentration are associated with medium- to long-term increases in wool growth. In general, these data suggest that elevated PRL concentrations

during late pregnancy *(Chapters Four and Six)* in conjunction with higher concentrations at parturition *(Chapters Three, Four and Six)* are linked to increases in wool growth rates over the next 3 months (Table 7.1). Within experiments, higher concentrations of prepartum and periparturient PRL were associated with the largest wool growth response. In contrast to the situation with non-pregnant ewes, changes in fibre diameter contributed more to the increased medium-term wool production than changes in fibre length *(Chapters Three, Four and Six).* The peak PRL concentration, rather than the magnitude of the increase from prepartum levels, appears to be more significantly associated with wool growth. PRL concentrations over lactation were also implicated in changes in wool growth. When plasma PRL concentrations were high in the lactating ewe, medium-term wool growth was increased *(Chapters Three, Four and Six).* This stimulatory effect appeared to be enhanced if the postpartum decline in PRL concentration was gradual as opposed to rapid.

The role of plasma PRL concentration in mediating wool production was also assessed using long-acting bromocriptine to suppress PRL levels under short *(Chapter Three),* natural *(Chapter Four)* and long *(Chapter Six)* daylength. In all experiments, the almost complete absence of endogenous pituitary PRL was associated with lower rates of long-term wool growth compared to their controls (Table 7.1). It is likely that the reduction in wool growth was due mainly to a decrease in fibre length growth rate rather than fibre diameter *(Chapter Four).* This could be confirmed by an extended period of radiolabelling of individual wool fibres. The suppression of plasma PRL concentration postpartum was also associated with reduced levels of long-term wool growth *(Chapters Three and Four).* More importantly, it indicated that the rise in PRL concentration at parturition does provide some stimulatory signal to the wool follicle as wool growth rates were higher in these groups compared to ewes whose PRL was suppressed prior to parturition *(Chapters Three, Four and Six).* Additionally, these data may also suggest that PRL is

likely to be partially responsible for the decline in wool growth following hypophysectomy (Ferguson *et al.*, 1965).

Table 7.1: Summary of prolactin and wool growth responses in pregnant ewes in all experiments.

Treatment	Experiment	Pregnancy PRL concentration[1]	Peripartum PRL concentration[2]	% CWG response[3]
SD-BrB	*Chapter Three*	low	low	58%
SD-BrA	"	low	medium	28%
SD	"	low	medium	76%
ND	"	medium	high	92%
ND-BrB	*Chapter Four*	low	low	–6%
ND-BrA	"	medium	medium	–2%
ND	"	medium	high	2%
LD	"	high	very high	83%
ND	*Chapter Six*	medium	high	53%
LD-Br	"	high	low	57%
LD	"	very high	very high	112%

[1]Pregnancy PRL: low= <10 ng/mL; medium= 10–30 ng/mL; high= 30–50 ng/mL; very high = > 50 ng/mL.
[2]Peripartum PRL: low = < 10 ng/mL; medium = 200–250 ng/mL; high= 250–300 ng/mL; very high= >300 ng/mL.
[3]Defined as the change in clean wool growth from parturition to weaning (3 months).

A later experiment provided indirect evidence that PRL may have a causal role in stimulating medium-term wool growth *(Chapter Six).* Prepartum treatment with bromocriptine to pregnant ewes in extended daylength modified spring wool production so that the pattern of wool growth resembled that of ND ewes rather than LD ewes (Figure 6.12). This suggests that the higher PRL concentrations over pregnancy provided a sufficient signal to elicit a wool response in contrast to ND bromocriptine-treated ewes, in which prepartum PRL levels were low (Table 7.1). Although the concentrations of other maternal hormones were not measured, there is no consistent evidence in the literature to suggest that bromocriptine has any effect on other maternal hormones in the pregnant ewe (Kann, 1976b; Martal & Lacroix, 1978; Gow *et*

al., 1983; Peterson *et al.*, 1997), with perhaps the exception of insulin (Johnsson *et al.*, 1985). Collectively, these findings suggest that elevated plasma PRL concentrations around parturition and throughout lactation are linked to stimulation of postpartum wool growth. In the absence of PRL, wool growth rate is depressed.

Future Research

It is important to identify which hormone, or combination of hormones, are causing the inhibition of wool growth observed during the early stages of gestation. Perhaps the first step in this process would be to ascertain the precise temporal changes of progesterone and placental lactogen relative to wool growth changes during pregnancy, using existing samples or in new trials under identical indoor experimental conditions. Alternatively, specific antagonists could be used in future trials to suppress the hormones in question. Examining the interaction of PRL with these hormones would provide a better understanding of the complexities of hormonally-induced control of the wool follicle in the pregnant ewe.

This research project has also provided a foundation for ongoing work on the role of plasma PRL concentration in regulating wool growth using a sheep model central to the New Zealand wool industry. The results from this study imply that photoperiod-induced increases in PRL have a significant impact on fleece production. In order to advance this field of knowledge, studies should be conducted to simulate the PRL profile of LD breeding ewes in ND breeding ewes. This could be achieved by the administration of exogenous PRL to winter-lambing ewes at parturition or exposure of spring-lambing ewes to an extended photoperiod prior to parturition. Furthermore, a holistic view of the PRL axis is required which also incorporates measurement of the seasonal and physiological changes in PRL receptor signal transduction. Understanding the timing of critical periods of wool follicle sensitivity to PRL may suggest an on-

farm treatment to mimic the effects of PRL, with direct benefits to the farmer and the wool industry by improving production efficiency and fibre quality.

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