THE ROLE OF PLASMA PROLACTIN CONCENTRATION IN SEASONAL FIBRE GROWTH CYCLES IN DOWN-PRODUCING GOATS AND WILTSHIRE SHEEP

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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The effort involved in this thesis is dedicated to my children Keshia and Ryan Melton who tolerated their working mother and fed the animals and never asked why.

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AND DOWN-PRODUCING GOATS

ERRATA

Insert as page 121b:

The radioimmunoassay of PRL was conducted using ovine PRL (NIDDK-oPRL-I-2) for standards and radioiodination, and ovine PRL antiserum (NIDDK-anti-oPRL-2). PRL was iodinated by the Iodogen technique (Pierce, Rockford, IL) using [125I]-iodide (New England Nuclear NE0033A). Separation of antibody-bound from free labelled PRL was by second antibody precipitation using excess goat antirabbit serum (SAR 265 generated at Ruakura Agricultural Centre). The assay was validated for caprine samples. Sensitivity was 0.6 ng/ml and assay range was up to 100 ng/ml. Intra-assay and inter-assay coefficients of variation at 32 ng/ml PRL concentration were 12.1% and 14.4% respectively.

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I wish to acknowledge the assistance of a number of people who have contributed in varying ways to the completion of these experiments.

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ABSTRACT

This study examined the role of plasma PRL concentration in regulating seasonal fibre growth following the transition from short to long day photoperiod. In three down goat genotypes higher proportions of Angora genes extended the duration of guard hair growth, decreased biannual down growth and reduced the period of secondary follicles inactivity. The timing of follicle reactivation in spring and seasonal changes in plasma PRL concentrations were similar in all genotypes. Plasma PRL concentration increase, in spring, was associated with primary, but not secondary, follicle reactivation. Secondary follicle reactivation produced down of less than 2 mm which was associated with the shedding of winter down. Plasma PRL concentrations were suppressed, in spring and long-photoperiods (16L:8D), by injections of 1-5 mg/goat/day of bromocryptine and 2-3 weekly injections of long-acting bromocryptine (Parlodel). Injections of 1-5 mg/goat/day of domperidone elevated plasma PRL concentrations for 12 hours by and shedding was advanced. The circulating half-life of PRL, in sheep and goats, was 42 ± 6 and 104 ± 14 minutes following PRL injection or constant infusion respectively. In down goats, the normal spring-rise in plasma PRL concentration was suppressed using Parlodel or advanced by long day photoperiod. Increased plasma PRL concentration in spring provided anagenic signals to telogen primary and secondary follicles and catagenic signals to anagen secondary follicles. Following a reversal from short to long photoperiod anagen follicles of both goats and sheep entered telogen. Shedding occurred when the follicles subsequently reactivated. The suppression of plasma PRL concentration using Parlodel, during long photoperiod reversal, prevented the catagenic effect of long-photoperiod on anagen Wiltshire sheep follicles. In goats, however bromocryptine did not prevent follicles entering catagen but delayed follicle reactivation. The intravenous infusion of PRL had no effect on fibre growth in down goats or Wiltshire sheep. While the direct infusion of PRL to the skin caused an extreme local tissue reaction. Plasma PRL concentration has a role in regulating seasonal fibre growth cycles in down-producing goats but it is not a simple causal relationship and is dependent on follicle growth stage.

SUMMARY

Chapter 1: The literature on fibre growth cycles in down and Angora goats and shedding sheep breeds was reviewed. Literature was presented on the regulation of plasma PRL concentration with special emphasis on seasonal factors regulating pituitary PRL secretion. The effects of plasma PRL concentration on fibre were also reviewed.

Chapter 2: Mixed-aged breeding does were categorised as having either no known Angora ancestry (F), a maximum of 25% Angora ancestry (C), or 50% Angora ancestry (G). The three genotypes were tested for differences in the quantity and timing of growth from primary and secondary follicles and seasonal plasma PRL concentrations. The sequence of primary follicle growth was similar in all three goat genotypes but the duration of growth of the guard hair fleece was 50-70 days longer in G compared to F goats. The timing of down growth was similar in all three goat genotypes. However, SA during July and August of 1991 and 1992 was more than 25% lower ($P<0.05$) in F goats compared to C or G goats. Summer down growth was identified in 78% of G, 97% of C and 100% of F goats. Plasma PRL concentrations were similar in all genotypes. It was concluded that, the inclusion of additional genes of the less seasonal Angora, extended the duration of growth from primary follicles and decreased biannual down growth and the expression of catagen in secondary follicles.

Chapter 3: In this experiment, the timing and correlation of fibre growth events and plasma PRL concentration events were determined for individual down-producing goats from July to February in natural photoperiod. PA began to increase on 17 September$\pm$7 days and primary follicles reached full activity on 1 January$\pm$10 days. Similarly, the mean date of secondary follicle activation was 17 September$\pm$11 days and rose to a summer peak of 38$\pm$7% on 31 October$\pm$9 days.

The increase in SA which eventually produced the winter down fleece commenced on 5 November$\pm$9 days. The date of primary follicle reactivation in spring was associated with the date at which plasma PRL concentration increased above 20 ng/ml ($r=0.78$, $P<0.01$). Associations between secondary follicle reactivation and plasma PRL concentration could not be found. In conclusion, it was found that the timing of maximum summer down SA was closely associated with maximum down fleece shedding and not associated with plasma PRL concentration.

Chapter 4: The effects on plasma PRL concentrations in down-producing goats of summer treatment with bromocryptine mesylate (BR) and spring and summer treatment with domperidone (DOM) were examined in two experiments. In experiment 1 (December), daily injections of BR (1-5 mg/goat/day) reduced mean plasma PRL concentration to less than 12 ng/ml compared with control concentrations of 87$\pm$7 ng/ml ($P<0.001$). A single BR injection suppressed plasma PRL concentration for 20 hours ($P<0.05$). DOM injections (1-5 mg/goat/day) increased mean plasma PRL concentrations to between 278 and 548 ng/ml for 12 hours. In experiment 2 (September), plasma PRL concentrations following DOM administered at 2.5 mg/day for 14 days either by subcutaneous injection (DOMinj), or by a subcutaneously fitted osmotic minipump (DOMosp) were 612$\pm$32, 73$\pm$35 and 60$\pm$34 ng/ml in DOMinj, DOMosp and control respectively ($P<0.001$). Mean plasma PRL concentrations immediately prior to the injection were lower ($P<0.01$) in DOMinj (33$\pm$6 ng/ml) goats compared with DOMosp (72$\pm$7 ng/ml) and control (60$\pm$8 ng/ml) groups. Shedding was advanced in DOMinj goats compared with control goats. It was concluded that plasma PRL concentration in goats can be successfully manipulated using single injections of bromocryptine and domperidone, but the effect is transitory.

Chapter 5: The circulating half-life of PRL was determined in sheep and goats, and methods for manipulating plasma PRL concentration, by PRL infusion, long-acting bromocryptine, and long-photoperiod were determined for sheep.

In sheep and goats the T½ of PRL was 42±6 or 105±14 minutes following PRL injection or infusion respectively. In sheep, T½ was longer ($P<0.01$) when PRL was infused at 0.1 (P1; 144±15 minutes) compared to 0.4 (P2; 55±13 minutes) mg oPRL/kgLW/day. In PRL-infused sheep, mean treatment plasma PRL concentration was higher in P2 (99±17 ng/ml), relative to P1 (60±17 ng/ml) and C (59±17 ng/ml) sheep ($P<0.05$). Plasma PRL concentration was suppressed to below 15 ng/ml in sheep for 21 days after the long-acting bromocryptine injection. Long photoperiod treatment increased mean plasma PRL concentration to LD sheep (170±33 ng/ml). In conclusion, plasma PRL concentration could be manipulated in sheep by PRL infusion, long photoperiod and long-acting bromocryptine treatment.

Chapter 6: The effect on fibre growth of down goats of either delaying or advancing the spring rise in plasma PRL concentration increase was studied from late July to middle of October. Goats were: maintained in natural spring photoperiod and received no further treatment (n=10, C); were injected with long-acting bromocryptine (n=10, BR); or were treated with long photoperiod (16L: 8D), with no further treatment (n=5, LD), or were injected with long-acting bromocryptine (n=5, BRLD). Mean overall plasma PRL concentration, in comparison to C goats (27 (23-31) ng/ml), was higher in LD goats (87 (69-109) ng/ml, $P<0.001$) and lower in both BR (4.2 (3.5-5.0) ng/ml, $P<0.001$) and BRLD (9 (7-12) ng/ml, $P<0.01$) goats. The mean date when all primary follicles became active in C goats was 26 December±8 days, which was similar to that of BR (16 January±8 days) goats but later than the 1 November±11 ($P<0.001$) and 14 November±11 days ($P<0.02$) when maximum activity was achieved in LD and BRLD goats. Mean SA increase to be 77±7% at the termination of treatment in BR goats (C 23±8%, $P<0.01$) while in LD goats SA increased to 42±9% after 41 days of treatment and then fell to be 19±10% at the end of the treatment period. Mean SA increased throughout the treatment period to reach 59±11% and 77±7% in BRLD and BR goats respectively at the end of the treatment period. At the termination of the bromocryptine treatment, SA decreased.

In BR goats, the early activation of secondary follicles was not associated with early summer down fleece emergence. The conclusion was that increased plasma PRL concentration provided anagenic signals to telogen secondary follicles and catagenic signals to anagen secondary follicles. The primary follicles, plasma PRL increase had an anagenic effect on telogen follicles.

Chapter 7: Following a reversal from short to long photoperiod the effects of suppressing the increase in plasma PRL concentration, using long-acting bromocryptine treatment, was studied from both down goats and Wiltshire sheep. Following 6 months pre-treatment short photoperiod down-producing does continued under short-photoperiod (SD goats, $n=6$), while Wiltshire sheep and goats were treated with long photoperiod (16L:8D) with either no further treatment (LD goats $n=6$, LD sheep $n=6$), or treatment with long-acting bromocryptine (BRLD goats, BRLD sheep) at two weekly intervals from 7 January until 31 March 1993 and then released onto pasture under natural photoperiod. Mean plasma PRL concentration during the treatment period was 6.0 (5.3-6.8 ng/ml), 8.5 (7.6-9.5) ng/ml and 97 (89-109) ng/ml in SD, BRLD and LD goats respectively ($P<0.001$) and 0.67 (0.74-0.87) ng/ml and 135 (123-148) ng/ml in BRLD and LD sheep respectively ($P<0.001$). In sheep compared to goats, mean plasma PRL concentration was 8 ng/ml lower during bromocryptine treatment ($P<0.0001$) and 38 ng/ml higher during long photoperiod treatment ($P<0.05$). During the treatment period mean PA and SA of SD goats remained high (PA 49-73%, SA >90%). However in LD sheep and goats, after the reversal from short to long photoperiod SA and PA fell to below 40% on 3 March 1993 before returning to be in excess of 73% on 31 March 1993. In BRLD sheep, mean PA and SA remained above 86% throughout the treatment period. In BRLD goats, mean PA and SA decline to be less than 40% at the end of the treatment period. In comparison to LD goats, follicle reactivation was delayed in BRLD goats. It was concluded was there was evidence that plasma PRL concentration mediated the catagenic effect of long photoperiod on anagen Wiltshire sheep follicles but not in anagen down-producing goat follicles.

In down goats, plasma PRL concentration may have a role in mediating the anagenic effect of long-photoperiod on telogen goat follicles.

Chapter 8: Following pre-treatment with short-photoperiod, the effects on follicle and fibre growth of elevating plasma PRL concentration by either whole body or local skin infusions were determined. From 2 February 1994 to 3 March 1994, goats were treated with short photoperiod and either received no further treatment (n=5, SD) or an infusion of PRL (0.5 mg/kg$^{0.75}$/day) via the jugular vein (n=5, P) or were treated with long photoperiod (n=5, LD). Mean plasma PRL concentration during the treatment period in SD (10 (8-13) ng/ml) goats was lower than in either LD (52 (42-72) ng/ml, $P<0.01$) or P (29 (23-36) ng/ml, $P<0.05$) goats. Mean PA reached minimal levels (21-30%) on 20 March in LD goats but not until 8 April in P and SD goats, with PA returning to levels in excess of 94% by 6 May in all treatments. The mean date of minimum SA in LD goats was 23 March$\pm$4 days which was earlier than in either P (10 April$\pm$4 days, $P<0.0.1$) or LD (6 April$\pm$4 days, $P<0.05$) goats. By 6 May 1994, mean SA in all three treatment groups was in excess of 99%. The timing in fibre growth (DL, GL, FGR, SS, NEDF) was 2 to 6 weeks earlier in LD goats compared to P and SD goats which were similar. When PRL was infused, via the descending iliac artery, directly to the skin, mean plasma PRL concentration was 9 (7-11) ng/ml, 110 (83-145) ng/ml and 43 (35-57) ng/ml in the venous jugular, PRL- and saline-infused iliac catheters respectively ($P<0.01$). The mean blood flow through the skin patch was 12$\pm$4 ml/min and 61 ml/min in goats and sheep respectively ($P<0.05$). There was no effect of PRL-infusion on either PA or SA. The direct infusion to the skin of PRL (from two different sources) resulted in the visible swelling of the proximal hind-leg 6.5$\pm$0.8 days after the start of the infusion. This effect was independent of species, PRL infusates established infusate sterility. In conclusion, follicle and fibre growth were unaffected by either systemic or local infusions of PRL in down-producing goats and Wiltshire sheep. However, the effects on the follicle of the infusion of PRL directly to the skin were confounded by the extreme local tissue reaction.

Chapter 9: The conclusions from the research programme are presented and future directions discussed.

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ABBREVIATIONS

DGR	Down growth rate
DL	Down length
DL_d	Mean down length decreased by more than 10 mm
DL_{min}	Mean down length first reached zero
DL_i	Mean down length began to increase.
DMFD	Down mean fibre diameter
$DUR_{max-min}$	Number of days between the date of maximum growth and date of minimum growth.
$DUR_{min-max}$	Number of days between the date of minimum growth and date of maximum growth.
FGR	Fibre growth rate
GHGR	Guard hair growth rate
GL	Guard hair length
GMFD	Guard hair mean fibre diameter
GL_{min}	Mean guard hair length reached a minimum.
NEDF	Newly emerged down fibres
$NEDF_e$	NEDF first appeared above the skin;NEDF score 1 or 2.
$NEDF_d$	NEDFs disappeared; NEDF returned to 6
NEWF	Newly emerged wool fibres
PA	Primary follicle activity
PAc+brush	Primary follicle containing both actively growing and inactive fibre
$PAct_f$	The last instance of primary follicles with both active and brush fibres
PA_{max}	Primary follicle activity reached 100%
PRL	Prolactin
PRL100	Plasma prolactin concentration reached 100 ng/ml
PRL_p	Plasma prolactin concentration reached peak concentrations
SA	Secondary follicle activity
SA_s	Peak in summer secondary follicle activity
SAc+brush	Secondary follicle containing both actively growing and inactive fibre
S225	Subgroup containing 225 does
S90	Subgroup containing 90 does
S30	Subgroup containing 30 does
S15	Subgroup containing 15 does
$SAct_w$	Second peak in proportion of secondary follicles with both active and brush fibres
SA_{max}	Secondary follicle activity reached 100%
SA_w	Secondary follicle activity increased indicating start of winter down growth
SS	Shedding score (1-5; 1=no shedding)
SS_i)	Shedding score first increased to 2 or greater
SS_{max}	Shedding score reached maximum levels
SS_f	Shedding score returned to 1.

CHAPTER 1
OVERVIEW AND REVIEW OF LITERATURE

1.1 OVERVIEW

The seasonality of follicle growth affects both fibre quality and quantity in sheep and goats. Seasonally derived variation in the rate of wool growth and hence wool strength is a major limitation to the development of consistent wool product of high staple strength. The down goat industry seeks to increase down production per head and harvest fibre at a more climatically favourable time. An understanding of the biological processes underlying the seasonality of fibre growth in sheep and goats will provide information which may be used in the future to manipulate seasonal fibre growth patterns.

Plasma prolactin (PRL) has a causal role in regulating the moulting and growth of pelage in mustalids (Martinet *et al.*, 1984) and there is increasing evidence of a regulating role for plasma PRL concentration on fibre growth in both sheep (Lincoln, 1990) and down-producing goats (Dicks, 1994). The studies of seasonal follicle cycles, described in this thesis, are focused predominantly on down-producing goats and to a lesser extent Wiltshire sheep. In both species the role of plasma PRL concentration in regulating seasonal fibre growth cycles was studied with particular emphasis on the fibre growth events which occur naturally during the progression from the winter to summer solstice.

The first study described the natural plasma PRL concentration and fibre growth cycles of groups of down-producing goats with differing seasonal fibre growth patterns. Secondly, circumstantial evidence was obtained which indicated that relationships exist between seasonal fibre events and plasma PRL concentration in down-producing goats. Methods were then examined for the manipulation of plasma PRL concentration in both goats and sheep. Finally, in Wiltshire sheep and down-producing goats, fibre and follicle growth was reported for a series of experiments where plasma PRL concentration was manipulated using photoperiod, bromocryptine treatment and systemic and local skin PRL infusions.

1.2 FIBRE GROWTH CYCLES IN GOATS AND SHEEP

1.2.1 Introduction

Fibres comprising the fleeces of sheep and goats grow from follicles which are tubular structures originating as downgrowths of the epidermis (Ryder and Stephenson, 1968). Structurally the follicle comprises a inner and outer-root sheath which is continuous with the basal dermal papilla, surrounding the outer and inner root sheaths which, in turn, surround the fibre (Figure 1.1).

Hair follicles can be classified into two distinct types, primary or secondary, based on the presence or absence of accessory structures. The accessory structures associated with a primary follicle include a sweat gland, an arrector pili muscle and often a bi-lobed sebaceous gland (Carter, 1955), while secondary follicles may have only mono-lobed sebaceous glands and neither a sweat gland nor an arrector pili muscle.

Though there are breed and species variations in this pattern (Schinckel, 1955; Lyne, 1961; Lambert *et al.*, 1984) typically the follicles form prenatally in clusters of three primary follicles with variable numbers of secondary follicles arranged between the primaries. Primary follicles are produced 60-70 days post conception in sheep and goats, and primary follicle maturation, (defined as the emergence of the newly growing fibre from the skin) is completed by 90 days of gestational age. Most secondary follicles are initiated by birth with many undergoing maturation postnatally (Ryder and Stephenson, 1968).

In the double-coated down-producing goat, the coarse outercoat is produced by the primary follicles and the inner down coat by the secondary follicles. However in single-coated Angora goats and modern sheep breeds the fibre type definition is not so equivocal.

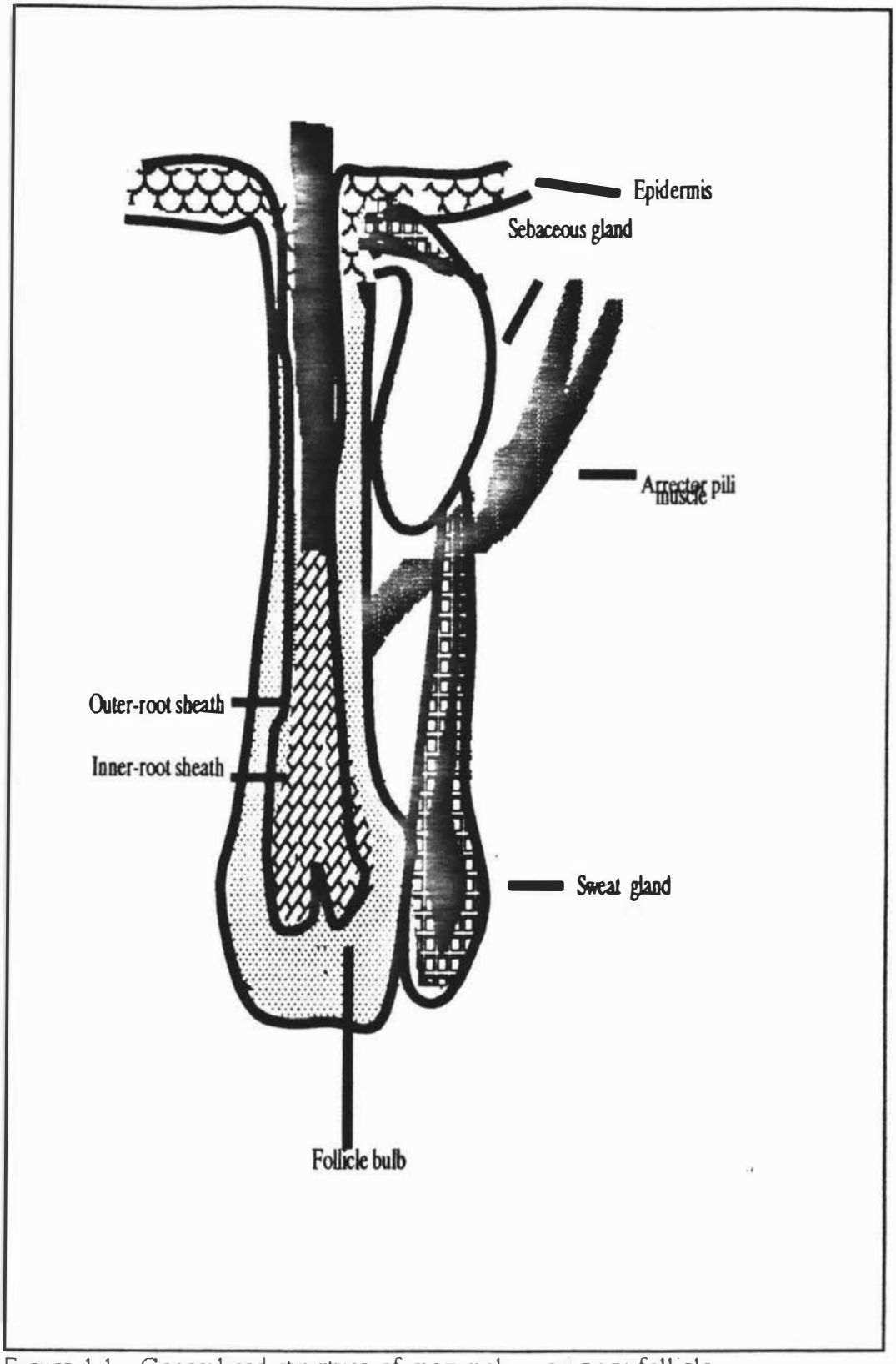

Figure 1.1: Generalised structure of mammalian primary follicle

In these animals the primary follicles may produce kemp, hair or medullated fibres or even true wool or mohair fibres. The secondary fibres produce wool, mohair or down fibres but can also produce medullated fibres (Nixon *et al.*, 1991).

All follicles undergo an endogenous cycle of growth which is followed by a period of regression. Photoperiod (the duration of light within a 24 h period) serves to synchronise this endogenous cycle with seasonal temperatures, provising the appropriate fleece for each season (Hutchinson, 1965; Ryder, 1978; Johnson, 1981). Follicles pass through three phases: anagen, the period of active fibre growth; catagen, the short period following anagen during which the follicle regresses, fibre growth ceases and a serrated "brush" end forms; and telogen, the resting stage of the cycle when the fibre is anchored in the follicle by a brush end. Less commonly the fibre is shed and the follicle is left 'shed empty' and resting until resumption of anagen (Dry, 1926; Chapman and Ward, 1979). The shedding mechanism still remains a subject of conjecture, the onset of new growth being closely synchronised with the shedding of the old fibre in most follicles but not in others (Nixon *et al.*, 1991a). Seasonal fibre growth cycles range from those possessing highly synchronised periods of secondary follicle telogen in down-producing goats with a clearly visible down shedding, to the less extreme decrease in follicle mitotic rate during winter, with little or no synchronised telogen, in the modern sheep breeds (Holle, 1993).

1.2.2 The fibre growth cycle of the down-producing goat

Much of the weight of the fleece of the down-producing goat (*Capra hircus laniger*) is made up of medullated fibres in excess of 80 microns diameter which are produced from primary follicles. This fibre type is commonly termed 'guard hair'. The other fleece portion is made up of fine fibres, commonly ranging in diameter from 10 to 30 microns, which are produced from secondary follicles and commonly termed 'down'. To be sold under the marketing name of "Cashmere" the down must have a mean fibre diameter of less than 18 microns. The down is typically non-medullated,

low in lustre and has no crimp.

Follicle development in the fetal down-producing goat is similar to that found in sheep. Primary follicle initiation and maturation are largely completed by birth. However, secondary follicle initiation continues after birth and is largely completed by 20 days of age (Lambert *et al.*, 1984; McCall and Fitzgerald, 1987). Approximately half the secondary follicles in down-producing goats undergo maturation after birth to 20 weeks of age. The secondary to primary (S:P) ratio in down-producing goats ranges from 5.7 to 7 (Henderson and Sabine, 1991b). Trio groups of primary follicles, form follicle groups with associated secondary follicles (or occasionally 4 or 5 primary follicles, personal observation). The follicle groups then progressively become entrained towards prevailing photoperiod. Following shedding in the first spring, the resultant fibre growth is synchronised with that of adult goats (Kloren, 1991).

Down-producing goats hair replacement cycles are seasonal (Ryder, 1966; McDonald *et al.*, 1987; Betteridge *et al.*, 1988). Down growth begins at the back of the animal and proceeds in a wavelike manner towards the shoulder (Norton, 1991). Both guard hair and down grow during summer and autumn, and are progressively shed during late winter and spring. Guard hair fibres are shed and replaced simultaneously which ensures coverage of guard hair throughout the year. In contrast, visible down disappears totally for two months in spring. It is clear, therefore, that the guard hair and down fleece components have different growth cycles which can be described in terms of length, weight or thickness and follicle activity (Table 1.1).

Secondary follicle activity in down-producing goats is high during summer-autumn and declines during winter (Figure 1.2). Soon after shedding in spring, short (less than 1 cm) replacement down (vellus) fibres cease growth and form brush ends, can be identified by microscopic examination. These fibres are then shed in summer prior to the onset of true down growth (Nixon *et al.*, 1991a). This spring subsidiary cycle

is thought to be a relic of the biannual pelage cycle found in other mammals (Ryder, 1966). Thus there is only a short period when down growth or mitotic activity is absent in secondary follicles (Henderson and Sabine,1992).

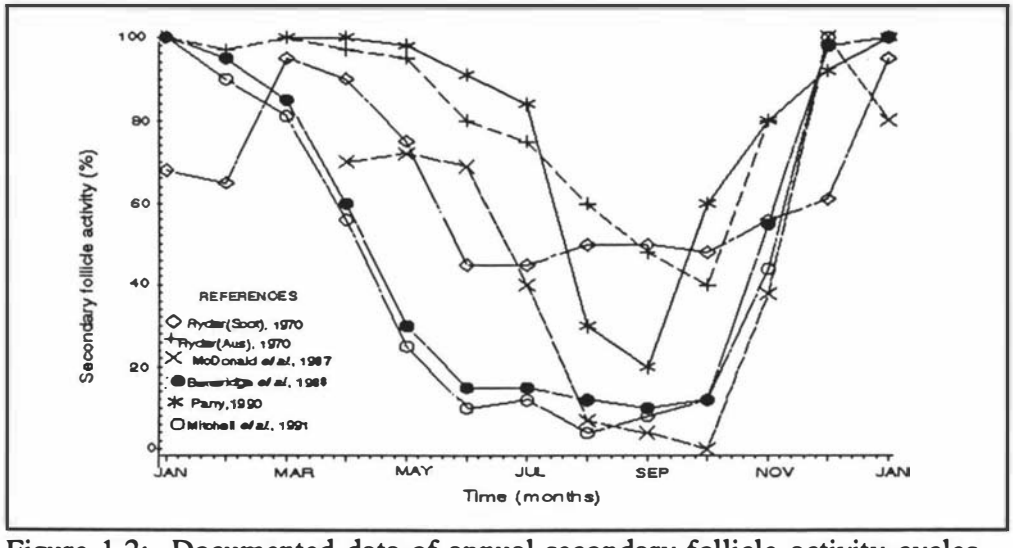

Figure 1.2: Documented data of annual secondary follicle activity cycles in down-producing goats.

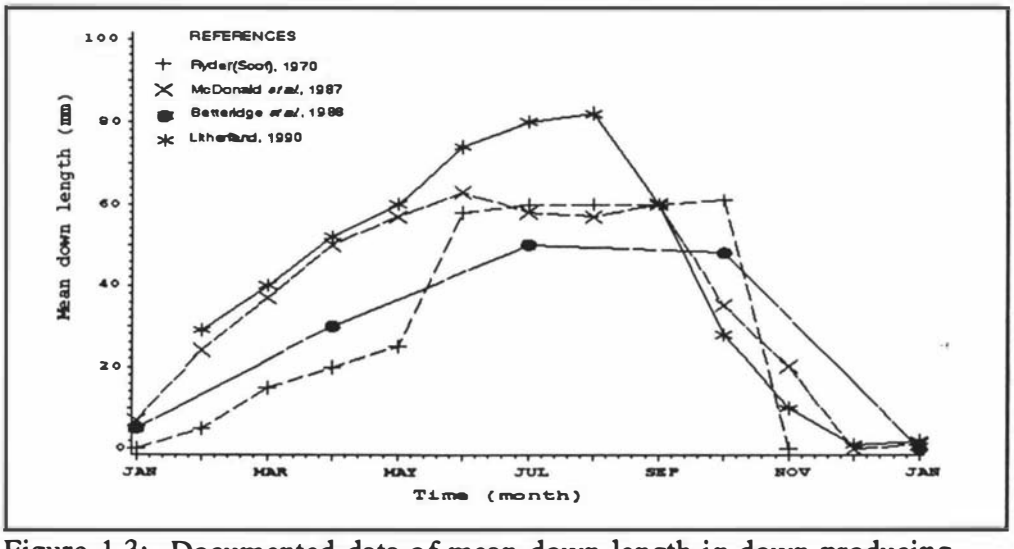

Figure 1.3: Documented data of mean down length in down-producing goats.

Little is known about the annual and between-animal variation of the subsidiary growth cycle. Visible 'true' down can generally be observed in the fleece from January[a] and it reaches a maximum length between July and September (Ryder 1970; McDonald *et al.*, 1987; Betteridge *et al.*, 1988; Litherland *et al.*, 1990; Nixon *et al.*, 1991a; Figure 1.3). A decline in down length marks the commencement to shedding (Figure 1.3). Phenotypically, down length and down weight are highly correlated (Couchman and McGregor, 1983; McDonald, 1988) and down length is a useful measure of down growth (Litherland *et al.*, 1990; Mitchell *et al.*, 1991).

In contrast to down, guard hair is present in the fleece throughout the year. Although it is seasonally replaced (Figure 1.4) the growth of new fibres is closely associated with the shedding of old fibres, and a cover of guard hair is always retained (Figure 1.5). Guard hair is shortest in summer and longest in winter (Ryder, 1966; McDonald, 1987; Betteridge *et al.*, 1988; Litherland *et al.*, 1990; Nixon *et al.*, 1991).

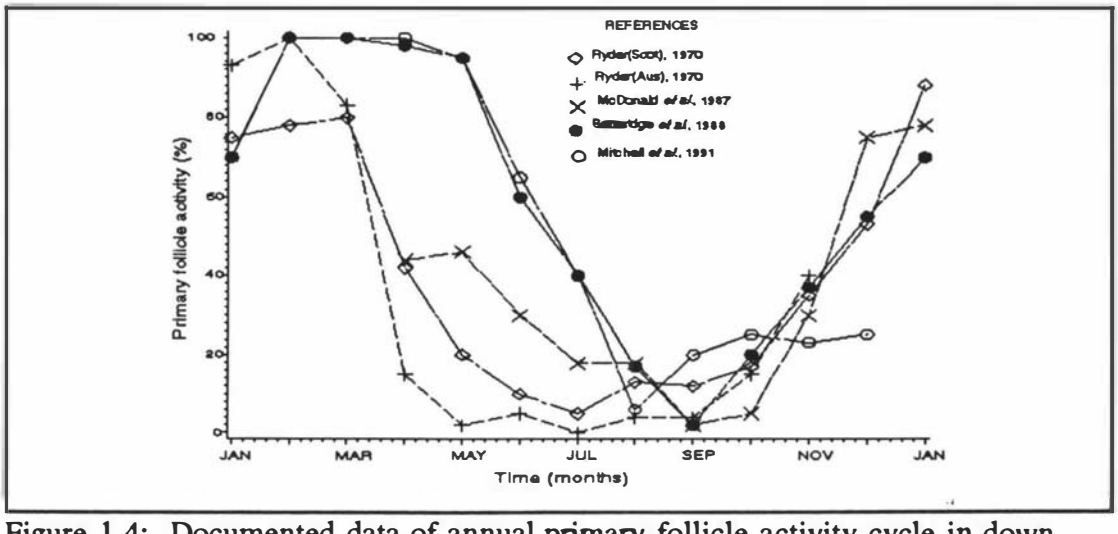

Figure 1.4: Documented data of annual primary follicle activity cycle in down-producing goats.

Mean fibre diameter of down is an important determinant of price in down fleeces. Some reports indicate that annual down fibre diameter cycles (Figure 1.6) are

bimodal, with peak fibre diameter occurring in October to December and again in March (McDonald, 1987), while others report only a single peak from March to June (Nixon *et al.*, 1991; Henderson and Sabine, 1992). The bimodal fibre growth cycle (or, more correctly, the decline in fibre diameter in January and February) described by McDonald (1987) contradicts other observations. It can be speculated that the techniques used by McDonald of cutting snippets 5 mm from the base for fibre diameter measurement captured some vellus fibres. If so, the peak of brush end formation during January and February noted by McDonald (1987) indicates that these fine fibres were then shed and true down commenced to grow. Fibre diameter fluctuations (Figure 1.6) reflect changes in follicle output or mitotic activity (Henderson and Sabine, 1991) which can be affected by physiological factors (Section 1.3.3). However, it is unlikely that such physiological factors can account for the bimodal fibre diameter changes reported by McDonald (1987) as the goats were fed a constant diet and were non-pregnant, with the same effect being observed in two consecutive years.

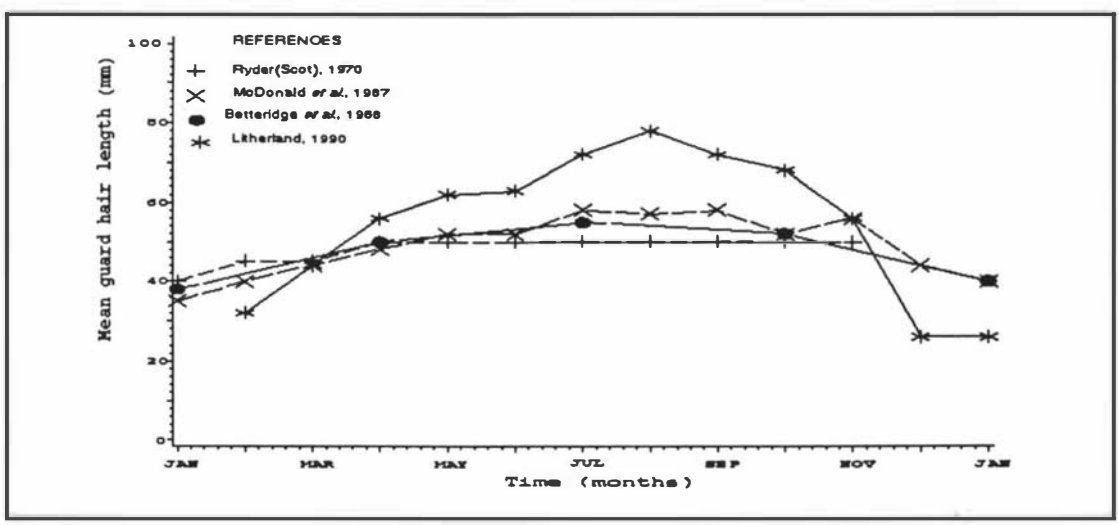

Figure 1.5: Documented data of guard hair length in down-producing goats.

A large difference in fibre diameter between guard hair and down is required for efficient separation of down from guard hair during processing (Bigham, 1992).

Maximum diameter of guard hair fibre occurs in December and January (Figure 1.7) and diameter declines to a minimum in August through to September (McDonald *et al.*, 1987).

Seasonal growth of fibre on a weight basis can be measured from repeated clipping of a patch within a defined area on the midside. In down-producing goats this patch is often then separated into the down and guard hair. Down growth rate rises rapidly from January to reach maximum in autumn, with levels declining over winter to reach a minimum in spring (McDonald *et al.*, 1987; Betteridge *et al.*, 1988; Mitchell *et al.*, 1991). In contrast the guard hair growth cycle is extended. With significant growth in spring and slowly building to peak growth rates in autumn before declining during winter (McDonald, 1987; Mitchell *et al.*, 1991).

In conclusion, the fleece growth of down follows a pattern in which the down portion of the fleece is grown in summer through to autumn, retained in winter and shed in spring. A study designed to clarify the physiological steps in the switch from telogen to anagen in goats would inevitably be directed at the spring. However, the subsidiary vellus cycle in spring complicates any such study. It cannot be assumed that vellus fibres occur in all goats or that they will respond to physiological manipulations in the same way as true down fibres. Further definition of this subsidiary cycle may provide interesting information on how animals have evolved from the double to single shedding pattern.

Examination of the pictorial presentation (Figures 1.1-1.7) of published studies on fibre growth cycles in down-producing goats shows that, while the pattern of growth is similar between studies, there are differences in the timing and magnitude of growth associated with differing physiological states, age, environment and genetic background.

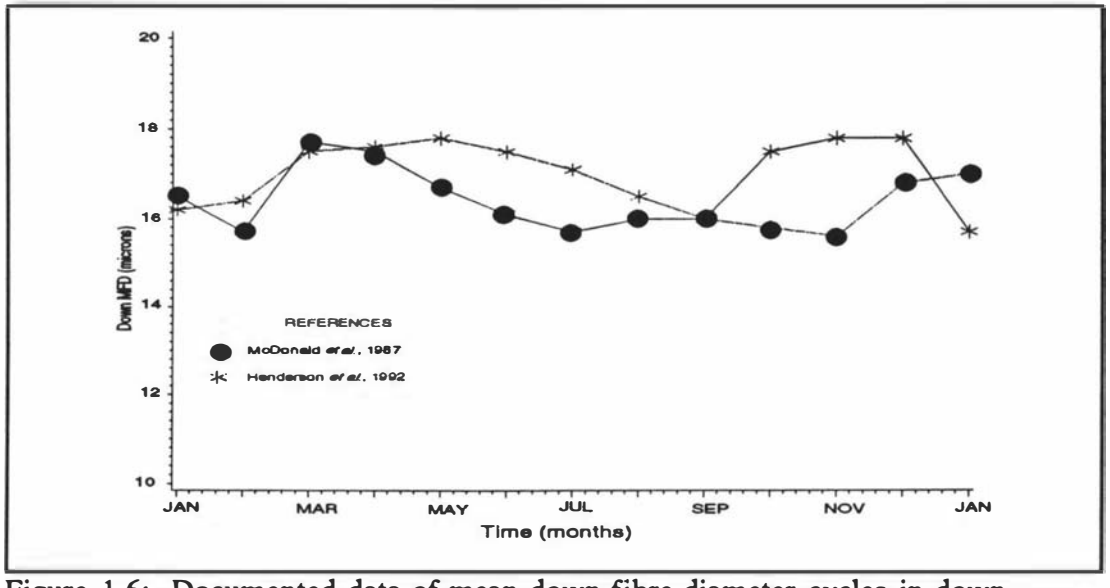

Figure 1.6: Documented data of mean down fibre diameter cycles in down-producing goats.

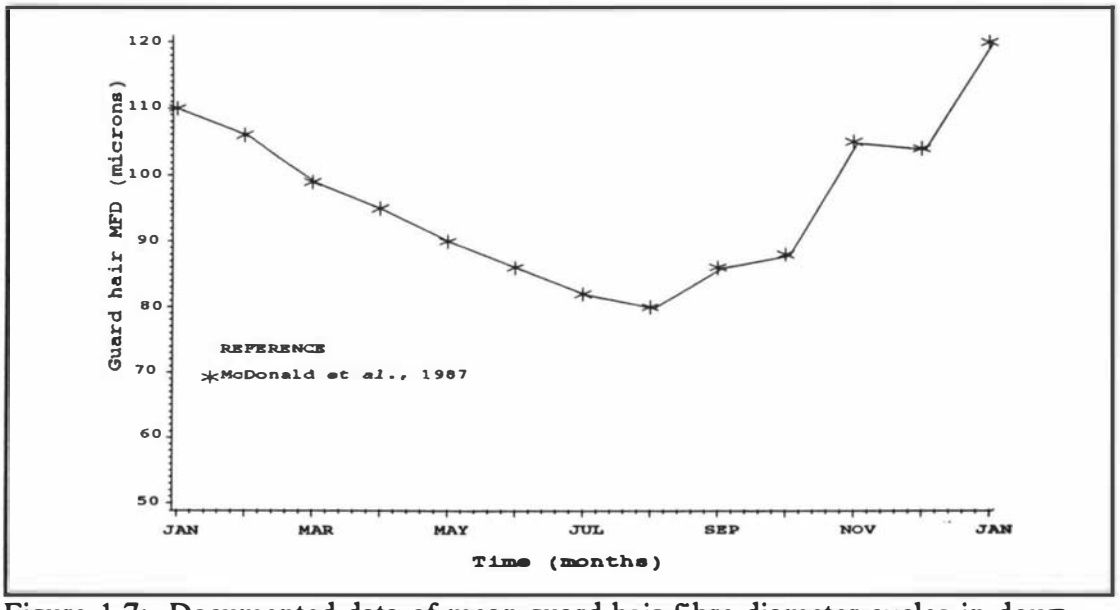

Figure 1.7: Documented data of mean guard hair fibre diameter cycles in down-producing goats.

Table 1.1: Experimental details of studies quoted on circannual growth patterns in down-producing goats

Reference[a]	Latitude	Breed	Sex	Reproductive Status	Age Adult,kid	Number of animals
Ryder, 1966a	30°S	Saanen x Angora	Doe	Pregnant/non-pregnant	Adult, kid	3
Ryder, 1966b	56°N	Saanen, Toggenburg	Doe	Pregnant/non-pregnant	Adult, kid	6
McDonald *et al.*, 1987	28°S	Down	Doe	Non-pregnant	Adult	10
Betteridge, 1988	38°S	Down	Doe	Pregnant	Adult	5
Litherland *et al.*, 1990	40°S	Down	Wether	N/A	Adult	16
Parry, 1990	40°S	Down	Wether	N/A	Adult	3
Mitchell *et al.*, 1991	38°S	Down	Doe	Pregnant	Adult	9
Nixon *et al.*, 1991	38°C	Down	Doe	Pregnant	Adult	5
Henderson and Sabine, 1992	35°S	Down	Doe and Buck	Non-pregnant	Adult	8

[a] Betteridge (1988), Mitchell *et al.* (1991), and Nixon *et al.* (1991) reported on various subsets of an experiment conducted on the same group of goats.

1.2.2.1 Photoperiod and melatonin influences on down growth: The circannual fibre growth cycle of down-producing goats can be manipulated by changing both photoperiod and circulating melatonin concentrations. Goats maintained in continuous light exhibit a compressed 8 month down growth cycle, largely because of a reduction in the duration of the telogen stage (McDonald, 1987).

Fibre growth in down-producing goats is responsive to continuous light treatments applied in September, December or February (McDonald and Hoey, 1987; Norton 1991). However, following a switch from continuous light to natural light in February, but not September and December, down fleece parameters continue to be influenced by previous continuous-light exposure. This indicates a carryover effect of continuous light treatment on pineal function or a photoperiod-sensitive window in the annual cycle to which fibre growth is responsive (McDonald and Hoey, 1987). Melatonin administered to down-producing goats in spring advances the onset of new down growth. The melatonin-induced down is then shed in autumn and a new cycle of growth is initiated which is then shed one month later than normal in spring (Betteridge *et al.*, 1987; Moore and Bigham, 1989; Scheurman *et al.*, 1989; Litherland *et al.*, 1990; Gebbie *et al.*, 1991; O'Neill *et al.*, 1992). In addition, down growth is responsive to a combination of long day and melatonin treatment (Gebbie *et al.*, 1991). It has been proposed that melatonin treatment promotes two down growth cycles within one season by causing vellus fibres to elongate into true down fibres (Betteridge *et al.*, 1989). Short term immunization against endogenous melatonin in July has a similar effect to melatonin treatment at this time (Norton, 1991). Light manipulations, melatonin immunisation, and melatonin treatment can all be used to alter down growth cycles. Until the nature of the photoperiod message delivered by continuous light, constant melatonin administration or melatonin abolition is clarified, the results will remain difficult to interpret.

1.2.2.2 Physiological factors affecting down growth: Entire down males are heavier and grow slightly more down per head than females goats (Pattie and Restall, 1988).

However, unlike sheep, the mean fibre diameter of down is not influenced by sex of the goats (Pattie and Restall, 1988; Gifford *et al.*, 1989). Down growth is initiated earlier in non-pregnant females than in males and, because this growth continues for a longer period, the date when down growth ceases is similar (Norton, 1991). Truly valid comparisons of sex classes are difficult, in practice, beyond puberty because the animals are invariably grazed separately.

When progressing from yearling to adult fleeces down-producing goats with no Angora ancestry can be expected to increase in fibre diameter by 2 microns and nearly double down production (Pattie and Restall, 1988; Gifford, 1989). However in goats with genotypes which include some Angora ancestry, yearling fleece diameter is coarser and fibre diameter can increase by up to 4 microns from yearling to adult fleeces (Paterson *et al.*, 1991).

It is generally accepted that pregnancy and lactation place nutritional stresses on fibre-producing animals which depress fibre growth. In addition, changes in hormonal status associated with pregnancy and lactation may have a direct effect on fibre growth (Reid and Sumner, 1991). In low producing down-producing goats, down growth is largely unresponsive to nutrition and therefore it can be assumed that the major effect on down growth, of pregnancy and lactation, is hormonal. Goats which are in late pregnancy or lactating during the period of active down growth from December to March show reductions of up to 60% in down production (Graham, 1988; Kloren, 1991). Kidding in July advances the cessation of down growth, while kidding in December delays the initiation of down growth (Kloren, 1991). A September kidding minimises the detrimental effect of pregnancy and lactation on down growth (Graham, 1988; Kloren, 1991).

The results of experiments to examine the effect of nutrition on down growth are conflicting which can be explained by differences in genetic potential to produce down. Studies conducted on low producing down-producing goats have failed to

show any increases in down production resulting from improved nutrition (Ash and Norton, 1984; Johnson and Rowe, 1984; Ash 1986; McCall and Fitzgerald, 1987). Conversely, McGregor (1988) increased down production in productive down wethers by 51% by increasing nutrition from 0.8 to 1.5 maintenance. However, it is somewhat surprising that down growth declined progressively from December in the study by McGregor (1988) when in the normal situation it would be expected to increase rapidly until autumn. However, it is possible that the duration of anagen can be affected by nutrition. To date most nutritional studies have been conducted during the active down growth period of summer and autumn. The possibility of nutritional responses outside this period cannot be discounted.

1.2.3 The fibre growth cycle of the Angora goat

The Angora goat (*Capra hircus angorienses*) developed as a separate goat breed in Asia minor where environment, perhaps more than human intervention, produced a goat with a white lustrous fleece which hangs in ringlets (Duerden and Spenser, 1930). Relative to those of the wild type of goat from which it developed, secondary follicles in the Angora goat have increased in size and number, while primary follicle number and size have declined. The ratio of secondary to primary follicles (S/P) of the Angora goat ranges from 7 to 10 (Shelton, 1968; Winklmaier, 1983: Eppleston and Moore, 1990; Nixon *et al.*,1991). The fleece comprises predominantly non-medullated fibres ranging in diameter from 20 to 60 microns.

In Australian and New Zealand Angora goats up to 20% of the fibres can be medullated (Bown *et al.*, 1990; Eppleston and Moore, 1990). Medullated fibres are categorised according to the medulla size and distribution along the fibre (ASTM, 1989). The coarsest of the medullated fibres, kemp fibres, are produced predominantly in the central primary and occasionally in the lateral primary follicles (Duerden and Spensor, 1930; Nixon *et al.*,1991). Kemp fibres have a continuous core of medulla which constitutes greater than 60% of the fibre diameter (Hardy, 1927)

and are shorter in length than mohair fibres. Kemp is thought to be a relic of the hair coat of the wild goat ancestor (Ryder, 1978; Winklemaier, 1983) and, similar to the guard hair coat of down-producing goats, is responsive to nutrition and sheds seasonally.

Medullated fibres with a 'continuous' medulla of less than 60% of the fibre diameter are sometimes called 'gare' fibres. Heterotype or 'partially' medullated (those in which medullation can be observed at some points along a fibre) fibres are found in some Angora goat fleeces. These fibres are produced in primary or original secondary follicles (Ryder, 1966; Nixon *et al.*,1991b) and are similar in length and seasonal growth cycle to non-medullated mohair fibres (Stapleton, 1978). The fibre diameter ranges of these three fibre type categories overlap (Dreyer and Marincowitz, 1967). However, above 100 microns in fibre diameter all medullated fibres are kemp, between 20 to 30 microna, all medullated fibres are gare or heterotype, and below 20 micron there are no medullated fibres in the Angora fleece (Lupton, 1992). Medullated fibres are considered undesirable by processors (Turpie, 1985) because they affect the dieing and handle characteristics of the fabric. While kemp fibres are readily seen in the fleece and can be removed during processing, gare fibres are difficult to view with the naked eye and cannot be removed. Medullation has a large influence on the price paid for mohair (Sinclair, 1988).

The timing and organization of follicle initiation and maturation in the Angora goat is very similar to that of the sheep (Stapleton, 1978). Additional lateral primaries may be initiated in some trio follicle groups, giving rise to follicle groups containing 4 to 6 follicles (Wentzel and Dreyer, 1967). Like many domestic sheep (Ryder and Stephenson, 1968), the Angora goat is born with a hairy birth coat which is shed by 3 months of age (Duerden and Spencer, 1930). In even the largely non-medullated South African Angora, the birth coat contains up to 50% medullated fibres of both primary and secondary follicle origin (Dreyer and Marincowitz, 1967). It has been hypothesised (Dreyer and Marincowitz, 1967) that this hairy birth coat demonstrates

the ancestral roots of the Angora goat. At birth, the S/P ratio, in the Angora goat, ranges from 2-3 indicating that only a small proportion of secondary follicles are mature and producing fibres (Wentzel and Vosloo, 1975).

The photoperiodic changes in mohair growth were first described as differences in autumn compared to spring fleeces. Detailed studies of seasonal mohair growth (Hutchinson, 1965; Stapleton, 1978; Winklemaier, 1983) show differences of up to 55% in amplitude of growth in summer compared with winter (Stapleton, 1978; McGregor, 1988) which is similar to that observed in British breeds of sheep (Nagorcka, 1979). Patch growth (Figure 1.8) and staple length are maximal in spring and summer and minimal in mid-winter, while correlated fibre diameter changes occur one month earlier (Stapleton, 1978; Winklemaier, 1983). In addition to the reduction of fibre volume in winter, up to 25% of secondary follicles may enter telogen (Figure 1.9) (Stapleton, 1977; Margolena, 1974) especially if nutrition is poor (Nixon *et al.*,1991b).

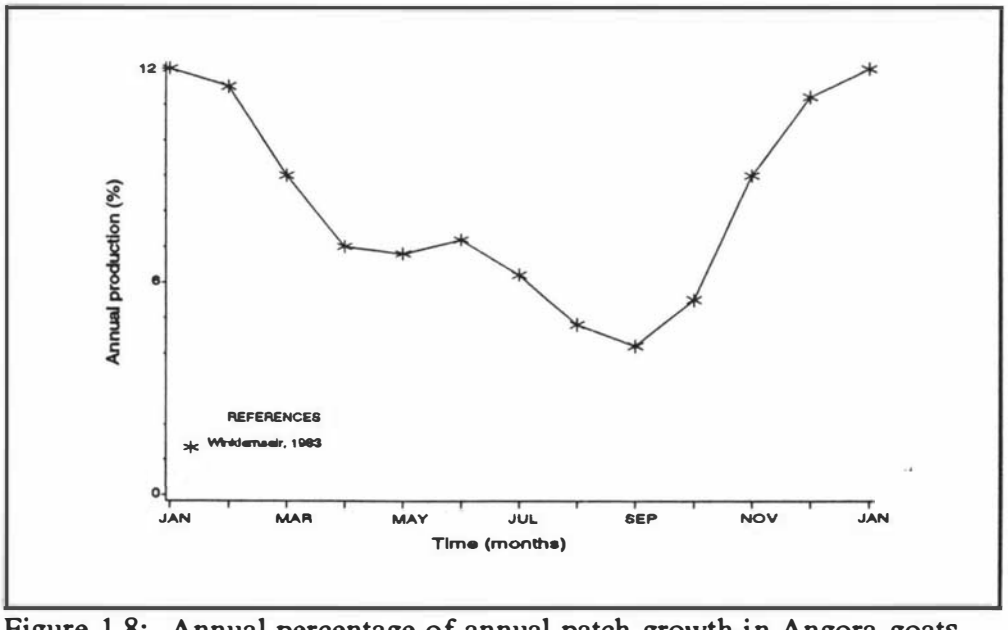

Figure 1.8: Annual percentage of annual patch growth in Angora goats

Gare fibres produced in the secondary follicles reflect the seasonal pattern of the non-medullated mohair fibres (Figure 1.10) (Dreyer and Marincowitz, 1967; Stapleton, 1978). In contrast, kemp fibres have a seasonal growth cycle with a synchronised period of telogen as in guard hair in the down-producing goat. Primary follicle activity in the Angora goat is high from September to May (Figure 1.9) but declines to 20% over winter (Nixon *et al.*,1991b). Maximum growth of kemp fibres occurs during October and November (Dreyer and Marincowitz, 1967) with brush end formation and shedding in late summer (Stapleton 1976; Winklmaier, 1983; Nixon *et al.*,1991b; Stapleton, 1991). Some workers have observed a small amplitude biannual pattern of kemp growth with peaks in both spring and autumn (Winklemaier, 1983; Nixon *et al.*,1991b). This is particularly apparent in the lateral primaries (Nixon *et al.*, 1991).

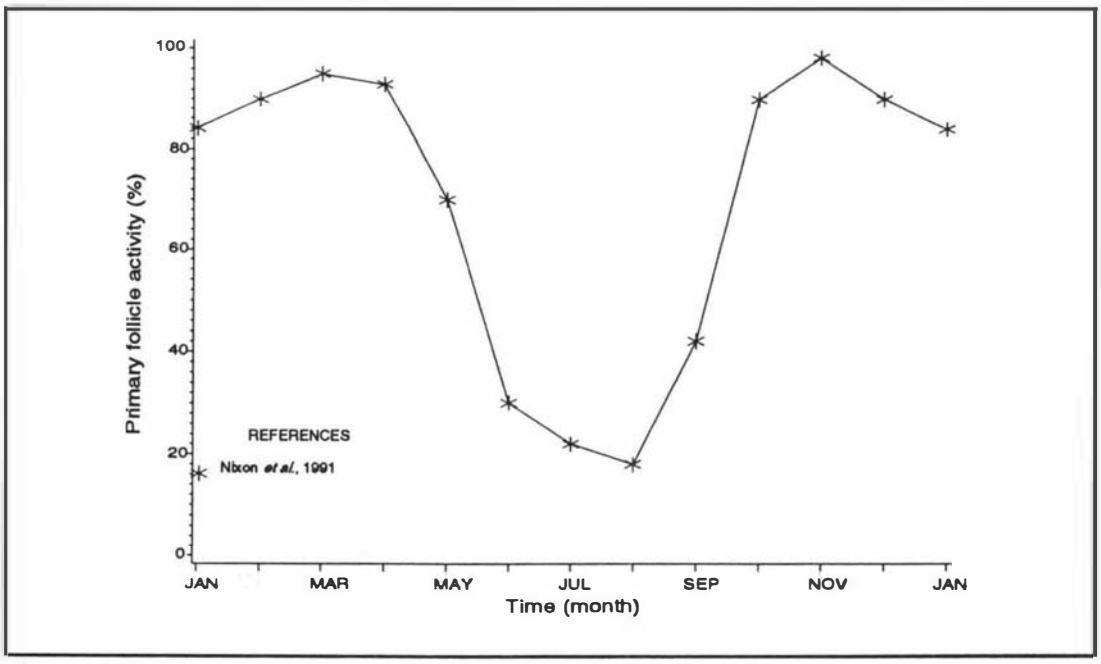

Figure 1.9: Annual primary follicle activity cycles in Angora goats.

1.2.3.1 Physiological effects on mohair growth: Angora bucks produce heavier and coarser fleeces (Jones, 1935; Ariturk *et al.*,1979) than wethers (Stapleton, 1977) or does (Nicol, 1985; Nicol, 1989; Gifford, 1990). As in sheep (Black and Reis, 1979),

this is attributable mainly to differences in liveweight and not to relative efficiencies of fibre production of the different sexes. Pregnancy reduces fibre production from 0 to 7 % (Stapleton, 1978; Winklemaier, 1983) while lactation can reduce fibre production by up to 14% (Stapleton, 1978; Ariturk *et al.*, 1979). The detrimental effect of pregnancy and lactation is greater in young compared to mature does (Jones *et al.*, 1935).As Angora goats age, fleece weights increase to a maximum at 4 years and then decline progressively, while fibre diameter continues to increase for the life time of the animal (Shelton, 1968; Uys *et al.*, 1985; Gifford *et al.*, 1990). The greatest increase in fibre diameter is seen from the first to the second year of age (Stapleton, 1977) when fibre diameter may increase by 6 microns.

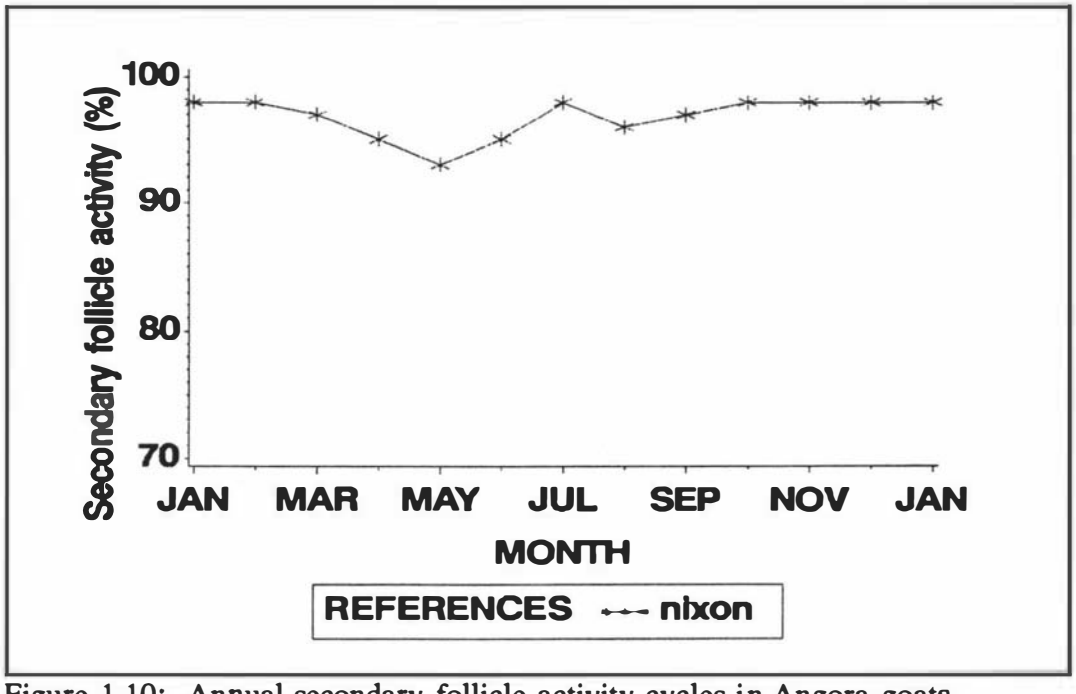

Figure 1.10: Annual secondary follicle activity cycles in Angora goats.

It is clear that Angora goats on poor levels of nutrition have lower fibre production (Malacheck and Leinweber, 1972; McGregor, 1988) however improved gross nutrition

has not always increased mohair growth (Bown *et al.*,1990). Responses in fibre production have been obtained by supplementing the diet of Angora goats with protected protein (Throckmorton *et al.*,1982; McGregor and Hodge, 1989) increased protein content (Deaville, 1989) and improved gross nutrition (McGregor, 1988). Some authors have suggested that there may be differential effects of nutrition on fibre diameter relative to length.

An increase in medullation in the Angora fleece can occur with improved nutrition (Lupton *et al.*,1986; Bigham and Baker, 1990; Bown *et al.*,1990; McGregor, 1990; Nixon *et al.*,1991) and with severe under-nutrition (McGregor, 1990) while others have failed to identify such an effect (Calhoun *et al.*,1988). The former experiments were conducted on goats with a high genetic potential to produce kemp and the latter on goats with a lower genetic potential. In responsive goats, improved nutrition induces kemp growth in previously gare-producing lateral primaries and gare growth in original secondaries which previously produced non-medullated mohair (Nixon *et al.*,1991b).

1.2.4 The fibre growth cycle of shedding sheep breeds

Sheep were domesticated about 9 000 BC and the fleece of the wild ancestor was composed of coarse kemp fibres originating from primary follicles and fine down fibres growing from secondary follicles. In comparison to modern sheep, wild sheep had a low S/P ratio, the fleece had an extended fibre diameter distribution and was coloured. In addition the fleece of the wild sheep was probably shed in an annual pattern much the same way as in down-producing goats. Today only the hair sheep breeds of tropical Africa and India retain this wild type fleece. After 7 000 years of domestication, the S/P ratio of sheep skin has increased, the fleece is white, fibre diameter distribution is condensed, and photoperiodically linked shedding is absent (Ryder, 1983).

Today there are a few recognised breeds of domesticated sheep, such as the Mouflon, Soay, and Wiltshire, which retain the ability to shed their fleece in an annual cycle. Interestingly, these breeds do not emanate from the original wild sheep ancestors but rather are derived from sheep which have escaped from domestication. The Mouflon 'escaped' approximately 3 000 years, the Soay 7 000 years and the Wiltshire 10 000 years, after sheep were first domesticated. There are numerous more recent instances where sheep have escaped domestication and rapidly evolved an ability to shed their fleece. For example, in New Zealand, pockets of feral sheep have developed the capacity to cast their fleece annually after less than 100 years of freedom from the influence of man. Fleece casting is more prevalent under poor nutritional conditions and during pregnancy as it is under (Pearson pers. comm., Wickham, pers. comm.) In these 'feral' sheep, fleece production is low and their skin contains high numbers of primary follicles (Meikle *et al.*, 1990) but both follicle inactivity and fibre breakage contribute to fleece casting (Bigham, 1984). Therefore, it is more appropriate to use the recognised fleece shedding breeds of sheep for studies on the physiological mechanism of shedding.

Shedding commences, in shedding sheep breeds, at the head and neck before extending to the belly and breech regions and finally upwards from the sides Slee, 1962; Ryder, 1971; Tierney, 1980; Parry *et al.*, 1991). In Wiltshire sheep, the extent of shedding varies greatly between individuals and often a 'toupee' of fleece is retained along the backbone. Variation in the timing of brush-end formation within the follicle bundle also occurs with follicles deactivating and activating in the order in which follicles are developed in the fetus (Ryder, 1971).

The Mouflon sheep (*Ovis musimon*) has a fleece of coarser-outer coat kemps and finer inner-coat down and most closely, of the three breeds described here, represents the original wild sheep fleece. Both kemp and down fleece components are shed around the spring equinox (Ryder, 1978). Kemp regrowth occurs shortly after the spring equinox and continues until the autumn equinox (Lincoln, 1990). Kemp-

producing follicles undergo an additional period of inactivity in late summer (Ryder, 1978). The majority of down appears above the skin surface around autumn equinox (Lincoln, 1990). Measurements taken to date have failed to identify a period of complete inactivity in secondary Mouflon follicles though such periods have been identified in primary follicles during both spring and autumn (Ryder, 1978).

Soay sheep have a two fibre type fleece structure. However,in some strains the primary follicles no longer produce medullated kemp fibres but rather produce a coarse non medullated 'wool' fibre. In spring, follicles are reactivated with loss of the existing fibre from the follicle (Ryder, 1971). Growth of fleece then continues until the autumn equinox (Ryder, 1971). However, during summer, subsidiary fibre growth cycles have been identified both in primary and secondary follicles (Ryder, 1971). These subsidiary decreases in follicle activity are also associated with decreases in fleece length suggesting that only the follicles with longer fibres are shed. Medullation rates in fibres emerging from the skin of Soay sheep are also highest during summer fleece growth (Ryder, 1971).

The Wiltshire sheep has the most 'modern' fleece type of all the shedding sheep breeds. It has a S/P ratio of between 2.5 and 4.8 (Slee and Carter, 1961). The fleece is largely white and has a condensed fibre diameter profile. However many fibres from both primary and secondary follicles can be medullated. In the British strain of Wiltshire sheep, follicles are activated in September and are all inactive by June (Ryder, 1978) and fleece casting is observed from November (Slee, 1959). Summer peaks of follicle inactivity have been identified but these were not found to be associated with visible shedding. However in NZ Wiltshire sheep, partial shedding was associated with summer subsidiary primary follicle inactivity cycles (Parry *et al.*, 1991). In the NZ Wiltshire the duration of the period of follicle inactivity was shorter than in the British strain and in some individuals follicles remained active during winter (Parry *et al.*, 1991). The NZ Wiltshire contains some Poll Dorset genes.

1.2.4.1: Physiological effects on fibre growth in shedding sheep: In Soay sheep, follicles are activated one month later and become inactive one month earlier in ewes compared to rams (Ryder, 1971; Ryder, 1978). The first seasonal moult of the one year old Soay is less complete than the second moult in the two year old Soay (Ryder, 1971). Similarly the in Wiltshire x Merino sheep the extent of shedding was greater in three year old sheep compared to two year old sheep (Tierney, 1980). Poor nutrition may impede shedding in Wiltshire sheep (Slee, 1962).

1.2.4.2: Photoperiod and fibre growth in shedding sheep: An intrinsic wool growth cycle, with a duration of 8 months, was recognised in halfbred Wiltshire x Merino ewes treated from the winter solstice with constant long photoperiod (Maxwell *et al.*, 1989). In constant short photoperiod, changes in wool growth could still be detected but the amplitude and frequency of the wool growth cycle decreased.

In skin from Wiltshire and Soay sheep, transposed by grafting, between winter and summer, it was found that follicle activity can be provoked in inactive follicles but it was impossible to restrain oncoming activity. However the period of anagen can be lengthened by several months by transplanting active autumn skin into inactive winter skin (Ryder and Priestley, 1977). However, these transplantation studies did have major effects on blood supply to the skin and therefore may not reflect only seasonal responses.

The wool growth cycles of the Soay and Wiltshire sheep are both highly responsive to photoperiodic manipulation. Constant dim light retarded shedding in Wiltshire lambs (Slee, 1965). Switches between long and short photoperiod are powerful stimuli for changes in the wool growth cycle. When Soay rams were treated with short photoperiod from February to April (Southern hemisphere dates) the decline in follicle activity, normally observed in winter, was advanced. But in April, when the Soay sheep were treated with long photoperiod, the follicles became activated (Ryder and Lincoln, 1976). It has been hypothesised that the intrinsic wool growth cycle is

synchronised by changes in photoperiod, especially lengthening photoperiod. In Soay and Wiltshire sheep, a transition from short to long photoperiod, while follicles were actively growing, resulted in an initial inhibition of wool growth followed by the initiation of a new wool growth cycle Parry *et al.*, 1993; Pearson *et al.*, 1994). In addition, in Soay sheep, when wool growth was already low at the time of photoperiod transition, wool growth was increased by long photoperiod treatment (Ryder, 1976; Lincoln *et al.*, 1980). To date studies have not been conducted to determine whether a transition from short to long photoperiod can activate inactive Wiltshire sheep follicles.

1.3 THE REGULATION OF PROLACTIN SECRETION FROM THE PITUITARY

1.3.1 Introduction

PRL is a protein hormone which, in its monomeric form, exists as a single polypeptide chain of about 200 amino acid residues. However, the PRL molecule in plasma varies both in molecular weight and isoelectric charge (Ostrom, 1990).

PRL is widely distributed in vertebrates (Fennessy and Suttie, 1984; Rillema *et al.*, 1988) but with a species variation of up to 40% in the amino acid sequence of the PRL molecule (Wallis, 1988). There are six regions of the PRL molecule, comprising about 146 amino acids, which are similar in structure to growth hormone (GH). This structural similarity is associated with some overlap in biological activity (Wallis, 1988).

The pars distalis region or anterior pituitary gland is the major source of PRL in the body. However, molecules with a similar structure to PRL are synthesised in the placenta (Yamakawa *et al.*, 1990; Tseng *et al.*, 1992), lymphocytes (Swarlo-Santo, 1992) and possibly also in the skin (Walker *et al.*, 1989). The *pars distalis* releases

six major hormones into the general circulation and these regulate various organs including the thyroid, adrenals, gonads and mammary gland. The *pars distalis* is made up of hormone-secreting cells whose staining characteristics reflect the nature of the hormone which they secrete (Martin, 1985).

The control of PRL secretion from the pituitary is highly complex and only partially understood but is thought to be co-ordinated at three levels. At the highest level, hypothalamic inhibitory and stimulatory molecules regulate secretion. PRL secretion is also modified by the action of peripheral hormones, which reach the pituitary from the systemic circulation (e.g. oestrogen) (Lamberts and MacCleod, 1990). At the lowest level, lactotrophs and other secretory cells of the *pars distalis* synthesise regulatory molecules which have autocrine (affect their own secretory activity) or paracrine (affect the secretory activity of neighbouring cells) effects. The lactotroph population is not homologous and subpopulations will respond differently to the various regulatory signals (Lamberts and MacCleod, 1990; Takahasi, 1992).

PRL release into the general circulation, like that of many hormones, is episodic. For example, in rats, pulses originate every 8 to 12 minutes. The pulsatile release of PRL from the *pars distalis* is due to an intrinsic inter-lactotroph communication system (Ben-Jonathan, 1985). The pulses are associated with activation of voltage-dependent calcium channels, calcium fluxes, and changes in intracellular calcium buffering capacity and potassium channels (Ben-Jonathan, 1985).

A variety of models have been used to study PRL regulation. Some of the common models include: the suckling-induced acute increase in plasma PRL concentration; the stress-induced acute increase (Milenkovic *et al.*, 1990); the pregnancy- and lactation-induced chronic increase in PRL and the diurnal fluctuations in plasma PRL concentration in rats (Jukubowski *et al.*, 1988; Mistry and Voogt, 1989; Arey and Freeman, 1992; Furudate *et al.*, 1992); and *in vitro* preparations of lactotroph cells. In cell culture, the lactotrophs are no longer under the inhibitory control of the

hypothalamus and the *in vitro* PRL secretion is much higher than the equivalent *in vivo* secretion. The cell culture system therefore does not reflect all the *in vivo* physiological regulatory pathways (Shin *et al.*, 1987). The regulatory pathways in the other models may also be different, complicating the formulation of an overall understanding of PRL regulation systems.

Key PRL regulatory molecules common to all systems and all species have yet to be identified. There is a great diversity of pituitary structure between species (reviewed Martin, 1985) and it is likely that equivalent species variation will be found in PRL regulatory mechanisms (Thomas *et al.*, 1989).

PRL has a wide range of physiological actions which have been characterised in a variety of species (Lamberts and MacCleod, 1990). These include: regulation of water and electrolyte balance; control of growth and development; metabolic effects (Eisemann *et al.*, 1984; Johnson and Hart, 1985); reproduction and steroid interactions (Sundquist *et a*l., 1988; Ryan and Robinson, 1989; Ciereszko and Dusza, 1993); effects on integumentary structures and fibre growth (Dicks, 1994; Ibraheem *et al.*, 1993); effects on behaviour (Bridges *et al.*, 1990); and effects on the immune system (Dardenne *et al.*, 1991; Swarlo-Santo, 1992). Many structures of epidermal origin are responsive to prolactin including amphibian skin, mammary glands, thymic epithelial cells, kidney, sweat glands and fibre follicles (Paus, 1991).

1.3.2 The structure of the hypothalamus and pituitary

The hypothalamus is formed from the floor and lower walls of the third ventricle of the brain and is highly complex with axons travelling to many parts of the brain controlling many bodily functions. The infundibular stem is a funnel shaped stem which links the hypothalamus to the pituitary. The bulging upper end of the infundibular stem is known as the median eminence. It contains axons originating

from the hypothalamus and the capillaries of the long portal blood vessels. It is in this zone that hormones and neurotransmitters synthesised in the perikarya of the hypothalamus are transferred into the long portal blood system servicing the *pars distalis*. These substances include dopamine (DA), thyrotropin releasing hormone (TRH), serotonin (5 HT), and gamma aminobutyric acid (GABA) (Figure 1.11).

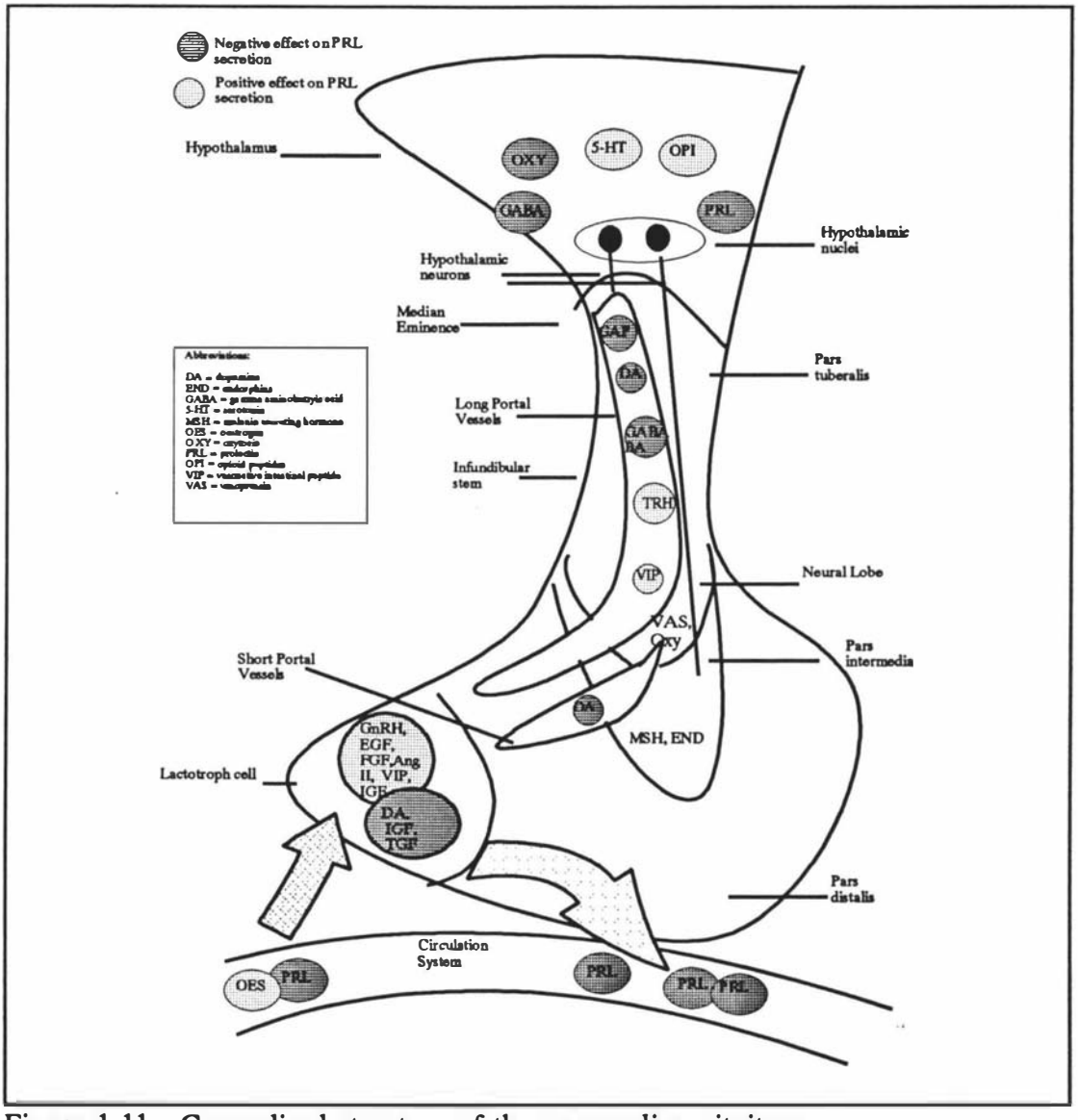

Figure 1.11: Generalised structure of the mammalian pituitary.

In addition to the median eminence and infundibular stem, the hypothalamus contains large and small neuron cell bodies grouped into nuclei. Large nuclei which have

relevance to the pituitary include the supraoptic nuclei (SON) which synthesise vasopressin and oxytocin, and the paraventricular nuclei (PVN) which synthesise oxytocin, TRH, follicle stimulating hormone (FSH), and thyrotropin stimulating hormone (TSH).The nerve tracts that terminate in the neural lobe originate largely from the SON and PVN. Smaller nuclei with relevance to the pituitary include the arcuate nuclei which synthesise luteinizing hormone releasing hormone (LHRH) and catecholamines, and ventromedial nuclei which are sources of gonadotrophin releasing hormone and somatostatin. These neurons terminate predominantly in the median eminence.

The pituitary, a gland of approximately 1 g gram weight in sheep, is located at the base of the brain. It has both blood and neural connections with the hypothalamus. The pituitary varies greatly in structure from species to species. However there are generalised zones which, while differing in size, shape and location, can be used to describe the pituitary.

The pituitary gland is described using two differing nomenclature systems. The first relies on locational and anatomical parameters of the gland (eg anterior and posterior pituitary), while the second is based on the different embryological origins and secretory functions of the components (eg *pars distalis*, *pars intermedia*). It is the latter classification system which is used in this review. The pituitary gland is divided into four regions: The *pars distalis*, *pars intermedia, pars tuberalis*, and the neural lobe.

The *pars distalis* is the largest component of the pituitary and is derived embryonically from the ectoderm originating from the roof of the mouth. In its mature form, it contains the cells which produce TSH, adrenocorticotropic hormone (ACTH), growth hormone (GH), FSH, LH, and PRL. The lactotrophs, which secrete PRL, are the largest cells in the *pars distalis*.

During embryonic development, neural ectoderm from the brain grows downward and forms a neural connection (infundibular stem) between the pituitary and hypothalamus. The *pars intermedia* forms adjacent to the infundibular stem. In some species it is a discrete zone while in others it appears as dispersed cells intermingled with the neural lobe. The *pars intermedia* secretes melanocyte stimulating hormone (MSH) and endorphins, and is largely avascular. It is widely dispersed with axons originating in the hypothalamus. The secretory capacity of the *pars intermedia* is dependent on the continuing attachment to the hypothalamus (Thomas *et al.*, 1986).

The *pars tuberalis* extends upwards from the main body of the pituitary and forms a collar around the neurohypophysis (Figure 1.11). The *pars tuberalis* is contiguous with the *pars distalis* and *pars intermedia* and contains cells with secretory granules characteristic of peptide secretory cells (Williams and Morgan, 1989). However, current knowledge suggests its main function is a conduit for the hypothalamo-hypophysial portal blood vessels (long portal vessels) travelling to the *pars distalis* and the hypothalamic axons travelling to the neural lobe and *pars intermedia.*

The neurohypophysis links the pituitary and the hypothalamus. It has three components: the neural lobe; the infundibular stem; and the median eminence. The neural lobe is considered to be part of the pituitary while the latter two structures form part of the hypothalamus. The neural lobe stores and releases neurohypophysial peptides which are transported from the hypothalamus by bundles of long axons. Hormones travelling along these axons include vasopressin and oxytocin. Short portal blood vessels connect the neural portion (*pars intermedia*, neural lobe) of the pituitary to the glandular portion (*pars distalis*).

1.3.3 The mechanism of prolactin secretion

The secretion of PRL from the lactotroph is a two step process involving the synthesis and then the release of PRL from the cell. These events are not inherently

linked and can respond differentially to hormonal signals (Shin *et al.*, 1987). For instance, low doses of oestradiol in rats increase pituitary PRL but not plasma PRL concentration. After higher doses of oestradiol both pituitary and plasma PRL concentrations are increased (Walro, 1991). PRL is synthesised on polyribosomes in the rough endoplasmic reticulum of lactotrophs, initially as a precursor with an amino-terminal extension which is subsequently cleaved. If PRL is not released immediately it is transferred to the golgi zone where it is packaged into secretory granules (Ben-Jonathan, 1985).

When PRL is not released from the membrane, it is stored within the lactotroph. The 'stored' PRL and 'newly synthesised' PRL can be released differentially. For instance, TRH stimulates differentially the release of 'stored' PRL while dopaminergic inhyibition controls release from the 'newly synthesised pool' (Lamberts and MacCleod, 1990).

Slow and fast releasable pools of PRL have been identified in cultured rat lactotrophs. The slow and fast releasable PRL pools could either be synonymous with the 'stored' and 'newly synthesised' pools of PRL found in individual lactotrophs, or originate from different subpopulations of lactotrophs, or even a combination of both. For instance, high density lactotroph cells containing a large number of secretory granules have a low spontaneous secretory capacity but a high intracellular PRL pool (Kazemzadeh *et al.*, 1992). It can be hypothesised that this lactotroph subpopulation would release PRL more slowly. But it is not clear whether lactotrophs comprise of distinct cell types or whether transformation between cell types occurs in vivo. this cell type is a readily convertable developmental stage or a different cell type.

There is some evidence that secretory cells of the *pars distalis* transform over time. There are cells in the *pars distalis* which secrete both GH and PRL. These cells are called 'mammosomatotrophs' and comprise 5 to 30% of the *pars distalis* cell population (Takahashi, 1992). In mammosomatotrophs, GH and PRL are either colocalized in the same secretory granules or contained in separate granules within

the same cell. The mammosomatotrophs are thought to be progenitor cells of the lactotroph as they appear before the lactotrophs in the developing foetal pituitary (Kineman *et al.*, 1992). However this cell type persists in the adult pituitary. There is strong evidence that the proportions of mammosomatotrophs and lactotrophs change and that hormonal treatment can facilitate this change (Lamberts and MacCleod, 1990; Inoue and Sakai, 1991; Kineman *et al.*, 1991; Takahashi, 1992). In times of high PRL secretion the mammosomatrophs are converted to lactotrophs (Kineman *et al.*, 1991). Therefore an increase in PRL secretion from the pituitary is associated with both an increase in secretion by individual lactotrophs and an increase in total lactotroph number.

The lactotroph population is dynamic and at any one time contains subpopulations which differ in morphological and sedimentary characteristics (Takahashi and Miyatake, 1991; Burris *et al.*, 1992), PRL secretion rate, type of PRL released and hormone responsiveness (Lamberts and MacCleod, 1990). Three lactotroph types have been described for rats based on the size of the secretory granules (Takahashi,1992). Cells with large secretory granules (300-700 um) are common during periods of high PRL secretion and have high basal rates of PRL secretion. Cells with small secretory granules have a low basal PRL secretion rate. The proportion of these cells types in the rat *pars distalis* can be decreased by bromocryptine treatment and increased by oestrogen treatment (Takahashi, 1992). Lactotrophs from different regions of the pituitary also respond differently to TRH and DA (Lamberts and MacCleod, 1990; Poole *et al.*, 1991; Takahashi, 1992).

1.3.4 Variations in the prolactin molecule

The PRL molecules secreted from the pituitary are heterogenous, differing in both molecular weight (Ben-Jonathan, 1985; Lamberts and MacCleod, 1990; Mana *et al.*, 1992) and net charge (Takahashi, 1992). It is not known whether the PRL variants have different target organs or different physiological roles. The monomeric form,

with a molecular weight of 23 kDa, is generally considered to be the primary biologically active form of PRL. This 'small' PRL is loosely coupled to organelles and is easily released from the cell. The dimer (56 kDa) and the oligomer (>100 kDa) are less biologically active in both mammary gland binding assays (Onstrom, 1990) and NB_2 cell binding assays (Stroud *et al.*, 1992). Aggregated PRL is also less immunoreactive (Stroud *et al.*, 1992). The 'big' forms of PRL are formed through thiol:disulphide linkages (Lorenson and Jacobs, 1987). This 'big' PRL is stored in secretory granules (Lamberts and MacCleod, 1990) and may be synonymous with 'stored' PRL. Glycosylated PRL has also been found in sheep. However, in contrast to other glycosylated hormones, it has reduced immunoreactivity (Ostrom, 1990) and bioactivity (Lamberts and MacCleod, 1990). Preferential release of the different PRL variants occurs in response to various acute stimuli (Farkoldh *et al.*, 1979) and photoperiod (Stroud *et al.*, 1992). The mechanism by which this occurs is not clear though it is known that changes in osmolality of the cell culture media disrupt the thiol:disulphide binding of 'big' PRL (Lorenson and Jacobs, 1987). The proportion of the monomeric form of PRL increases during times of high PRL secretion rate (Farkoldh *et al.*, 1979). Each lactotrophic cell secretes only one type of PRL variant at one time (Ostrom, 1990), and each variant is secreted in its complete form directly from the lactotroph (Stroud *et al.*, 1992). The varying ratios of the PRL variants identified in the plasma arise from secretion by different subpopulations of lactatrophs. The D2 receptor which binds DA, to inhibit PRL secretion, has two subtypes which may be linked to the release of specific PRL variants (Takahashi, 1992).

1.3.5 Prolactin inhibiting factors

Dopamine and Noradrenaline: PRL is unique among pituitary hormones as its secretion is spontaneous in the absence of hypothalamic influences (Ben-Jonathan, 1985; Lamberts and MacCleod, 1990). The hypothalamus exerts a largely inhibitory influence on the pituitary lactotrophs while the neural lobe secretes PRL releasing

factors (Dymshitz and Ben-Jonathan, 1991). When the lactotroph is removed from the influence of the hypothalamus, either by hypothalmic-pituitary stalk section or by *in vitro* cell culturing, PRL secretion increases (Nagy *et al.*, 1988). Catecholamines, especially DA, inhibit PRL secretion both *in vivo* and *in vitro,* and are currently considered to be the main hypothalamic inhibitory factors. However, in rats, hypothalamic extracts with DA removed still inhibit PRL release (Shin *et al.*, 1987) and DA cannot completely inhibit PRL secretion (Stirling and Shin, 1990). Hence it is likely that additional inhibitory molecules regulate PRL secretion in the rat (Enjalbert *et al.*, 1978; de Greef and van der Schoot, 1985).

The role of DA as a major hypothalamic molecule inhibiting PRL secretion from the *pars distalis* remains controversial. Infusion of DA in ovariectomised, pituitary stalk sectioned ewes reduces plasma PRL concentration (Thomas *et al.*, 1989; Donnelly and Dailey, 1991). However, DA does not necessarily act directly on the ovine pituitary (Martin, 1985). In sheep, DA cannot be identified in the portal vessels while noradrenalin (NA) is detected. In rats both DA and NA can be detected in the portal vessels. NA may be the major PRL inhibiting factor in sheep while in rats it is likely to be DA (Thomas *et al.*, 1989). It is possible that DA induces the release of other PRL inhibitory factors which then act on the *pars distalis* (Martin, 1985). However, DA receptors do exist in ovine lactotrophs and presumably serve some functional purpose. It is also possible that DA reaches sheep lactotrophs from the alternative route, via short portal vessels or the *pars intermedia* (Ssewannyana and Lincoln, 1990).

The synthesis of DA in the hypothalamus involves the conversion of tyrosine into dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase (the rate limiting step) and then conversion of DOPA to DA (Martin, 1985). DA is synthesised in tuberoinfundibular neurons (TIDA) of the arcuate nuclei (ACN) and periventricular nuclei (PVN) of the hypothalamus. Noradrenergic neurons further process DA into NA. DA is also synthesised in peripheral nerves and in mast cells (Martin, 1985).

The TIDA neurons of the hypothalamus terminate in the *pars intermedia*, neural lobe and the median eminence (Ben-Jonathan, 1985). In the median eminence, 15-20% of the DA synthesised by the TIDA neuron enters the hypophysial long portal vessels and is transported to the *pars distalis* (Ben-Jonathan, 1985; de Greef and van der Schoot, 1985). DA is also synthesised in the neural lobe of the pituitary and reaches the *pars distalis* via the short portal blood vessels. Between 20 and 30% of the blood reaching the *pars distalis* comes via the short portal vessels (Ben-Jonathan, 1985). These two DA pathways are interdependent (Ben-Jonathan, 1985) and may be involved in the regulation of PRL secretion in different physiological systems.

A high affinity and a low affinity D_2 receptor subtype have been identified from DA agonist binding studies. DA antagonists bind only to the high affinity receptor subtype (Ben-Jonathan, 1985). D_2 receptors are also found in the vascular smooth muscle, endocrine glands, kidneys, peripheral nervous system and in the *pars intermedia* of the pituitary. On binding to the receptor, DA is internalised and becomes incorporated into PRL granules (Martin, 1985).

When binding to the cell surface, hormones require a second messenger system to couple the extracelluar signal to the cellular secretory machinery. Guanosine triphosphate (GTP) regulatory proteins (G proteins) are involved with the transduction of the signal generated by the occupancy of the cell membrane receptor by PRL regulatory hormones (Martin, 1985; Kanyicska *et al.*, 1991; Liedo *et al.*, 1991). DA interacts with the G_i protein which is linked to the adenylate cyclase second messenger to suppress PRL synthesis (Cook *et al.*, 1988; Burris *et al.*, 1992). There are a variety of other second messenger systems (Denef, 1988) and three are implicated in D_2 receptor binding. The D_2 receptor is negatively coupled to the adenylate cyclase and Ca^{2+} second messenger systems (White *et al.*, 1989; Elsholt *et al.*, 1991). There is also some evidence that the phosphoinositide phosphorylation pathway is involved with the inhibitory effect DA has on PRL secretion (Jarvis *et al.*, 1988). It is also postulated that the internalisation of DA has a direct autocrine effect

on PRL secretion (Martin, 1985; Lamberts and MacCleod, 1990). The eventual effect of DA binding to the lactotrophs is a reduction in PRL gene transcription (Elsholt *et al.*, 1991). DA also reduces monomeric PRL release from the lactotroph leading to the formation of a less soluble, higher molecular weight isoforms (Shin *et al.*, 1987) with a correlated increase in secretory granule volume (Poole *et al.*, 1991).

Different subpopulations of lactotrophs are variably responsive to DA. In cells differentiated by density, low density lactotrophs were more responsive to DA (Kazemzadeh *et al.*, 1992). However in contrast DA was found to have a greater effect on lactotrophs with large secretory granules (Takahashi, 1992). Lactotrophs from the peripheral regions of the pituitary were unresponsive to DA while those from the central region were highly responsive (Poole *et al.*, 1991) which may arise from variations in the concentration of DA reaching various locations of the *pars distalis* (de Greef and van der Schoot, 1985).

It has recently been discovered that DA can have a stimulatory effect on PRL secretion of the rat lactotroph. A subset of the rat lactotroph population was found to increase PRL secretion when treated with low doses of DA (Burris *et al.*, 1992). This PRL stimulatory capacity of DA is not associated with either D_1 or D_2 receptors which suggests the existence of a third DA receptor subtype (Burris *et al.*, 1991; Hanna and Shin, 1992). It remains to be seen whether stimulation of PRL secretion by DA is physiologically significant.

The suppressive effect of DA on PRL secretion is potentiated by other hormones. For example, oestrogen can decrease the sensitivity of lactotroph DA receptors in rats, but increase the effect of DA in the monkey (de Greef and van der Schoot, 1985). Melatonin decreases the pituitary responsiveness to DA in wallabies (Loudon and Brinklow, 1990).

A variety of both peripheral and pituitary hormones feed back on TIDA activity,

thereby modulating DA secretion. Acute oestrogen administration increases DA concentrations in TIDA neurons while long term oestrogen treatment decreases DA release into the long portal vessels (Martin, 1985). These effects provide a partial explanation for the higher based plasma PRL concentrations in females compared to males. Serotonin also decreases DA synthesis in the hypothalamus (de Greef and van der Schoot, 1985). PRL itself acts via a short loop feedback on the TIDA neurons to increase DA secretion. PRL reaches the hypothalamus by retrograde movement in the long portal blood vessels (Enjalbert *et al.*, 1978; Ben-Jonathan, 1985; Ostrom, 1990).

DA can also potentiate the effects, on the *pars distalis*, of other hormones. The transitory reduction in DA potentiates the response of PRL releasing factors such as serotonin, TRH (Pan and Teo, 1989), oxytocin, vasoactive intestinal polypeptide (Arey and Freeman, 1989), and neurotensin (Martin, 1985; Pan and Teo, 1989; Login *et al.*, 1990; Hanna and Shin, 1992).

Gamma aminobutyric acid (GABA): GABA is an amine which is synthesised from glutamic acid by glutamate decarboxylase. It is a hypothalamic neuromodulator which acts on both the higher brain centres and the pituitary. GABA is transported to the median eminence by the TIDA neurons and from there enters the long portal blood vessels. GABA inhibits PRL secretion from lactotrophs in cell culture and enhances DA secretion from TIDA neurons (de Greef and van der Schoot, 1985; Martin, 1985; Lamberts and MaCleod, 1990). Like DA, GABA concentration is elevated following cerebroventricular injections of PRL (Thomas *et al.*, 1986).

Somatostatin: Somatostatin inhibits PRL secretion from the *pars distalis*. Receptors exist for somatostatin on the lactotroph (Martin, 1985) but the exact mechanism of action of somatostatin is unknown (de Greef and van der Schoot, 1985).

Gonadotropin releasing hormone-associated peptide (GAP): GAP is a precursor of

GnRH and is transported from the median eminence to the *pars distalis* via the long portal vessels. It inhibits PRL secretion in a more potent fashion than DA in rats but may have no effect in sheep (Martin, 1985; Vacher *et al.*, 1991).

1.3.6 Prolactin releasing factors

Oestrogen: In some species, such as primates, PRL secretion is unresponsive to oestrogen treatment (Lamberts and MacCleod, 1990). In others, oestrogen acts on PRL secretion from the *pars distalis* directly and via the hypothalamus. In the hypothalamus of rats, oestrogen modulates TIDA activity and DA concentration via hypothalamic oestrogen receptors (de Groot and van der Schoot, 1985; Lamberts and MacCleod, 1990; Ostrom, 1990). In the rat, short term oestrogen treatment can increase DA concentration in TIDA neurons while long term oestrogen treatment results in a decrease in DA in the long portal vessels. As a consequence female rats have a higher basal PRL secretion rate than males. In addition, PRL concentrations increase in castrated rams when they are treated with oestradiol (Sanford and Robaire, 1990).

In the rat pituitary, there is a two phase response to oestradiol. First, PRL is released rapidly from the lactotroph and, second, PRL gene transcription increases, resulting in a sustained elevation in PRL secretion. The first phase is mediated by the *pars intermedia* and may involve MSH or endorphin. It has been established that lactotrophs close to the termination points of the short portal vessels are more responsive to oestradiol, MSH and endorphin (Porter and Frawley, 1992) than those located at a distance from the short portal vessels. The second phase is mediated directly by oestrogen.

In the *pars distalis*, oestrogen treatment increases the proportion of lactotroph secretory cells with large granules and high PRL secretory capacity and decreases lactotroph sensitivity to DA (Lamberts and MacCleod, 1990). It also increases the

number of PRL secreting cells, by conversion of mammosomatotrophs to lactotrophs, and by proliferation of lactotrophs (Pasolli *et al.*, 1992; Takahashi, 1992). In contrast, oestrogens reduce the bioactivity of the PRL by increasing in the proportion of 'big' PRL produced by rat lactotrophs in culture (Pasolli *et al.*, 1992). These effects could be caused by a reduction in DA uptake by the rat lactotroph rather than by a direct effect of oestrogen (de Groot and van der Schoot, 1985).

Oxytocin: Oxytocin can either stimulate or inhibit PRL secretion. Oxytocin is released into the *pars intermedia* and the neural lobe by hypothalamic neurons and reaches the *pars distalis* via the short portal vessels. Therefore an intact connection to the hypothalamus is required for oyxtocin to affect plasma PRL concentrations (Thomas *et al.*, 1988). Oxytocin acts directly on the *pars distalis* to stimulate PRL release in rats (Arey and Freeman, 1989) but reduces PRL secretion at the level of the hypothalamus by reducing the amount of VIP entering the portal circulation (Mogg and Samson, 1990).

Serotonin: Serotonin is an amine which is synthesised from tryptophan in a two-step process involving the rate limiting enzyme, tryptophan hydroxylase. It is a regulating substance, neurotransmitter and a precursor of other hormones, including melatonin. Serotonin is synthesised in the pineal gland, some brain neurons and mast cells of many species (Martin, 1985). Serotonin causes increases in the activity of MSH-secreting cells in the *pars intermedia* (Martin, 1985), and decreases in DA and increases in TRH and VIP concentrations in the long portal vessels (Martin, 1985; de Greef and van der Schoot, 1985). All these effects have potential implications for the regulation of prolactin release.

Serotonin is a PRL releasing factor associated with suckling in both rats (Tomogane *et al.*, 1992) and sheep (Thomas *et al.*, 1988). Suckling increases serotonin concentrations in the anterior hypothalamic nuclei (Ostrom, 1990). This increase in serotonin concentration elevates PRL secretion in the lactotroph only when there is

an intact hypothalamus (Thomas *et al.*, 1988; Donnelly and Dailey, 1991). Serotonin, does not act directly on the pituitary but rather through some other PRL releasing factor or inhibitory factor (Martin, 1985; Lamberts and MacCleod, 1990).

Thyrotropin-Releasing Hormone (TRH): TRH is a tripeptide (pyroglutamyl-histidyl-prolinamide) which is found in the brain, both in hypothalamic and extrahypothalamic sites (including the pineal gland), spinal cord, and other organs. Little is known about the pathway by which it is synthesised (Martin, 1985) and the site of synthesis. It is a regulator of TSH secretion by thryotrophs in the *pars distalis* and also increases both synthesis and release of PRL from lactotrophs *in vitro* (Hanna and Shin, 1992) and *in vivo* (Bjoro *et al.*, 1990) and elevating plasma PRL concentrations (Donnelly and Dailey, 1991). TRH binds directly to receptors on the lactotroph (Bjoro *et al.*, 1990).

In sheep a continuous infusion of TRH elicits an initial acute elevation in PRL secretion which then falls over a 3 day period (Klindt *et al.*, 1979). Plasma PRL concentrations cannot be repeatedly elevated by repeated daily injections of TRH (Klind *et al.*, 1979). In addition, immunisation of sheep against TRH fails to affect basal PRL concentrations (Fraser *et al.*, 1982) and TRH pulsatility is not associated with PRL pulsatility (Thomas *et al.*, 1989). All this evidence suggests that, while TRH has an initial pharmacological effect on PRL secretion, it is not likely to be a key PRL-regulating molecule in sheep.

The effect of TRH treatment in rats is different. In this species, TRH stimulates the release of proportionally more 'stored' PRL (Ben-Jonathan, 1985; Takahashi, 1992) and newly synthesised PRL than in sheep (Stirling and Shin, 1990). This latter PRL release is sustained over a longer period due to a sustained increase in PRL synthesis by the lactotroph (Niimi *et al.*, 1985; Bjoro *et al.*, 1990).

Binding of TRH in the rat lactotrophs triggers the phospoinositide secondary

messenger system in the first phase of its release pattern and the adenylate cyclase secondary messenger system in the second phase (Bjoro *et al.*, 1990). TRH binding with the membrane, coupled with the required G_{ps} protein, increases intracellular phospholipase and arachidonate concentration (Lamberts and MacCleod, 1990).

Rat lactotroph cell populations respond differentially to TRH treatment. Lactotrophs from the peripheral regions of the *pars distalis* are highly TRH responsive while those in the inner zone respond minimally (Boockfor and Frawley 1987; Takahashi, 1992). Further, TRH responsiveness is altered by the action of other hormones. In rats, DA reduces the release of PRL from lactotrophs following TRH treatment (Enjalbert *et al.*, 1978; Stirling and Shin, 1990; Hanna and Shin, 1992) and the abrupt removal of DA enhances the TRH responsiveness observed during suckling in rats (Martin, 1985; Ostrom, 1990). Pre-treatment of rat lactotrophs with serotonin and VIP has no effect on the subsequent response to TRH treatment (Pan and Teo, 1989). However, simultaneous treatment with VIP potentiates TRH responsiveness (Haisenleder *et al.*, 1988; Pan and Teo, 1989).

TRH has been implicated in suckling- and oestrogen-induced PRL surges in studies using exogenous TRH treatments but controversy remains regarding the biological significance of endogenous TRH to PRL secretion (de Greef and van der Schoot, 1985; Shin *et al.*, 1987).

Vasoactive intestinal polypeptide (VIP): VIP is a polypeptide hormone which is synthesised in the intestinal tract and the brain (Martin, 1985). Within the brain it is synthesised in the hypothalamus and lactotrophs. VIP is a factor which stimulates PRL release ***in vivo*** and ***in vitro*** (Shin *et al.*, 1987). In both an endocrine and autocrine manner (Martin, 1985; Shin *et al.*, 1987). When cultured rat lactotrophs are treated with VIP antibodies, the high rate of spontaneous PRL secretion disappears (Nagy *et al.*, 1988). In the rat, VIP potentiates the stimulatory action of TRH and domperidone (Haisenleder *et al.*, 1988) and is required for increased PRL secretion

following suckling (Abe *et al.*, 1985).

Receptors for VIP on the rat lactotroph are linked to an adenylate cyclase second messenger system (Martin, 1985). Unlike the PRL release profile from rat lactotrophs following TRH treatment, the VIP-induced profile is monophasic and constant (Niimi *et al.*, 1985). High density rat lactotrophs are more responsive to VIP than low density cells (Kazemzadeh *et al.*, 1992).

A physiologic role for VIP in the regulation of PRL in sheep has not yet been established. For instance, VIP has no effect on PRL secretion in either intact or hypothalamic-pituitary disconnected ewes (Thomas *et al.*, 1988).

Endogenous opioid peptides: Endogenous opioid peptides increase PRL secretion from the pituitary (Barb *et al.*, 1991). They act at the level of the hypothalamus, possibly via a temporary reversal of DA inhibition. This DA inhibition is observed in the long portal vessels but not in the neural lobe. Opioids may also stimulate the release of a PRL releasing factor from the hypothalamus as well as acting directly on lactotroph receptors (de Greef and van der Schoot, 1985). Opiates are likely to be important in the stress-induced release of PRL (Martin, 1985).

1.3.7 Autocrine and paracrine factors affecting prolactin secretion

Some hormone-secreting cells produce autocrine or paracrine messengers. Cell-to-cell interactions play a role in the normal regulation of PRL release. Neuropeptides that have been implicated as paracrine regulators include angiotensin II (ANGII), gonadotrophin releasing hormone (GnRH), neurotensin, secretin and VIP. Gonadotrophs and thyrotrophs are required for the PRL stimulatory effect of GnRH and ANGII (Lamberts and MacCleod, 1990). Regulatory endothelin peptides decrease PRL secretion *in vitro* (Kanyicska *et al.*, 1991). Growth factors are synthesised in

the *pars distalis*. Epidermal growth factor (EGF) and fibroblast growth factor (FGF) increase PRL synthesis while, IGF1 has been shown to both increase and decrease PRL synthesis (Lamberts and MacCleod, 1990). Transforming growth factor beta (TGFβ) decreases PRL secretion in GH4 cells by inhibiting PRL gene transcription (Delidow *et al.*, 1991). Basic fibroblast growth factor (bFGF) decreases basal PRL secretion in cultured rat pituitary cells (Larson *et al.*, 1990). Kallidin, a neuropeptide, acts as a paracrine agent which increases PRL secretion by increasing both cell secretion rate and cell number of cultured rat lactotrophs. It acts via the activation of the phosphoinositide second messenger system (Stirland *et al.*, 1991)

1.3.8 Autoregulation of prolactin secretion

PRL exerts a feedback on its own secretion (Milenkovic *et al.*, 1990) both at the hypothalamus (Thomas *et al.*, 1986) and at the pituitary (Bazan and Fernado-Bazan, 1989). Retrograde movement of blood in the long portal vessels carries PRL to the median eminence where it binds to PRL receptors (Chiu *et al.*, 1992). This short loop feedback system results in the elevation of DA and GABA secretion (Kolbinger *et al.*, 1992) in the TIDA neurons (Greef and Schoot, 1985; Lamberts and MacCleod, 1990). Elevation of blood PRL concentration in the blood increases DA synthesis in the median eminence but has no effect on DA synthesis in the neural lobe (Ben-Jonathan, 1985).

PRL synthesis is also autoregulated directly at the pituitary. It has been shown that a build up of PRL in the culture medium reduces the synthesis of PRL from the lactotroph (Bazan *et al.*, 1989). PRL receptors are present in both the *pars distalis* and neural lobe (Chie *et al.*, 1992; Krown *et al.*, 1992).

1.4 SEASONAL REGULATION OF PROLACTIN SECRETION FROM THE PITUITARY

PRL release from the pituitary is highly cyclic over three separate time frames. It is released in pulses which span minutes and also released in characteristic diurnal and circannual patterns. Changes in both pulsatile release and diurnal patterns are associated with the circannual PRL cycle observed in the plasma of animals (Rhind *et al.*, 1991). These circannual cycles are found in most seasonal vertebrates. This suggests that the circannual PRL cycle emerged early during evolution and has remained in photoperiodically sensitive animals (Curlewis, 1992).

Plasma PRL concentrations are characteristically high during summer and low during winter. This pattern is observed in sheep (Pelletier, 1973; Thimonier *et al.*, 1978; Brown *et al.*, 1980; Munro *et al.*, 1980; Kennaway *et al.*, 1983; Leshin and Jackson, 1987; Poulton and Robinson, 1987; Lincoln, 1990) and goats (Buttle, 1973; Mori *et al.*, 1985; Maeda *et al.*, 1988; Tamanin *et al.*, 1988 Grasselli *et al.*, 1992). The capacity to express circannual plasma PRL cycles is apparent in new born animals and cycles are even expressed *in utero* (Ebling *et al.*, 1989; Seron-Ferre *et al.*, 1989; Adam *et al.*, 1992; Basset, 1992).

There is also a seasonal variation in PRL variants. A greater proportion of PRL in winter is the higher molecular weight, disulphide linked PRL (Stroud *et al.*, 1992). This is not detected as readily by radioimmunoassay as the more imnuroreactive and biologically active monomeric form, so exaggerating the extent of the observed circannual pattern of PRL. The lower immunoactivity of 'big' PRL could be due to a lower binding affinity for the primary antibody.

The timing of summer elevation and winter suppression of plasma PRL concentration is similar in most animals. This similarity of pattern was demonstrated in sheep ranging from highly seasonal breeds such as the Mouflon and feral breeds , to less

seasonal domesticated sheep breeds, which were grazed together (Lincoln, 1990). In these sheep, the patterns of plasma PRL concentration identical but there were large differences in the magnitude of the maximum and minimum PRL concentrations achieved during summer and winter respectively. Sheep with less seasonal wool growth patterns had a less marked winter depression in PRL concentrations.

The similarity in the pattern of the PRL cycle between breeds and species indicates control by environmental cues. Both photoperiod (duration of light within 24 hours) and temperature fluctuations (Tamanini *et al.*, 1988; Grasselli *et al.*, 1992) are associated with changing PRL concentrations. High ambient temperatures, in particular, elevate plasma PRL concentration and may be important in summer increases in PRL concentration (Wettemann *et al.*, 1982; Howland *et al.*, 1983). However, temperature cues can be overridden by photoperiod. This is observed under experimentally derived 6 monthly light cycles in which PRL changes mimic the light and not the temperature cycles (Howland *et al.*, 1983).

It is not the spectral properties of light which affect PRL secretion (Petitclerc and Zinn, 1991) . Rather it is the photoperiod which regulates circannual PRL cycles. Long days, up to 16 hours in length, elevate plasma PRL concentrations while short days reduce plasma PRL concentration. However, the duration of signal can be modified by the presence or absence of light in key photosensitive 'windows'. An afternoon photosensitive window has been identified in a variety of species. The presence of light at this time increases plasma PRL concentrations irrespective of preceding periods of dark (Petitclerc and Zinn, 1991). In sheep and cows the photosensitive phase is usually 13-17 hours after dawn (Thomonier *et al.*, 1978; Evans, 1987). In rats and mink the presence of light at 8 to 10 hours after dawn is interpreted as a long day signal (Evans, 1987). Others maintain that there are two photosensitive phases, one in the morning and the other in the afternoon (Occhio and Suttie, 1992).

Plasma PRL concentrations are generally low during the light phase and increase as darkness approaches. In long days, there is a peak in plasma PRL concentration both at dusk and dawn while only a dusk peak exists during short days (Lincoln *et al.*, 1978; Kennaway *et al.*, 1983). In goats, a nocturnal peak of PRL concentration is observed in long days but not under short days (Mori *et al.*, 1985; Maeda *et al.*, 1988). The nocturnal pulse of PRL released in long days is the critical factor in the inhibition of the corpus luteum in the tammer during seasonal quiesense (Hinds, 1989). The assumption that diurnal cycles are regulated in a similar fashion to circannual cycles may be spurious in some species. In pinealectomised sheep, seasonal cycles in PRL are abolished, but the dark-induced peak at dusk remains. In this case, it appears that the circadian and diurnal pattern may be under independent control (Deveson *et al.*, 1992).

The first step in the relay of the photoperiodic message to the pineal gland occurs when light strikes the retina. This light signal is transmitted via the retinohypothalamic tract to the suprachiasmatic nucleus (SCN). The SCN is thought to be the major site at which endogenous circadian rhythms are generated in mammals. PRL exhibits an endogenous circannual cycle which is expressed during constant environmental cues in sheep (Kennaway *et al.*, 1983; Karsh *et al.*, 1989), goats (Mori *et al.*, 1985), and mink (Boissen *et al.*, 1991; Martinet *et al.*, 1992). This endogenous cycle is not synchronous between animals and the amplitude of the cycle varies between individuals and may be absent in some individuals (Curlewis, 1990; Matt, 1993). It is not known whether the SCN is involved with the endogenous circannual PRL cycle. It is clear, however, that light cues are essential for the full and synchronised expression of the seasonal cycle.

From the SCN, the transduced signal travels along neurons to the paraventricular nucleus. This nucleus has been identified as a key centre in the regulation of PRL secretion (see section 1.3.2). From the paraventricular nucleus, neurons pass through the medial forebrain bundle and reticular formation to the intermediolateral cell

column in the spinal cord. These projections innervate preganglionic cells which in turn innervate the superior cervical ganglion (SCG). Post-ganglionic noradrenergic cells in the SCG project to the pineal gland via the inferior carotid nerve. Noradrenaline is released from these nerve endings during dark and binds to receptors which initiate the synthesis and release of melatonin from pinealocytes (Petitclerc *et al.*, 1991). The duration of melatonin secretion provides an internal clock by which changes in photoperiod can be sensed (Kennaway *et al.*, 1987; Chemineau *et al.*, 1988). This simplistic `duration' mechanism is complicated by the existence of the photosensitive window in which the presence of melatonin is interpreted physiologically as long days (Pelletier and Thimonier, 1987; Chemineau *et al.*, 1988). In addition the interpretation of this message is dependent upon the preceding photoperiodic history (Deveson *et al.*, 1992).

The pathway leading from light to melatonin secretion involves the PVN and SCN, both of which are known to be involved in PRL regulation in the pituitary (see section 1.3.2). It is possible that the light signal travelling through these centres affects PRL secretion directly. For instance, in hamsters, light deprivation reduces plasma PRL concentration by 90% while pinealectomy reduces PRL by only 40-50%. However, the light-deprived and pinealectomised animals in that experiment had different levels of oestrous activity which could account for differences in PRL concentrations (Massa and Blask, 1990). There is considerable evidence that circannual changes in PRL concentrations are driven by changes in melatonin secretion (Kusakari *et al.*, 1991). Perhaps the most categorical evidence is the necessity for an intact pineal gland for the expression of photoperiodically aligned changes in PRL concentrations.

The mechanism by which the photoperiod message is processed by the animal is well understood, but how this is transcribed into changes in PRL secretion is poorly understood. It is known that an intact pineal gland is required for the photoperiodic entrainment of PRL secretion (Brown *et al.*, 1980; Munro *et al.*, 1980; Schulte *et al.*,

1981; Synder *et al.*, 1983; Brinklow and Forbes, 1984; Occhio and Suttie, 1992; Matt, 1993). Furthermore it is a change in duration of melatonin secretion which is expressed in changes in plasma PRL concentrations and not the absolute duration of melatonin secretion (Ebling *et al.*, 1989).

Continuous treatment with exogenous melatonin has become a commonly used method of manipulating seasonality in animals. It is thought that continuous melatonin treatment sends the animal a short day signal (O'Callaghan *et al.*, 1991). In the majority of experiments, treatment of sheep (Kennaway *et al.*, 1983; Lincoln and Ebling, 1985; Foldes *et al.*, 1990; Robinson *et al.*, 1992), goats (Prandi *et al.*, 1987; Gebbie *et al.*, 1990), and other species (Adam *et al.*, 1989; Forsberg and Madej, 1990; Petticlerc, 1991; Domingue *et al.*, 1992) with continuous exogenous melatonin suppressed plasma PRL concentrations. This effect is not observed in cattle (Petticlerc, 1991). Sheep and goats may eventually become refractory to this signal at which time the endogenous PRL cycle is expressed (Poulton *et al.*, 1987; Lynch and Russell, 1989; Robinson *et al.*, 1991).

Evidence that melatonin does directly affect PRL secretion is provided by a short period of melatonin infusion to simulate a long photoperiod in goats. These infusions induce the characteristic long day nocturnal peaks of PRL while the long infusions of melatonin ablate nocturnal peaks (Maeda *et al.*, 1988). Furthermore, melatonin infusions in goats (Maeda *et al.*, 1988) and sheep (Robinson *et al.*, 1991; Robinson *et al.*, 1992) can cause changes in plasma PRL concentrations independent of the prevailing photoperiod.

The mechanism by which the short day-melatonin signal is eventually translated to a reduction in PRL synthesis rate from individual lactotrophs and, to a lesser extent, in lactotroph number (Curlewis, 1992; Zinn *et al.*, 1991; Matt, 1993) is unknown. There is a considerable amount of knowledge about the regulation of PRL secretion in physiological states such as pregnancy and lactation (see sections 1.3.4, 1.3.5).

Processes such as lactotroph recruitment, PRL feed back, and hypothalamic regulation, which are observed during pregnancy and lactation, should also be involved in photoperiodic changes in plasma PRL concentrations. In fact, photoperiod-induced changes in PRL do interact with other PRL release systems. For instance, suckling induces greater release of PRL during summer than during spring and the suckling-induced PRL release is greater in the afternoon than in the morning (Stern and Reichlin, 1990; Brunet and Sebastian, 1991; Newbold *et al.*, 1991). Photoperiod-induced changes in PRL are not immediate. Rather, changes occur a week after a switch from short days to long days and are not fully expressed for four weeks (Lincoln *et al.*, 1978; Ryan and Robinson, 1989; Steger and Bartke, 1991). This delay may reflect the requirement for lactotroph recruitment and up-regulation of receptors in the pituitary (Petitclerc, 1991).

Melatonin suppresses PRL secretion from cultured lactotrophs by 10 to 50% and decreases lactotroph sensitivity to TRH, VIP and oestradiol. However, these changes do not approach the magnitude of the *in vivo* seasonal changes in PRL secretion rate. Therefore, the main physiological target of melatonin must be elsewhere (Griffiths *et al.*, 1987). Melatonin receptors exist throughout the brain of the sheep, in the hypothalamus of rodents but not at all in the central nervous system of mustelids. However, all species have high concentrations of receptor binding in the *pars tuberalis* of the pituitary (Williams and Morgan, 1988; Morgan et al., 1989; Bittman and Weaver, 1990; Stankov et al., 1991; Ebling and Hastings, 1992). Five percent of the *pars tuberalis* cells are gonadotrophs and thyrotrophs (Morgan and Williams, 1989; Bittman and Wecue, 1990). The remainder of the unidentified cells have the structural features of peptide secretory cells but as yet the secreted peptide has not been identified. These cells appear to be relatively unresponsive to endocrine perturbations but they do undergo ultrastructural changes in response to photoperiod (Morgan and Williams, 1989). It is possible that the *pars tuberalis* is the site of action for melatonin and causes the release of a yet unidentified secretory product.

Melatonin treatment in certain locations within the hypothalamus will effect changes in PRL secretion from the pituitary. In sheep, treatment with melatonin in the medial basal area of the hypothalamus suppresses plasma PRL concentrations (Lincoln and Maeda, 1992), while in the mink the preoptic-anterior hypothalamus may be a site of melatonin action (Bonnefond *et al.*, 1990; Kaplan *et al.*, 1991). In hamsters and rats it is claimed that the SCN is implicated in the transduction of the melatonin signal to reproductive cycles (Cassone, 1990). However, it does not appear to be an essential area for the action of melatonin in the photoperiodic control of prolactin secretion in mustelids (Bonnefond et al., 1990; Maywood et al., 1990).

Given the current knowledge of PRL regulation, it is reasonable to look to the hypothalamus as a source of melatonin-induced modulatory molecules. Hypothalamo-pituitary disconnection increases PRL concentrations by ten fold in winter but by only two fold in summer (Thomas et al., 1986).

Studies have examined the seasonal aspects of a variety of hypothalamic neuromodulators. The hypothalamic hormones which may be involved with the seasonal plasma PRL cycle include DA, TRH, serotonin, opioids and GABA. GABA has been implicated in seasonal PRL cycles because high concentrations of GABA occur in the hypothalamus of mink during short days (Boisin *et al.*, 1991). In the rabbit, cortex melatonin treatment potentiates the action of GABA on neural activity (Stankov *et al.*, 1992). Endogenous opioids augment the inhibitory activity of DA in short days (Ssewannyana and Lincoln, 1990). However opioid antagonists do not elevate PRL secretion during long days and are unlikely to be the cause of increased PRL secretion during summer (Schillo *et al.*, 1985). Neither is there any evidence to suggest that serotonin or TRH drive seasonal PRL cycles. Serotonin levels in the arcuate nucleus are unaffected by long days (Zinn *et al.*, 1992) and the long-day increase in plasma prolactin concentrations cannot be accounted for by increases in TRH secretion in the ewe (Leshin and Jackson, 1987). However, the prolactin response to TRH is greater in ewes during long days (Howland *et al.*, 1983).

Dopamine is a potent PRL inhibiting factor (see section 1.3.3) but there is conflicting evidence regarding its role in seasonal regulation of PRL concentrations in plasma. While reductions in dopamine concentration in the hypothalamus have been identified during long days (Ssewannyana and Lincoln, 1990) others have failed to find such differences (Zinn *et al.*, 1991; Matt, 1993). In mink, tyrosine hydroxylase concentrations were found to be high during short days (Boisin *et al.*, 1991). In fact, in long days, dopaminergic activity is enhanced in the median eminence (Curlewis, 1992; Matt, 1993) and is not likely to be contributing to the long day elevations in plasma PRL concentrations (Steger and Bartke, 1991). Dopamine inhibited PRL secretion equally in both long and short days in Soay rams (Ssewannyana and Lincoln, 1990). Dopamine immunisation increases PRL secretion in both summer and winter (Matt, 1993). Interestingly, pinealectomy of hamsters prevented the efficacy of a DA agonist but not of the antagonist in altering plasma PRL conentrations under a short-day photoperiod. In female hamsters, some aspects of the short-day induced decrease in PRL synthesis are independent of the pineal gland (Blask *et al.*, 1986) and may involve direct photoperiod-dependent changes in neuropeptides with prolactin-releasing properties. The recent discovery that low dose concentrations of dopamine stimulate PRL release via a D1 receptor (Curlewis, 1992) does not provide a mechanism by which dopamine regulates seasonal PRL cycles. If dopamine does have an effect on seasonal PRL cycles it must either interact with another regulatory compound or act in a pathway independent of the long portal vessels. The short portal vessels are a potential source of dopamine (Section 1.3.3). To date, dopamine concentrations have not been determined in these vessels.

There may be seasonal cycles in lactotroph sensitivity to DA. In hamsters afternoon injections of melatonin increase the lactotroph sensitivity to dopamine suppression (Steger and Gay-Primel, 1990). Some groups have identified seasonal responses to dopamine antagonists and agonists (Curlewis, 1988; Zinn *et al.*, 1991) but this is not universal (Badura and Goldman, 1992). The role of dopamine in the seasonal regulation of PRL remains perplexing. The few studies that have been conducted

examining dopamine and seasonal PRL cycles have been conducted have involved a wide range of species so it is not surprising that a clear picture has yet to emerge.

The effect of steroids on seasonal PRL release is controversial, with some claiming no effect (Matt, 1993) while others claim that plasma PRL concentrations are increased during darkness in ovariectomized ewes. In ovariectomised ewes, melatonin and oestradiol treatment partially negate this effect (Maxwell *et al.*, 1988). However, as discussed previously, the night surges of PRL may be under control independent to that of circannual PRL cycles. As gonadectomy and adrenalectomy have no effect on the timing of the PRL cycles it seems unlikely that steroids are mediators of the seasonal PRL cycle (Curlewis, 1992).

PRL is regulated by a short loop-feed back and the loop sensitivity could potentially vary with season (see section 1.3.8). However, intracerebroventricular injections of PRL are equally effective in suppressing plasma PRL concentrations in summer and winter. The high PRL concentrations in summer are not due, therefore, to the absence of short-loop feedback control (Curlewis and McNeilly, 1991).

In conclusion, circannual PRL cycles are generated primarily by changes in photoperiod. Photoperiod is translated into a melatonin message which affects PRL secretion. However at this stage it has not been established how melatonin affects PRL secretion in the *pars distalis*. It is possible that melatonin regulates PRL secretion by the secretion of an as yet unidentified compound synthesised in the *pars tuberalis*.

1.5 PROLACTIN AND FIBRE GROWTH

1.5.1 Introduction

PRL has a role in regulating a wide variety of physiological processes in organs of

epidermal origin (Lamberts and MacCleod, 1990; Paus, 1991). Examples include the production of milk in the mammary gland (Onstrom, 1990), secretory activity in the pigeon crop (Rillema *et al.*, 1988); thymulin production in epithelial thymus cells (Dardenne *et al.* 1989); sebum production in the sebaceous gland (Paus, 1991); proliferation of lymphocytes (Skwarlo-Sonta, 1992); and fibre growth cycling in the mammalian hair follicle (Badura and Goldman, 1992; Ibraheem *et al.*, 1993). Many cells of epidermal origin possess PRL receptors and the hair follicle is no exception (Choy *et al.*, 1995).

In addition to PRL secreted from the *pars distalis* region of the pituitary gland, PRL-like compounds are synthesised by uterine tissue, peripheral lymphocytes, sweat glands and connective tissue (Chaptis *et al.*, 1989; Paus, 1991; Sabharwal *et al.*, 1992; Arkins *et al.*, 1993). Accordingly, PRL may act as an endocrine, paracrine and autocrine modulator of epithelial cell growth. It is hypothesised that many substances (eg. VIP, EGF, substance P, histamine) produced in the skin could also affect PRL secretion in the pituitary and in part regulate plasma PRL concentrations (Morhenn, 1988; Paus, 1991).

It is the objective of this section to review in depth the effects of PRL on fibre growth cycling in small mammals, goats, deer and sheep and review the mode of action of PRL in the immune system and mammary gland and Other photoperiodically dependent hormones, which may also affect fibre growth cycling, are beyond the scope of this review.

1.5.2 Prolactin and fibre growth cycling in small mammals

Many small mammals undergo an annual cycle in pelage colour and characteristics, exhibiting darker, less dense and shorter pelages in summer and whiter, denser and longer pelages in winter (Smale *et al.*, 1988). In small mammals the provision of the appropriate seasonal pelage involves seasonal synchronisation of keratinocycle

production, melanogenesis and follicle activation.

In 1935, it was first noted that the timing of the transition from one pelage to another (moulting) in ferrets was related to day length (Bissonnette, 1935). In mustelids, increasing day length prompts the spring moult while decreasing day length stimulates the autumn moult (Martinet *et al.*, 1984). The disconnection of the melatonin-producing pineal gland prevents photoperiodically induced moulting in foxes, voles and hamsters, indicating the pivotal role of melatonin in seasonal changes in the pelage (Duncan and Goldman, 1984; Smale *et al.*, 1988). However, in voles pinealectomy has no effect on follicle density and therefore not all facets of seasonal pelage recruitment are regulated via melatonin (Smale *et al.*, 1988). Treatment with systemic exogenous melatonin, in small mammals, induces moulting of the summer fleece and regrowth of a fleece with winter colouration and length (Rose *et al.*, 1987; Slayden *et al.*, 1989).

Disconnection of the hypothalamus, the major regulator of the pituitary, has a similar effect to pinealectomy in negating seasonal moulting in mink and ferrets (Martinet *et al.*, 1984; Badura and Goldman, 1992). In mink (Martinet *et al.*, 1983; Martinet *et al.*, 1992), hamsters (Badura and Goldman, 1992), and voles (Smale *et al.*, 1988), increasing and decreasing plasma PRL concentrations are associated with spring and autumn moults respectively in both natural and photoperiodically induced fibre growth cycles. Even when mink are kept under constant photoperiod, moulting is observed only after 19-25 weeks when plasma PRL concentrations eventually spontaneously increase (Martinet *et al.*, 1992). In hamsters, exposure to several weeks of short-day photoperiods of less than 10 hours of light in a 24 hour period (10L:14D) reduces plasma PRL concentrations to below 10 ng/ml and subsequently induces the growth of a winter pelage (Badura and Goldman, 1992). Plasma PRL concentrations also increase approaching parturition which serves to prompt the development of the mammary gland and onset of milk secretion (Ostrom, 1990). In ferrets, an out-of-season lactation also results in an out-of-season moult (Rust and Shackelford, 1965).

PRL release from the *pars distalis* region and the pituitary is largely under the control of the inhibitory, hypothalamic, metabolite chormone dopamine (section 1.3.5). Treatment of hamsters and foxes with bromocryptine (a dopamine agonist) during spring delays the spring moult and subsequent development of the summer pelage (Smith *et al.*, 1987). In hamsters (Badura and Goldman, 1992) and mink (Rose *et al.*, 1987), treatment with bromocryptine during short, or declining, day length accelerates the growth of the winter pelage, while treatment with pimozide (a dopamine antagonist) prevents the development of the winter pelage following a switch from long to short day length (Badura and Goldman, 1992).

Further evidence for plasma PRL concentration being a major trigger for pelage cycling in small mammal, comes from the direct administration of bioactive PRL. Chronic, continuous treatment with exogenous PRL prevents the moulting of the summer fleece in short-day treated hamsters (Duncan and Goldman, 1984) and stimulates anagen and visible melanogenesis indicative of summer pelage growth in hamsters (Duncan and Goldman, 1984) and mink (Badura and Goldman, 1992) maintained in short days.

Melanin pigments, which are responsible for differences in pelage colouration, are produced by follicular melanocytes. The pigments are transferred into the keratin-fibre-producing keratinocytes during anagen (Slominski and Paus, 1993). In fact, in the mouse, a decline in melanogenesis is one of the earliest, detectable markers for the onset of catagen (Strail *et al.*, 1961; Slominski and Paus, 1993). It is thought that white winter pelages are produced in the absence of melanogenesis. However, the current method for the identification of active follicular melanocytes relies on the visualisation of pigment granules (Slominski and Paus, 1993). It is possible that melanocytes are active but not producing dark melanin pigments. In hamsters, tyrosinase, a key enzyme in melanin production, is found to be equally high during the growth of pigmented summer and light winter pelage (Ebling and Hale, 1983). It is hypothesised that melanocytes may regulate fibre growth cycling in small

mammals by the synthesis of growth factors (Slominski and Paus, 1993) and that PRL may be involved with this process. High plasma PRL concentrations stimulate the production of visible melanin pigmentation while bromocryptine inhibits melanogenesis in isolated hair follicles of young mice (Rollag and Adelman, 1992).

Clearly, changes in plasma PRL concentrations modify seasonal pelage growth in small mammals. Elevated plasma PRL concentrations promote the growth of summer pelages while low plasma PRL concentrations encourage the growth of winter pelages. Small mammals are responsive to PRL manipulation irrespective of the stage of pelage growth, which demonstrates that the seasonal pelage growth cycle is largely under endocrine control and endogenous follicle growth is of lesser importance.

1.5.3 Prolactin and fibre growth in goats

The down growth cycle (see detailed description in Section 1.2) initially appears more complex than the pelage replacement cycle of the small mammals described in the preceding section. Growth of down from secondary follicles can be separated into five distinct events, (Figure 1.12) any of which may be responsive to photoperiodically based endocrine signals. The five down events are described below with their associated photoperiod and plasma PRL concentrations:

1. **The spring anagen phase.** This occurs during declining photoperiods from the summer to winter solstice (Nixon *et al.*, 1991; Henderson and Sabine, 1992). The greatest growth rate of down occurs during reducing photoperiods associated with the autumn equinox (McDonald *et al.*, 1987; Betteridge *et al.*, 1988) and rapidly reducing plasma PRL concentrations (Kloren and Norton, 1993c).

2. **The catagen and telogen phases which signal the end of the growth of the winter down fleece.** After the autumn equinox, photoperiod slowly declines

approaching the winter solstice. Plasma PRL concentrations decline to minimal levels (Kloren and Norton, 1993c) and secondary follicles progressively enter catagen and then telogen (McDonald *et al.*, 1987; Betteridge, 1988).

3. **The loss of fibres from telogen follicles.** Photoperiod increases rapidly approaching the spring equinox and plasma PRL concentrations begin to increase (Gebbie *et al.* 1992a; Kloren and Norton, 1993c). Fibres are shed from telogen follicles, possibly in association with the anagen phase of the summer down fleece.

4. **The anagen phase which produces the summer down fleece.** Secondary follicles undergo a short asynchronous anagen phase which produces short vellus fibres of less than 5 mm length in at least some of the secondary follicles (Nixon *et al.*, 1991). The summer anagen phase occurs after the spring equinox during rapidly increasing photoperiod and plasma PRL concentrations (Nixon *et al.*, 1991).

5. **The catagen, telogen phase and shedding of the summer down fleece.** Growth of the summer fleece ceases and summer down fibres may be shed following, in comparison to the winter fleece, a greatly shortened period of telogen (Nixon *et al.*, 1991). This occurs as photoperiod and plasma PRL concentrations approach maximal values around the summer solstice (Kloren and Norton, 1993c).

There is currently no published information examining the association between changes in plasma PRL concentrations and down events 1 to 5. In a study of only the winter down fleece, increases in plasma PRL concentrations, after the spring equinox, were associated with the loss of fibres from telogen follicles (Kloren and Norton, 1993c).

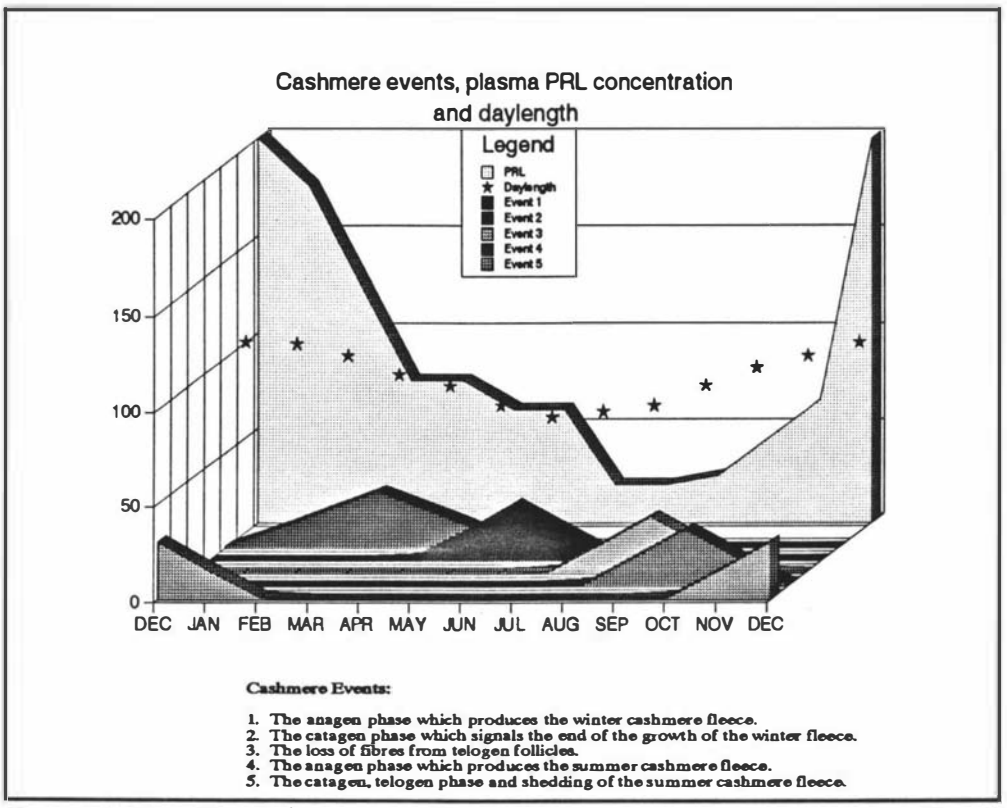

Figure 1.12: Pictorial representation of frequency of occurence in follicles of cashmere events summarised from Section 1.3, plasma PRL concentrations (ng/ml) (Kloren, 1991) and day length (% relative to December in Palmerston North, New Zealand).

Growth of fibre from primary follicles, in the down-producing goat, follows a relatively simple annual pattern (Section 1.2). Active fibre growth occurs from the spring equinox, during increasing plasma PRL concentrations, and ceases after the autumn equinox associated with rapidly falling plasma PRL concentrations (Ryder, 1970; Betteridge *et al.*, 1988). The primary follicles undergo a relatively long period of telogen, compared to secondary follicles, and shed fibres a month later than secondary follicles. The loss of fibres from primary follicles is associated with the growth of new fibres during the spring equinox (McDonald *et al.*, 1987; Betteridge *et al.*, 1988).

Fibre growth of secondary follicles of down-producing goats is less responsive to nutrition (Ash and Norton, 1984; Johnson and Rowe, 1984; Ash, 1986; McCall and Fitzgerald, 1987) but highly responsive to photoperiod (McDonald and Hoey, 1987; Norton, 1991; Gebbie *et al.*, 1991b) and endocrine manipulation (Betteridge *et al.*, 1989; Litherland *et al.*, 1990; Lynch and Russell, 1990a; Gebbie *et al.*, 1992a, 1992b). Pregnancy and lactation, noted for elevations in plasma PRL concentrations, have little effect on either guard hair growth or linear growth rate of down but do affect secondary follicle activation (Kloren and Norton, 1993b). With pregnancy and lactation in summer, the increase in down length indicative of winter fleece growth is delayed (Graham, 1988; Kloren and Norton, 1993b). It is not clear whether this is because of an extension in the summer fleece growth period or a delay in the activation of follicles for winter fleece growth. Kidding in winter advances the reduction in down length symptomatic of shedding of the winter down fleece (Kloren and Norton, 1993b).

In down-producing goats, treatment with melatonin induces an out-of-season shedding of down following the cessation of treatment (Litherland *et al.* 1991). A continuous melatonin treatment is interpreted by the animal as a constant, short day signal following which the subsequent natural photoperiods are perceived as an increase in day length (O'Callaghan *et al.*, 1991). After cessation of melatonin treatment, plasma PRL concentrations increase (Parry *et al.*, 1992) and may be the trigger for out-of-season shedding.

The continuous treatment of down-producing goats with exogenous melatonin during spring advances the growth of the guard hair and extends the anagen phase of the spring down cycle. The long down fleece is shed in autumn and simultaneously regrows (Litherland *et al.*, 1991; Gebbie *et al.*, 1992). This second long down fleece is shed one month later than a normal winter fleece (Betteridge *et al.*, 1989; Litherland *et al.*, 1991; O'Neill *et al.*, 1992). Spring treatment of down-producing goats with melatonin (Lynch and Russell, 1989; Nixon *et al.*, 1993) and at other times

of the year (Mori and Okahama, 1986; Prandi *et al.*, 1987; Maeda *et al.*, 1988; Gebbie *et al.*, 1990) suppresses plasma PRL concentrations during the treatment period. The only contrary report follows the treatment of down-producing goats with melatonin from the winter solstice until the autumn equinox. In that study, plasma PRL concentrations increased earlier in treated compared with control goats and the winter fleece was shed earlier (Lynch and Russel, 1989). But the melatonin treatment regime was inappropriate as the interval between melatonin implant replacement was longer than the published efficacy periods for the implants (Parry *et al.*, 1990).

The absolute change in plasma PRL concentrations, associated with spring melatonin treatment, is small. For example, in goats treated with exogenous melatonin in September, plasma PRL concentrations are reduced from 15 ng/me (controls) to 2 ng/ml. However, this change is still associated with a early stimulation of guard hair growth (Nixon *et al.*, 1993) and winter down growth (Litherland, unpubl.). It can be hypothesised that the **rate** of change in plasma PRL concentrations may be an important physiological trigger. A reduction of 13 ng/ml over a two to three day period is similar to the rapid daily reduction in plasma PRL concentrations which occurs naturally during autumn (Kloren et al., 1993c).

Bromocryptine, a dopamine agonist (see Section 1.3), has been used to suppress the spring rise in plasma PRL concentrations in down-producing goats. In goats treated with bromocryptine from early spring to the summer solstice, down fibres continue to lengthen and shedding is delayed (Lynch and Russel, 1990a; Kloren and Norton, 1993d). Following cessation of the bromocryptine treatment, the down fleece appears to undergo a rapid shedding with a concurrent initiation of winter fleece growth (Kloren and Norton, 1993d). In the untreated fleece, there is an extended period between shedding of the old winter fleece and growth of the new winter fleece. In this period a vestigial summer down fleece grows (Nixon *et al.*, 1991). In the above reviewed experiments, no measurements have quantified either follicle activity changes or the presence of down growth. This makes it difficult to interpret the

effects on down growth of suppressing spring rises in plasma PRL concentrations.

Bromocryptine treatment from January until June has no effect on length growth rate of down when winter down fleece growth has already been activated. However, in a goat in which winter down fleece growth has not yet started, bromocryptine treatment completely suppresses down length for the duration of winter (Kloren and Norton, 1993d) suggesting that an increase in plasma PRL concentration is required to activate follicles. Guard hair growth is unaffected by either bromocryptine treatment regime (Kloren and Norton, 1993d).

In Saanen goats treated with long days (20L:4D) for 8 weeks in spring, subsequent melatonin treatment, but not bromocryptine treatment, stimulated winter fleece growth (Gebbie *et al.*, 1992a). It is possible that declines in plasma PRL concentrations act in synergy with another melatonin-dependent hormone, to stimulate winter fleece growth.

Subcutaneous injections of ovine PRL with and without concurrent bromocryptine treatment advance the spring moult in down-producing goats (Lynch and Russel, 1990). Following the addition of PRL in excess of 200 ng/ml to the media, the length growth rate of cultured secondary down-producing follicles is increased by 20% (Ibraheem *et al.*, 1993).

In conclusion, there is sufficient evidence presented above to show that plasma PRL concentration does have an effect on down growth, particularly in the period bounded by the spring equinox and summer solstice. Any model to account for these data should take into consideration the complexities of the interactions between treatment regime, pretreatment follicle growth stage and photoperiodic message interpretation by the goats. A simplistic model is that increasing plasma PRL concentrations cause the shedding of the winter fleece and growth of the summer fleece, while declining plasma PRL concentrations cause the shedding of the summer fleece and growth of

the winter fleece.

To date, there is a stronger case for changes in plasma PRL concentrations inducing winter down fleece shedding than for them stimulating winter down growth, while summer fleece growth is yet to be studied. It also unclear whether magnitude, rate of change, or direction of change in plasma PRL concentrations affords the stimulus for down growth changes. There is some evidence that down growth could be regulated by plasma PRL concentrations in synergy with another photoperiodically mediated hormone. More information is required before the relationship between plasma PRL concentrations and down growth can be described.

1.5.4 Prolactin and fibre growth of deer

As in goats, the coat of the deer undergoes intervals of synchronous growth and shedding which results in a characteristic downy winter undercoat (Webster and Barrell, 1985). The growth and shedding of antlers of deer also follows a photoperiodically dependent cycle which can be disturbed by pinealectomy (Synder *et al.*, 1983).

The timing of the seasonal PRL cycle is similar in many different species, including deer (Lincoln, 1990; Curlewis, 1992; Grasseli *et al.*, 1992). However, in Père David deer (*Elaphurus davidianus*) plasma PRL concentrations increase two months earlier than in the red deer (*Cervus elapus*). Interestingly, in comparison to red deer, summer coat growth of the Père David deer is also advanced by two months (Loudon *et al.*, 1989).

Melatonin treatment of red deer after the summer solstice results in a 'patchy' appearance of the winter fleece (Milne *et al.*, 1990). It is likely that this patchy appearance is caused by an out-of-season shedding which is also observed in down-producing goats treated with melatonin at the same time (Litherland *et al.*, 1991).

Winter coat growth (Bubenik *et al.*, 1985; Webster and Barrell, 1985; Milne *et al.*, 1990) and antler growth (Bubenik *et al.*, 1985) of deer is stimulated by bromocryptine treatment, while spring bromocryptine treatment delays the development of the summer coat (Curlewis *et al.*, 1988). In red deer, treatment with domperidone (a dopamine antagonist) in autumn increases plasma PRL concentrations but has no effect on coat growth (Milne *et al.*, 1990).

The linear fibre growth rate of summer red deer hair follicles actively growing *in vitro* is increased by 17% when 100 ng/ml of PRL is added to the media (Thomas *et al.*, 1993). Combined PRL and triiodothyronine treatment produces an apparent synergistic stimulation of hair shaft growth.

1.5.5 Polactin and fibre growth in shedding sheep breeds

Most sheep studied of shedding breeds such as the Soay, Mouflon and Wiltshire, goats, have a simple fibre growth cycle with one period of catagen, telogen and anagen annually (Ryder, 1978; Lincoln *et al.*, 1980; Parry *et al.*, 1992). In most Wiltshire sheep small subsidiary fibre growth cycles are observed during summer (Parry *et al.*, 1992; Pearson *et al.*, 1993; Person et al, unpub).

The seasonal changes in fibre growth, in shedding sheep, are dependent on photoperiod (Lincoln *et al.*, 1980). Ganglionectomy disrupts the function of the pineal gland and interferes with the synchrony between photoperiod and PRL secretion from the *pars distalis* region of the pituitary (Lincoln *et al.* 1979) as well as render the fibre growth cycle less responsive to photoperiod (Lincoln, 1980).

As in down-producing goats, the growth of fleece in shedding sheep ceases after the autumn equinox following rapidly declining plasma PRL concentrations (Parry *et al.*, 1992). Regrowth begins around the spring equinox though some follicles become active prior to the spring increase in plasma PRL concentrations (Ryder, 1978;

Pearson *et al.*, 1993). However, active follicles enter catagen and fibres are shed from telogen follicles, when plasma PRL concentrations increase in spring (Parry *et al.*, 1992). As PRL receptors have been identified in the Wiltshire wool follicle (Choy *et al.*, 1995) it is likely that PRL acts directly on the wool follicle to induce catagen.

In Wiltshire sheep, during natural or induced short photoperiods (8L:16D), plasma PRL concentrations remain below 20 ng/ml (Pearson *et al.*, 1993, 1994). However, following a switch from short to artificial long or natural long but declining day length, plasma PRL concentrations increase by five-fold. When switched to constant long days (16L:8D) follicle activity is reduced and remains suppressed until the cessation of long day treatment. However, when the sheep are released into long but naturally declining day lengths, the majority of follicles enter a condensed period of catagen, telogen and fibre loss approximately one to two months after release (Parry *et al.*, 1993; Pearson *et al.*, 1993). The follicles then reactivate and resume fibre growth. If the duration of the preceding short day treatment is longer than 3 months then changes in the follicle growth phase are highly synchronous both within and between animals.

Melatonin treatment of Soay sheep alters the timing of shedding (Lincoln and Ebling, 1985). In Wiltshire sheep, melatonin treatment during spring maintains both follicle activity and plasma PRL concentrations at low levels. However, after melatonin treatment ceases, plasma PRL concentrations and follicle activities increase (Parry *et al.*, 1992). If Wiltshire sheep are treated with bromocryptine following a switch from short to longs, follicles fail to enter catagen until after cessation of the bromocryptine treatment (Pearson *et al.*, 1993).

To date circumstantial evidence indicates that PRL may have a role in regulating fibre growth in shedding sheep breeds. It is claimed by Pearson *et al.*, (1993, 1994) that "increases in plasma PRL (in Wiltshire sheep) are inhibitory to follicle growth and

that entrainment of the pelage cycle with season is an outcome of the associated induced follicle growth cycle". The dilemma is that, following an abrupt artificial change from short to long days, plasma PRL concentrations increase, and catagen is provoked whereas catagen normally occurs during times of decreasing plasma PRL concentrations.

1.5.6 Prolactin and fibre growth in non-shedding sheep breeds

While domestic breeds of sheep, such as the Romney, do not display a visible fleece shedding they do have a photoperiodically dependent rhythm in rate of wool growth with a trough in winter and a peak in summer (Ryder and Stephenson, 1968; Hutchinson 1976; Hawker *et al.*, 1985). Domestic sheep also possess a seasonal cycle in plasma PRL concentrations which is similar in timing, though not in amplitude, to that of shedding sheep breeds (Lincoln *et al.*, 1990). As in shedding sheep breeds, the wool growth pattern of domestic sheep is correlated to the cycle in plasma PRL concentrations (Dolling *et al.*, 1986). The winter wool growth suppression in Romney sheep is predominantly the result of a decrease in mitotic rate in the follicle bulb and a reduction in the proportion of proliferating follicular cells entering the fibre. In domestic sheep there is only a small increase in telogen follicles during winter (Holle *et al.*, 1993) and the selection process has greatly extended the anagen phase of the annual fibre growth cycle.

In a study of modern and shedding sheep breeds and their crosses it has been found that high winter plasma PRL concentrations are associated with more continuous wool growth during winter (Lincoln, 1990; Pearson *et al.* 1994). An increase in winter wool growth relative to summer wool growth is one of the consequences of selection for high fleece weight in Romney sheep (McClelland *et al.*, 1987; Hawker *et al.* 1988; Wuliji *et al.*, 1993). Provisional data indicate that the amplitude in seasonal PRL concentrations is reduced in high, compared to low, fleece weight lines of Romney sheep (Clarke *et al.*, 1993). These reductions in the amplitude in seasonality

of plasma PRL concentrations may be an associated effect and not the direct cause of the increase in winter wool growth seen in sheep selected for high fleece weight.

Melatonin, the endocrine mediator of the seasonal clock, is associated with most seasonal cycles (see reviews by Bittman, 1984; Petitclerc and Zinn, 1991; Deveson et al., 1992; Occhio and Suttie, 1992). However, to date, the seasonal wool growth cycle in domestic sheep has been surprisingly resistant to melatonin treatment (Kennaway and Gilmore, 1985; Harris *et al.*, 1989; Foldes *et al.*, 1990). Melatonin treatments given at each of 6 weekly intervals throughout the year had no effect on wool growth of Merinos (Foldes *et al.*, 1990) and melatonin administered in November had no effect on wool growth in Romney sheep though there was a tendency for an increase in grease production (Harris *et al.*, 1989). There is some evidence that melatonin and PRL have a role in the initiation of follicles during fetal development of modern sheep breeds. Suppression of maternal (and foetal) melatonin concentrations by immunization against melatonin increases the rate of secondary follicle development in Merinos (Foldes *et al.*, 1991), while continuous treatment of Merino ewes with exogenous melatonin retards follicle development (Foldes *et al.*, 1991). In fetal Merino skin grafted on to nude mice, the number of secondary derived follicles was increased three-fold following PRL infusion (McCloughry *et al.*, 1992).

Considerable shedding in the largely aseasonal Merino and other domestic sheep breeds occurs soon after birth when coarse hairs in primary follicles tend to moult. Maternal plasma PRL concentrations are elevated in late gestation (Peterson *et al.*, 1991) but it is not known whether inceases in plasma PRL concentration cause birth-coat shedding in young lambs. The processes of pregnancy and lactation depressed annual wool growth, compared to that of non-pregnant sheep, by up to 28% (Sumner and McCall, 1989; Betteridge *et al.*, 1992).

Bromocryptine treatment has no effect on the wool growth (Dolling *et al.*, 1986) of domestic sheep breeds when treatment is administered in late summer through to winter (McCloghry *et al.*, 1993) or from winter to spring (Curlewis *et al.*, 1991). However, in the both these latter experiments, the spring increases in plasma PRL concentrations were not completely negated. In lambs treated with bromocryptine and PRL in combination with various light regimes it is found that the weight of the pelt is inversely related to circulating PRL concentrations (Eisemann *et al.*, 1984).

Neither the administration of exogenous PRL in hypophysectomized sheep (Ferguson *et al.*, 1965) nor the 4 day infusion of PRL directly into the skin (Kelly *et al.*, 1993) of Romneys has any effect on wool growth. In addition, English Leicester and Drysdale wool follicles maintained *in vitro* are unresponsive to the inclusion of PRL in the medium in concentrations ranging up to 10,000 ng/ml (Winder pers comm.). Downes and Wallace (1964) found that low and high doses rates of intradermally injected PRL increased wool length growth rate and cysteine uptake while intermediate doses decreased wool length growth rate. PRL receptors have been located on the wool follicle of the Romney sheep (Choy *et al.* 1995) but to date their function is unknown.

In conclusion, there is limited evidence that changes in plasma PRL concentrations regulate the seasonal wool growth pattern in modern sheep breeds. This is perhaps not surprising, as the main role of PRL in other breeds appears to be regulation of the catagen and telogen phases. It is these phases which are either entirely missing or greatly shortened in the follicle of sheep of modern breeds.

1.5.7 Prolactin and the immune system

It is now known that fibre growth cycling is associated with changes in the activity in components of the immune system in skin. In mouse follicles in which hairs have

been plucked to initiate anagen, mast cell concentrations increase during early anagen and low concentrations of histamine and high concentrations of macrophages occur during catagen (Ebling and Hale, 1983; Gibson *et al.*, 1991). The most visible involvement of the immune system in fibre growth cycling occurs during catagen when the components of the follicle below the level of the sebaceous gland are surrounded by macrophages and resorbed (Gibson *et al.*, 1991). It has recently been claimed that this is an active process involving the recognition of class II Major Histocompatibility markers which protect the follicle from the immune system above the level of the sebaceous gland. Following an as yet unspecified catagenic trigger, the protective sheath, possibly made of proteoglycans, is disrupted, allowing macrophages to attack cells which do not possess the appropriate expression of self (Gibson *et al.*, 1991; Westgate *et al.*, 1991; Messenger, 1993). In the mice, PRL administration increases the capacity of the mammary gland to attract and retain antibody-containing lymphoblasts (Shiu and Friesen, 1980). PRL could also serve a similar role in fibre growth by 'attracting' immune cells such as macrophages to the catagen follicle.

Another example of the link between the immune system, PRL and fibre growth is found in the athymic or nude mouse. In the athymic mouse, both fibre growth and function of the thymus (a gland largely concerned with immune regulation) impaired and plasma PRL concentrations are low. If thymi are implanted into the nude mouse, plasma PRL concentrations return to normal (Skwarlo-Sonta, 1992). Hair growth can be stimulated in the nude mouse by the immunosuppressive drug Cyclosporine A (Paus *et al.*, 1989). Cyclosporine A (CsA) inhibits the function of immune cells and in mice and humans, and provokes anagen in telogen hair follicles (Paus *et al.*, 1989). PRL and CsA are thought to compete for a common receptor (Paus, 1991) and CsA acts on keratinocytes, primarily through its action on cytokine-producing T cells (Elder, 1993). There have been few studies of the effects of cytokines on *hair* growth, but one, EGF is inhibitory (Duncross, 1993).

Psoriasis, a common skin disease of man, is characterised by abnormal epithelial proliferation and hair formation and involves keratinocytes, langerhans cells, dermal fibroblasts, endothelial cells and T lymphocytes (Paus, 1991). The clinical signs of this disease can be reduced by treatment with either bromocryptine or CsA, implicating PRL in this disease (Paus and Link, 1988; Morkawa *et al.*, 1993).

Lymphocytes move in and out of the skin in response to immune and possibly other signals. Bromocryptine treatment impairs antibody production, reduces thymus weight, and decreases macrophage and lymphocyte activity (Morkawa *et al.*, 1993). Exogenous PRL treatment reverses these effects (Skwarlo-Sonta, 1992;Arkins *et al.*, 1993). In addition, lymphocytes are capable of synthesising their own PRL-like molecule which may have some autocrine function (Sabharwal *et al.*, 1992; Arkins *et al.*, 1993). Therefore PRL may participate in the regulation of the immune system as an endocrine, paracrine or autocrine regulator.

PRL receptors have been found on T and B lymphocytes. However, at only 360 receptors per cell, the receptor expression is low compared to that of liver and mammary gland (Arkins *et al.*, 1993). Nevertheless, there is increasing evidence of a role for PRL, other somatolactogens and IGF-1 in adult lymphocyte function and T and B lymphocyte development (Paus, 1991).

The action of PRL on lymph cells has been studied in the mutant Nb2 lymph node line of rats. Following binding to the PRL receptor on lymphocytes, PRL is internalised and a cascade of secondary messenger systems eventually results in an increase in the synthesis of polyamines and cell proliferation. A similar system is observed in the mammary gland, another organ of epidermal origin. The intracellular signalling and effects on polyamine synthesis may be common to all PRL-responsive tissues (Skwarlo-Sonta, 1992). PRL action in Nb2 lymphoma cells is enhanced by IGF-1, which alone has no intrinsic mitogenic activity.

PRL treatment changes immune cell concentrations in the circulation. Chickens immunized against bovine PRL have higher serum anti-SRBC agglutinin titres and peripheral lymphocyte numbers (Skwarlo-Sonta, 1992). Exogenous PRL injected into chickens at various times after daybreak has differing effects on lymphocyte numbers (Skwarlo-Sonta, 1992). In the chicken PRL also affects antigen perception in lymphocytes. The immunomodulatory effect of PRL is less marked in young animals (Skwarlo-Sonta, 1992) as is the synchrony of fibre growth cycles (Restall *et al.*, 1994).

1.5.8 Prolactin and other epidermal structures

The role of PRL is most widely studied in the mammary gland, which is phylogenetically related to the skin sweat gland (Paus, 1991). The specialised mammotrophic action of PRL may be adapted from its more generalised action on other structures of epidermal origin such as the sweat gland and the crop sac of birds. Therefore a brief summary of the action of PRL on the mammary gland is warranted and similarities with actions in the skin are noted.

In a cycle similar to fibre growth, milk secreting cells in the mammary gland undergo cycles of alveolar emptying and refilling. Short thickened 'resting' milk- secreting cells full of milk, contain no intracellular PRL despite possessing PRL receptors. Following alveolar emptying, which initiates a new cycle, "resting cells" are rapidly transformed into tall columnar cells. Intracellular PRL, some of which is found in the nucleus, apparent in clusters in these newly activated cells, while in mid cycle, intracellular PRL becomes more randomly dispersed through the intracellular domain and enters the milk (Nolin, 1979).

PRL receptor number in mammary cells alters under a variety of physiological conditions including pregnancy and lactation. The up- and down- regulation of PRL receptor numbers may have physiological importance in both mammary glands

(Rillema *et al.*, 1988) and hair follicles. PRL is internalised and may act directly on the nucleus (Nolin, 1979), but it is the binding of PRL receptors on the external cell surface which initiates the first biochemical changes in mammary cells (Rillema, 1988). In mammary gland explants, 8 to 12 hours after the addition of PRL, genes for production of milk components are activated. Initially following PRL receptor binding it is likely that phosphatidylinositol-specific phospholipase C is stimulated followed by an increase in protein kinase C, an elevation in intracellular calcium ion concentration, and probably an increased rate of prostaglandin formation (Rillema, 1988). As in lymph cells, this results in an increase in orthinine decarboxylase (ODC) and polyamine concentration 30 minutes after the initial PRL treatment of mammary gland explants. ODC has a higher activity in anagen compared to telogen follicles (Paus, 1991) and ODC concentration in the skin is increased following fibre plucking in rats (Paus and Link, 1988).

The crop cells of the pigeon produce a milk-like substance in response to the presence of PRL. In the pigeon it has been determined that a liver-derived serum factor potentiates the effect of PRL on crop sac proliferation (Rillema *et al.*, 1988). IGF-1 production in the liver is stimulated by PRL and dermal fibroblasts can synthesise IGF-1 (Clemmons, 1984: Paus, 1991). It is interesting to note that the proliferation of Nb2 lymphoma cells by PRL is also enhanced by IGF-1 (Skwarlo-Sonta, 1992).

The effect of PRL on casein mRNA production in the mammary gland is altered by the potentiating effects of other hormones (oestrogen and progesterone) and growth factors (Fulkerson *et al.* 1976; Matusik and Rosen, 1978; Plaut, 1992). The interaction of PRL and epidermal growth factor (EGF) is essential for full mammary gland development (Plaut *et al.*, 1992; Vonderhaar, 1993). However, prolonged PRL treatment of mammary cells down-regulates EGF receptor numbers (Fenton and Sheffield, 1993). Receptors for EGF are found in the fibre follicle and differ in distribution during the different stages of the fibre growth cycle (Green and Couchman, 1984; Wynn *et al.*, 1989). Venous systemic infusion of EGF in sheep

causes a shortening of the follicle and a temporary termination in wool growth which results in fleece shedding (Moore *et al.*, 1984). However, the infusion of EGF has no effect on plasma PRL concentration. A cautionary note is that EGF treatment does not produce a true 'brush end' which is characteristic of normal catagen (Hollis *et al.*, 1987).

1.5.9 Conclusions

Despite fibre growth cycling occurring in the vast majority of mammals, this review shows that little is known of its physiological basis. Information which is presented indicates that plasma PRL concentration is involved in pelage transitions in small mammals, goats, and shedding sheep but little is known either of the mechanism of action of PRL or the nature of the PRL message.

Until recently, it has been hypothesised that shedding in goats and sheep was triggered by increasing plasma PRL concentration in spring. In sheep, this was thought to be associated with follicle activation. However, studies conducted in Wiltshire sheep, during the time span of this PhD, have revealed a catagenic role for increasing plasma PRL concentration. It is possible that changes in plasma PRL concentrations may act in conjunction with another photoperiodic hormone which fluctuates inversely with PRL. This hormone or growth factor may be largely permissive and act in combination with plasma PRL. A certain ratio of the two hormones may be a catagenic stimulus. The characteristic of the resultant fleece growth could be a function of the changing hormone ratio. Increasing PRL and reducing concentrations of another hormone may produce summer fleece growth and the reverse in winter. This ratio hypothesis could be applied to most of the reviewed experimental data however there is little data to substantiate such an hypothesis.

In the second hypothesis a rapid change (whether it is an increase or a decrease) in plasma PRL concentration may be a trigger for catagen in responsive follicles. The

fleece growth subsequent to catagen may be characteristic of the prevailing **changing** plasma PRL concentrations. Constant plasma PRL concentrations could maintain the follicle in its current phase. Physical shedding of fibres from follicles may be linked with follicle activation. This may be due to either a physical pushing of the fibre from the follicle or due to a shared physiological stimulus. Telogen follicles might be activated by an increase in plasma PRL concentration.

In a natural cycle of fibre growth the rapidly changing plasma PRL concentrations that occur during the autumn and spring equinoxes may be catagenic stimuli. In autumn, following a progressive transition to catagen, follicles remain in telogen during relatively constant plasma PRL concentrations. Follicles are activated in spring and in summer, the anagen phase of the down-producing goat and small mammals may be sufficiently advanced to be responsive to a catagenic signal, which may occur during rapidly increasing photoperiods after the spring equinox.

In animals treated with short days and then long days, the rapid increase in plasma PRL concentration triggers catagen. The resultant follicle growth is dictated by the prevailing photoperiod. If the animal is 'released' from short days into long but slowly declining day lengths then it immediately grows a winter fleece. In animals treated with melatonin in spring, plasma PRL concentrations are rapidly reduced and then maintained at low concentrations. The subsequent increase in plasma PRL concentrations, following cessation of melatonin treatment in autumn induces catagen and winter fleece regrowth.

In a single follicle, once a new phase of growth is initiated, a certain fixed cascade of metabolic activities eventuates. During this time the follicle is probably insensitive to seasonal physiological keys. The duration of insensitivity (sometimes called endogenous cycle) varies for both the type of phase and the species of animal. The telogen phase appears to be a 'waiting for physiological stimulus' phase and can be shortened greatly. However, anagen follicles (particularly large follicles or

developmentally older follicles) have a longer period of insensivity.

Outside these insensitivity periods the follicle of the seasonal shedding animal may be highly responsive to changes in plasma PRL concentration. Maybe it is our lack of understanding of factors such as the duration of the responsive phase of the follicle, the interaction between preceding and proceeding photoperiod conditions on signal interpretation of the animal, and the required duration and rate of change in the plasma PRL signal that leads to the confusion in interpretation of many experimental results.

Plasma PRL concentrations may affect fibre growth in shedding animals by possessing both anagenic and catagenic effects. In addition, PRL concentrations also determines seasonal fibre characteristics via melanogenesis and keratinisation.

With current information, one can only speculate about the possible mechanisms which lead to the lack of seasonal shedding in modern sheep breeds. Modern sheep breeds may still possess the PRL-shedding system but express it only during large changes in plasma PRL concentration which occur only during late pregnancy when relatively more maternal and offspring follicles are in a responsive stage of anagen. Elevations in winter plasma PRL concentration seen in modern sheep breeds may be causal in preventing seasonal shedding, an evolutionary association, or in fact an indication that the aseasonal skin enhances plasma PRL concentration either at the pituitary or by synthesis of its own PRL, thereby avoiding rapid changes in PRL concentrations in the skin. Finally the wool follicle of the modern sheep may have lost the ability to respond to changing plasma PRL concentrations.

CHAPTER 2

FIBRE GROWTH AND PLASMA PRL CONCENTRATIONS IN DOWN-PRODUCING GOATS.

2.1 ABSTRACT

Mixed aged breeding does were categorised as having either no known Angora ancestry (F), a maximum of 25% Angora ancestry (C), or 50% Angora ancestry (G). Fibre growth (length, patch growth, mean fibre diameter, fibre diameter distribution, follicle activity, and summer down growth) was measured in up to 225 does, for varying periods, from August 1990 until November 1992. The three genotypes were tested for differences in the quantity and timing of growth from both primary and secondary follicles.

The mean ratio of secondary to primary follicles was 5.9±0.3 in both C and F goats, and 7.3±0.3 in G goats ($P<0.001$). The annual guard hair fleece of G goats was shorter (40±0.3 mm) and finer (77±4 micron) than the 104±4 micron and 46.0±0.3 mm guard hair fleece of F and G goats. The sequence of primary follicle growth was similar in all three goat genotypes but the duration of growth of the guard hair fleece was 50-70 days greater in G compared to F goats.

The down fibres produced by G goats had a mean annual fibre diameter of 23.0±0.3 microns and a mean annual down length of 75.0±0.9 mm. This down fleece was coarser and longer than that produced by F goats (17.5±0.3 micron, 34.6±0.9 mm). The 18.7±0.3 micron and 44.7±0.9 mm fleece of the C goats was intermediate to that of the other two genotypes. The timing of down growth was similar in all three goat genotypes. However, secondary follicle activity during July and August of 1991 and 1992 was more than 25% lower ($P<0.05$) in F goats compared to C or G goats. Summer down growth was identified in 78% of G, 97% of C and 100% of F goats. In those G goats with a biannual down growth cycle, the duration of summer fleece growth was 35 days shorter compared to C and F goats ($P<0.01$).

In conclusion, a greater proportion of Angora genes, extended the duration of growth from primary follicles but decreased biannual down growth and the expression of catagen in secondary follicles.

2.2 INTRODUCTION

Many mammals, including the Cashmere goat, exhibit seasonal changes in their

pelage. The winter coat often has a denser longer down and a hair coat which traps air for winter warmth and a less dense, shorter hair coat for coolness in summer (Ryder, 1978; Bubenik *et al.*, 1985; McDonald *et al.*, 1987). The provision of such pelages is achieved by seasonally linked periods of either biannual or annual pelage growth and shedding (Ryder, 1978; Smale *et al.*, 1988). The selection by humans for desirable pelage characteristics in sheep and goats has, in some breeds, resulted in the elimination of a seasonally linked pelage shedding. Angora goats (*Capra hircus angorienses*) no longer shed their fleece annually but they do retain a seasonally dependent rhythm in fibre growth rate (Wilkelmaier, 1983), while Cashmere goats (*Capra hircus laniger*) shed their fleeces annually (McDonald *et al.*, 1987; Nixon *et al.*, 1991).

In the double-coated Australiasian down goat, the coarse, outer, guard hair coat is produced from primary follicles. These fibres grow from the spring to autumn equinox and shedding occurs in synchrony with the initiation of new guard hair growth in spring (McDonald *et al.*, 1987; Betteridge *et al.*, 1988). The down under-coat is produced from secondary follicles which have a biannual growth pattern (Ryder, 1970; Nixon *et al.*, 1991). From the spring equinox to the summer solstice, microscopic 'vellus' down fibres grow and are shed (Nixon *et al.*, 1991). After the summer solstice, the winter down fleece begins to grow and growth continues until the autumn equinox, after which the follicles progressively enter a 'resting phase' until spring when fibres are shed (McDonald *et al.*, 1987; Kloren and Norton, 1993c).

In the single-coated Angora goat, fleece growth is largely continuous throughout the year with no visible shedding of the fleece (Stapleton, 1977; Margolena, 1979). However, as in crossbred sheep (Hawker *et al.*, 1985), maximum fleece growth occurs during spring and summer with minimum growth in mid-winter (Stapleton, 1978; Winklemaier, 1983). In comparison to those of the double-coated Cashmere goat, secondary follicles in the Angora goat are more numerous and bigger, while primary follicle numbers and size are reduced. Normally fewer than 5% of Angora primary

follicles produce medullated kemp fibres, and in these follicles a biannual seasonal growth cycle, with synchronised periods of growth and shedding, has been retained (Jones *et al.*, 1935; Winklemaier, 1983; Nixon *et al.*, 1991).

Recently in New Zealand and Australia, Cashmere and Angora goats have been crossed in order to develop a new 'Cashgora' down type and to enhance the down-producing capabilities of Cashmere goats (Gretton and Bigham, 1984). It is widely accepted by farmers of Cashgora goats that, in comparison to Cashmere goats, the down growth period of the Cashgora goats is lengthened. Information is presented in this Chapter on the fibre growth cycles of down-producing goats with varying proportions of Angora and Cashmere ancestry. This information is of importance for the development of efficient fibre harvesting regimes for cashgora goats. In addition, it may also provide some useful information on the physiological basis for the evolution from shedding to non- shedding fibre growth patterns in goats.

The fibre growth cycle of the down-producing goat is responsive to photoperiod (McDonald and Hoey, 1987; Gebbie *et al.*, 1991b; Norton, 1991) and the physiological recognition of photoperiod involves the endocrine system (Kennaway *et al.*, 1987; Chemineau *et al.*, 1988). Recently, plasma PRL concentration has been identified as having a causal role in regulating the moulting and growth of pelage in mustalids (Martinet, et al, 1984; Rose *et al.*, 1987; Badura and Goldman, 1992), sheep (Lincoln, 1990), and Cashmere goats (Lynch and Russell, 1989; Kloren and Norton, 1993d). It has been suggested that, in sheep, less seasonal fibre growth patterns are associated with higher winter plasma PRL concentrations (Lincoln, 1990; Clarke *et al.*, 1993; Pearson *et al.*, 1994). Seasonal plasma PRL concentrations in goats with potentially differing seasonal fibre growth patterns are compared in this Chapter.

2.3 METHODS

2.3.1 Animals

Ancestry records of the Wanganui Research Station down goat flock were examined and mixed age does of 2 years or older were categorised as having no known Angora ancestry (F) or a maximum of 25% Angora ancestry (C). Half-bred down x Angora does (G) were purchased from 5 farms in the North Island and all goats were grazed at Wanganui Research Station (40°S 175°E). The experiment was conducted on seventy five, unshorn does of each of the three genotype categories from August 1990 until November 1992. The goats were grazed on pasture following normal farm management practises, except during the 24 hour blood sampling period. During that period the goats were individually penned indoors under natural illumination for a 24 hour acclimatization period and a 24 hour sampling period. While indoors the goats were fed hay and maize with *ad libitum* access to water.

The does were single sire mated (1 buck: 25 does) to either feral, cashgora or half-bred feral x Angora bucks in 1990, 1991 and 1992 breeding seasons. Each sire was mated to does of all 3 genotypes to and kids were tagged and identified to dams within 24 hours of birth.

2.3.2 Field Measurements

225 (S225) experimental does were randomly allocated, within genotype groups, into a subgroup of 90 does (S90) and this group was further divided into a 30 doe subgroup (S30) and then again into a 15 doe group (S15). The more detailed fibre measurements were conducted on the subgroups containing fewer goats (Table 2.1).

2.3.2.1 Fibre length. Monthly measurements of down and guard hair length were made from viewing staples on S225 goats. In addition, two weekly fibre length measurements were collected on S30 goats between July and December inclusive. Fibre length measurements of guard hair and cashmere were taken at four sites (neck, front shoulder, midside and hind shoulder) of the goats. A ruler was placed on the skin of the goat, the adjacent staple was gently stretched and the lengths of the longest down and guard hairs were recorded. A mean length for the four sites was then calculated for down and guard hair.

2.3.2.2 Fibre diameter. Diameters of fibres less than 80 micron were measured at bimonthly and monthly intervals in S90 and S30 goats respectively. Guard hair fibres of greater than 80 micron were measured at monthly intervals only in S30 goats. Fibre diameter was measured on samples collected from August 1990 until November 1991.

Fibre diameter measurement was conducted on fibre samples clipped, at the level of the skin, using Oster animal clippers. A 15x15cm grid, containing nine, 5x5 cm squares, was centred over the left midside. At the start of the experiment, the goats were randomly allocated a square number (1-9) within the grid and that square was cut. At the next sample collection time, the adjacent square, in a clock wise direction, was cut.

2.3.2.3 Patch growth. Fibre growth rates were measured at bimonthly intervals from August 1990 until March 1992 in S30 goats. Fibre regrowth rates were estimated by clipping the same area of fleece repeatedly, with Oster animal clippers, in a $100cm^2$ measured square patch, on the right midside of the goats. A 0.08 gram subsample of fibre pieces, selected by random grid sampling, was manually separated into down and guard hair and the average weekly patch growth rates of down and guard hair were calculated.

2.3.2.4 Skin samples. Skin biopsy samples were collected from S30 goats from 21 August 1990 to 04 December 1990 and from 02 July 1991 to 25 November 1992. Samples were collected at monthly intervals from December to August and fortnightly from September to November. The animals were restrained in an upright position, the fibre on the sampling site was trimmed to 5 mm using surgical scissors and 1 ml of local anaesthetic (Lopaine: Lignocaine hydrochloride, USP 20 mg/ml Ethical Agents, South Auckland, N.Z.) was injected subcutaneously. Skin samples, taken by snip biopsy, were immediately fixed in 10% buffered Formalin. The wound was treated with topical antiseptic (Oxytetrin aerosol, Pitman-Moore N.Z. Ltd., Upper Hutt, N.Z.) to prevent infection.

2.3.2.5 Blood samples. Blood samples were collected over a 24 hour period, in S30 goats, on 19-20 March 1991, 19-20 June 1991, 19-20 September 1991 and 19-20 December 1991. Cannulae were inserted into the external jugular vein and kept patent by flushing with isotonic saline. To avoid chewing by the goats, the catheter tubing was extended, under tape, to the front shoulder blades of the goat. The tape was fixed to the goat's trimmed skin with glue (Loctite 454 Gel, Loctite UK, Herts Al7 1JB, UK). On the day following catheterisation, blood samples were collected into EDTA vacutainers, at hourly intervals for the four hour periods centred on both dawn and dusk and at two hourly intervals for the remainder of the 24 hr period. Serum was separated by centrifugation and stored at -15°C.

The samples were thawed and a 200 μl sample was drawn from vortex-mixed samples and pooled into categories of day, night, dusk, and dawn. Dawn was categorised as being the two hours before and up to and including sunrise, and dusk was categorised as being from sunset and the two hours following.

Table 2.1: Number of goats sampled and frequency of sample in an experiment examining fibre growth cycles in feral (F), cashgora (C) and first cross feral x Angora (G) breeding does.

Measurement	Number of goats sampled		
	F	C	G
Monthly down length (S225)	75	75	75
Fortnightly down length (S30)	10	10	10
Bimonthly fibre diameter (S90)	30	30	30
Monthly fibre diameter (S30)	10	10	10
Bimonthly midside growth (S30)	10	10	10
Fortnightly newly erupted down fibres (S30)	10	10	10
Monthly follicle activity (S15)	5	5	5
Blood sample (S30)	10	10	10

2.3.3 Laboratory procedures

2.2.3.1 Fibre Diameter. Two thousand measurements of the diameter of single fibres were conducted on randomly selected 1-2 mm snippets of the fleece using the Fibre Fineness Distribution Analyser (CSIRO, Ryde, Sydney, Australia). Mean diameter of down fibre was calculated from fibres measuring less than 40 μm. Fibres of greater than 40 μm but less than 80 μm were classified as intermediate fibres.

On each fleece sample, 250 individual measurements of fibre diameter were conducted on medullated fibres of greater than 80 micron diameter (guard hair). Fibre snippets were aligned on paraffin-mounted slides using an electrical charge and fibre diameter measured at 10x10 magnification using a video image analysing system (Ensor Scientific, Auckland, New Zealand).

2.3.3.2 Newly erupted down fibres. The presence of the intact fibre tip was used to score the skin sample for the presence of newly erupted down fibres (NEDF). Fortnightly and monthly skin samples of S30 goats were removed from the formalin fixative, rinsed well with water and blotted dry. The epidermal surface of the skin sample was viewed edgewise under a dissecting microscope with a contrasting colour background. The form of the emergent fibres from the secondary follicles was scored as follows: 1=newly erupted fibres (fibre of less than 2 mm and characterised by an arrow-like tip) and long down (fibre in which the tip exceeds 5 mm and hence the tip was not present); 2=newly erupted fibres only; 3=newly erupted fibres and short down (fibres 2 to 5 mm with arrow-like tip); 4=short down only; 5=short and long down; 6=long down only.

2.3.3.3 Skin histology. Monthly skin samples from S15 goats were processed overnight through graded concentrations of ethanol to parafin wax (Table 2.2). The samples were embedded, epidermal surface uppermost, in paraffin polymer wax (Polymer embedding wax, Tissue Tek Ltd, Auckland, N.Z.). Using a rotary microtome, each wax block was serially transverse sectioned at 8 μm, beginning at the skin surface and continuing down until only primary follicles were visible in the section. Ribbons were divided into three-section groups and every second group discarded. Sections were floated onto a water bath at 40 °C and 4 section groups were mounted on each slide using egg albumin as an adhesive. The slides were dried and stained (Table 2.3) using a modification of the Sacpic method (Auber, 1952; Nixon, 1993) and then permanently mounted (DPx, Product Number 36029, Ajax Chemicals, P.O. Box 12645, Penrose, Auckland).

Approximately 10 follicle groups, of predominantly three primary follicles and associated secondary follicles, were scored. The follicles were scored, based on the procedures of Nixon (1993), as being either actively growing (anagen) or inactive (telogen). Follicles which contained both active and inactive fibres were also noted.

The anagen follicle had a well-defined, round, darkly red stained inner-root sheath with round outer-root sheath nuclei. The follicle bulb was darkly stained. The fibre of an anagen follicle had a circular outline and the presence of pigmentation and medullation was symptomatic of the actively growing state.

A telogen follicle had a serrated, pale red stained inner root sheath with elongated radially arranged outer-root sheath nuclei. The follicle bulb was lightly stained. The telogen follicle may also contain a fibre with a serrated 'brush' fibre.

The presence of medullated fibres in the secondary follicles was also noted.

2.3.3.4 PRL Assay. The standard double-antibody competitive binding radioimmunoassay, conducted at Massey University, used rabbit anti-oPRL as the antiserum (NIADDK-anti-oPRL-I-2, AFP-973269, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health (NIADDK), National Hormone and Pituitary Programme (NIH), Bethesda, Md, USA) and lyophilized ovine PRL (NIADDK-oPRL-I-2, AFP-973269, NIADDK,NIH) for the radioiodination (Greenwood *et al.*, 1963). The standards were biological grade ovine PRL (NIADDK-oPRL-18, AFP-8277E, NIADDK, NIH) in the range of 1-1200 ng/ml. Separation of the antibody-bound from free labelled PRL was by donkey anti-rabbit serum (APPT 1, Lot 116556, IDS, Tyne and Wear, England). The displacement of labelled PRL by caprine samples was in parallel with ovine standards and internal recovery was 82%. Intra- and inter-assay coefficients of variation were 9.7% and 16.6% respectively.

2.3.4 Statistical Analysis

For each individual goat, the size and time of maximum and minimum growth parameters were identified. The date selected was that at which the maximum or minimum values were first achieved. Primary follicle activity and guard hair mean

fibre diameter had a bimodal annual pattern, with a major peak associated with spring and lesser peaks in autumn. This analysis reports only the changes associated with the spring peak.

Two durations of growth were defined: $DUR_{max-min}$ was the the number of days between the date of maximum growth and date of minimum growth and $DUR_{min-max}$ the number of days between the date of minimum growth and date of maximum growth. The difference in size between the maximum and minimum growth was called the "magnitude of growth".

Data on fibre length and newly erupted down fibres were collected for two years while fibre diameter, down and guard hair growth rate data were collected for one year. No skin samples were collected from January 1990 to July 1991 and therefore estimates of maximum and minimum primary and secondary follicle activity, for two years, were not always possible. However, because of a difference in the timing of secondary follicle activity, two year estimates of minimum follicle activity were possible. Separate analyses were conducted on fibre length measurements collected on S225 goats and S30 goats (Table 2.1).

The effect of genotype of goat, on the timing and mass of growth, was determined by general linear models procedures with genotype contrasts (SAS, 1987). The single year data were analysed using ANOVA procedures and two year data were analysed with repeated measures in time and the Wilks' Lambda test of significance. Data quoted in the text, unless otherwise indicated, are least square means and standard errors. Raw data are presented graphically.

Table 2.2: Procedure for processing skin samples from fixative into paraffin wax blocks.

Step	Solvent	Time
1.	70% Alcohol	1 hour or more
2.	70% Alcohol	1 hour
3.	95% Alcohol	1 hour
4.	95% Alcohol	1 hour
5.	100% Alcohol	1 hour
6.	100% Alcohol	2 hours
7.	Chloroform	3 hours
8.	Xylene	1 hour
9.	Xylene	1 hour
10.	Paraffin wax	2 hours
11.	Paraffin wax	2 hours

Table 2.3: Procedure for staining follicles for determination of follicle activity based on procedures of Nixon (1993).

Step	Solvent	Time
1.	Xylene	5 minutes
2.	Xylene	5 minutes
3.	Absolute alcohol	2 minutes
4.	95% alcohol	1 minute
5.	70% alcohol	1 minute
6.	50% alcohol	1 minute
7.	Tap water rinse	10 seconds
8.	Gill's Haematoxylin[a]	5 minutes
9.	Tap water rinse	5 seconds
10.	Scott's tap water rinse	1 minute
11.	50% alcohol rinse	10 seconds
12.	Safranin solution[b]	5 minutes
13.	Tap water rinse	30 seconds
14.	50% alcohol rinse	10 seconds
15.	70% alcohol rinse	10-40 seconds
16.	50% alcohol rinse	10 seconds
17.	Tap water rinse	10 seconds
18.	Picric acid (aqueous)	2 minutes
19.	Tap water rinse	10 seconds
20.	Picro-indigo carmine[c]	4 minutes
21.	Tap water rinse	10 seconds
22.	50% alcohol rinse	10 seconds
23.	70% alcohol	2 minutes
24.	95% alcohol	2 minutes
25.	Absolute alcohol	2 minutes
26.	Absolute alcohol rinse	2 minutes
27.	Xylene	5 minutes
28.	Xylene	5 minutes

[a] Gill's Haematoxylin (C.I. 75290, Gurr stains, BDH Ltd, Broom Rd, Dorset BH12 4NN, England)
[b] Safranin Solution (Product Number 34312, C.I. 50240, Gurr Stains, BDH Ltd)
[c] Picro-indigo carmine (Product Number 34038, C.I. 73015, Gurr Stains, BDH Ltd)

2.4 RESULTS

2.4.1 Production parameters

2.4.1.1 Kidding Performance: In 1990, 1991 and 1992, 38, 66 and 67% of the does produced kids (Table 2.4). In 1990 and 1991 the mean date of kidding was 16 September±1 days (mean±se) but in 1992 the does kidded 18 days earlier. Of the does kidding, the mean birth kidding percentage was 130±5% (mean±se) in 1990 and 1991, and 157±4% (mean±se) in 1992 with a mean death rate to weaning of 5%. The mean birth weight of kids was similar in all years at 2.65±0.05 and 2.18±0.05 kg for single and twin kids respectively. There was no effect of genotype on any kidding parameters.

Fibre traits and plasma PRL concentrations were similar in non-pregnant and pregnant does and in does which weaned kids.

Table 2.4: Percentage of does kidding in 1990, 1991 and 1992 in cashgora (C), feral (F) and first cross Angora feral (G) goats.

Genotype of Goat	Year		
	1990	1991	1992
C	46	68	67
F	29	64	72
G	39	68	80

***2.4.1.2 Liveweight and Fleece production*:** Throughout the experiment G goats were 0.9-2.1 kg heavier ($P<0.01$) than C and F goats which were similar in weight. Genotype mean liveweights at mating and weaning in 1991 and 1992 ranged from 27.7 to 32.2 kg.

The secondary to primary follicle ratio (S/P) was 5.9±0.3 in both C and F goats, however G goats had an additional 1.4 secondary follicles for each primary follicle (P<0.001). The secondary follicles of the G goats produced down fibres which had a mean annual fibre diameter of 23.0±0.3 microns and a mean annual down length of 75.0±0.9 mm while the down produced by F goats was both finer (17.5±0.3 micron) and shorter (34.6±0.9 mm) (both, P<0.0001). The 18.7±0.3 micron and 44.7±0.9 mm down fleece of C goats was intermediate between that of F and G goats (contrasts, P<0.001). Conversely, the primary follicles of the G goats produced guard hair fibres which were shorter (40.0±0.3 mm) and finer (77±4 micron) than the 104±4 micron and 46.0±0.3 mm guard hair fleeces of F and C goats.

2.4.2 Primary follicle growth

2.4.2.1 Guard Hair Length (GL). In S30 and S225 goats (Table 2.5, 2.6; Figure 2.1, 2.2), minimum mean GL was similar in C and F goats and in S30 goats the minimum mean GL of G goats was 4 to 11 mm shorter (Table 2.5) than that of the other two genotypes (P<0.05). Maximum mean GL was also 6 to 9 mm shorter (Table 2.5, 2.6) in G compared to C and F goats (P<0.001). This trend for a reduction in GL in G goats compared to C and F goats (Figure 2.1, 2.2) was, in S225 goats, also manifest as a 5 to 8 mm reduction (P<0.001) in the magnitude of GL (Table 2.6). In S30 goats, minimum mean GL occurred on 5 December±5 days and it was unaffected by genotype (Table 2.5). In S225 goats the date of minimum GL in C and F goats was 21 December±3 days with the minimum in G goats (Table 2.6) occurring 24 days and 13 days earlier in year 1 and year 2 respectively (P<0.01).

The mean date of achieving maximum mean GL in the first year ranged from 11 August to 1 September and was unaffected by genotype (Table 2.5, 2.6). However, in the second year, maximum mean GL occurred earlier (P<0.001), ranging from 5 May to 27 July, and F goats reached maximum mean GL more than 30 days earlier

than the other two genotype groups (P<0.001).

In S225 goats, the $DUR_{min-max}$ GL was longest in G (229±5 days) goats, medium in C (203±5 days) and shortest in F (179±5 days) goats (genotype effect, P<0.001). The time taken for GL to fall from maximum to minimum mean GL ranged between 89 and 144 days and was similar in all three genotypes (Table 2.5, 2.6).

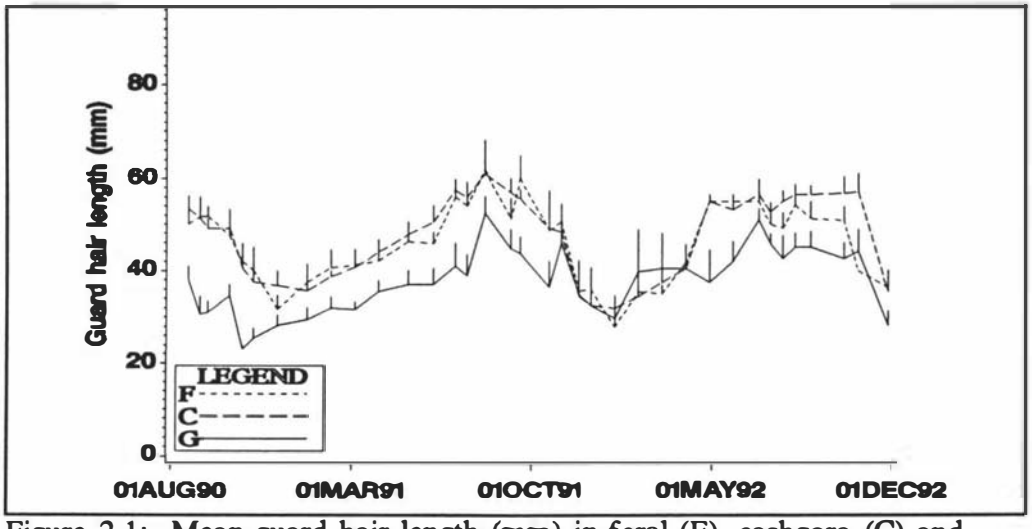

Figure 2.1: Mean guard hair length (mm) in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S30. Bars represent standard errors.

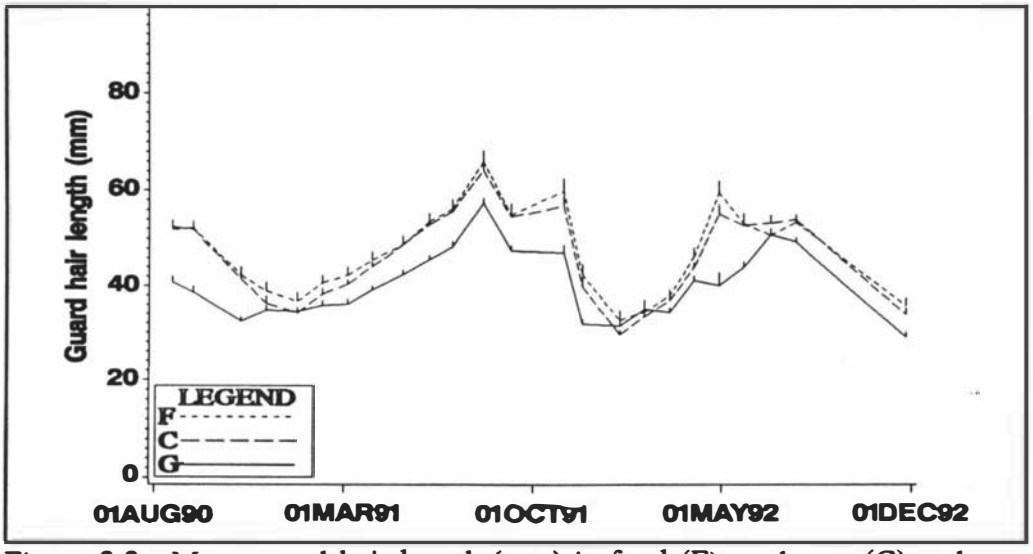

Figure 2.2: Mean guard hair length (mm) in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S225. Bars represent standard errors.

Table 2.5: Measurements of guard hair length (GL) including magnitude (Maximum GL-Minimum GL), $DUR_{max-min}$ (number of days between the date of maximum and minimum GL), $DUR_{min-max}$ (number of days between the date of minimum and maximum GL), date of maximum and minimum GL (days.month), maximum and minimum GL in cashgora (C), feral (F) and first cross Angora x cashmere (G) does in subgroup S30. Data are means and standard errors. Significance (*** P<0.001, ** P<0.01, * P<0.05, NS not significant) indicates effects of year (Yr) and genotype (Ge) and their interaction (Yr*Ge) following repeated measures by time analysis.

Guard hair length measurement	Genotype of Goat			Yr	Yr * Ge	Ge
	C	F	G			
Magnitude of GL (mm)						
Year 1	30±3	26±3	32±4	NS	NS	NS
Year 2	32±3	32±2	27±2			
$DUR_{max-min}$ of GL (days)	89±14	109±15	109±16	na	na	NS
$DUR_{min-max}$ of GL (days)						
Year 1	280±30	278±30	241±32	*	NS	NS
Year 2	240±15	182±15	232±16			
Date of Minimum GL (day.month)						
Year 1	24.11±6	21.11±6	09.10±7	***	NS	NS
Year 2	4.12±12	14.12±11	30.11±13			
Minimum GL (mm)						
Year 1	33±2^{a}	30±2^{a}	20±3^{b}	NS	*	*
Year 2	28±2	25±2	23±3			
Date of Maximum GL (day.month)						
Year 1	1.09±8	28.08±9	11.08±10	***	*	NS
Year 2	27.07±10^{a}	16.06±11^{b}	20.07±12^{ab}			
Maximum GL (mm)						
Year 1	64±3^{a}	56±3^{b}	52±3^{b}	NS	NS	**
Year 2	60±2^{a}	56±2^{a}	50±2^{b}			

Values with different superscripts in each row for each fibre measurement in each year indicate where genotypes are significantly different at the 5% level. No superscript is present when genotype differences are not significant.

Table 2.6: Measurements of GL including magnitude, $DUR_{max-min}$, $DUR_{min-max}$, date of maximum and minimum GL, maximum and minimum GL in cashgora (C), feral (F) and first cross Angora x cashmere (G) does in S225 subgroup. Data are means and standard errors. For full table description see Table 2.5.

Guard hair length measurement	Genotype of Goat			Yr	Yr * Ge	Ge
	C	F	G			
Magnitude of GL (mm)						
Year 1	37 ± 2^{b}	35 ± 1^{b}	31 ± 2^{a}	***	NS	***
Year 2	32 ± 3^{b}	32 ± 1^{b}	24 ± 1^{a}			
$DUR_{max-min}$ of GL (days)	132 ± 3	144 ± 5	130 ± 5	na	na	NS
$DUR_{min-max}$ of GL (days)						
Year 1	252 ± 7^{b}	242 ± 7^{b}	278 ± 7^{a}	***	NS	***
Year 2	153 ± 7^{b}	116 ± 7^{c}	180 ± 7^{a}			
Date of Minimum GL (day.month)						
Year 1	8.12 ± 5^{a}	21.12 ± 5^{a}	06.11 ± 5^{b}	***	**	***
Year 2	28.12 ± 3^{b}	02.01 ± 3^{b}	19.12 ± 3^{a}			
Minimum GL (mm)						
Year 1	32 ± 1^{b}	32 ± 1^{b}	27 ± 1^{b}	***	*	NS
Year 2	28 ± 1	28 ± 1	27 ± 3			
Date of Maximum GL (day.month)						
Year 1	21.08±5	15.08±5	15.08±5.0	***	***	***
Year 2	04.06 ± 7^{b}	03.05 ± 7^{c}	23.06 ± 7^{a}			
Maximum GL (mm)						
Year 1	69 ± 2^{a}	67 ± 2^{a}	59 ± 2^{b}	***	NS	**
Year 2	58 ± 2^{a}	59 ± 2^{a}	52 ± 2^{b}			

Values with different superscripts in each row for each GL measurement in each year indicate where genotypes are significantly different at the 5% level. No superscripts are present when genotype differences are not signficant.

2.4.2.2 ***Primary Follicle Activity (PA).*** The size and time of achieving maximum and minimum mean PA, and the proportion of follicles containing both active and inactive fibres (PAct+brush) were unaffected by genotype (Table 2.7). The mean $DUR_{min-max}$ of PA was longer in G goats (182±18 days) than in F goats (112±16 days), the latter being similar to C goats (121±18 days) (contrasts, $P<0.05$).

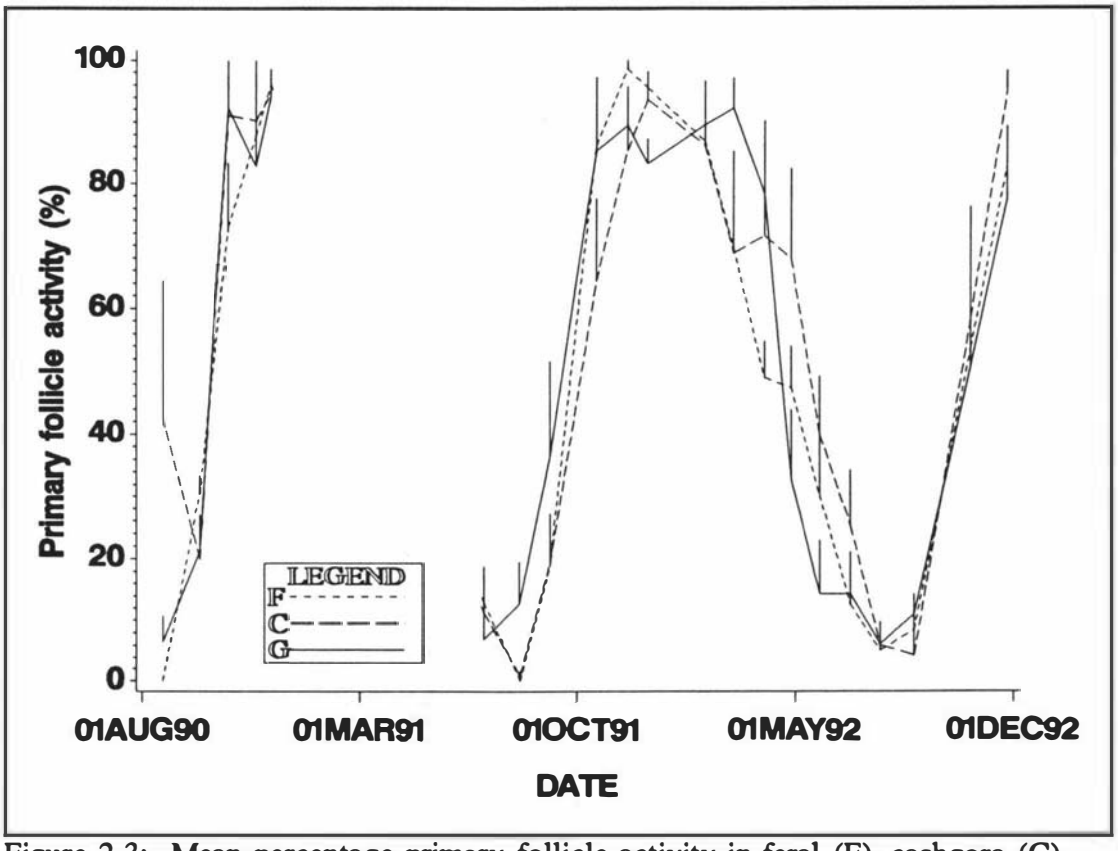

Figure 2.3: Mean percentage primary follicle activity in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S15. Bars represent standard errors.

Mean PA increased from September, reaching maximal levels on 9 December±12 days (Figure 2.3). However, 90% of goats in all genotypes, then underwent a subsidiary activity cycle during November to April (Figure A2.1). These subsidiary cycles were characterised by small peaks and troughs in PA but, more conclusively, distinct peaks in the percentage PAct+brush follicles (Figure A2.2).

Table 2.7: Measurements of percentage primary follicle activity (PA) including magnitude, $DUR_{min-max}$, date of maximum and minimum PA, maximum and minimum PA, in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S15. Data are pooled means and standard errors. For full description of statistical parameters see Table 2.5.

Primary follicle activity	Genotype of Goat			Ge
	C	F	G	
Magnitude of PA (%)	100±2	96±2	97±1	NS
$DUR_{min-max}$ of PA (days)	121 ± 18^{ab}	112 ± 16^{b}	182 ± 18^{a}	*
Date of Minimum PA (day.month)	31.07±12	22.07±11	19.07±12	NS
Minimum PA (%)	1±2	5±2	2±1	NS
Date of Maximum PA (day.month)	28.11±16	21.11±14	09.01±16	NS
Maximum PA (%)	100±0	100±0	100±0	NS

Values with different superscripts in each row indicate where genotypes are significantly different at the 5% level.

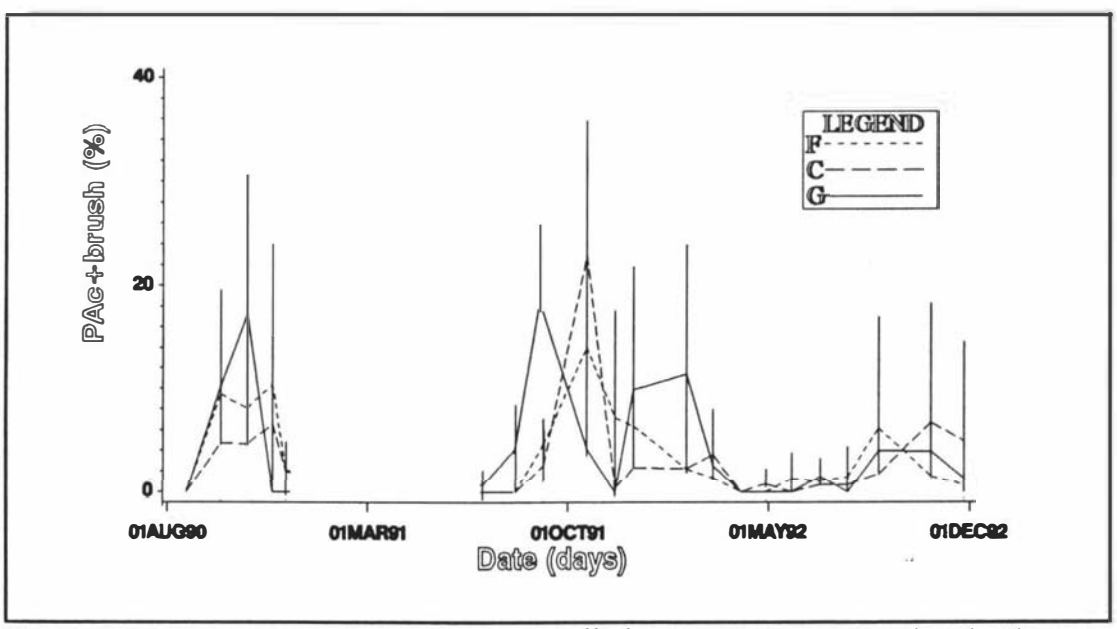

Figure 2.4: Mean percentage of primary follicles containing active+brush fibres (PAC + brush) in feral (F), cashgora (C), and halfbred Angora x feral (G) breeding does in the S15 subgroup. Bars represent standard errors.

The first peak in PAct+brush follicles was synchronised between individuals and occurred during September and October (Figure 2.4). However the timing of the second peak, while predominantly observed in only a single month, varied between individual goats and occurred from November to April. This pattern was observed in both central and lateral primary follicles but central primaries had lower (P<0.01) PA from December to May (Figure 2.5). After the subsidiary cycle, mean PA declined progressively from April and reached minimal values on 27July±6 days.

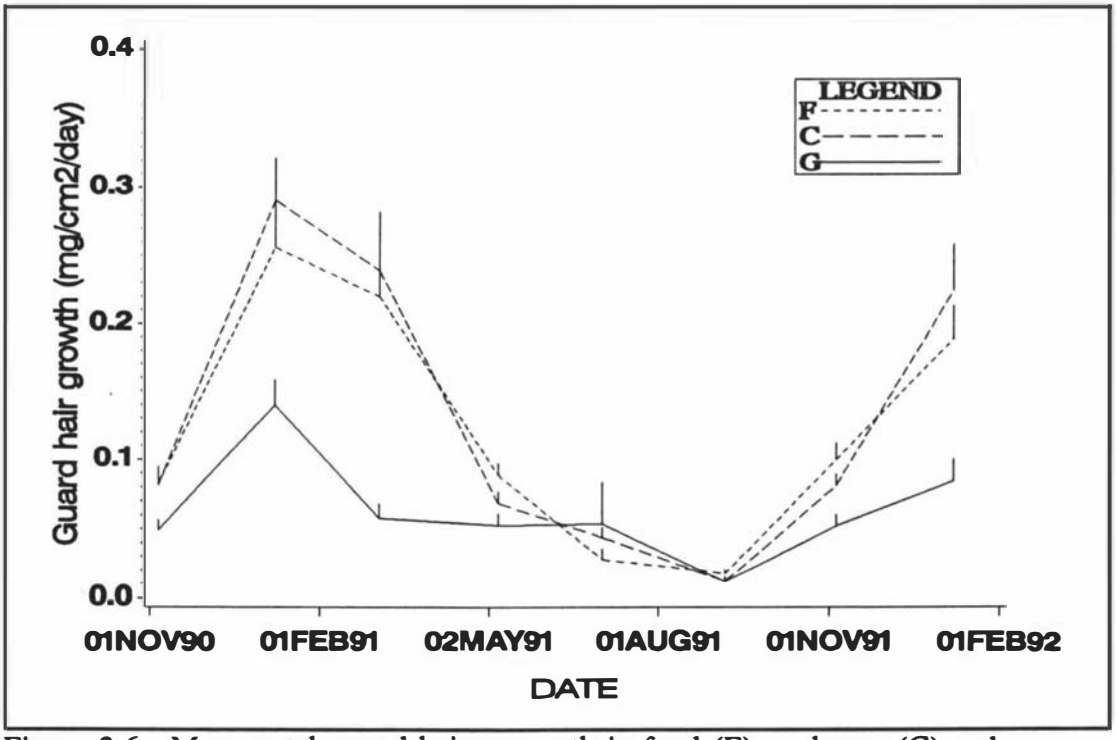

Figure 2.6: Mean patch guard hair regrowth in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S30. Bars represent standard errors.

2.4.2.3 Guard hair growth rate (GHGR). Maximum mean GHGR (Table 2.8) was lower in G goats (0.13±0.03 mg/cm^2/day) than in either C (0.32±0.03 mg/cm^2/day) or F (0.29 ±0.03 mg/cm^2/day) goats (P<0.01). All genotypes achieved maximum mean GHGR during January (Figure 2.6). Minimum mean GHGR occurred on 5 September±8 days in C and G goats but on 3 August ±7 days in F goats (P<0.01).

This resulted in F goats having a shorter $DUR_{min-max}$ of GHGR (186±10 days) than C (224±11 days) and G (240±11) goats (P<0.01).

2.4.2.4 Guard hair mean fibre diameter (GMFD). In C and F goats mean GMFD had a bimodal pattern with troughs in both spring and autumn (Figure 2.7). Maximum and minimum mean GMFD were 23 and 16 microns (P<0.01) respectively greater in C and F goats than in G goats. However, the mean magnitude of change was similar in all genotypes (Table 2.9). The mean $DUR_{max-min}$ of GMFD was 257±10 days and was unaffected by genotype (Table 2.9). The date of achieving maximum and minimum GMFD was unaffected by genotype with mean maximum GMFD occurring on 17 December±4 days and the guard hair fleece being at its finest on 28 August±10 days (Table 2.9).

Table 2.8: Measurements of guard hair growth rate (GHGR) including magnitude, $DUR_{max-min}$, date of maximum and minimum GHGR, maximum and minimum GHGR in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S30. Data are least square means and standard errors. For full description of statistical parameters see Table 2.5.

Guard hair growth	Genotype of Goat			Ge
	C	F	G	
Magnitude of GHGR (mg/cm^2/day)	0.30±0.03^a	0.27±0.03^a	0.12±0.04^b	**
$DUR_{max-min}$ of GHGR (days)	224±11^a	186±10^c	240±11^a	**
Date of Minimum GHGR (day.month)	5.09±8	3.08±7	5.09±8	**
Minimum GHGR (mg/cm^2/day)	0.010±0.003	0.015±0.003	0.009±0.004	ns
Date of Maximum GHGR (day.month)	24.01±9	29.01±8	8.01±9	ns
Maximum GHGR (mg/cm^2/day)	0.32±0.03^a	0.29±0.03^a	0.13±0.03^b	**

Values with different superscripts in each row indicate where genotypes are significantly different at the 5% level. No superscripts are present when genotype differences are not significant.

Table 2.9: Measurements of guard hair mean fibre diameter (GMFD) including magnitude, $DUR_{max-min}$, date of maximum and minimum GMFD, maximum and minimum GMFD in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S30. Data are least square means and standard errors. For full table description see Table 2.5.

Guard Hair Fibre Diameter	Genotype of Goat			Ge
	C	F	G	
Magnitude of GMFD (microns)	30±5	27±5	23±5	NS
$DUR_{max-min}$ of GMFD (days)	254±18	268±19	245±19	NS
Date of Minimum GMFD (day.month)	26.08±17	09.09±18	22.08±18	NS
Minimum GMFD (microns)	$88±3^{b}$	$91±4^{b}$	$73±4^{a}$	**
Date of Maximum GMFD (day.month)	16.12±7	18.12±7	18.12±7	NS
Maximum GMFD (microns)	$118±5^{a}$	$119±5^{a}$	$96±5^{b}$	**

Values with different superscripts in each row for each GMFD measurement indicate where genotypes are significantly different at the 5% level. No superscripts are present when genotype differences are significant.

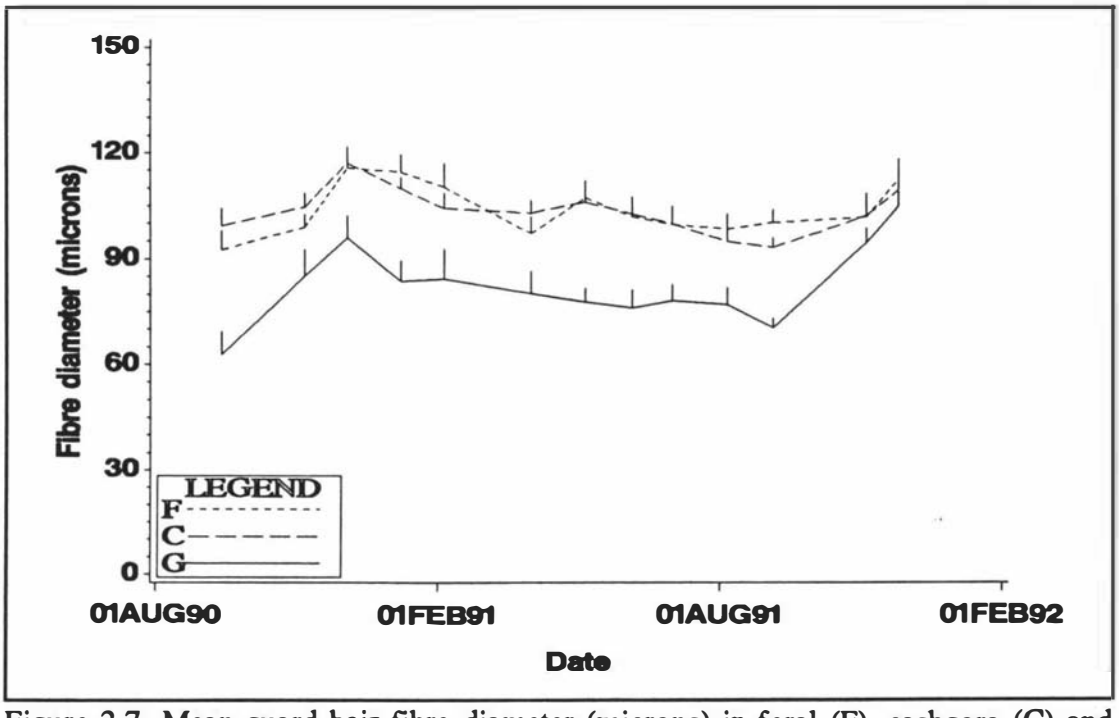

Figure 2.7: Mean guard hair fibre diameter (microns) in feral (F), cashgora (C) and half-bred Angora x feral (G) does in subgroup S30.

2.4.3 Secondary follicle growth

2.4.3.1 Down length (DL). An increase in the proportion of Angora ancestry in goats resulted in longer down fleeces (Figure 2.8, 2.9). The mean maximum DL was longer in G (range 132-161 mm) fleeces than in C (range 86-100 mm) fleeces which were longer than F (66 to 74 mm) fleeces (P<0.001). Mean minimum DL was similar in C and F fleeces (range 0.3 to 1.4 mm) but was longer in G (6 to 16 mm) fleeces (P<0.01). Mean magnitude of DL, which was different for each genotype group, was: G goats, 126-145 mm; C goats 85-99 mm; and F goats, 66-73 mm (Table 2.10 to 2.11).

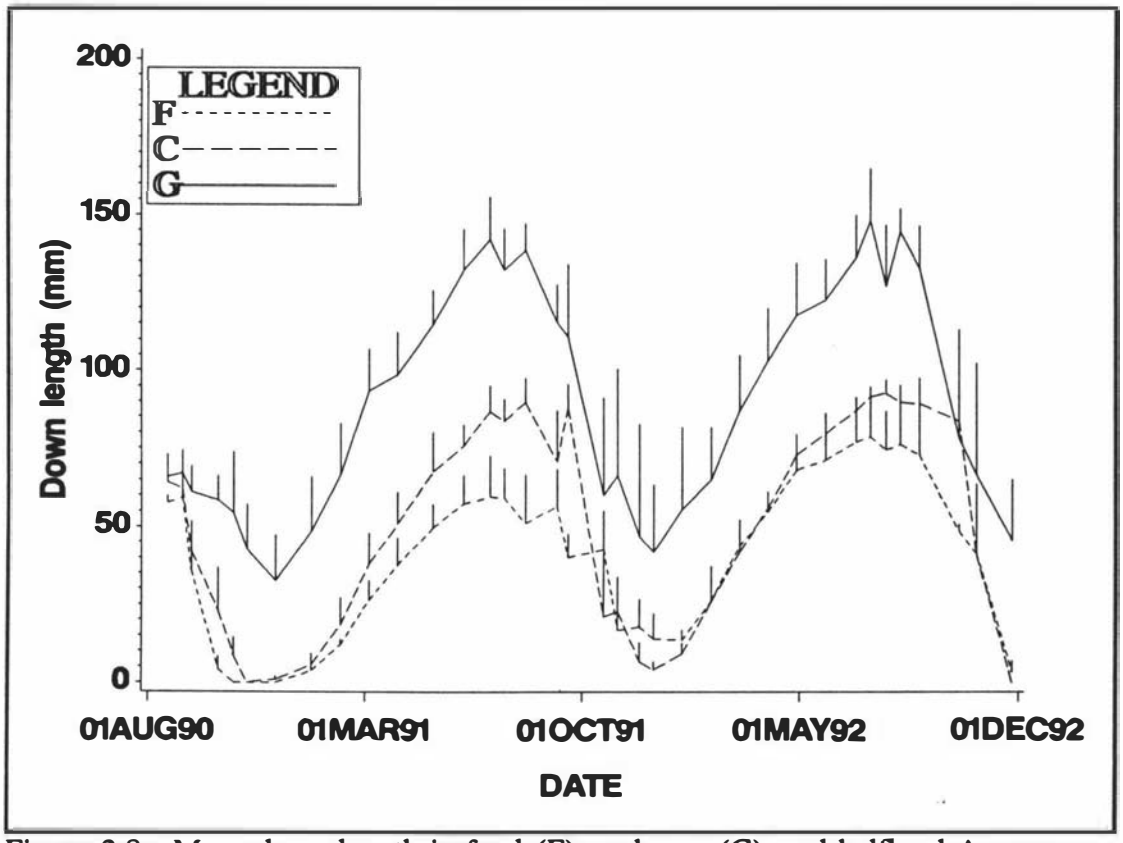

Figure 2.8: Mean down length in feral (F), cashgora (C), and halfbred Angora x feral (G) breeding does in subgroup S30. Bars represent standard errors.

In S30 goats, genotype had no effect on the timing of the DL growth cycle (Figure

2.8). Maximum mean DL and minimum mean DL occurred on 28 July±3 days and 27 October±3 days respectively. Mean DL increased for 273±4 days from minimum to maximum length and then declined for 108±3 days to achieve minimal DL (Table 2.10).

In S225 goats, minimum mean DL occurred during November (Figure 2.9) with an interaction between genotype and year ($P<0.01$). In Year 1, G goats achieved minimum mean DL, 8 and 10 days later than C and F goats respectively ($P<0.001$) but no genotype differences occurred in Year 2. F goats reached maximum mean DL on 12 July±2 days, which was 9 and 12 days than C and G goats respectively. However, this effect was expressed mostly in Year 2, when F goats achieved maximum DL 16 to 32 days earlier than G and C goats respectively ($P<0.001$). There was both a genotype ($P<0.01$) and genotype by year interaction ($P<0.001$) in the duration of the DL cycle. In the first year, G goats (249±2 days) had a shorter mean $DUR_{min-max}$ of DL growth than the other two groups (260±3 and, 266±3 days in C and F goats respectively) while in the second year the mean $DUR_{min-max}$ of DL growth of F goats (218±4 days) was shorter than that of the other two genotype (C, 237±4 days; G, 241±4 days).

2.4.3.2 Secondary Follicle Activity (SA). SA reached a mean minimum level of 10±4% on 21 August±7 days after which it increased and reached a maximum SA of 100±0% on 1 December±4 days. From December until April, SA remained above 94% activity (Figure 2.10). Genotype had no effect on the size or time of minimum and maximum mean SA (Table 12). However, in July and August of both 1991 and 1992 (Figure 2.10) mean SA was more than 25% lower in F goats than in C or G goats ($P<0.05$).

Table 2.10: Measurements of down length (DL) including magnitude, $DUR_{max-min}$, $DUR_{min-max}$, date of minimum and maximum DL, maximum and minimum DL in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S30. Data are least square means and standard errors. For full description of statistical parameters see Table 2.5.

DL measurement	Genotype of Goat			Yr	Yr * Ge	Ge
	C	F	G			
Magnitude of DL (mm)						
Year 1	94 ± 6^{b}	68 ± 6^{c}	130 ± 6^{a}	**	NS	***
Year 2	99 ± 7^{b}	71 ± 7^{c}	145 ± 8^{a}			
$DUR_{max-min}$ of DL (days)	88±9	98±9	111±10	na	na	NS
$DUR_{min-max}$ of DL (days)						
Year 1	280±8	279±8	256±9	NS	NS	NS
Year 2	271±8	279±8	265±9			
Date of Minimum DL (day.month)						
Year 1	25.10±6	16.10±6	2.11±7	NS	NS	NS
Year 2	28.10±5	30.10±5	5.11±5			
Minimum DL (mm)						
Year 1	0 ± 3^{b}	0 ± 3^{b}	12 ± 3^{a}	NS	NS	**
Year 2	2 ± 4^{b}	3 ± 4^{b}	15 ± 5^{a}			
Date of Maximum DL (day.month)						
Year 1	2.08±7	23.07±7	17.07±8	***	NS	NS
Year 2	29.07±7	5.08±7	30.07±8			
Maximum DL (mm)						
Year 1	94 ± 7^{b}	68 ± 7^{c}	142 ± 8^{a}	**	NS	***
Year 2	100 ± 7^{b}	74 ± 7^{c}	161 ± 8^{a}			

Values with different superscripts in each row for each DL measurement in each year indicate where genotypes are significantly different at the 5% level. No superscripts are present when genotype differences are not significant.

Table 2.11: Measurements of down length (DL) including magnitude, $DUR_{max-min}$, $DUR_{min-max}$, date of maximum and minimum DL, maximum and minimum DL (mm) in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S225. Data are least square means and standard errors. For full description of statistical parameters see Table 2.5

DL measurement	Genotype of Goat			Yr	Yr * Ge	Ge
	C	F	G			
Magnitude of DL (mm)						
Year 1	97 ± 3^{b}	73 ± 3^{c}	126 ± 3^{a}	***	**	***
Year 2	85 ± 3^{b}	66 ± 3^{c}	126 ± 3^{a}			
$DUR_{max-min}$ of DL (days)	103±5	113±3	110±5	na	na	NS
$DUR_{min-max}$ of DL (days)						
Year 1	266 ± 3^{a}	260 ± 3^{a}	249 ± 2^{b}	***	**	**
Year 2	237 ± 4^{a}	218 ± 4^{b}	241 ± 4^{a}			
Date of Minimum DL (day.month)						
Year 1	11.11 ± 2^{b}	09.11 ± 2^{b}	19.11 ± 2^{a}	NS	**	NS
Year 2	15.11±3	19.11±3	14.11±3			
Minimum DL (mm)						
Year 1	0 ± 2^{b}	0 ± 2^{b}	16 ± 2^{a}	**	***	***
Year 2	1 ± 1^{b}	0 ± 1^{b}	6 ± 1^{a}			
Date of Maximum DL (day.month)						
Year 1	4.08 ± 2^{a}	27.07 ± 3^{b}	26.07 ± 3^{b}	***	**	***
Year 2	27.07 ± 3^{a}	25.06 ± 4^{b}	14.07 ± 3^{a}			
Maximum DL (mm)						
Year 1	98 ± 3^{b}	74 ± 3^{c}	142 ± 3^{a}	***	NS	***
Year 2	86 ± 3^{b}	66 ± 3^{c}	132 ± 3^{a}			

Values with different superscripts in each row for each DL measurement in each year indicate where genotypes are significantly different at the 5% level. No superscript are present when genotype differences are non significant.

Only half of the goats (Figure A2.3) displayed a decrease in SA activity during summer which may be indicative of the shedding of summer down fleece. Double peaks in the proportion of secondary follicles with active and brush fibres (SAct+brush) could be identified only in 30% of the goats (Figure A2.4). SAct+brush follicles were identified from August to February (Figure 2.11) with no differences between genotypes.

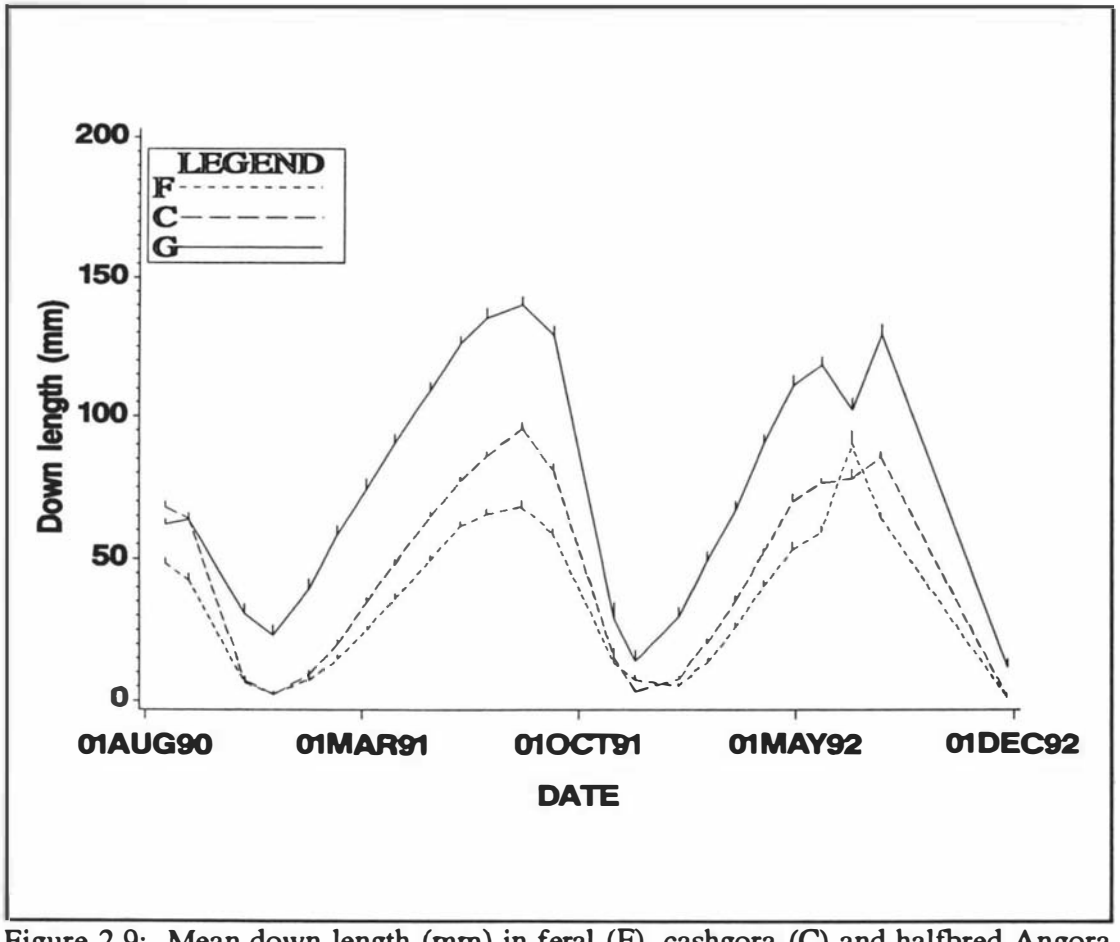

Figure 2.9: Mean down length (mm) in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S225. Bars represent standard errors.

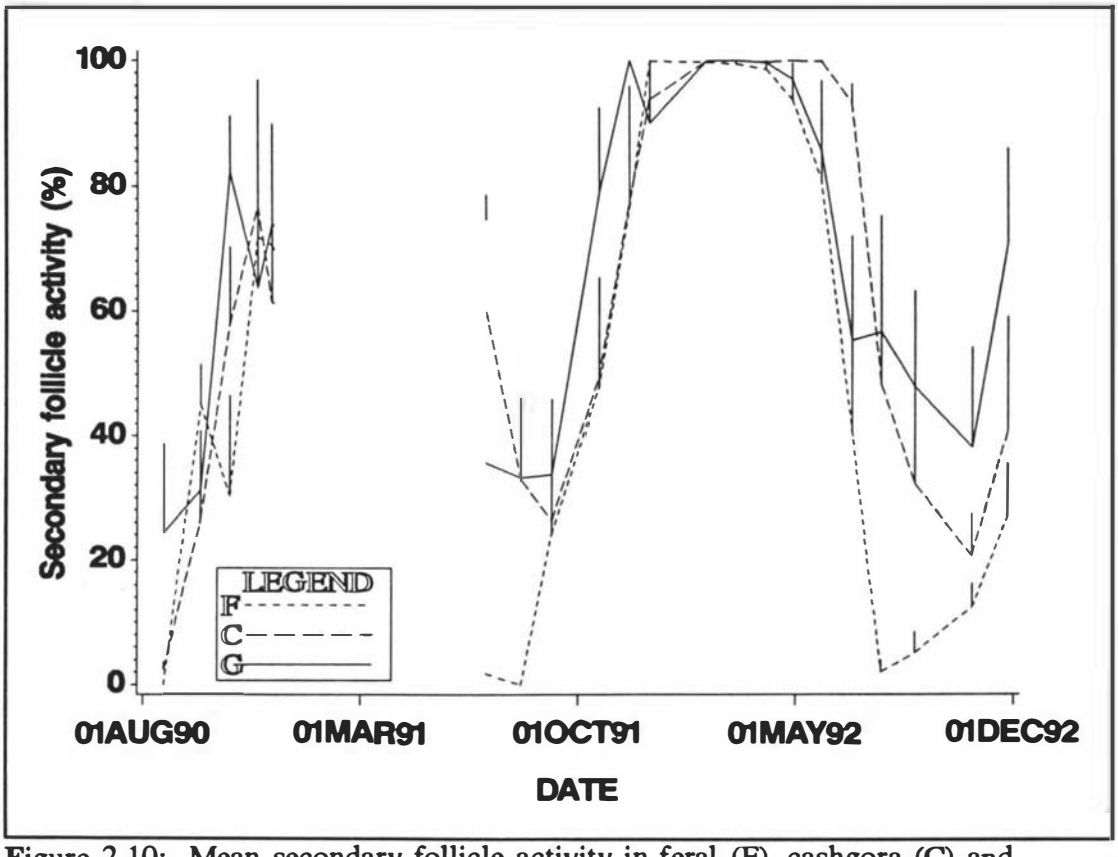

Figure 2.10: Mean secondary follicle activity in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S15. Bars represent standard errors.

2.4.3.3 Medullation in fibres of secondary follicles. The secondary follicles producing medullated fibres were located on the fringe of the follicle group most distant from primary follicles. Medullated secondary fibres were identified only during November and December and occurred in 40% of goats in each of the three genotype groups. The percentage of medullated downe fibres at one time in an individual goat ranged from 1 to 16%. Only one goat had medullated down fibres in both years.

Table 2.12: Measurements of percentage secondary follicle activity (SA) including magnitude, $DUR_{min-max}$, $DUR_{max-min}$, date of maximum and minimum SA, maximum and minimum SA in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S30. Data are pooled means and standard errors.

Secondary follicle activity	Genotype of Goat			Yr	Yr * Ge	Ge
	C	F	G			
Magnitude of SA (%)	89±7	95±7	88±7	na	na	NS
$DUR_{max-min}$ of SA (days)	262±30	247±27	322±30	na	na	NS
$DUR_{min-max}$ of SA (days)	154±20	119±18	107±20	na	na	NS
Date of Minimum SA (days.month)						
Year 1	27.07±131	5.08±12	5.08±13	*	NS	NS
Year 2	7.09±20	7.08±18	9.10±20			
Minimum SA (%)						
Year 1	11±7	5±7	12±7	NS	NS	NS
Year 2	13±7	0±6	23±7			
Date of Maximum SA (days.month)	12.01±13	17.12±11	5.12±12	na	na	NS
Maximum SA (%)	100±0	100±0	100±0	na	na	NS

Values with different superscripts in each row for each SA measurement in each year indicate where genotypes are significantly different at the 5% level. No superscripts are present when genotypes are not signficant.

2.4.3.4 ***Newly erupted down fibres (NEDF).*** Down fibres emerged above the skin in spring at the same time as SA increased and mean DL rapidly decreased (Figure 2.8, 2.10, 2.12,). NEDF's were detected in 100% of F, 97% of C and 78% of G4 goats. In the goats in which NEDF's had been identified, genotype had no effect on the mean date of first identification on 7 October±3 days or mean date of disappearance on 10 November±6 days (Table 2.13). However, the duration of presence of NEDF's was 35 days shorter in G goats compared to C and F goats ($P<0.01$).

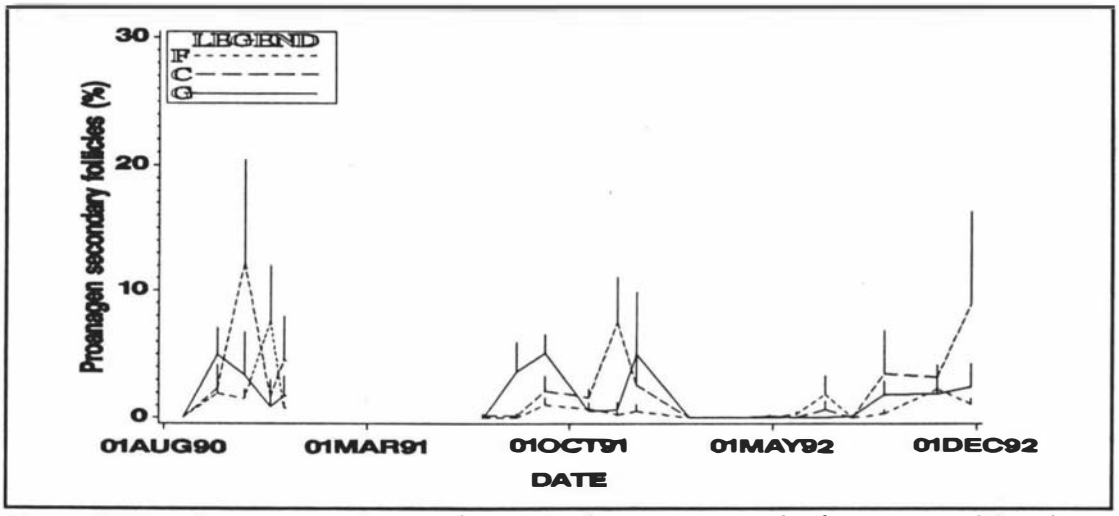

Figure 2.11: Percentage of secondary follicles containing both active and brush fibres in feral (F), cashgora (C) and halfbred Angora x feral (G). Bars represents standary errors.

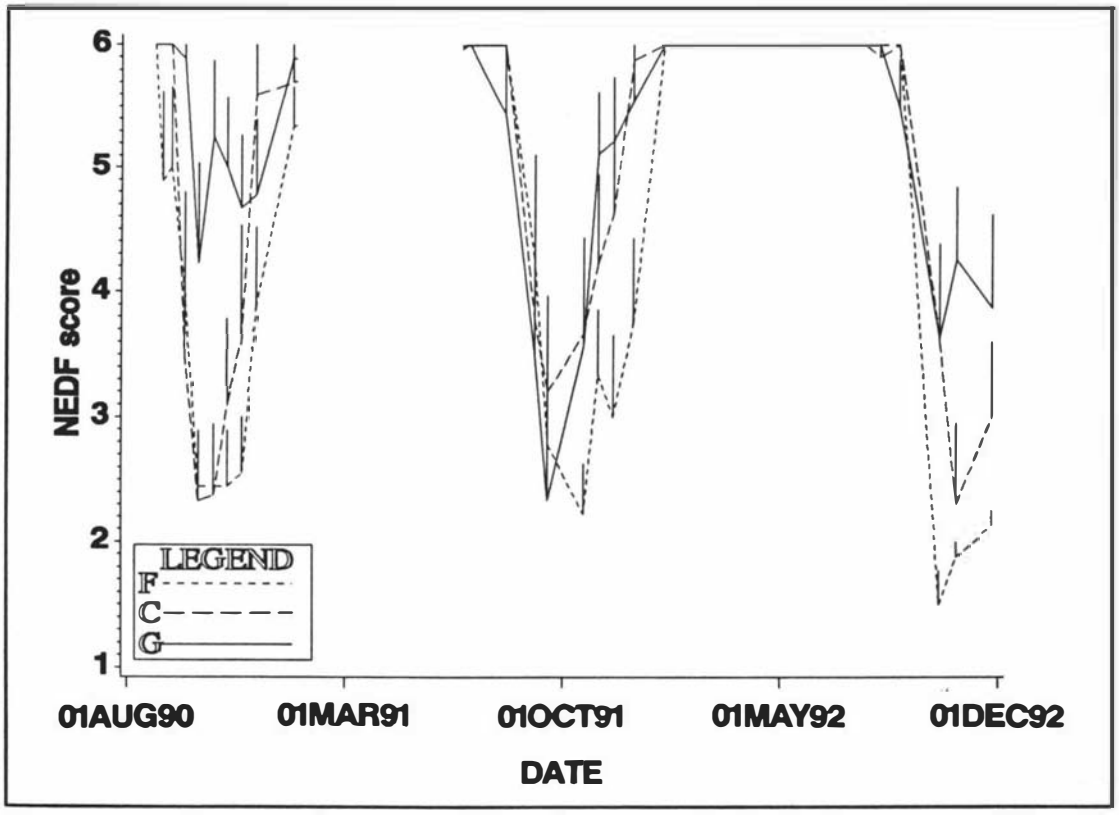

Figure 2.12: Mean newly emerged down fibre score in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S30. Bars represent standard errors.

2.4.3.5 ***Down growth rate (DGR).*** Relative to F goats, G goats had over 4 times greater maximum, minimum and magnitude of mean DGR ($P<0.01$). C goats grew similar amounts of down to F goats (Table 2.14). There was no difference between genotypes in the timing of the DGR (Figure 2.13). DGR was minimal on 19 October±12 days and maximal on 21 March±8 days with a mean $DUR_{max-min}$ of 212±12 days.

2.4.3.6 Down mean fibre diameter (DMFD). The three genotypes differed only in maximum and minimum mean DMFD with down of G goats being more than 4 microns coarser than that of C and F goats (Table 2.15). Mean DMFD was minimal on 2 December±11 days and reached a maximum on 24 April±15 days, and $DUR_{max-min}$ was 145±17 days.

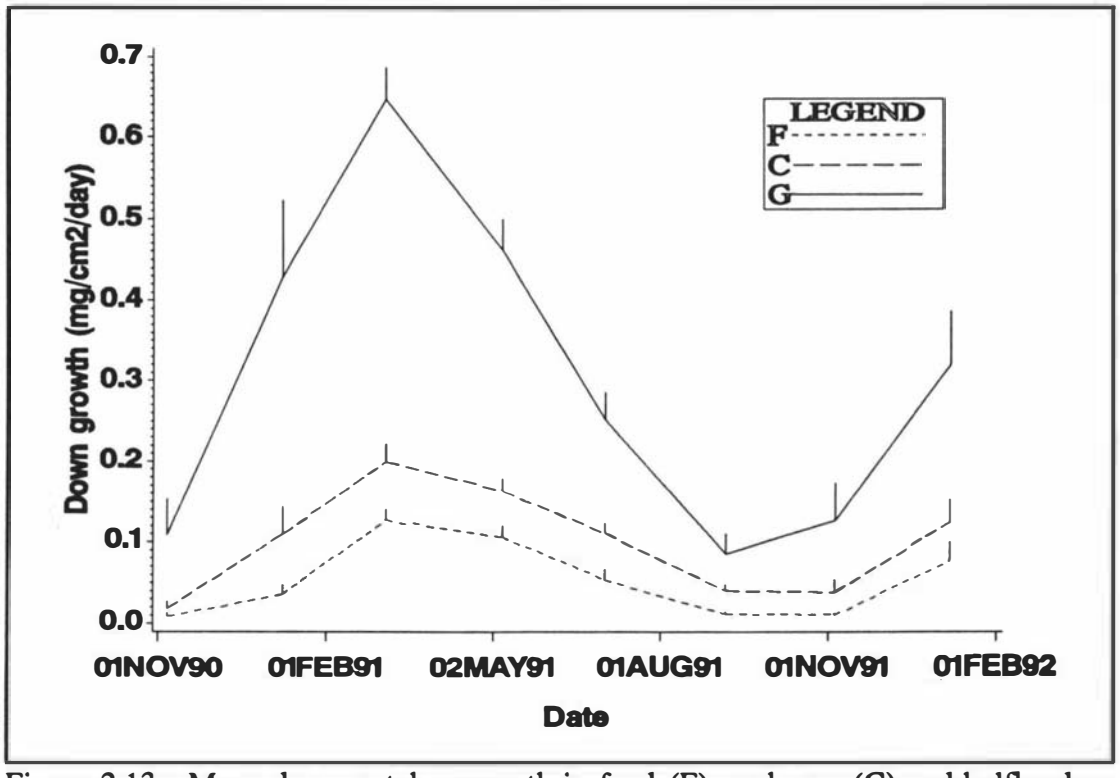

Figure 2.13: Mean down patch regrowth in feral (F), cashgora (C) and halfbred Angora x feral breeding does in subgroup S30. Bars represnet standard errors.

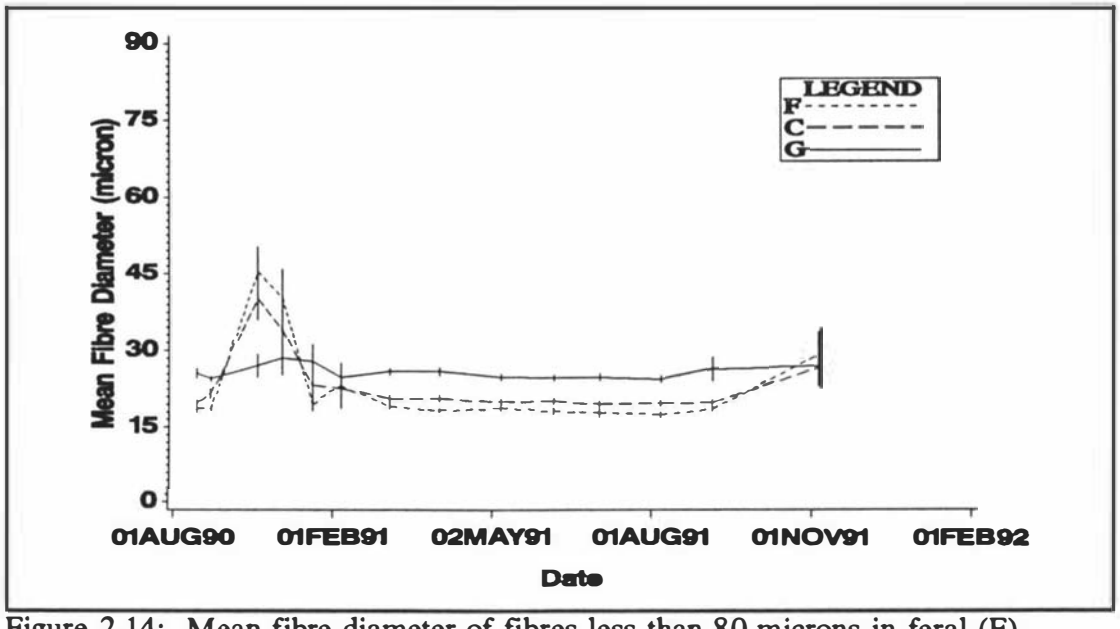

Figure 2.14: Mean fibre diameter of fibres less than 80 microns in feral (F), cashgora (C) and halfbred Angora x feral breeding does in subgroup S30. Bars represent standard errors.

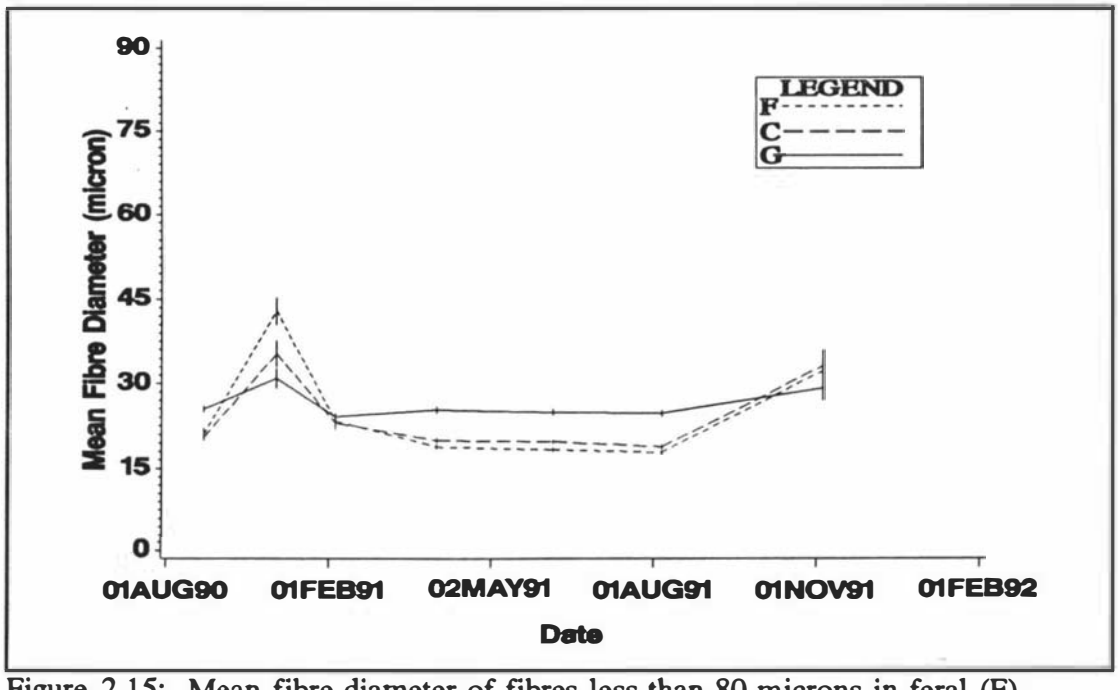

Figure 2.15: Mean fibre diameter of fibres less than 80 microns in feral (F), cashgora (C) and halfbred Angora x feral breeding does in subgroup S90. Bars represent standard errors.

There was an increase in MFD during summer which existed in all three genotypes, but the increase was expressed to a greater extent in C and F goats (Figure 2.14-2.15). During summer there was a higher proportion of fibres between 40 and 80 microns than at other times of the year (Figure 2.16, 2.17). In comparison to C and F goats, G goats had an extended pattern of fibre diameter distribution (Figure 2.16, 2.17).

Table 2.13: Measurements of newly emerged down fibres (NEDF) including date of appearance and date of disappearance and duration (days when NEDF can be observed) in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S30. Data are least square means and standard errors.

Newly emerged down fibres	Genotype of Goat			Yr	Yr * Ge	Ge
	C	F	G			
Date of appearance of NEDF (days.month)						
Year 1	1.10±4	26.09±4	14.10±7	**	NS	NS
Year 2	1.09±9	16.09±8	16.09±13	*		
Date of disappearance of NEDF (days.month)						
Year 1	8.12±15	3.12±12	18.11±11	*	NS	NS
Year 2	8.11±11	18.11±15	18.10±11			
Duration of NEDF (days)						
Year 1	64±12	67±10	30±14	NS	NS	**
Year 2	52±11^{ab}	59±9^{a}	21±13^{b}			

Values with different superscripts in each row for each NEDF measurement in each year indicate where genotypes are significantly different at the 5% level. No superscript are present when genotype differences are not significant.

Table 2.14: Measurements of down growth rate (DGR) including magnitude, $DUR_{max-min}$, date of maximum and minimum DGR, maximum and minimum DGR in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S30. Data are least square means and standard errors.

Down growth rate	Genotype of Goat			Ge
	C	F	G	
Magnitude of DGR (mg/cm^2/day)	0.19±0.03^{b}	0.13±0.03^{b}	0.58±0.03^{a}	***
$DUR_{max-min}$ of DGR (days)	214±13	198±13	224±15	NS
Date of Minimum DGR (days.month)	21.10±14	21.10±14	15.10±8	NS
Minimum DGR (mg/cm^2/day)	0.02±0.01^{a}	0.01±0.01^{a}	0.07±0.01^{b}	*
Date of Maximum DGR (days.month)	20.03±9	6.04±9	5.03±11	NS
Maximum DGR (mg/cm^2/day)	0.21±0.02^{ab}	0.14±0.02^{b}	0.65±0.03^{a}	***

Values with different superscripts in each row for each DGR measurement in each year indicate where genotypes are significantly different at the 5% level. No superscripts are present when genotype differences are not signficant.

Table 2.15: Measurements of down mean fibre diameter (DMFD) including magnitude, $DUR_{max-min}$, date of maximum and minimum DMFD, maximum and minimum DMFD in cashgora (C), feral (F) and first cross Angora x feral (G) does in subgroup S30. Data are least significant means and standard errors.

Down mean fibre diameter	Genotype of Goat			Ge
	C	F	G	
Magnitude of DGR (mg/cm²/day)	3.0±0.7	2.9±0.8	4.9±0.7	NS
$DUR_{max-min}$ of DGR (days)	161±28	101±29	168±28	NS
Date of Minimum DGR (days.month)	07.12±19	01.12±19	28.11±19	NS
Minimum DGR (mg/cm²/day)	16.3 ± 0.5^{b}	15.9 ± 0.5^{b}	20.5 ± 0.5^{a}	***
Date of Maximum DGR (days.month)	14.05±25	9.03±26	13.05±24	NS
Maximum DGR (mg/cm²/day)	19.3 ± 0.8^{b}	18.8 ± 0.8^{b}	25.4 ± 0.7^{a}	***

Values with different superscripts in each row for each DMFD measurement in each year indicate where genotypes are significantly different at the 5% level. No superscripts are present when genotype differences are not signficant.

2.4.4 Plasma PRL Concentrations

The mean 24 hour plasma PRL concentrations were higher at the summer solstice (166±8 ng/ml) than at the winter solstice (22±8 ng/ml) with intermediate values at the autumn (54±8 ng/ml) and spring (53±8 ng/ml) equinoxes (season effect, P<0.001). This pattern and the ratio of winter to summer plasma PRL concentrations (6 to 7.8:2) were unaffected by genotype.

A diurnal pattern in plasma PRL concentrations was evident at all four sample dates (time of day, P<0.05) but it was less apparent at the winter solstice (Table 2.16). Plasma PRL concentrations were higher during dusk than during the day (P<0.05). All genotypes had a similar diurnal pattern in three seasons but not during the autumn equinox (Time*genotype, P<0.001). In autumn the diurnal fluctuations were diminished in F compared to C or G goats (Table 2.17).

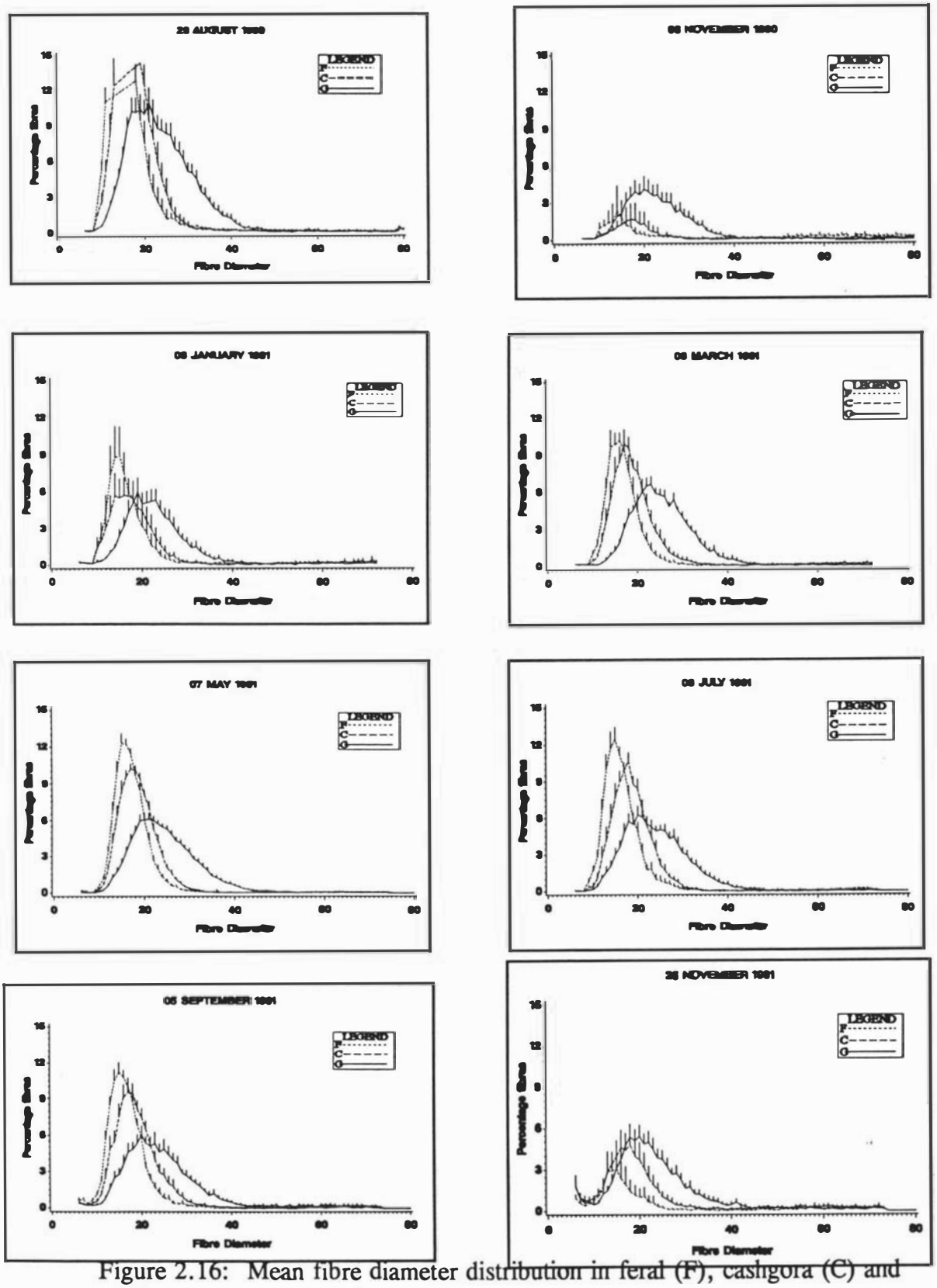

Figure 2.16: Mean fibre diameter distribution in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S30. Bars represent standard errors.

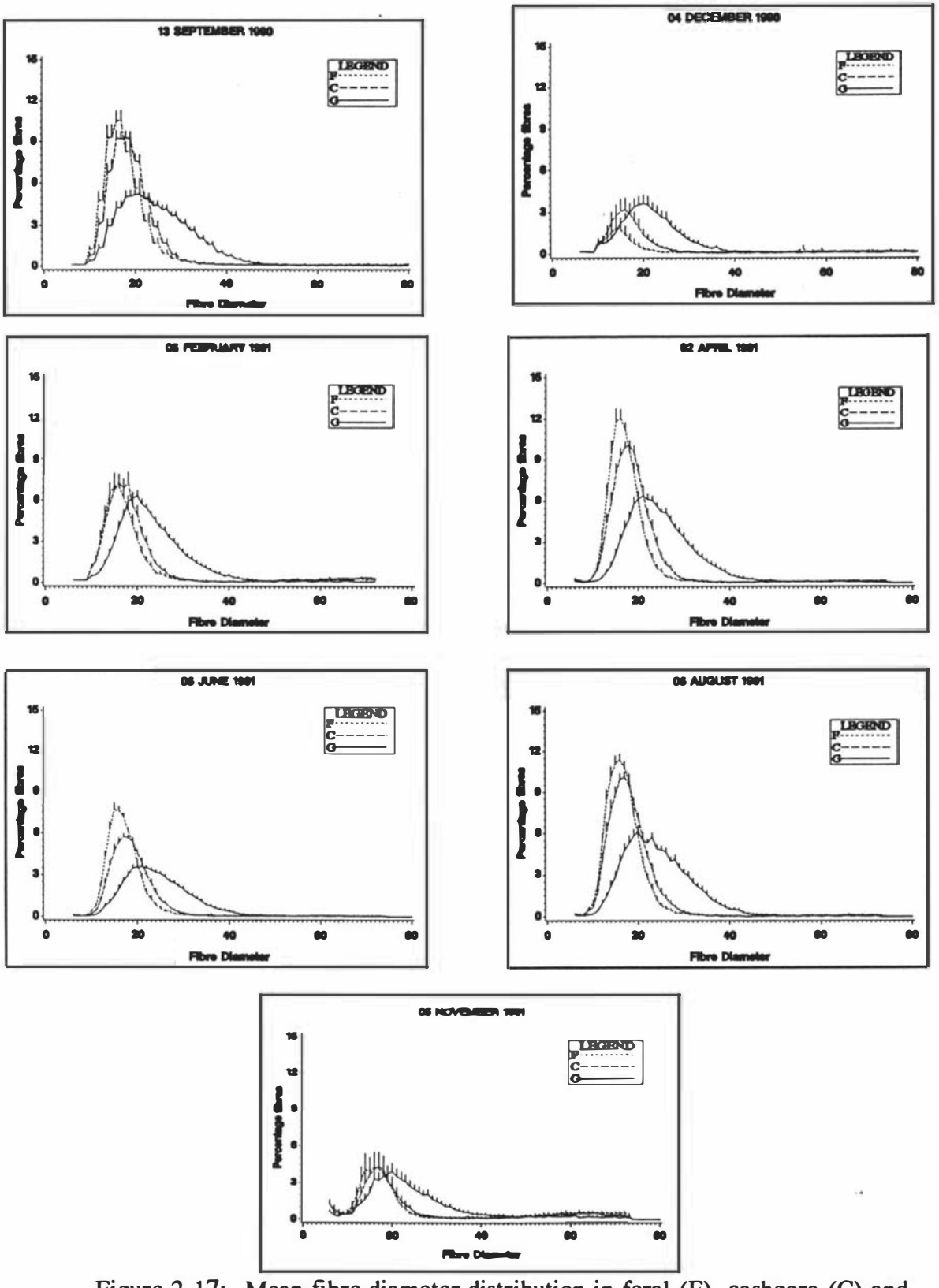

Figure 2.17: Mean fibre diameter distribution in feral (F), cashgora (C) and halfbred Angora x feral (G) breeding does in subgroup S90. Bars represent standard errors.

Table 2.16: Least square (mean±se) plasma PRL concentrations pooled for the periods of dawn, day, dusk, and night on 19 March 1991, 19 June 1991, 19 September 1991 and 19 December 1991 in down-producing goats.

Diurnal period	Plasma PRL Concentrations (ng/ml)			
	19 March	19 June	19 September	19 December
Dawn	22 ± 7^{c}	23 ± 3^{ab}	41 ± 7^{b}	151 ± 21^{bc}
Day	39 ± 7^{bc}	18 ± 3^{b}	51 ± 7^{b}	121 ± 21^{c}
Dusk	99 ± 7^{a}	28 ± 3^{ab}	76 ± 7^{a}	178 ± 21^{ab}
Night	54 ± 7^{b}	21 ± 3^{ab}	43 ± 7^{b}	214 ± 21^{a}
Significance	***	*	***	***

Values with different superscripts in each column of plasma PRL data indicate where diurnal periods are significantly different at the 5% level. Significance of diurnal period effect *** P<0.001, * P<0.05.

Table 2.17: Pooled mean±se plasma PRL concentrations in cashgora (C), feral (F) and first cross Angora x feral (G) goats during dawn, day, dusk and night on 19 March 1991 showing effect of diurnal period (DP) and Genotype (Ge).

Genotype of Goat	Plasma PRL Concentrations (ng/ml)				DP	Ge	DP * Ge
	Dawn	Day	Dusk	Night			
C	23±4	40±7	108±19	53±12			
F	23±5	52±8	73±20	55±12	***	NS	**
G	22±5	114±20	114±20	58±12			

Significance *** P<0.001, ** P<0.01.

2.5 DISCUSSION

2.5.1 Fibre growth cycles in three genotypes of goats

The inclusion of additional Angora ancestry in down-producing goats alters the follicle structure of the skin. The S/P ratio in the purebred Angora goat ranges from 7 to 10 (Shelton, 1968; Clarke, 1977; Winklmaier, 1983: Eppleston and Moore, 1990; Nixon *et al.*,1991) and the feral and cashgora goats in this study fell within the published means of 4 to 6 for feral goats (Henderson and Sabine, 1991b; Jonen, 1991; Nixon *et al.*, 1991). In comparison to C and F goats, the skin of G goats in this study and another study (Jonen, 1991), had a S/P ratio 1.4 unit higher.

The processing of down for garment manufacture requires the separation of the guard hair (>80 micron) from the down (<40 micron) portion of the fleece. This dehairing process requires a good divergence in weights of individual guard hair and down fibres with few fibres of intermediate fineness (40-80 micron) (Bigham, 1992). In comparison to feral goats, and consistent with other studies (Dadabaev, 1984; Gretton and Bigham, 1988; Scheurmann *et al.* 1990; Jonen, 1991) down goats with additional Angora ancestry produced down which was coarser and longer and guard hair which was thinner and shorter. In addition, the fibre diameter distribution of G goats was more extended, with higher proportions of intermediate fibres. Therefore, as the weight components, one of which is fibre diameter, of guard hair and down fibres are more convergent, in G compared to C and F goats, it is not suprising that G fleeces can be more difficult to dehair (Bigham, 1992). Unfortunately the diameter distribution of fibres less than 80 micron, down and guard hair mean fibre diameter remains relatively consistent from January to September. Therefore it is unlikely that the dehairing characteristics of the winter down fleece of the G goats could be improved by altering the timing of shearing.

2.5.2 Primary follicle growth. The pattern of guard hair growth in F and C goats was similar to that in other published studies (Ryder, 1970; McDonald *et al.*, 1987; Nixon *et al.*, 1991a). However, the guard hair growth cycle reported by McDonald *et al.* (1987) occurred one month earlier, while that of Nixon *et al.*, (1991a) occurred one month later, than that observed in this study. In this study, all primary follicles of all genotypes were quiescent in July when the guard hair fleece reached its maximum length. Primary follicles become progressively activated from September (Ryder, 1970; Nixon *et al.*, 1991) and old fibres are pushed from the follicle and shed. The guard hair fleece is not, however, completely lost (Nixon *et al.*, 1991; Kloren *et al.*, 1993) but it is shorter and thinner during summer (McDonald *et al.*, 1987). In comparison to other studies (Ryder, 1970; Nixon *et al.*, 1991) PA in this study increased more rapidly from September and reached maximum levels in December. Shortly after December, the guard hair fleece was maximal for both GMFD and GHGR. PA and GHGR began to decline in April (Ryder, 1970; Nixon *et al.*, 1991) approximately two months earlier than the equivalent decrease in SA.

In this study, during November to April, both central and lateral primary follicles underwent a period of quiescence and then activity with a short (or absent?) period of telogen. This autumn cycle may be a vestige of the biannual spring and autumn pelage growth cycle. Autumn subsidiary cycles in PA are observed in Soay sheep (Ryder, 1971), Mouflon sheep (Ryder, 1978), Limousin sheep (Rougeot, 1961), Wiltshire sheep (Slee and Carter, 1962; Ryder, 1978; Parry *et al.*, 1991) and medullated fibre-producing, central and lateral primary follicles of the Angora goat (Nixon *et al.*, 1991b). Such a cycle has not been reported previously for Cashmere goats. The subsidiary cycle is not easily observed when PA is averaged because, unlike the spring activation of primary follicles, the timing of catagen and follicle reactivation is not highly synchronised between individuals and the telogen period appears to be very short. The absence of synchrony between animals suggests that this cycle of growth may be an intrinsic growth pattern, inherent in the individual

primary follicles of goats in this study and not modified by a common photoperiodically dependent, endocrine modulator.

There were similarities in the sequence of primary follicle growth in all three genotypes of goats but the duration of the primary fibre growth was lengthened by increasing proportions of Angora ancestry. In comparison to F goats, G goats had a $DUR_{min-max}$ 70 and 50 days longer for PA and GL growth and a 54 day longer $DUR_{max-min}$ in GHGR. The primary follicles of the Angora goat retain a seasonal cycle in PA, achieving maximum levels in October and minimal levels in August (Nixon et al., 1991b) which, from averaged data, appears to be of a longer duration than that of F goats. In this study, the growth of fibre from primary follicles was longer in duration in goats with more Angora influence in growth. In a cross between Mouflon (a shedding sheep breed) and the Merino (largely aseasonal wool growth pattern) the primary follicles of the crossbred behave like those of the Mouflon parent while the secondary follicles behave like those of the Merino parent, having a low incidence of shedding (Ryder, 1978).

In comparison to shedding sheep breeds, the less seasonal fibre growth cycles of modern sheep breeds are characterised by longer periods of active growth and shorter periods of follicular quiescence (Lincoln, 1978). In the Angora goat large medullated fibres in primary follicles have a biannual growth pattern while smaller non-medullated fibres in primary follicles have an annual pattern of growth and shedding (Nixon *et al.*, 1991b). It could be speculated that, in the primary follicles of goats, a decrease in size is associated with a reduction in seasonality of growth. As the proportion of Angora ancestry increases the number and size of primary follicles decrease.

2.5.3 Secondary follicle growth: Contrary to generally accepted dogma, a greater proportion of Angora ancestry in down-producing goats does not consistently extend the duration of winter down growth. The contention that winter down growth

commences earlier and fibres are shed later, in down goats with Angora ancestry, is an optical illusion. This illusion is created because down does not completely disappear from the fleece of many G goats and neither does SA fall to zero in spring as in F goats. It is possible that some secondary follicles in the G goats either no longer possess a photoperiodically synchronised catagen phase or have a telogen stage that is relatively fleeting such that the monthly sample regime used in this study failed to identify a period of complete follicle quiescence. A failure to achieve full follicle inactivity is associated with a reduced expression of physical shedding in Wiltshire (Parry *et al.*, 1991) and Cheviot (Ryder, 1978) sheep, while expression of fleece shedding through fibre breakage and not follicle quiescence is observed in Shetland sheep (Ryder, 1978). A decline in follicle inactivity may be an evolutionary stage towards continuous fleece growth. Certainly in the Angora goat secondary follicles no longer possess a synchronised follicle inactivity stage. However, all facets of the timing of changes in winter down growth in this study were identical in all three goat genotypes. This may indicate that the timing of fibre growth in secondary follicles, in both shedding and non-shedding goats, shares a common physiological mechanism even when follicles no longer have a catagen stage.

The timing of down growth in this study is identical to that previously published for New Zealand feral goats (Nixon *et al.*, 1991) and the $DUR_{min-max}$ of the DL growth cycle is within the range of 224 to 281 days published for Australian does (Kloren *et al.*, 1993c). As in primary follicles, maximum SA occurs in December, but in contrast to primary follicles, this is associated with reducing DMFD. As in a previous study (Nixon *et al.*, 1991a) maximum DGR occurred in March and winter DMFD was highest in April. SA declined from June but the remaining active secondary follicles continued to produce longer fibres which continued to lengthen the down fleece until the end of July. Secondary follicles, relative to primary follicles, had a longer anagen (Ryder, 1971) and a shortened telogen phase and reached minimal activity at the end of August, one month after primary follicles. In contrast to the results of Ryder (1971), this study confirmed that both primary and

secondary follicles become activated in September consistent with the results of Nixon *et al.*, (1991a) and Henderson and Sabine (1993), and both may be triggered by the same physiological event.

Subsequent to the activation of secondary follicles in September, DGR and DL reduce and reach a minimum in October. The activation of secondary follicles does not produce visible down in the fleece and appears to be associated with winter down shedding. NEDF's are found in the fleece from October to November as was also observed by Nixon *et al.* (1991a). In this study the appearance of NEDF's occurred at the same time as the rapid reduction in DL which is symptomatic of the loss of down from the follicle. Growth of the summer down fleece may push the winter down fibres from the follicle. However, Nixon *et al.*, (1991a) found that some down fibres are shed prior to the initiation of new growth. Therefore loss of fibres from secondary follicles and summer down fleece growth may simply share a common physiological trigger.

Secondary follicles contained both anagen and brush fibres from August to February however the percentage of occurrence of such follicles, at any one instance of time in an individual, was below 30% and they had usually disappeared within a month. A period of active growth and quiescence in secondary follicles during summer could be confirmed by follicle activity measurement in only 50% of the goats in this study. Kloren *et al.*, 1993c established that winter DL grows at a rate of 0.2 to 0.3 mm per day. If a similar DL growth rate occurred in the summer fleece then the brush fibre in an individual follicle may disappear after 2 days and anagen could cease after ten days. In this experiment it is possible that the follicle measurement regime was not sufficiently frequent to accurately demonstrate the existence of the shedding of the summer fleece.

This study identified a bimodal pattern in DMFD in C and F and, to a lesser extent, G goats which also was also found in Australian feral goats (McDonald *et al.*, 1987).

The down which appeared in the fleece during October to November was coarser than that which grew during winter and there was an increase in the proportion of fibres between 40 and 80 microns. During October and November, medullated fibres were found in the outer fringes of the secondary follicles of goats in all three genotype groups in this experiment, as also reported in cashmere (Nixon *et al.*, 1991a) and Angora (Nixon *et al.*, 1991b) goats. In theory, it is possible that the coarser DMFD and medullation are characteristics of summer fleece growth. However, the measurement system used may have contributed to the summer peak in MFD. One thousand fibres of less than 80 micron were measured. In summer there is very little down in the fleece and therefore intermediate fibres, while not increasing in absolute number, become a greater proportion of the "less than 80 micron" fleece.

NEDF's during summer are less prevalent in G goats and occur for a shorter time than in the C and F goats. The inclusion of additional Angora ancestry has decreased the propensity for biannual down growth in secondary follicles.

2.5.4 Plasma PRL concentrations: Plasma PRL concentrations, in this experiment exhibited the classic pattern which was found in goats (Buttle, 1973; Mori *et al.*, 1985; Maeda *et al.*, 1988; Prandi *et al.*, 1988; Grasselli *et al.*, 1992) and other animals (deer: Kelly *et al.*, 1982, Bubenik *et al.*, 1985; Loudon *et al.*, 1989; sheep: Lincoln, 1979; Leshin and Jackson, 1987; cattle: McNatty *et al.*, 1984; Zinn *et al.*, 1986) of low concentrations during short days and high concentrations during long days. Plasma PRL concentrations during the autumn and spring equinox were similar.

As in other studies with sheep (Lincoln *et al.*, 1978; Kennaway *et al.*, 1983) and goats (Sugawara *et al.*, 1990), plasma PRL concentrations were lower during daylight but, following reducing light levels during dusk, plasma PRL concentrations increased. In the goats in this experiment and in others (Mudulli *et al.*, 1979; Sugawara *et al.*, 1990), diurnal patterns in plasma PRL concentrations were magnified

during summer compared to winter photoperiods. In addition, during the summer solstice, plasma PRL concentrations were high throughout the whole nocturnal period. In other studies in goats, a nocturnal peak of plasma PRL concentrations is observed in long but not in short days (Muduuli *et al.*, 1979; Mori *et al.*, 1985; Maeda *et al.*, 1988). In sheep, a a peak in plasma PRL concentrations is found at dusk and dawn during long days while only a dusk peak exists during short days (Lincoln *et al.*, 1978; Kennaway *et al.*, 1983). It is not known whether the seasonal differences in diurnal pattern in plasma PRL concentration form part of the message which modifies seasonal fibre growth patterns.

In this experiment, the only effect of genotype of goat on plasma PRL concentrations was the decrease in the size of fluctuations in the diurnal pattern in F goats in March. This is an unexpected result which was not reflected in diurnal patterns during other seasons. Whether this is a chance effect or has physiological significance is not clear.

Additional Angora ancestry had no effect on mean seasonal plasma PRL concentrations. In contrast, in two down genotypes with different patterns of seasonal fibre growth it was found that early shedding was associated with an early spring increase plasma PRL concentration (Rhind, 1994). In sheep (Lincoln, 1990; Clarke *et al.*, 1993), there was no evidence that the magnitude of seasonal plasma PRL concentrations differs for genotypes differing in seasonal patterns of fibre growth. However, the genotype differences in this study were not as extreme as in the study in sheep which compared shedding sheep breeds and the largely aseasonal Merino sheep.

2.6 CONCLUSIONS

It can be postulated that in goats there is an interaction between follicle size and seasonality. The largest primary follicles and the smallest secondary follicles retain

the most primitive seasonal patterns. As primary follicles decrease, and secondary follicles increase, in size their pattern of fibre growth becomes less seasonal.

In the Angora, feral goats and their crosses it appears that selection for fibre growth has progressively shifted growth from a primitive biannual shedding of short duration, to an annual shedding of longer duration, and then to a seasonal amplitude in growth with no shedding. Presumably with further selection the amplitude in growth would eventually disappear as it has in the Merino. The first stage of this selection is reflected in the first cross of the Angora and feral goat, where the biannual growth pattern of secondary follicles is reduced in G goats and the duration of growth seen in the primary follicles is increased. Second, it appears in this study that some secondary follicles of G goats no longer express a catagen phase. However, it could be speculated that the lack of differences in the timing of secondary follicle growth in all three genotypes reflects a physiological mechanism which links fibre growth with photoperiod and has been largely retained in the goat genotypes used in this experiment, despite the absence of catagen in some secondary follicles.

CHAPTER 3

FIBRE GROWTH AND PLASMA PRL CONCENTRATIONS IN DOWN-PRODUCING CASTRATE MALE GOATS.

3.1 ABSTRACT

Plasma PRL concentration and fibre growth (length, shedding score, newly emerged down fibres, follicle activity) were measured in ten adult down-producing castrate wethers at two weekly intervals from 18 July until 22 November 1991 and thereafter at monthly intervals until 20 February 1992. The timing and correlation of fibre growth and plasma PRL concentration events were determined for both primary and secondary follicles.

Follicle activity of primary follicles began to increase on 17 September±7 days and follicles reached full activity on 1 January±10 days. The date of primary follicle reactivation in spring was associated with the date at which plasma PRL concentration increased above 20 ng/ml ($r=0.78$, $P<0.01$).

Secondary follicles were activated on 17 September±11 days and SA reached a summer peak of 38±4% on 31 October±9 days. The date of summer peak in SA was correlated with other summer down events such as the date of first peak in SAc+brush follicles ($r=0.85$, $P<0.01$), date of emergence of NEDF's ($r=0.92$, $P<0.001$), date of reduction in DL ($r=0.87$, $P<0.01$), and date of maximum shedding score ($r=0.85$, $P<0.01$). The increase in SA, which resulted in winter down production, commenced on 5 November±9 days and it was correlated with the date of disappearance of NEDF's ($r=0.82$, $P<0.01$) and the date of second peak in SAc+brush follicles ($r=0.82$, $P<0.01$). Associations between secondary follicle reactivation and plasma PRL concentration could not be found though some aspects of winter down growth were correlated with plasma PRL concentration events.

Both primary and secondary follicles commenced growth during the rapidly increasing photoperiods of the spring equinox. While primary follicles continued a sustained rise in activity up to a maximum at the summer solstice, secondary follicles underwent an additional down growth cycle during summer. All goats exhibited a summer down growth phase but it was not clear whether all secondary follicles participated. The timing of onset of shedding could not be conclusively linked to activation of summer down growth but maximum shedding did occur in synchrony with peak summer down growth. Individual variation in the timing of primary

follicle activation was partially explained by variation in the date of increase in plasma PRL increase to 20 ng/ml. Some aspects of the timing of winter down growth were also associated with plasma PRL concentration events.

3.2 INTRODUCTION

Grafting studies have demonstrated that the fibre follicles of rats (Ebling and Johnson, 1959; Ebling, 1965; Ebling and Johnson, 1961) and sheep (Priestley, 1965) possess an endogenous growth cycle which takes several cycles before reflecting the growth of the surrounding recipient skin. In addition, endogenous follicle cycling can be demonstrated in sheep (Lincoln *et al.*, 1980; Allain *et al.*, 1984; Allain *et al.*, 1986), mink (Marinet *et al.*, 1985; Boissin-Agasse *et al.*, 1988; Lincoln *et al.*, 1989; Martinet *et al.*, 1992) and Cashmere goats (McDonald and Hoey, 1987) following pinealectomy, ganglionectomy or exposure to constant photoperiod. During continuous-light treatment, but not temperature control, the fibre cycles of down-producing goats are repeated at 8 month intervals and this is thought to reflect the nature of the endogenous cashmere fleece cycle (McDonald and Hoey, 1987). Relative to the down growth cycle in natural photoperiod, the telogen period of the endogenous cycle shorter (McDonald and Hoey, 1987). The telogen period is also shorter in down goats which have been immunized against melatonin (Sutherland *et al.*, 1990).

Follicle reactivation, or perhaps alternatively the prevention of follicle reactivation, may prove to be the key processes which synchronise the endogenous follicle down cycle with season. In natural temperate photoperiods, follicle reactivation occurs in spring (Nixon *et al.* 1991a, Henderson and Sabine, 1993; Chapter 2). It has already been established that the follicles of down-producing goats are responsive to manipulations of photoperiod (McDonald and Hoey, 1987; Norton 1991) and melatonin (Betteridge *et al.*, 1989; Moore *et al.*, 1989; Scheurman *et al.*, 1989; Litherland *et al.*, 1990; Gebbie *et al.*, 1991; O'Neill *et al.*, 1992) when treatments are

applied between the spring equinox and the summer solstice. It is during this period that the winter fleece is shed, the summer fleece is grown and shed, and the winter fleece begins to grow (Nixon *et al.*, 1991a). However, to date, little is known about the timing and association of these fibre events.

The physiological recognition of photoperiod involves the endocrine system (Kennaway *et al.*, 1987; Chemineau *et al.*, 1988), though which facets of this system act to synchronise the down follicles, with photoperiod, is unclear. In goats, plasma PRL concentration has a seasonal pattern with high concentrations during summer, low concentrations during winter and increasing concentrations in spring (Buttle, 1973; Mori *et al.*, 1985; Maeda *et al.*, 1988; Tamanin *et al.*, 1988; Grasselli *et al.*, 1992). It has been suggested that plasma PRL concentration has a causal role in regulating the moulting and growth of pelage in mustalids (Martinet, *et al.*, 1984; Rose *et al.*, 1987; Badura and Goldman, 1992), sheep (Pearson *et al.*, 1994), and down goats (Lynch and Russell, 1989; Kloren and Norton, 1993d). Studies in down goats, where plasma PRL concentration has been manipulated after the spring equinox, suggest that physical shedding of the winter down fleece is associated with increasing plasma PRL concentration during spring (Lynch and Russell, 1990; Kloren and Norton, 1993d). To date it has not been established whether summer down growth is coupled with the shedding of 'winter' down but, in many species, shedding of fibres coincides with the new growth phase in the follicle.

In the study reported in this Chapter, the natural pattern of fibre growth, from both primary and secondary follicles, was described for the period from July 1991 to February 1992. A detailed knowledge of the timing and associations of natural fibre events in down-producing goats forms the basis against which treatments in later studies can be interpreted. In this Chapter, the timing and inter-correlations of winter fleece shedding, summer fleece growth and winter fleece activation are described. The association between primary and secondary growth events are also reported. Finally it is determined whether changes in plasma PRL concentration, in natural

photopheriod, were associated with the timing of fibre growth events.

3.3 METHODS

Ten mixed-age castrate male down-producing goats (mean liveweight 33±4 (±se) kg) were housed in two pens from 5 July until 31 October 1991 and then released on to pasture at the Flock House Agricultural Centre, Bulls, New Zealand (40° 11′ S 175° 23′ E). While housed the goats were each fed 250 g per goat of maize between 0800 and 0900 h daily with meadow hay and water on offer *ad libitum*. Goats were kept under natural photoperiod and temperature with no additional lighting. These animals formed the control group used in the experiment described in Chapter 6.

3.3.1 Measurements

Various fibre growth measurements were conducted at two weekly intervals from 18 July until 22 November 1991 and thereafter at monthly intervals until 20 February 1992. Stretched down and guard hair length were measured on the neck, front shoulder, midside and hind shoulder (Section 2.2.2.1) from which mean down (DL) and guard hair (GL) length were calculated.

The amount of fleece shedding was determined by visually scoring the amount of fibre plucked from the fleece. The amount of plucked fibre was scored in a shedding score (SS) from 1 (none) up to 5 (profuse).

At each fibre measurement date, skin snip biopsy samples (Section 2.2.2.4) were collected and fixed in formalin. All skin samples were viewed under a dissecting microscope (Section 2.2.3.2) and scored for the presence of newly emerged down fibres (Section 2.2.3.2). Skin samples were embedded in wax, serial sectioned and stained using an adapted Sacpic stain (Section 2.2.3.2). Approximately 10 follicle groups in each sample were studied and the guard hair-producing (primary) and

down-producing (secondary) follicles were scored as either being active (anagen), inactive (telogen) or active+brush (Section 2.2.3.3). Primary follicle activity (PA), secondary follicle activity (SA), active+brush primary (PAc+brush) and secondary (SAc+brush) follicles were calculated as a percentage of total number of either primary or secondary follicle types.

Blood samples were collected in heparinised vacutainers by jugular venipuncture at 1100 h on a day adjacent to fibre measurements. Plasma was separated from blood by centrifugation at 3000 g at room temperature and plasma samples were frozen at -8°C pending assay for PRL concentration.

The radioimmunoassay of plasma PRL concentration was conducted at AgResearch Ruakura using the protocol outlined in Section 2.3.2.3.

3.3.2 Statistical Analysis

For each individual goat the timing of various events relating to fibre growth and plasma PRL concentrations was determined (Table 3.1) and are presented in the text as mean date±se days. Then the association between fibre growth and plasma PRL concentration events was determined by calculating Pearson correlation coefficients (r) (SAS, 1987).

3.4 RESULTS

3.4.1 Primary follicle growth events

PA increased after winter in individuals at the mean date of 17 September± 7 days (range 14 August-10 October) and follicles had reached full activity by 1 January±10 days (20 November-20 February). New growth in primary follicles, i.e. follicles containing both active and brush fibres, was initiated from 26 September until 4 December (Table 3.1).

Table 3.1: Fibre and plasma PRL concentration events and mean date (day.month) of their occurrence (in date order) for down-producing wethers. Measurements were conducted from July 1991 to February 1992.

Date±se (range) (days)	Fibre event descriptions
26.08 ± 4 (14.08-12.09)	Plasma PRL concentration reached 20 ng/ml (PRL_{20})
08.09 ±11 (30.07-20.11)	Fleece Shedding score first increased to 2 or greater (SS_i).
17.09 ± 7 (14.08-09.10)	Primary follicle activity first increased by 10% from minimum winter levels (PA_i).
17.09 ± 6 (30.07-20.11)	Secondary follicles with both active and brush fibres were first identified (SAc_d).
22.09 ±11 (14.08-06.11)	Secondary follicle activity first increased by more than 10% from minimum winter levels indicating start of summer down growth. (SA_d)
26.09 ± 6 (12.09-23.10)	Active primary follicles with brush fibres also were first identified (PAc_a).
06.10± 7 (12.09-06.11)	Plasma PRL concentration reached 50 ng/ml (PRL_{50}).
06.10± 6 (12.09-20.11)	The first peak in secondary follicles with both active and brush fibres (SAc_s).
09.10± 8 (01.10-12.12)	Fleece shedding score reached maximum levels (SS_{max}).
11.10± 9 (12.09-20.12)	First peak in secondary follicle activity (SA_s).
24.10±12 (12.09-17.01)	Newly emerged down fibres first appeared above the skin; NEDF score 1 or 2 ($NEDF_a$).
28.10±10 (12.09-20.12)	Mean down length decreased by more than 10 mm (DL_d).
08.11± 9 (09.10-17.01)	Secondary follicle activity increased indicating start of winter down growth (SA_w)
27.11± 9 (24.10-17.01)	Mean down length first reached zero (DL_{min}).
24.11± 6 (06.11-17.01)	Second peak in secondary follicles with both active and brush fibres (SAc_w).
4.12± 6 (06.11-17.01)	The last instance of primary follicles with both active and brush fibres (PAc_i).
14.12± 9 (09.10-17.01)	Shedding score returned to 1 (SS_i).
20.12± 7 (09.10-17.01)	Plasma prolactin concentration reached 100 ng/ml (PRL_{100}).
29.12± 8 (20.11-20.02)	Newly emerged down fibres disappeared; NEDF score returned to 6 ($NEDF_d$).
31.12± 8 (20.11-20.02)	Secondary follicle activity reached 100% (SA_{max}).
31.12±10 (09.10-17.01)	Plasma PRL concentration reached peak concentrations (PRL_p).
01.01± 9 (20.11-20.02)	Primary follicle activity reached 100% (PA_{max}).
01.01±11 (06.11-20.02)	Mean guard hair length reached a minimum (GL_{min}).
22.01± 7 (20.12-20.02)	Mean down length began to increase (DL_i)

Table 3.2: Correlation coefficients between primary fibre growth and shedding events in down-producing castrate male wethers from July 1991 to February 1992. Abbreviations are described in Table 3.1.

	SS_i	SS_f	SS_{max}	GL_{min}	PA_i	PA_{max}	PAc_i	PAc_f
SS_i	1.0	0.43	0.05	-0.20	0.07	0.09	0.37	0.48
SS_f		1.0	0.44	0.20	**0.70***	0.30	0.49	0.51
SS_{max}			1.0	0.17	0.20	0.27	0.12	0.07
GL_{min}				1.0	0.311	-0.35	-0.09	-0.18
PA_i					1.0	-0.20	0.11	0.34
PA_{max}						1.0	0.41	**0.78****
PA_{max}							1.0	**0.72***
PAc_f								1.0

*P<0.05, **P<0.01

Table 3.3: Correlation coefficients between primary and secondary follicle activity events in down-producing castrate male goats measured from July 1991 to February 1992. Abbreviations are described in Table 3.1.

	PAc_i	PAc_f	PA_i	PA_{max}
SAc_s	0.02	0.13	0.26	0.17
SAc_w	0.40	0.62	0.49	**0.67***
SAc_i	0.43	0.62	-0.08	0.53
SA_s	0.37	0.40	0.27	0.38
SA_{si}	0.58	**0.76****	-0.08	**0.74***
SA_{wi}	0.47	0.61	0.28	**0.74***
SA_{max}	0.37	**0.77****	0.57	**0.86*****

* P<0.05, ** P<0.01, *** P<0.001

The guard hair fleece reached its minimal summer length on 11 January±11 days. In comparison to secondary fibre events, the primary fibre events differed in timing and were not consistent between individuals (Table 3.2).

The timing of primary and secondary follicle growth events during the spring reactivation period were not correlated (Table 3.3). However, they were linked in the late summer period, when there was an association between the date of achieving maximum PA and the date of maximum SA ($r=0.86$, $P<0.001$), the date of increase in SA for winter down growth ($r=0.74$, $P<0.05$) and the date of the second peak in SAc+brush follicles ($r=0.67$, $P<0.05$). In addition, the date of achieving maximum SA was associated with the last date when new growth (PAc+brush follicles) was identified in primary follicles ($r=0.77$, $P<0.01$).

3.4.2 Secondary follicle down growth events

New down growth (SAc+brush follicles) was first identified in secondary follicles on 17 September±6 days and significant increases in SA, from winter minimum values, were detected on 22 September±11 days. The percentage of secondary follicles with new growth peaked on 6 October±6 days and subsequently on 24 October±12 days this new growth could be identified above the skin surface as NEDF's.

Under the two weekly measurement regime, summer down growth was described as having a mean peak in SA of 38±4% and a mean duration (SA_w-SA_{si}) of 46±10 days. On the external surface of the skin, NEDF's were present for a period of 66±7 days. The duration of the presence of NEDF's was more closely related to the time of emergence ($r=-0.78$, $P<0.01$) than to the time of disappearance ($r=-0.25$, NS). Similarly, the duration (SA_w-SA_{si}) of the summer down growth measured within the follicle was more highly correlated with the date of first SA activation ($r=-0.66$, $P<0.05$) than with the onset of winter down growth ($r=0.05$, NS).

Table 3.4: Correlation coefficients between secondary fibre growth events in down-producing castrate male wethers measured from July 1991 to February 1992. Events are presented in date order. Abbreviations are described in Table 3.1.

	SAc_i	SA_{si}	SAa_s	SS_{max}	SA_s	$NEDF_e$	DL_d	SA_w	DL_{min}	SAc_w	SS_f	$NEDF_d$	SA_{max}	DL_f
SS_i	0.30	0.16	0.14	0.05	0.22	0.04	0.04	0.0	0.04	0.05	0.43	0.09	0.19	0.59
SAc_i		0.41	**0.65***	0.38	**0.72***	0.61	0.39	**0.67***	0.32	**0.78****	0.35	0.60	**0.64***	0.43
SA_{si}			0.002	0.24	0.39	0.43	0.17	**0.72***	0.39	0.51	0.21	0.47	0.62	0.19
SAc_s				**0.72***	**0.85****	**0.67***	**0.69***	**0.62***	0.59	**0.75****	0.34	**0.65***	0.42	0.45
SS_{max}					**0.85****	**0.70***	**0.89*****	**0.69***	**0.80****	**0.66***	0.44	**0.68***	0.34	0.50
SA_s						**0.92*****	**0.87****	**0.85****	**0.80****	**0.87****	0.60	**0.84****	0.60	**0.64***
$NEDF_e$							**0.90*****	**0.70***	**0.78****	**0.82****	0.61	**0.80****	0.49	**0.65***
DL_d								**0.64***	**0.88****	**0.64***	0.56	0.62	0.24	0.55
SA_w									**0.77****	**0.93*****	0.45	**0.82****	**0.80****	0.39
DL_{min}										**0.71***	0.55	0.55	0.40	0.41
SAc_w											0.54	**0.84****	**0.81****	0.53
SS_f												**0.65***	0.60	**0.86****
$NEDF_d$													**0.80****	**0.67***
SA_{max}														0.49

*P<0.05, **P<0.01, ***P<0.001

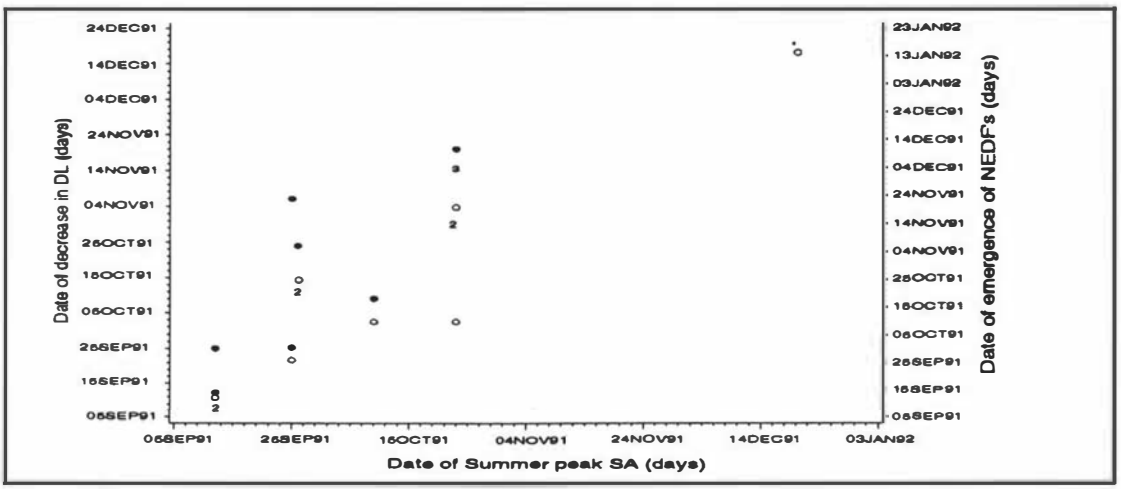

Figure 3.1: Relationship between the date of peak summer secondary follicle activity and either the date when down length decreased by more than 10 mm (●) (r = 0.87) or the date when newly emerged down fibres were first identified above the skin surface (○) (r = 0.92) in individual down-producing wethers. Numbers indicate the number of individuals with data falling at this point. No number indicates one data point.

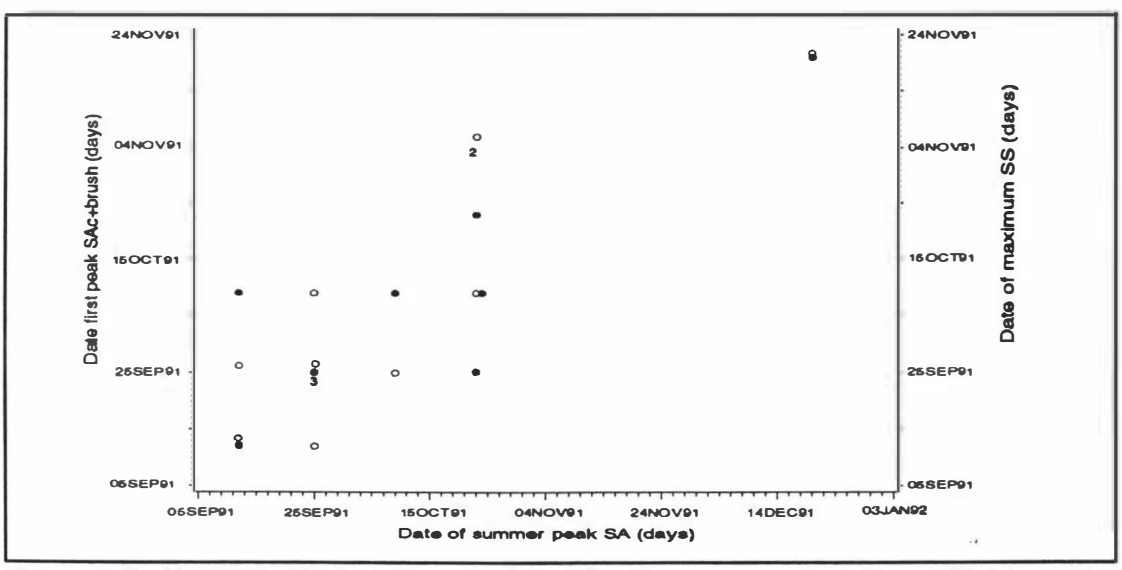

Figure 3.2: Relationship between the date of peak summer secondary follicle activity and either the date of the first peak in SAc+brush follicles (●) (r = 0.72) or the date of maximum shedding score (○) (r = 0.85) in individual down-producing wethers. Numbers indicate number of individuals with data falling at this point. No number indicates one data point.

The increase in SA associated with the onset of winter down growth commenced on 8 November±9 days and reached a maximum on 24 November±6 days (Table 3.1). By 27 November±9 days the previous winter down fleece could no longer be detected in the fleece. On 29 December±8 days NEDF's had disappeared, and shortly after, on 31 December±8, secondary follicles reached full activity and were producing winter down which had a measurable length by 22 January±7 days (Table 3.1).

The date of SA increase leading to winter growth (Figure 3.3) was correlated with both the date of disappearance of NEDF's ($r=0.82$, $P<0.01$) and the date of second peak in proportion of SAc+brush ($r=0.84$, $P<0.01$). In addition the date of NEDF disappearance was associated with the date of appearance of measurable DL (Table 3.4) in the fleece ($r=0.67$, $P<0.05$).

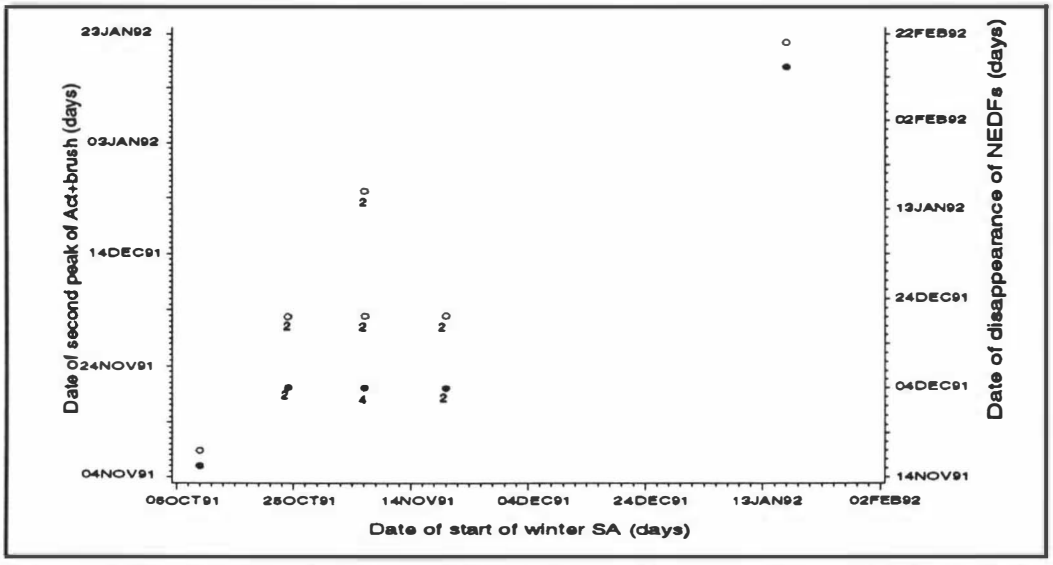

Figure 3.3: Relationship between the date when secondary follicle activity increased to produce winter down and either the date of second peak in SAc+brush follicles (●) ($r = 0.62$) or the date of disappearance of newly emerged down fibres (○) ($r = 0.82$) in down-producing wether goats. Numbers indicate number of individuals with data falling at this point. No number indicates one data point.

3.4.3 Shedding

An increase in SS on 8 September±11 days was the first detectable change in the winter fleece as spring approached (Table 3.1). Fleece shedding continued for 96±11 days, ceasing on 14 December±9 days. Maximum SS occurred on 9 October±8 days.

The date of onset of shedding was not correlated with primary (Table 3.2) or secondary (Table 3.4) fibre events. However, the date of cessation of shedding was correlated with the date of disappearance of NEDF's ($r=0.65$, $P<0.05$), the date of DL increase ($r=0.86$, $P<0.01$) and the date on which PA first increased ($r=0.70$, $P<0.05$). The date of maximum SS was associated with summer down growth, exhibiting significant correlations (Table 3.4) with the date of peak summer SA (Figure 3.2; $r=0.85$, $P<0.01$), the date of decreasing DL ($r=0.89$, $P<0.001$) and the emergence of NEDF's on the skin surface ($r=0.70$, $P<0.05$).

3.4.4 Plasma PRL concentration

Plasma PRL concentration increased to 20 ng/ml at a mean date of 26 August±4 days. By 6 October±7 days plasma PRL concentration had increased to 50 ng/ml and then reached 100 ng/ml on 20 December±7 days. Plasma PRL concentration was at a peak of 158±23 ng/ml on 31 December±10 days.

The date at which PA first increased from winter minimum levels was associated with the date at which plasma PRL concentration increased above 20 ng/ml (Figure 3.4; $r=0.78$, $P<0.01$). No other primary follicle events were associated with preceding plasma PRL concentration events (Table 3.5).

Table 3.5: Correlation coefficients between primary fibre growth and plasma PRL concentration events in down-producing castrate male goats.

	SS_i	SS_f	SS_{max}	GL_{min}	PA_i	PA_{max}	PAc_i	PAC_f
PRL_{20}	0.38	**0.64***	0.29	0.43	**0.78***	0.33	0.27	0.43
PRL_{50}	-0.11	0.41	0.33	0.37	0.41	0.09	-0.07	0.09
PRL_{100}	0.44	0.55	0.18	0.51	0.05	-0.25	0.29	0.27
PRL_p	0.32	0.39	0.13	**0.61***	-0.05	-0.52	0.05	-0.04

* P<0.05

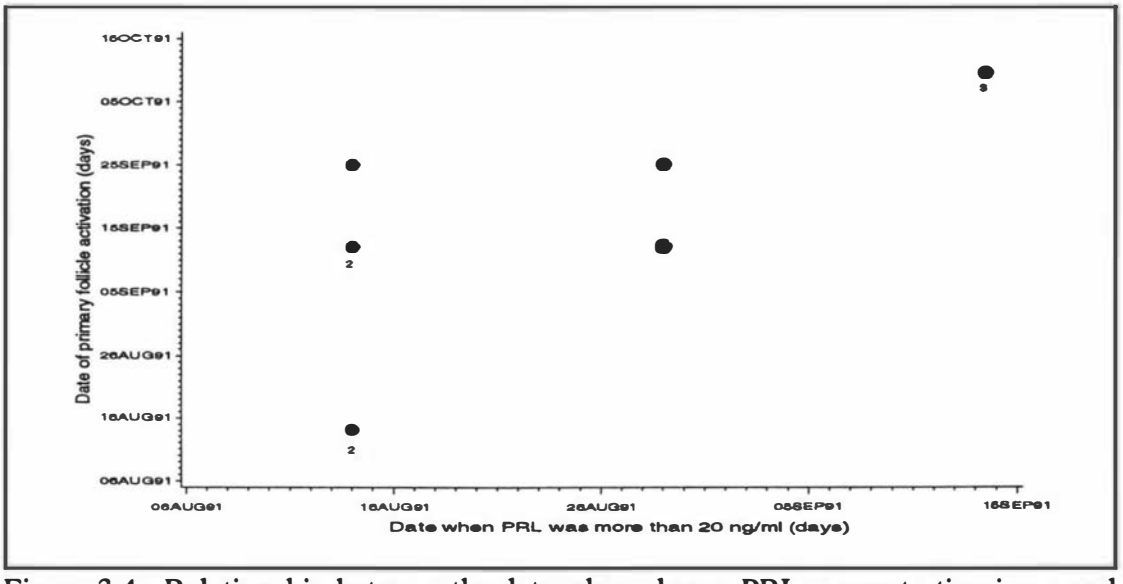

Figure 3.4: Relationship between the date when plasma PRL concentration increased above 20 ng/ml and the date when primary follicle activity first increased. Numbers indicate number of individuals with data falling on this point. No number indicates one data point.

There were three significant associations (Table 3.6) between the dates of plasma PRL concentration events and subsequent secondary fibre growth events. First, there was an association between the date when plasma PRL concentration reached 50 ng/ml and the date of disappearance of NEDF (r=0.64, P<0.05) and second the date when plasma PRL concentration reached 100 ng/ml was correlated (r=0.70, P<0.05) with the date of increase in DL. Finally, the date at which plasma PRL concentration reached 20 ng/ml was correlated (r=0.69, P<0.05) with the date of eventual increase in DL.

Shedding events were largely uncorrelated with plasma PRL events, with the exception of the date of cessation of shedding which was correlated (r=0.64, P<0.05) with the date when plasma PRL concentration increased beyond 20 ng/ml (Table 3.2). As these two events were separated by more than 3 months, a direct physiological link appears unlikely.

Table 3.6: The association between secondary fibre growth and plasma PRL concentration events in down-producing castrate male wether goats. Abbreviations are described in Table 3.1.

	SAc_{si}	SA_{si}	SAc_s	SA_s	$NEDF_e$	DL_d	SA_w	DL_{min}	SAc_w	$NEDF_d$	DL_1
PRL_{20}	0.61	-0.10	0.40	0.40	0.40	0.31	0.34	0.17	0.50	0.40	**0.69**
PRL_{50}	**0.64***	-0.08	0.55	0.55	0.58	0.42	0.41	0.10	0.54	**0.64***	0.45
PRL_{100}	0.32	0.01	0.30	0.46	0.59	0.49	0.03	0.26	0.28	0.34	**0.70**
PRL_p	0.08	-0.27	0.40	0.30	0.40	0.44	0.09	0.20	0.12	0.19	0.52

*P<0.05

Table 3.7 Correlation coefficients between plasma PRL concentration events in down-producing castrate male goats.

	PRL_{20}	PRL_{50}	PRL_{100}	PRL_p
PRL_{20}	1.0	0.59	0.39	0.16
PRL_{50}		1.0	0.43	0.30
PRL_{100}			1.0	**0.89****
PRL_p				1.0

** P<0.01

3.5 DISCUSSION

3.5.1 Primary follicle growth

In comparison to secondary follicles, primary follicles had a relatively simple growth pattern. As in other goats at similar latitudes, primary follicles were activated around the spring equinox and reached full activity soon after the summer solstice (Nixon *et al.*, 1991a; Chapter 2). However, the timing of primary follicle growth events varied in individuals by up to 2 months.

On 20 February, some time after achieving full PA, two goats had 21% and 2% of primary follicles in the PAc+brush stage. This further demonstrated the presence of a subsidiary primary follicle cycle in late summer, as first reported and discussed in Chapter 2.

Both primary and secondary follicles reactivated around the spring equinox but the timing of events in the two follicle types were not consistent. This implied that interpretation of the increasing day-length message which activated follicles, may differ between primary and secondary follicles. However there was a greater association in late summer between primary and secondary follicle events.

3.5.2 Secondary follicle growth

With the exception of Nixon *et al.*, (1991a), experiments examining secondary follicle growth have reported only the growth of the long winter down fleece (McDonald et al., 1987; Kloren, 1991). In this experiment, winter down growth was deemed to have occurred when SA showed a sustained increase up to full activity and the time of winter growth initiation was identified. In addition at this time, a second (for winter) peak in SAc+brush follicles was also identified. The mean date of onset of winter down growth occurred in early November but it ranged, between individuals, from 9

October to 17 January. Peak onset of new winter growth occurred in late November and by late December the last of the NEDF's had disappeared. Measurable DL appeared in the fleece in the latter half of January. The timing of winter down growth events are associated but greater lag phases existed between follicle changes and gross fibre indicators than in summer down growth. This was expected as the emergence of winter down growth will contribute to the presence of NEDF's, albeit fleetingly. In addition, it was not practical to measure the length of the down fleece until it grew to be in excess of 10 mm. This introduced a delay in recording DL growth for minimum of one month or more. Finally, from late November the sampling regime reverted to monthly rather than fortnightly sampling intervals, resulting in a loss of precision in the measurement of the timing of winter down growth events.

In other studies, summer down growth patterns have been identified, in some but not all goats, using monthly estimation of SA by either follicle form (Nixon *et al.*, 1991a) or mitotic rate (Henderson and Sabine, 1993) observations. In this experiment, estimates based on follice form were made at two weekly intervals during spring and summer down growth cycles were identified in all ten goats. This study and that of Nixon *et al.* (1991a) report conclusively the presence of a summer down growth cycle in New Zealand down-producing goats. It remains to be established whether down-producing goats from other countries and perhaps also down-producing sheep, possess a comparable growth phase.

As discussed in the Introduction, the presence of endogenous down cycles (McDonald *et al.*, 1987) shows that follicles are capable of progressing through a cycle of growth independent of photoperiodic signals. In this experiment, the timing of some summer and winter down growth events were highly correlated. This could indicate that either a common physiological cue has triggered the progression from summer to winter down growth or that the follicles of individual goats retain a consistent responsiveness to the increasing photoperiod signal. It was interesting to note that goats with a

longer duration of summer down growth achieved this via an early activation of growth and not a delay in the onset of winter growth, suggesting a dissociation between summer and winter down growth. To determine whether summer and down growth are linked it will be necessary to impose physiological manipulations which are likely to alter the timing of summer relative to winter down growth. In one study, continuous light treatment of Australian down-producing goats prevented the appearance of a small peak in brush-ends normally found in down fibres during December to March (McDonald *et al.*, 1987). Possibly the summer down, and not the winter down, cycle was suppressed by continuous light treatment.

As in the study of Nixon *et al.* (1991a) the mean date of secondary follicle activation which resulted in the summer down fleece occurred around the spring equinox. However, considerable between-goat variation existed with reactivation occurring in individuals from 30 July to 20 November. Goats in this study possessed two definable peaks in the proportion of SAc+brush follicles over the spring and summer period (Figure A3.1-A3.10). The first mean peak in SAc+brush follicles coincided with maximum mean SS and maximum mean summer SA during early October. Similarly in Australian down-producing goats during October secondary follicle mototic rates are two thirds those of peak autumn rates (Henderson and Sabine, 1993). By late October, NEDF's had emerged from the skin and DL began decreasing. These summer down events (DL_d, SAc_s, SA_s, $NEDF_e$, SS_{max}) were highly associated. The determination of follicle activity is a time-consuming process (approximately 1 hour per sample). The high correlation between follicle activity changes and the quicker gross fibre measurements such as NEDF, DL and SS make these latter measurements practical alternatives for determining the timing of the summer down growth cycle. However, care should still be taken to quantify growth within the follicle when physiological manipulations of short duration are applied as differences in gross fibre production may not always reflect the subtle changes within the follicle (see Chapter 6).

It is not clear from these data whether all secondary follicles underwent a summer cycle of down growth as the peak in summer SA only averaged 38±4%, a similar level to that of Nixon *et al.*, (1991a). Either only a third of follicles underwent a period of secondary follice growth or some follicles enter catagen prior to other follicles becoming active which implies a short anager period. In support of a short anagen period, there was an interval of less than 20 days between the mean time of increase in SA and achievement of maximum summer SA. In addition, if summer down grew at the winter down rate of 0.2-0.3 mm per day (Kloren, 1991) then summer down fibre lengths could be achieved in less than 20 days. In comparison to winter, shorter growth and resting phases have been observed on the legs of Merinos in summer follicle cycles (Pancretto, 1979).

Greater insight into the proportion of secondary follicles undergoing summer down growth could be achieved by synchronising the onset of summer down growth either by switching from short to long photoperiod or by providing a strong endocrinological signal for follicle reactivation hence increasing the peak summer SA. If all secondary follicles do undergo a period of summer down growth then summer down growth may prove to be the mechanism by which winter down fleece sheds.

3.5.3 Shedding

Manipulation of the time of shedding in Cashmere goats has important practical implications. If harvesting is by shearing it must occur prior to the onset of shedding; in many countries climatic conditions are often unfavourable for newly shorn goats. The process of winter down shedding in New Zealand goat involves, during June and July (Chapter 2), a period of catagen during which the follicle regresses and a serrated brush-end forms. The follicle then enters telogen when the fibre remains anchored in the follicle by the brush-end. In many species, the reactivation of the follicle occurs at the time of physical loss (shedding) of the fibre from the follicle (Dry, 1926; Chase, 1954; Chapman and Ward, 1979; Allain *et al.*, 1994). In goats, the

guard hair fleece sheds coincidentally with primary follicle reactivation (Ryder, 1970). In contrast, the down fleece of both the Mouflon and Soay sheep (Ryder, 1978) and Cashmere goats (McDonald *et al.*, 1987) sheds in the apparent absence of new down growth. However, it is possible that a vellus growth cycle may induce winter down shedding and that the growth of the vellus fibres was missed.

With the progression to spring, the first detectable change in the goat fleece was an increase in the amount of fleece that could be plucked from the goat. There was no association between the time of this increase in SS and other fibre events. This is perhaps not surprising as SS is a measure of fleece shedding from both primary and secondary follicles, and little association was found between the timing of primary and secondary follicle reactivation. Future studies should include a separate SS for the down and guard hair fleeces.

In individual goats, the date of onset of shedding did not precede secondary follicle reactivation by more than 2 weeks. In primary follicles observed in longitudinal section, a synchronised change from telogen to anagen was induced by melatonin treatment. Melatonin-treated follicles progress from telogen to anagen III within 14-20 days (Nixon *et al.*, 1993). Anagen III is the first stage of follicle reactivation when a keratinized growing fibre can be observed in transverse section (Nixon *et al.*, 1993). Follicle changes preceding anagen III are difficult to observe in transverse section, but may loosen fibres making them easier to pluck from the follicle. This possibly accounts for the up to 45% of transverse telogen down follicles in which fibres had been lost prior to reactivation (shed empty) observed in New Zealand down goats by Nixon *et al.* (1991). However it is unlikely, in natural photoperiod, that 45% of follicles would be passing from telogen to anagen III at one sample time. Possibly either brush-end fibres in late telogen are prone to being pulled from the follicle by rubbing, or New Zealand goats have differing genetic propensities to shed prior to follicle reactivation. Shed empty telogen follicles in this experiment were rare events and were not recorded. This apparent lack of association between the timing of onset

of shedding and follicle reactivation does indicate the possibility that physical shedding and follicle reactivation are not necessarily co-dependent processes. Nevertheless such co-dependence between follicle reactivation and pelage moulting is found in a wide variety of species and the alternative that the methodology used was too insensitive to detect early changes in SA seems more likely. Mitotic rate increased just prior to the onset of the loss of down fibres in the secondary follicles of Australian down-producing goats, indicating that, at least in these goats, follicles were active prior to the onset of shedding (Henderson and Sabine, 1992).

The date of maximum fleece shedding was correlated with the date of peak summer down SA, the summer peak in SAc+brush follicles, and the emergence of NEDF's. A reduction in DL of the fleece implies that longer down fibres are being shed and thus is also an index of down shedding. The timing of the reduction in DL was also highly correlated with the timing of summer down growth.

In this study, while the timing of onset of shedding could not be conclusively linked with follicle reactivation, peak shedding occurred in synchrony with peak summer down growth activity.

3.5.4 Plasma PRL concentration

Photoperiod drives the circannual pattern in plasma PRL concentrations and a pattern of high concentration around the summer solstice and low concentrations around the winter solstice is common in a wide range of mammalian species (Section 1.4). In Cashmere goats, day-time plasma PRL concentration during short days and low temperature are characteristically less than 20 ng/ml (Buttle, 1974; Mori *et al.*, 1985; Chapter 2). In this experiment, plasma PRL concentration increased from minimal winter levels to reach 20 ng/ml in late August. In common with other experiments (Buttle, 1974; Mori *et al.*, 1985; Tamanini *et al.* 1988; Grasselli *et al.*, 1992; Kloren *et al.*, 1993) plasma PRL concentration increased rapidly during the spring equinox,

passed 50 ng/ml in early October and reached a mean peak 10 days after the summer solstice.

In individual animals, the date of activation of primary follicles was correlated with the date on which plasma PRL concentration increased to 20 ng/ml. In fact, PA and plasma PRL concentration increased in synchrony over the spring/summer period, reaching peaks at comparable times. However, there were no other correlations between the dates of primary follicle growth and plasma PRL concentration events. A rise in plasma PRL concentration in spring may activate primary follicles, thereby promoting a physical shedding of the guard hair fleece and hence synchronising guard hair growth with season. In Wiltshire sheep, fleece regrowth commenced around the spring equinox. Follicles in some animals activated prior to the spring increase in plasma PRL concentration but shortly after they entered winter telogen (Pearson *et al.* 1993). In the Wiltshire sheep, it is thought that increased plasma PRL concentration during spring entrains wool growth by inducing catagen in active follicles and bringing all follicles into the same growth stage for the onset of the next cycle (Pearson *et al.,* 1994). In this experiment in goats, follicle activity measurements were not conducted during winter so it was difficult to determine whether there was an association between the increase in plasma PRL concentration and the decrease in PA.

As plasma PRL concentration rose from August and reached a peak in December, the goats had initiated the new growth which eventually produced both summer and winter down fleeces. Mitchell *et al.* (1991) also found that winter down growth was initiated prior to the summer solstice and therefore prior to the peak plasma PRL concentration. In contrast, in mink, (Martinet *et al.*, 1983; Rouget *et al.*, 1984; Rose *et al.*, 1985; Martinet *et al.*, 1992), hamsters (Badura and Goldman, 1992), and voles (Smale *et al.*, 1988), increasing and decreasing plasma PRL concentrations are associated with spring and autumn moults respectively. In the goats of this study there was no evidence that the transition between summer and winter down pelages

was triggered by falling plasma PRL concentrations.

The only associations that existed between plasma PRL concentration and secondary follicle growth involved aspects of winter down growth. The date of PRL reaching 100 ng/ml and date of DL increase were correlated. In addition, there was an association between the date at which plasma PRL concentration reached 50 ng/ml and the date at which NEDFs disappeared. If secondary follicles were activated by increasing photoperiod (and associated endocrinological changes) and then deactivated by high plasma PRL concentrations then short down fibres would then issue. Treatments suppressing the rise in plasma PRL concentration should result in the production of long down fibres during spring. Betteridge *et al.*, (1989) treated goats with mealtonin in spring which would suppress the PRL spring rise and observed the production of long down fibres.

Chronologically, in this experiment and others (Kloren, 1991), the spring increase in plasma PRL concentration preceded the initiation of summer down growth and winter fleece shedding. In addition, in two genotypes of down-producing goats, the genotype which shed two weeks earlier also had a similarly advanced rise in plasma PRL concentration (Rhind, 1994). In this experiment, variation between individuals in the timing of the rise in plasma PRL concentration to 20 ng/ml (PRL_{20}) did not contribute to the variation in the timing of secondary follicle reactivation or the date of commencement of shedding.

There are some inherent problems in the measurement of plasma PRL concentration which may have contributed to the failure of plasma PRL changes to account for individual variation in secondary down growth activation events. First, the plasma PRL concentration events were somewhat arbitrarily selected and perhaps other trigger concentrations or rates of change in plasma PRL concentration may have been more appropriate. Secondly, the pattern in plasma PRL concentration has a simple graduation associated with photoperiod but acute changes can also be attributable to

other stimuli such as temperature (Howland *et al.*, 1983; Mori *et al.*, 1985; Tamanini *et al.*, 1988) and stress (Fitzgerald *et al.*, 1981; Greef, 1985). A single blood sample collected at two weekly intervals probably does not accurately describe plasma PRL concentration changes associated with photoperiod in individual goats. Finally, there are multiple pathways (eg. PRL receptor changes and growth factor production) that may exist between the sensing of photoperiod, and changes in plasma PRL concentration and follicle fibre output which are likely to contribute to the variability between individuals. In order to study the hypothesis that plasma PRL concentrations modify seasonal fibre growth changes, experiments in goats should be conducted that modify only plasma PRL concentrations.

3.6 CONCLUSIONS

Both primary and secondary follicles became activated during the rapidly increasing photoperiod of the spring equinox. While primary follicles continued a sustained rise in PA up to maximum activity at the summer solstice, secondary follicles underwent an additional down growth cycle during summer. All goats were observed to possess a summer down growth phase but it was not clear whether all secondary follicles participated. The timing of onset of shedding could not be linked conclusively to activation of summer down growth but maximum shedding did occur in synchrony with peak summer down growth. Individual variation in the timing of primary follicle activation could be partially explained by variation in the date on which plasma PRL increased to 20 ng/ml. Some aspects of the timing of winter down growth were also associated with plasma PRL events.

CHAPTER 4

THE MANIPULATION OF PROLACTIN CONCENTRATIONS IN PLASMA DURING SPRING AND SUMMER IN DOWN-PRODUCING GOATS.

4.1 ABSTRACT

The effects on plasma PRL concentrations, in Cashmere goats, of summer treatment with bromocryptine mesylate (BR) and spring and summer treatment with domperidone (DOM) were examined in two experiments.

In experiment 1, BR and DOM were administered by subcutaneous injections at 1000 h for ten days commencing on 1 December 1990. BR dose rates were 1 (BR1), 2.5 (BR2.5) and 5 (BR5) mg/goat/day while DOM dose rates were 1 (DOM1), 2.5 (DOM2.5), and 5 (DOM5) mg/goat/day. A further DOM treatment group received two injections of 2.5 mg/goat (DOM2.5x2) at 1000 h and 1500 h daily and a control group (C) received daily injections of diluent. Ten day mean plasma PRL concentrations at 1500 h were 11.9±0.7, 9.4±0.7 and 8.5±0.7 ng/ml in BR1, BR2.5 and BR5 respectively compared with control concentrations of 87±7 ng/ml ($P<0.001$). There was no effect of dose rate of BR on ten day mean plasma PRL concentration. In comparison to C, the injection of BR2.5 suppressed plasma PRL concentrations for 20 h ($P<0.05$). DOM1, DOM2.5, DOM2.5x2 and DOM5 increased plasma PRL concentrations to 278±23, 548±65, 336±39, 332±30 ng/ml respectively. There was no effect of dose rate of DOM. The DOM2.5 injection at 1000 h increased plasma PRL concentrations for 12 hours. The second injection given at 1500 h failed to extend the duration of plasma PRL elevation relative to the single injection.

In experiment 2, DOM was administered at 2.5 mg/day for 14 days either by subcutaneous injection at 1000 h (DOMinj) or by a subcutaneously fitted osmotic minipump (DOMosp) and fibre growth was monitored. The treatments commenced on 13 September 1991. Mean daily plasma PRL concentrations at 1200 h were 612±32, 73±35 and 60±34 ng/ml in DOMinj, DOMosp and control respectively ($P<0.001$). Immediately prior to the injection at 1000 h, mean daily plasma PRL concentrations were lower ($P<0.01$) in the DOMinj (33±6 ng/ml) in comparison with DOMosp (72±7 ng/ml) and control (60±8 ng/ml) groups.

The response in plasma PRL concentrations two hours after a single daily injection of DOM was greater ($P<0.01$) in summer (743±44 ng/ml) than in spring (536±40 ng/ml).

There was a tendency ($P<0.10$) for mean down length (DL) over the measurement period to be reduced in DOMinj goats (33±13 mm) when compared with control

goats (46±13 mm). A greater percentage of fleece by weight was shed earlier in DOMinj compared to control groups ($P<0.001$) and PA increased earlier ($P<0.001$) in DOMinj goats. There was no effect of DOMosp on fibre growth and no overall effect of DOMinj on GL, shedding score, presence of NEDF's, or SA.

It is concluded that BR and DOM could be used to manipulate circulating PRL concentrations during summer and spring. Following a daily injection of DOM for 14 days primary, and but secondary follicles, were activated.

4.2 INTRODUCTION

Circulating PRL concentrations are controlled in a multifactorial manner with hormones either stimulating or inhibiting secretion of PRL by the pituitary (Enjdlbert *et al.*, 1978; Ben-Jonathan, 1985). It is generally accepted, however, that dopamine is a primary inhibitor of PRL secretion (Ben-Jonathan, 1985; Lambert and MacCleod, 1990). Dopamine produced in the hypothalamus activates receptor sites on the lactotrophs of the par distalis (Goldsmith *et al.*, 1979) resulting in a suppression of PRL secretion (Thomas *et al.*, 1989).

Domperidone (DOM), a benzimidazol derivative (Figure 4.1), is a potent and selective (Laduron and Leysen, 1979) D2 dopamine receptor antagonist which elevates plasma PRL concentrations in rats (Laduron and Leysen, 1979; Haisenleder *et al.*, 1988; Pohl *et al.*, 1988; Mogg and Samson, 1990), deer (Milne *et al.*, 1990), sheep (Deaver *et al.*, 1987; Thomas *et al.*, 1989), wallabies (Loudon and Brinklow, 1990) and humans (Nappi *et al.*, 1987; Devesa *et al.*, 1988). It does not pass the blood-brain barrier (Laduron and Leysen, 1979) but will act on peripheral dopamine receptors including those of the pituitary. DOM is a registered drug (Motilium: Janssens Pharmaceuticals) used for its anti-nauseant properties in humans while in animals it is used to elevate circulating PRL concentrations and as a research tool for the study of the role of dopamine in PRL regulation (Milne *et al.*, 1990). DOM is insoluble in water but has a solubility of 2.6 mg/ml in methanol.

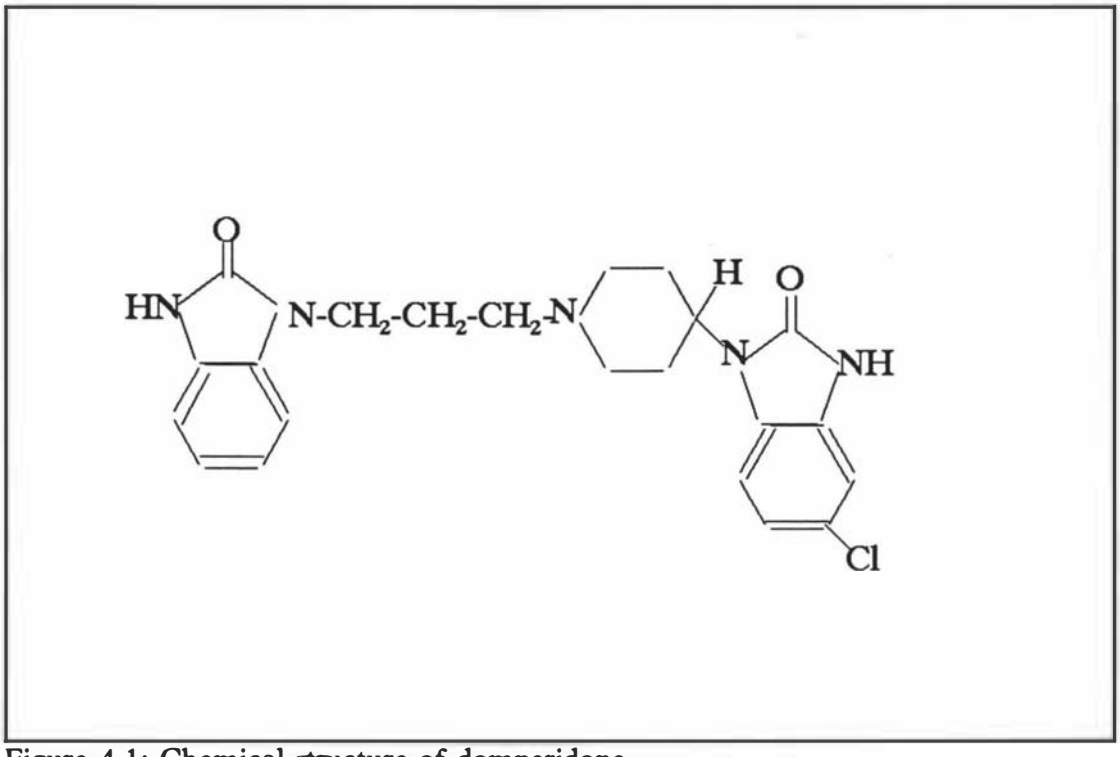

Figure 4.1: Chemical structure of domperidone

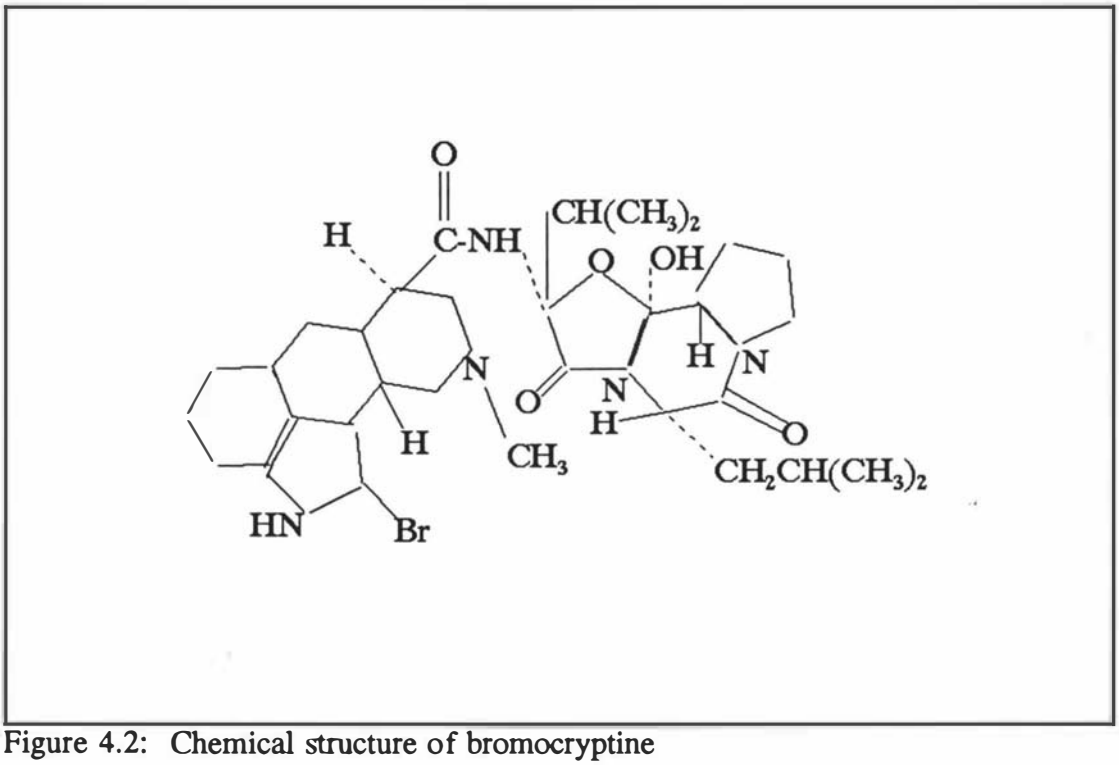

Figure 4.2: Chemical structure of bromocryptine

DOM, either administered as an intravenous or intramuscular injection at doses ranging from 0.04 to 0.1 mg/kg/day, has been effective in elevating plasma PRL concentrations in sheep (Deaver *et al.*, 1987; Thomas *et al.*, 1989), while in deer an intramuscular injection of 0.07 mg/kg/day was also effective (Milne *et al.*, 1990). DOM elevates circulating PRL concentrations as a result of increased PRL pulse frequency and the pulse amplitude (Pohl *et al.*, 1988). The magnitude of this increase is dependent on basal plasma PRL concentrations (Devesa *et al.*, 1988) and therefore on season (Loudon and Brinklow, 1990). It is known that DOM will activate dopamine receptors throughout the body and on other hormone-producing cells within the pituitary. For instance, DOM increases both the pulse frequency and pulse amplitude of plasma luteinizing hormone concentrations (Dever *et al.*, 1988).

Bromocryptine is a potent D_2 dopamine receptor agonist (Jarvis *et al.*, 1988) which interferes with the phosphorylation pathway within the lactotroph, thereby stimulating the action of dopamine. It is an ergot alkaloid (Figure 4.2) with a solubility of 910 mg/ml in methanol and 23 mg/ml in water. The suppressive effect of bromocryptine on PRL secretion is achieved by stimulating dopamine receptors in the anterior pituitary of sheep (Lowe *et al.*, 1979; Eiseman, 1982; Rodway *et al.*, 1983; Takahashi, 1986; Peclaris, 1988), goats (Hart and Morant, 1980; Forsyth *et al.*, 1985; Taverne *et al.*, 1988; Lynch and Russell, 1989) and deer (Milne *et al.*, 1990). Bromocryptine is widely used for PRL suppression in both humans and animals. Administration can be by either intramuscular or subcutaneous injection. Doses ranging from 0.03 to 0.17 mg/kg/day have been effective in suppressing plasma PRL concentrations in goats (Hart and Morant, 1980; Forsyth *et al.*, 1985; Taverne *et al.*, 1988; Lynch and Russell, 1989). Dose-response relationships to bromocryptine have been determined in sheep, with the optimum dose rate being 1 mg/sheep/day (Niswender, 1974).

The many and varied effects of bromocryptine on parameters other than PRL secretion are reviewed by Thorner *et al.* (1980). Only the effects which may interact

with PRL, to affect fibre growth, are described in this Chapter. Some controversy exists regarding the effects of bromocryptine on plasma growth hormone concentrations. In some studies bromocryptine has failed to modify growth hormone release (Thorner *et al.*, 1980; Jarvis *et al.*, 1988) while in others the effect of growth hormone treatment on growth has been reduced by bromocryptine treatment. In another, growth hormone concentrations were elevated following bromocryptine treatment in lambs (Forsyth *et al.*, 1985). Bromocryptine treatment appears to have a suppressive effect on plasma concentrations of thyroxine (Milne *et al.*, 1990), melanotropin secretion (Munemura *et al.*, 1980), and plasma insulin (Johnsson and Hart, 1985), and β-endorphin (Ssewannyana and Lincoln, 1990) concentrations. Bromocryptine has no effect on plasma concentrations of gonadotrophic hormones in sheep or goats (Land *et al.*, 1980; Thorner *et al.*, 1980).

Bromocryptine has many effects on the central nervous system (Thorner *et al.*, 1980). The reported effects, which might affect fibre growth in goats, include a suppression of blood pressure in cats, dogs, hypertensive rats (Tan and Hutchinson, 1987) and hypertensive humans (Thorne *et al.*, 1980; Sowers *et al.*, 1982). Body temperature of rats and rabbits can also be manipulated using bromocryptine (Thorne *et al.*, 1980).

Fibre growth, in Cashmere goats, has seasonally driven hair replacement cycles (Ryder, 1966; McDonald *et al.*, 1987; Betteridge *et al.*, 1988) and this circannual fibre growth cycle can be manipulated by changing both photoperiod (McDonald and Hoey, 1987; Norton, 1991) and circulating melatonin concentrations (Betteridge *et al.*, 1989; Gebbie *et al.*, 1991). The most responsive season for manipulation of cashmere growth with melatonin is spring (Litherland *et al.*, 1990). Continuous melatonin treatment, in spring, initiates new growth in primary follicles after only 14 days of treatment (Nixon *et al.*, 1991a). The physiological events induced by the melatonin treatment, which result in the initiation of new fibre growth, are unknown. It is known that plasma PRL concentrations are reduced by spring melatonin treatment

(Nixon *et al.*, 1993), and PRL plasma concentration is thought to play an important role in the regulation of cashmere growth cycles (Lynch and Russell, 1990; Kloren and Norton, 1993c,d). Treatment with PRL from mid-winter advances shedding while suppression of PRL delays shedding in cashmere goats (Lynch and Russell, 1989; Kloren and Norton, 1993d). Once new winter down growth has been activated, suppression of plasma PRL concentrations has no effect on cashmere growth (Kloren and Norton, 1993d).

The objective of this Chapter is to present information from two experiments examining methods of manipulating plasma PRL concentrations in Cashmere wethers in two seasons. The information from these studies was then used to design appropriate treatment regimens to examine the effects on fibre growth of manipulating plasma PRL concentrations. The first experiment determined the effect of three dose rates each of BR and DOM on circulating PRL concentrations in summer. In addition, the duration of effective PRL manipulation using the two drugs was determined over a 24 hour period. Finally, the effects on plasma concentrations of other hormones and glucose were also studied. In the second experiment, the effects on plasma PRL concentration and fibre growth of two methods of DOM administration were measured, in spring. The plasma PRL concentrations two hours after a daily DOM injection given in either spring or summer were compared.

4.3 METHODS

4.3.1 Experiment 1

Forty goats were assigned randomly to eight indoor pens. Each pen contained five, mixed- age, adult cashmere castrate male goats of 30 kg mean weight, balanced for liveweight across pens. The goats were each fed 250 g of maize between 0800 and 0900 h daily. Meadow hay and water were on offer *ad libitum*. The goats were housed under natural daylight at the Flock House Agricultural Centre in Bulls, New

Zealand (40° 14′ S, 175° 16′ E). Sunrise on 1 December 1991 occurred at 0445 hours and sunset at 1930 h.

Each drug treatment was randomly allocated to a single pen. BR (Catalog No. B2134, Sigma Chemical Company, St Louis, Mo, USA) and DOM (Catalog No. B8910, Sigma Chemical Company) were administered by subcutaneous 2 ml injections at 1000 h for twelve days commencing on 1 December 1990. The BR dose rates were 1 (BR1), 2.5 (BR2.5) and 5 (BR5) mg/goat/day in a diluent of (v/v) 14 ethanol: 14 propylene glycol: 72 water. DOM dose rates were 1 (DOM1), 2.5 (DOM2.5), and 5 (DOM5) mg/goat/day in propylene glycol (Catalog No., P1009, Sigma Chemical Company). A further treatment group received two injections of 2.5 mg/goat (DOM2.5x2) at 1000 and 1500 h daily. A control (C) group received daily injections of the diluent used for BR injections. Solutions were prepared every three days in glassware rinsed with 70% ethanol.

Daily blood samples were taken by jugular venipuncture at 1500 h for 4 days prior to treatment and for the first 10 treatment days. Catheters were fitted in the jugular vein of the goats in groups C, BR2.5, DOM2.5 and DOM2.5x2 on day 11 of treatment. Blood samples were drawn hourly (0400 to 2200 h) or two hourly (2200 to 0400 h) on the following day. Plasma was removed after centrifugation and stored at -15 °C. All samples were assayed for PRL concentrations. Aliquots of 0.2 ml volumes were taken from each plasma sample collected over the 24 hour period, pooled within goat, and frozen until assayed for thyroxine, glucose, insulin and growth hormone concentrations.

4.3.2 Experiment 2

Seventeen mixed-aged wether goats (mean liveweight 33 kg) were randomly allocated to treatment groups. The goats were maintained indoors, under natural light, in pens of either 3 or 5 goats and fed 250 g per goat of maize between 0800 and 0900 h daily

with meadow hay and water on offer *ad libitum*. The experiment was conducted at the same location as experiment 1.

Domperidone was administered to groups of six goats, at a rate of 2.5 mg/goat/day, by either injection or osmotic pump (Model 2 ML 2, Alzet minipumps). There was an untreated control group of five goats. The treatments commenced on 13 September 1991 and continued for 14 days.

DOM, in a 1 ml diluent of dimethylsulfoxide (DSO) (higher solubility than proplylene glycol in experiment one), was injected subcutaneously, into the anterior neck of goats at 1000 h daily. Osmotic pumps (delivering 5 μl/hr) containing 35 mg of domperidone, in 2 ml of DSO, were placed subcutaneously on the inside left back leg of the goats. The pump was placed plunger inwards in the upwardly extending subcutaneous pocket (3 cm by 6 cm) closed by suturing. The pumps were removed after 14 days.

Catheters were fitted to the external jugular vein of goats on 12 September and blood samples were drawn at 1000 h, immediately prior to the DOM injection, on treatment days 2, 3, 9, 10, 11 and 14 and at both at 1000 and 1200 h on days 1, 4, 5 to 9, and 13. Due to the action of other goats, half of the catheters were lost by day 8 of the experiment. The remainder were then removed and blood samples were collected in heparinised vacutainers by jugular venipuncture. After the treatment period, blood samples continued to be collected at 3 weekly intervals at 1100 and 1300 h until 10 November 1992. Plasma samples were assayed for PRL concentration.

Fibre growth was measured at two weekly intervals by a variety of methods commencing one day prior to the first treatment day and continuing until 07 November 1991. Length of stretched guard hairs and down fibres (Section 2.2.2.1) were measured at the neck, front shoulder, mid side and hind shoulder on each goat. Mean lengths of down (DL) and guard hair (GL) were calculated.

The amount of fibre being lost from the fleece (shedding) at each sampling time was assessed both by combing the goat and by visual scoring. A hand comb was drawn horizontally and then vertically through the fleece in a single pass over the whole left side of the goat (Section 2.3.2.1). The shed fibre captured by the comb was weighed and summed over the period of collection. The percentage of weight of fleece shedding at any one time was then calculated. Shedding was also assessed on the right side of the goat by plucking the fleece prior to combing on the left side of the goat. The amount of fibre plucked from the goat was scored from 1 (no shedding) up to 5 (large amount of shedding).

Skin biopsy samples were taken from the left midside region of the goat (Section 2.2.2.4). The skin samples were viewed under a dissecting microscope and scored for the presence of newly emerged down fibres (NEDF, Section 2.2.3.2). The skin samples were processed (Table 2.2), and embedded, epidermal surface uppermost, in paraffin wax. The samples were then serially sectioned in transverse orientation and stained (Nixon, 1993; Table 2.3). Approximately 10 follicle groups were scored according to the three phases of the hair cycle; anagen, telogen or active +brush (Section 2.2.3.3).

4.3.3 Hormone assays

The radioimmunoassay of PRL was conducted at Ruakura Agricultural Centre (Section 2.3.2.3).

Plasma thyroxine concentrations were measured using a solid phase radioimmunoassay human kit ("Coat-a-Count", Diagnostic Products, Corporation, Los Angles, USA, 1991). The assay showed 100% internal recovery and was parallel to kit standards for caprine samples. The sensitivity of the assay was 0.25 μg/dl. The intra-assay and inter-assay coefficients of variation were 3% and 8% respectively.

The radioimmunoassay of insulin was conducted using crystalline bovine insulin (Catalog No. I-5500, Sigma Chemical Company) for standards and radioiodination. Iodination of the crystalline bovine insulin was based on the procedures of Greenwood *et al.* (1963). The guinea pig first antibody was raised used insulin (crystalline bovine pancreatic insulin) polymerised with diethyl pyrocarbonate (Wolf *et al.*, 1970). The first antibody-hormone complex was agglutinated using a second antibody of sheep anti-guinea pig gamma globulin ("Colin", 08.08.85). The assay was based on the method of Hales and Randle (1963). The displacement of labelled insulin by caprine samples was not in parallel and as a consequence the internal recovery (132%) was overestimated. The assay sensitivity was 48 pg/ml and intra-assay coefficient of variation was 8.2% while inter-assay coefficient of variation was 12.4%. Plasma from five goats exhibited insulin concentrations below the sensitivity of the assay and were assigned a zero insulin concentration. The radioimmunoassay of growth hormone was conducted using bovine growth hormone (USDA-bGH-B1: USDA Reproduction laboratory) for standards and radioiodination. The iodination procedure was based on the method of Greenwood et al. (1963). The first antibody (GP1, 12.06.74) was raised in guinea pigs and sheep anti-guinea pig gamma globulin ("Colin", 08.08.85) was used as the second antibody (Frohman *et al.*, 1970). The assay method was based on that of Hart *et al.* (1975). The validation for caprine samples indicated reasonable parallelism with the standards and had a mean internal recovery rate of 111%. Assay sensitivity was 2 ng/ml and the intra-assay and inter-assay coefficients of variation were 8.6% and 13.2%, respectively.

Samples were assayed for glucose using the Glucose PAP kit (Roche Products, NZ, Limited) and a Cobas Fara II autoanalyser (Bergmeyer, 1974; Caraway, 1976). The intra-assay and inter-assay coefficients of variation were 1.9% and 4.6%, respectively.

4.3.4 Statistical analysis

Due to unequal variances in both experiments the PRL concentrations were $\log_{10}$

transformed. Daily PRL concentrations were statistically analysed using the GLM procedure of SAS (SAS, 1987) with repeated measures in time using the Wilks' Lambda test of significance. The mean of the four pre-treatment days in experiment 1 and a single pre-treatment day in experiment 2 were used as covariates. Data in the text are presented as least square means and least square standard errors of the mean.

4.3.4.1 Experiment 1: In order to prevent the data from individual animals being deleted from the repeated measures analysis due to missing sample values, plasma PRL concentrations from the preceding day, for that animal, were used to replace missing values (9 such cases in total). Comparisons of dose rates within drug types, and individual treatment and control comparisons, were made using orthogonal contrasts. Twenty four hour treatment comparisons of plasma PRL concentrations were analysed using repeated measures analysis on 2 hourly data to ensure equal time intervals. Effects on plasma concentrations of glucose and hormones other than PRL were analysed using general linear models ANOVA (SAS, 1985).

Daylight was defined as being between 0700 and 2000, dusk 2100 to 2200, dawn 0500 to 0600 and dark 2300 to 0400 h inclusive. Concentrations within these categories were averaged and differences analysed using the General Linear Models ANOVA procedures of SAS (SAS, 1987). The raw data are presented graphically.

4.3.4.2 Experiment 2: General Linear Model ANOVA procedures were used to determine the effect of December and September conditions on the response in plasma PRL concentrations to a 2.5 mg/day injection of DOM. The mean daily plasma PRL concentration at 1200 h in spring was compared to the sample collected at 1200 h during the 24 hour sampling in summer.

Fibre growth measurements were analysed using the GLM procedures of SAS (1987) with repeated measures in time using the Wilks' Lambda test of significance. Fibre

growth parameters measured on 12 September 1991 were fitted as a covariate in the model. Comparisons of DOM and control treatment groups were made using orthogonal contrasts. Where missing data existed (2 values in the fibre length data) a data point was fitted mid way between the preceding and following data point. The data are presented as least square means and standard errors.

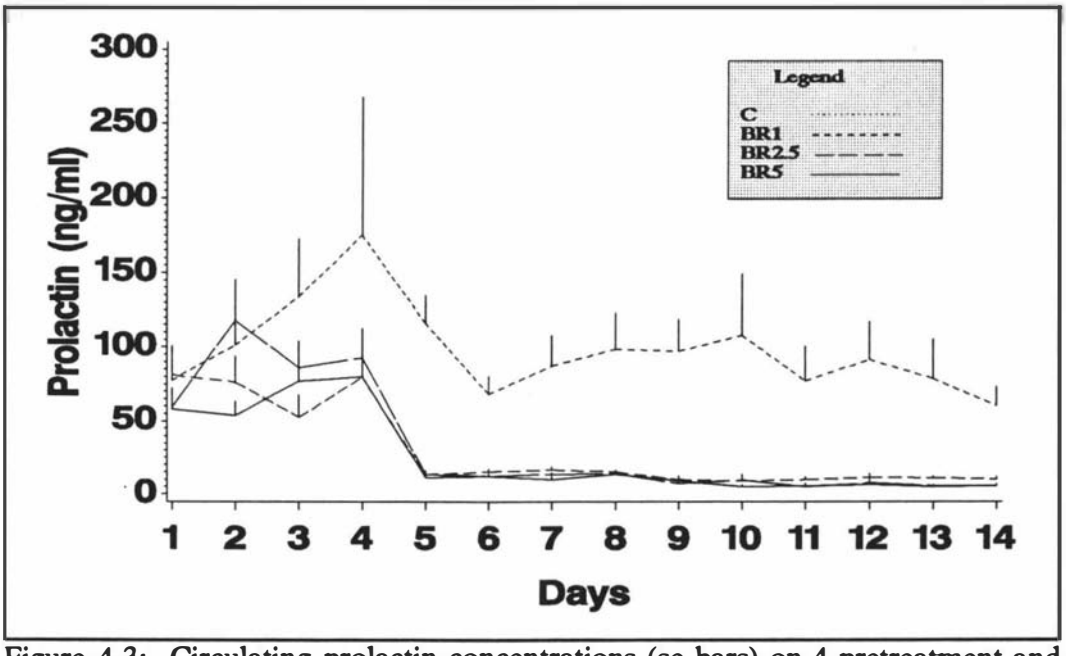

Figure 4.3: Circulating prolactin concentrations (se bars) on 4 pretreatment and 10 treatment days in untreated control (C) goats and goats treated with bromocryptine mesylate at 1 (BR1), 2.5 (BR2.5) and 5 (BR5) mg/day.

4.4 RESULTS

4.4.1 Experiment 1

4.4.1.1 Daily plasma PRL concentrations: Plasma PRL concentrations averaged over the 10 day treatment period were 87±7 ng/ml in the C animals (Figure 4.3). Over the same period concentrations in the BR-treated goats were: BR1, 11.9±0.7 ng/ml; BR2.5, 9.4±0.7 ng/ml; and BR5, 8.5±0.7 ng/ml. All were significantly different from concentrations in the control goats ($P<0.001$). There was no overall effect of BR

dose rate on plasma PRL concentrations although on days 13 and 14 concentrations were lower (P<0.05) in BR2.5 (5.9 ng/ml) and BR5 (3.9 ng/ml) than in BR1 (11.2 ng/ml).

Mean daily PRL concentrations in DOM-treated goats were: DOM1, 278±23 ng/ml; DOM2.5, 548±65 ng/ml; DOM2.5x2, 336±39 ng/ml; DOM5, 322±30 ng/ml (Figure 4.4). All were significantly (P<0.001) greater than in C goats. There was no difference between the DOM dose rates although the DOM2.5 group had higher plasma PRL concentrations than the DOM1 group on days 5, 8, 9, 10, 13 and 14 (P<0.05). Pre-treatment basal plasma PRL concentrations were not correlated with post-treatment plasma PRL concentrations.

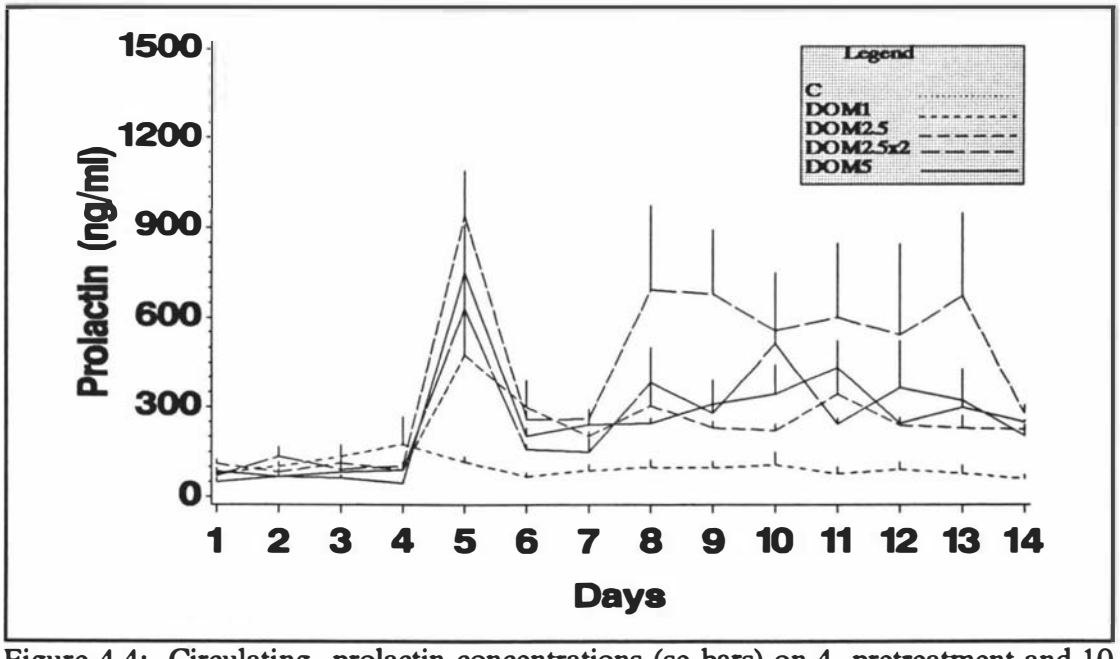

Figure 4.4: Circulating prolactin concentrations (se bars) on 4 pretreatment and 10 treatment days in untreated control (C) goats and goats treated with domperidone at 1 (DOM1), 2.5 (DOM2.5) and 5 (DOM5) mg/day and 2.5 (DOM2.5x2) mg/twice daily.

4.4.1.2 Plasma PRL concentrations over 24 hours: Night-time PRL concentrations (125±15 ng/ml) in C goats were higher than day-time (79±5 ng/ml) concentrations ($P<0.001$). The highest plasma PRL concentrations of 185±13 ng/ml occurred during dusk, while dawn concentrations were similar to night-time concentrations (Figure 4.5).

A BR injection of 2.5 mg/day given at 1000 h significantly suppressed plasma PRL concentrations relative to those in C animals until 0600 h the following day ($P<0.05$). The suppressive effect of BR occurred within 2 hours of the injection and concentrations remained low until 1800 h after which PRL concentrations slowly returned to control levels (Figure 4.5).

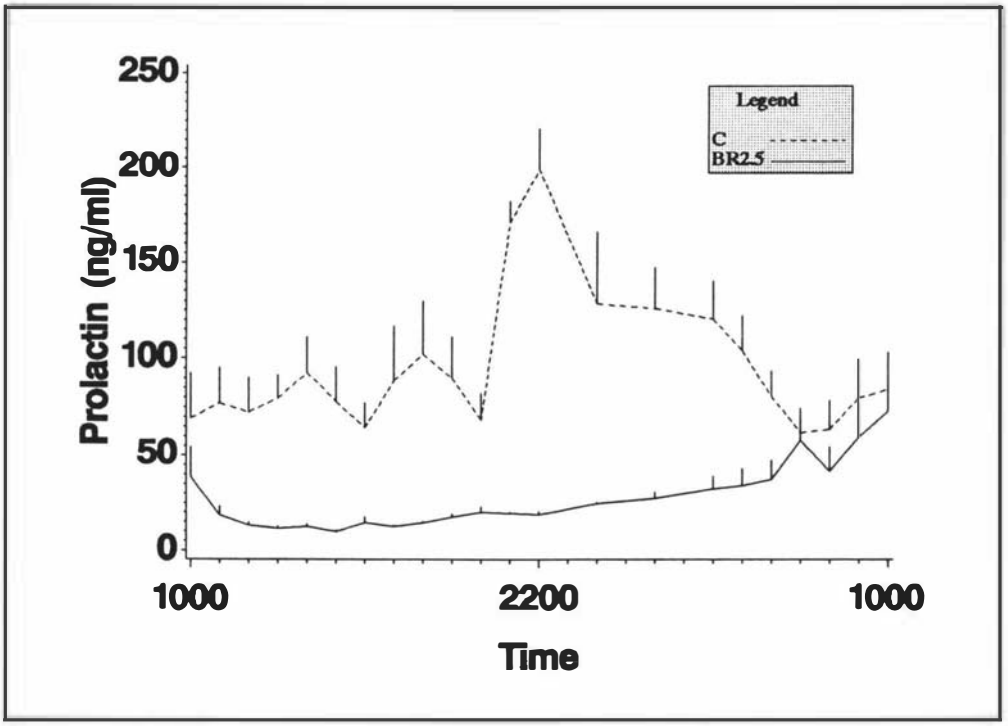

Figure 4.5: Circulating prolactin concentrations (se bars) over 24 hours in untreated control (C) goats and goats treated with 2.5 mg/day of bromocryptine at 1000 h (BR2.5).

Goats injected with DOM at 1000 h achieved peak PRL concentrations of 1029±110

ng/ml 2.6±0.1 hours later. Thereafter, the concentrations declined in an asymptotic fashion with significant differences being no longer be detected from 2200 h onwards (Figure 4.6).

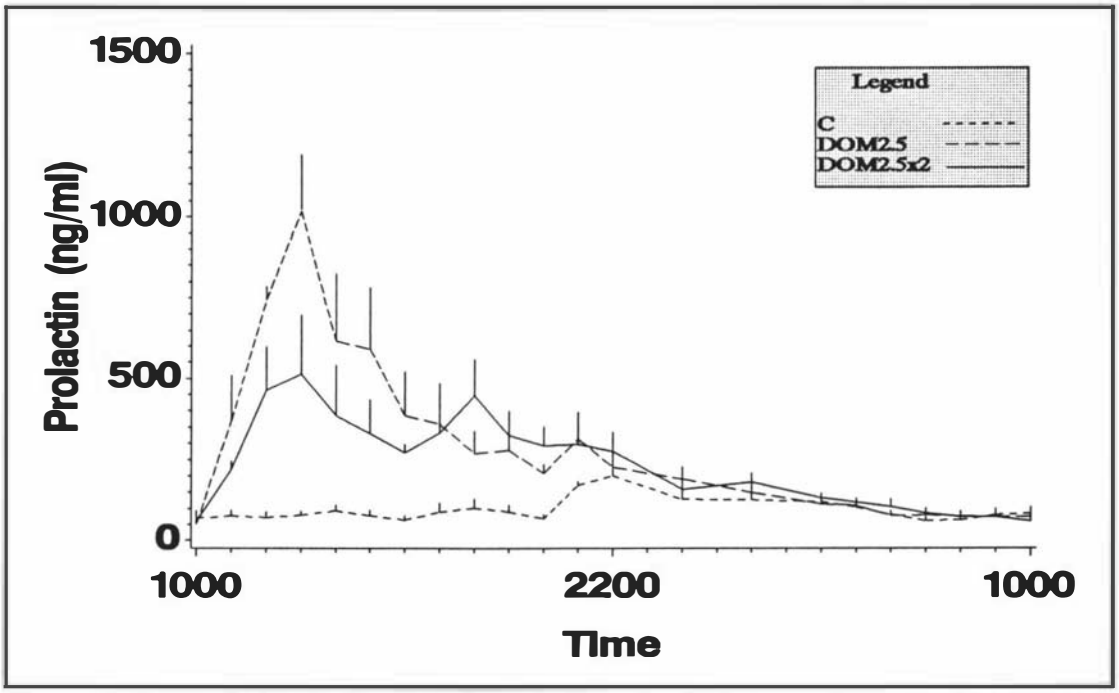

Figure 4.6: Circulating prolactin concentrations (se bars) over 24 hours in untreated control (C) goats and goats treated with domperidone at 2.5 mg/day at 1000 h (DOM2.5) or 2.5 mg at 1000 h and again at 1500 h (DOM2.5x2).

Two injections of DOM at 1000 and 1500 h failed to extend significantly the duration of elevated plasma PRL concentrations beyond the 12 hours observed following the single injection ($P<0.05$). However, plasma PRL concentrations did respond to the second injection as evidenced by the different slope of the regression line of plasma PRL concentrations against time from 1500 to 1800 h ($P<0.05$) in treatment group DOM2.5 versus DOM2.5x2. In addition, there was a treatment by time interaction ($P<0.05$) between DOM2.5 and DOM2.5x2 in circulating PRL concentration.

4.4.1.3 Effects on other hormones and metabolites: BR and DOM treatment had no effect on plasma concentrations of thyroxine, glucose, insulin or growth hormone.

Plasma thyroxine concentrations ranged from 6.4 to 7.3 μg/dl; glucose concentrations ranged from 3.1 to 3.6 mM; insulin concentrations from 190 to 355 pg/ml; and growth hormone concentrations from 1.7 to 7.0 ng/ml.

4.4.2 Experiment 2

4.4.2.1 Daily plasma prolactin concentrations: Mean daily plasma PRL concentrations in samples collected two hours after the injection of DOM (Figure 4.7) were greater ($P<0.0001$) in DOMinj (612±32 ng/ml) than in control (60±34 ng/ml) goats. However, 24 hours after the injection of DOM (Figure 4.8), mean daily plasma PRL concentrations had dropped to below that of the control goats (33±6 vs 60±8 ng/ml, $P<0.01$). Both these effects were consistent over the duration of the experiment and no post-treatment effects of DOMinj were detected.

Treatment with DOM at 2.5 mg/day, using an osmotic pump, had no effect on plasma PRL concentrations at any sampling time (Figure 4.7, 4.8). Neither was there an effect of the osmotic pump treatment on plasma PRL concentrations in the post-treatment period. Careful observation of the pump showed that the liquid had been discharged from the pump at the expected rate.

As expected, plasma PRL concentrations over the 40 day post-treatment period increased with time in all treatment groups ($P<0.001$) (data not shown).

4.4.2.2 Plasma prolactin concentrations in different seasons: The plasma PRL concentrations 2 hours after a DOM injection of 2.5 mg/day were greater ($P<0.01$) in December (743±44 ng/ml) compared to September (536±40 ng/ml). However the plasma PRL concentrations in control goats at 1200 h were similar in summer (72±14 ng/ml) compared to spring (57±14 ng/ml).

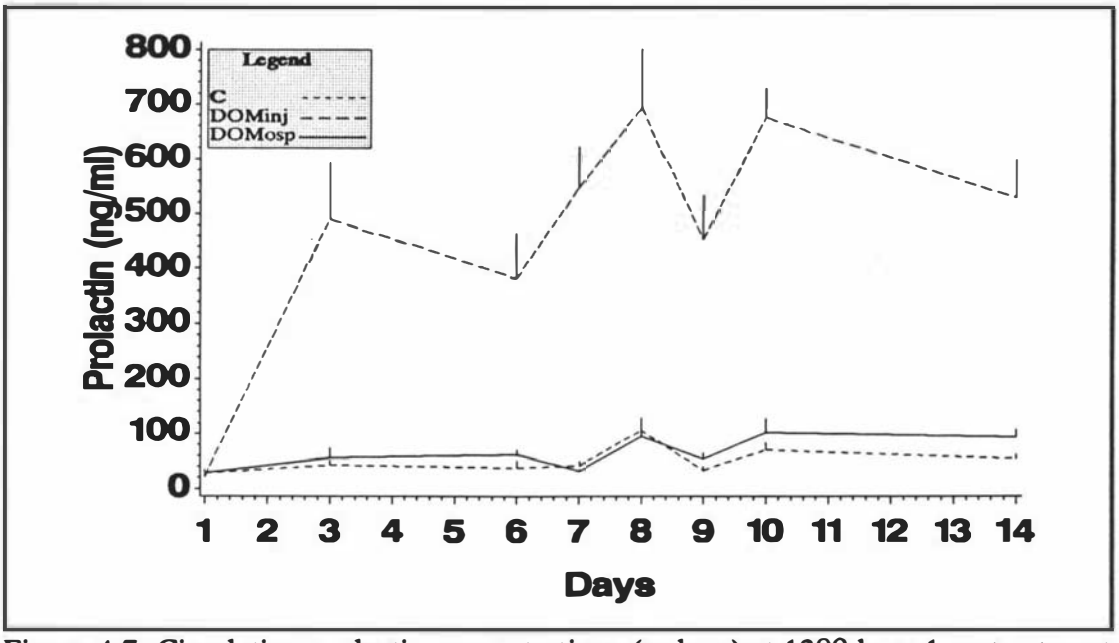

Figure 4.7: Circulating prolactin concentrations (se bars) at 1200 h on 1 pretreatment day and 14 days of treatment in untreated control goats (C) and goats treated with domperidone at 2.5 mg/day by injection (DOMinj) or by osmotic pump (DOMosp).

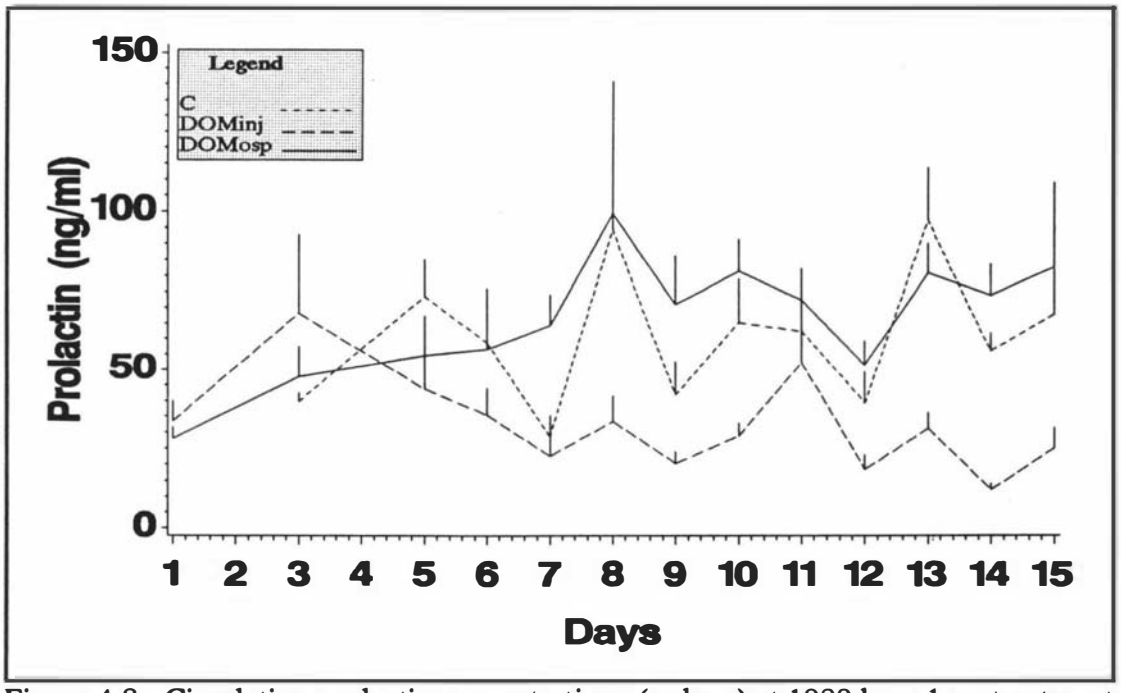

Figure 4.8: Circulating prolactin concentrations (se bars) at 1000 h on 1 pretreatment day and 14 days of treatment in untreated control goats (C) and goats treated with domperidone at 2.5 mg/day by injection (DOMinj) or by osmotic pump (DOMosp).

4.4.2.3 Fibre measurements: There was a small effect of treatment on mean DL ($P<0.05$) but not on mean GL (Table 4.1). In contrast to the C goats (46±4 mm), there was a trend ($P<0.10$) for overall mean period DL to be reduced in the DOMinj goats (34±4 mm). There was no overall effect of treatment on SS. However, on 26 September, the SS was greater ($P<0.05$) in DOMinj goats (4.4±0.4) than in C goats (2.8±0.4, Table 4.1). This advance in shedding was also observed in the percentage weight of fibre shed in DOMinj compared with control goats, both as a trend for an interaction with time ($P<0.10$) and as an increase ($P<0.01$) in the percentage of fleece shedding on 10 October (Table 4.1).

The advance in shedding seen in the gross fibre measurements was observed in the detailed observations of the primary but not secondary follicles. PA increased more rapidly in the DOMinj than in control goats. This was expressed as an increase in PA ($P<0.01$) on 10 October (Table 4.1) and as an overall interaction of treatment with time ($P<0.001$). There was, however, no effect of treatment on SA or on NEDF's (Table 4.1).

4.5 DISCUSSION

This study has demonstrated that BR and DOM can be used to manipulate circulating PRL concentrations in down-producing goats during summer. However, effects of dose rate, in the ranges examined in experiment 1, could not be identified. Dose-response relationships have been found for DOM (Deaver *et al.* 1987) and BR (Milne *et al.*, 1990) using ranges much greater than in this experiment. In hindsight the dose rate ranges, while encompassing the range of effective dose rates quoted in the literature, may either be above the maximal effective dose rate or not extend over a large enough range to detect differential efffects in animals not treated on a liveweight basis.

The effective dose rate of BR, as defined by statistically significant changes in

circulating PRL concentrations, varies with season (Loudon and Brinklow, 1990; Curlewis *et al.*, 1991), possibly due to an interactive brain dopaminergic mechanism (Delitala *et al.*, 1980) or a change in lactotroph sensitivity (Curlewis *et al.*, 1990). When dopamine concentrations or lactotroph sensitivity are low, higher dose rates of BR appear to be required to suppress plasma PRL concentrations and *vice versa* (Curlewis, 1990). This experiment coincided with a period of high plasma PRL concentrations, so dose rates of BR in excess of 1 mg/goat/day should be effective at suppressing circulating PRL concentrations throughout the year. DOM was effective in elevating circulating PRL concentrations but the effect was erratic and variable between animals and between days, as has been found in other studies (Devesa *et al.*, 1988; Mogg and Samson, 1990). The variability in PRL responses was unaffected by the range of dose rates used in this experiment. However, lower dose rates than those used in this study may reduce the variability as the plasma PRL concentration was well above normal physiological levels.

The continuous treatment with DOM from the osmotic pump was not effective at elevating plasma PRL concentrations. The actual dose rate reaching the pituitary from the osmotic pump may have been too low to be effective, or the DOM may have come out of solution in the osmotic pump. Because of its low solubility, DOM does not lend itself readily to low volume infusions.

The PRL content of pituitaries is reduced during short days (Curlewis, 1990) and therefore it is not surprising that this study found DOM to be more effective in summer compared to spring. A similar effect has been observed in wallabies where DOM was more effective in summer compared to winter (Loudon and Brinklow, 1990). In contrast, Craven *et al.* (1993) found that, in sheep, plasma PRL concentrations were similarly responsive to DOM in winter and summer.

Table 4.1: Fibre growth (ls means±ls stder.), in untreated control goats and goats treated with 2.5 mg/day of domperidone by injection (DOMinj) or osmotic pump (DOMosp) from 13 September until 26 September 1991.

	Fibre Measurement Date			
	26.9.91	10.10.91	24.10.91	07.11.91
Down Length (mm)				
Control	73±8	46±8	31±4^{b}	16±10^{ab}
DOMosp	61±7	64±6	54±3^{a}	37± 8^{a}
DOMinj	53±7	47±6	27±3^{b}	11± 9^{b}
Guard hair length (mm)				
Control	64±4	65±3	58±3	56±4
DOMosp	59±4	61±3	61±3	59±4
DOMinj	62±4	65±3	60±3	60±3
Percentage of combed shed fibre (%)				
Control	24±8	12±4^{b}	12±5	8±5
DOMosp	23±7	19±4^{ab}	14±5	18±5
DOMinj	28±7	29±4^{a}	11±5	4±4
Shedding score				
Control	2.8±0.4^{b}	3.2±0.8	2.1±0.5	1.6±0.5
DOMosp	2.5±0.4^{b}	1.7±0.7	1.9±0.4	1.9±0.4
DOMinj	4.4±0.4^{a}	3.5±0.7	2.5±0.5	1.3±0.4
Newly emerged down fibre score				
Control	4.0±0.9	3.6±0.9	2.6±0.9	2.8±0.9
DOMosp	2.9±0.9	3.2±0.8	3.2±0.8	3.5±0.8
DOMinj	4.5±0.9	2.0±0.9	2.3±0.8	2.2±0.8
Primary follicle activity (%)				
Control	27±7	27±9^{b}	60±8^{a}	60±12
DOMosp	22±6	35±8^{b}	33±7^{b}	53±11
DOMinj	30±7	68±8^{a}	53±8^{a}	68±11
Secondary follicle activity (%)				
Control	25±8	21±8	29±8	22±6
DOMosp	13±6	29±7	19±8	18±5
DOMinj	30±6	17±7	18±8	24±5

Values with different superscripts in each column within each fibre measurement trait are significantly different at the 5% level.

In Cashmere goats, DOM can be used effectively to elevate PRL concentrations throughout the year.

The single daily injection of BR in experiment 1 was effective at suppressing PRL concentrations for only 20 h. A similar length of suppression following a BR injection was reported by Niswender (1974) and Kann *et al.* (1977). The summer daily injection of DOM elevated plasma PRL concentrations for only 12 hours. The different responses could reflect differences in drug half-life in combination with the three-fold greater dopamine D2 receptor binding affinity of BR compared with DOM (Nappi *et al.*, 1987). This variation in the duration of responsiveness to these drugs highlights the risk of using a single daily blood sample to describe drug-induced changes in blood hormone concentrations.

As seen in this experiment, and in others (Lincoln *et al.*, 1978, 1979; Brown 1980; Kennaway *et al.*, 1983; Mori *et al.*, 1985; Maeda *et al.*, 1988), plasma PRL concentration has a diurnal cycle in summer. The physiological significance of the diurnal variation in plasma PRL concentration is unknown. It is known that suppression of PRL with BR injections during daylight differs from dusk injections in stimulating oestrus in rats (Sanchez-Criado *et al.*, 1988). It is unclear whether diurnal fluctuations in PRL concentrations are important physiological regulators of fibre growth. Either repeated injections or the use of slow release technology (Curlewis *et al.*, 1991) may ensure stable plasma PRL concentrations. However, while the second injection of DOM clearly resulted in a small elevation of plasma PRL concentrations it failed to significantly extend the duration of PRL elevation. Plasma PRL concentrations exert an autoregulatory effect on PRL secretion both at the pituitary level and at the hypothalamic level (Thorne *et al.*, 1980; Lamberts and MacCleod, 1990). It is possible that an initial DOM-induced PRL surge increases hypothalamic dopamine secretion, and reduces PRL synthesis and release in the lactotrophs, thereby reducing the effectiveness of a subsequent injection.

In this study, the suppressive effect of plasma PRL concentration on PRL secretion and release from the pituitary was greater in spring relative to summer. Following a single injection of DOM in spring, plasma PRL concentrations 24 hours post-injection were half those found in control animals. No such effect was observed in the summer experiment.

In summer, BR had a tendency to become slightly more effective as the experiment progressed. This improvement in effectiveness of BR following repeated injections can be explained by a progressive reduction in pituitary mitotic rate and DNA synthesis (Fluckiger *et al.*, 1978; 1985). Because of this interaction with time, the duration of effectiveness of BR over 24 hours may have differed at the beginning compared to the end of experiment 1.

There was no indication that DOM effectiveness changed during either the 10 or 14 day treatment periods in summer or spring. This may not be the case over a more extended treatment period as found by Milne *et al.*, (1990) when DOM effectiveness reduced after two weeks of repeated dosing.

In experiment 1, the effects of BR and DOM on the plasma concentrations of other hormones other than PRL, and glucose, appeared to be small. Contrary to other studies using similar dose rates, neither drug used in experiment 1 had detectable effects on plasma concentrations of thyroxine (Milne *et al.*, 1990), glucose, insulin (Johnsson and Hart, 1985) or growth hormone (Forsyth *et al.*, 1985). Pooling of blood samples may have prevented the detection of any short term effects and as this result is in direct conflict with other work, it should be treated with caution.

It was expected that an elevation in plasma PRL concentration, in spring, would advance the shedding of the winter fleece of the goat. By the start of the experiment in September, plasma PRL concentrations had already increased to concentrations ranging between 20 and 80 ng/ml and follicle activity was low. Primary follicles

became activated 2 weeks earlier in response to the DOM injections and there was an associated shedding of guard hairs.

In experiment 2, the diurnal cycle of plasma PRL concentration was initially increased and then decreased by a single DOM injection. In Cashmere goats, treated with melatonin in September, plasma PRL concentrations were suppressed and primary follicles were activated after 24 days of treatment (Nixon *et al.*, 1993). As reported by Nixon *et al,* (1993), SA varied considerably during September. The similarity of the primary follicles responses in these two experiments might imply that the DOM-injected goats were responding to the reduction in plasma PRL concentration rather than the increase.

In Cashmere goats, which had been treated with BR to suppress plasma PRL concentrations from July, shedding of secondary follicles was delayed, but primary follicle growth was unaffected (Kloren *et al.*, 1993). It is clear that the state of the follicles at the time of treatment is crucial to the treatment response. *In vitro,* actively growing secondary cashmere follicles responded to increasing dose rates of PRL by increasing fibre length growth rate (Ibraheem *et al.*, 1993). The failure of the secondary follicles to respond to the DOM injection in this experiment could be due to the complex changes in plasma PRL concentrations, the lack of synchronisation of the secondary follicles or the short duration of the treatment.

4.6 CONCLUSIONS

In summer, injections of 2.5 mg of BR were effective at reducing plasma PRL concentrations for 20 hours in cashmere wethers. DOM was effective in elevating PRL concentrations for 12 hours in cashmere goats exposed to long days. DOM was also effective in goats in spring photoperiods. Due to the short period of effectiveness over 24 hours of these drugs, alternative methods of slow release administration are indicated. However, DOM administered by osmotic minipump at

2.5 mg/day did not elevate plasma PRL concentrations. Given the development of effective slow release methods for administration of BR, it may be a useful tool for the study of the role of PRL in ruminant fibre physiology. However, the DOM treatment regimens studied in this Chapter did not result in consistent, controlled elevations in plasma PRL concentrations. Other methods for increasing plasma PRL concentrations should be identified in further studies.

Fibre growth in only primary follicles and not secondary follicles was perturbed by DOM administered at 2.5 mg/day for 14 days by injection, but not by osmotic pump.

CHAPTER 5

HALF-LIFE OF CIRCULATING PROLACTIN IN SHEEP AND GOATS AND MANIPULATION OF PLASMA PROLACTIN CONCENTRATION IN LIGHT-TREATED ROMNEY SHEEP.

5.1 ABSTRACT

Two studies were conducted to examine the half-life of circulating prolactin (PRL) in sheep and goats, and the manipulation of circulating PRL concentration by treatment with PRL or with bromocryptine. In the first study (May 1992), a single sheep and goat were each treated, on alternate weeks, with a bolus iv injection of one of three sources of ovine PRL (oPRL). A further sheep and goat were treated with a continuous 2 day infusion of oPRL (0.18 mg/kg LW/day). Blood samples were taken at 60, 30 minutes and immediately prior to the start of the PRL treatment. Thereafter the injected animals were sampled at 15, 30, 45, 60, 120, 180, 240, and 600 minutes post injection while the infused animals were blood sampled at 1000 and 1500 h for the duration of the infusion.

In animals injected with PRL, there was no effect of source of oPRL or species of animal on circulating half-life (T½). T½ was 42±6 minutes in injected animals and 105±14 minutes in infused animals. The dispersion volume of PRL as a percentage of liveweight was 30%.

In the second study, commencing on 6 March 1992, sheep were either housed under natural photoperiod (N, n=6) or treated with 16 weeks of short photoperiod (8L:16D) and 10°C ambient temperature (n=14). Six short-photoperiod sheep continued under short photoperiod and were infused for three days with 0 (C), 0.1 (P1), and 0.4 (P2) mg oPRL/kg LW/day. Each sheep received all treatments, over three infusion dates, on alternate weeks. Eight, short photoperiod-primed sheep were transferred to long photoperiod (16L:8D) and 20°C ambient temperature for 34 days. Half were treated with 100 mg of long acting bromocryptine (Parlodel LA) by im injection (BR) and half remained untreated (LD).

In PRL-infused sheep, mean treatment plasma PRL concentration was higher in P2 sheep (99±17 ng/ml), than in P1 (60±17 ng/ml) and C (59±17 ng/ml) sheep ($P<0.05$). T½ was longer ($P<0.01$) in P1 (144±15 minutes) than in P2 (55±13 minutes) infused sheep.

Plasma PRL concentration was suppressed to below 15 ng/ml in BR sheep for 21 days post-injection. After 34 days, PRL concentrations were equivalent to those of LD sheep. Mean plasma PRL concentration of LD sheep (170±33 ng/ml) was higher than that of N sheep (80±27 ng/ml, $P<0.05$). Following the change from short to

long days, plasma PRL concentrations increased and the increase was proportionally higher in samples collected at 1700 h, 1100 h and 0500 h than in those collected at 1500, 1300, and 1000 h ($P<0.01$).

In conclusion, circulating-half life of PRL was similar in both sheep and goats but it was affected by mode of administration and dose rate of PRL. In sheep, in long photoperiods, Parlodel LA was effective at suppressing plasma PRL concentrations for 21 days.

5.2 INTRODUCTION

Plasma prolactin (PRL) concentration may have a role in regulating seasonal fibre growth cycles in sheep. In order to conduct experiments to elucidate this role, methods need to be developed to manipulate plasma PRL concentrations in sheep. Plasma PRL concentration can be manipulated either by changing the endogenous pituitary PRL secretion rate or by administering exogenous PRL into the circulatory system.

Endogenous PRL secretion from the pituitary pars distalisis is controlled by a variety of hypothalamic inhibitory and stimulatory factors (see Sections 1.3.5 and 1.3.6). In many species, hypothalmic dopamine (DA), is a major inhibitor of PRL secretion from the pituitary (Martin, 1985; Shin *et al.*, 1987; Stirling and Shin, 1990). The role of DA in regulating PRL secretion in sheep remains controversial as DA is undetectable in the long portal blood vessels which link the hypothalamus and the pituitary (Thomas *et al.*, 1989). However, there are dopamine receptors in the pituitary gland of sheep and it is possible that DA reaches these receptors via short portal vessels which link the neural lobe and the pars distalis of the pituitary (Martin, 1985). Binding of pituitary DA receptors by the receptor agonist bromocryptine mesylate (BR) suppresses plasma PRL concentrations in sheep (Niswender, 1974; Land *et al.*, 1980; Eisemann *et al.*, 1984; Pijoan and Williams, 1985). It has been reported that the effectiveness of BR in sheep is reduced during long photoperiods when plasma PRL concentration is high (Curlewis *et al.*, 1991;

Pelletier, 1973; Thimonier *et al.*, 1978; Lincoln, 1990), while others have found BR to be equally effective in both long and short photoperiods (Eisemann *et al.*, 1984; Pijoan and Williams, 1985; Ssewannyana and Lincoln, 1990). Treatment regimens for Parlodel LA need to be developed for sheep which reduce plasma PRL concentration to below 15 ng/ml in long photoperiods.

A single BR injection suppresses plasma PRL concentrations for 20 hours in goats (Chapter 4) and for 18 hours in sheep (Niswender, 1974). In sheep, suppression of plasma PRL concentrations for 24 hours is achieved by 12 hourly BR injections (Niswender, 1974; Land *et al.*, 1980; Pijoan and Williams, 1985; Takahashi, 1986). Alternatively, long-acting forms of BR have been developed for use in humans (Rolland *et al.*, 1986; Kocijanicic, 1990), and a single 50 mg injection of long acting BR (Parlodel LA) suppresses plasma PRL concentrations for 4 weeks (Rolland *et al.*, 1986; Kocijanicic, 1990). In sheep, injections of 18 mg of Parlodel LA, given at weekly intervals, only reduced mean plasma PRL concentrations to 30 ng/ml in summer while in winter plasma PRL concentrations were maintained at less than 15 ng/ml (Curlewis *et al.*, 1991). In addition, monthly injections of 50 mg of Parlodel LA failed to completely suppress the normal spring rise in plasma PRL concentrations in sheep (McCloghry, 1992; Parry *et al.*, 1993).

Plasma PRL concentration has been elevated in sheep (Brinklow and Forbes, 1984; Eisemann *et al.*, 1984) and other animals (Spoon and Hallford, 1989; Kjemacke *et al.*, 1990; Lynch and Russel, 1990; Jacquemet and Prigge, 1991; Xia and Yang, 1991; Ciereszko and Dusza, 1993) by the administration of exogenous PRL into the circulatory system. PRL plasma concentration is the net result of the secretion rate of PRL from the pituitary, clearance rate from the plasma and size of the pools into which PRL distributes. If it is assumed that inputs of exogenous PRL and pituitary PRL, plasma clearance and plasma PRL concentration are in equilibrium, then these relationships can be described mathematically by steady state equations (Shipley and Clark, 1972). The effects treatment of exogenous PRL either as a single, dose, or

continuous infusion, can be used to determine the mathematic parameters of this equilibrium.

Clearance of exogenous PRL from plasma is described by the "rate constant", "half-life in circulation" and "metabolic clearance rate". The rate constant (k) is the fraction of the PRL lost from plasma per unit of time; half life (T½) is the time required for half of the PRL in the circulation to disappear; metabolic clearance rate (MCR) is the rate of PRL removed from the circulation per unit of time.

PRL is removed from plasma by enzymic breakdown in organs such as the liver, kidneys, and mammary gland. The mammary gland also excretes PRL directly into the milk (Martin, 1985). PRL clearance is affected by physiological status of the animal; lactation increases (Blake *et al.*, 1973; Grosvenor *et al.*, 1977; Akers *et al.*, 1980) and ovariectomy decreases (Blake *et al.*, 1973) clearance of PRL from plasma while it is unaffected by the sex of young animals (Blake *et al.*, 1973). Some have found that clearance is greater at higher dose rates of PRL (Van der Gugten and Kawa, 1970; Grosvenor *et al.*, 1977) while others have failed to find a dose rate-response relationship (Akers *et al.*, 1980). These differences in clearance rates of PRL from the body may contribute to the variation in plasma PRL concentrations seen in a variety of physiological states. It is not known whether differences in clearance rate of PRL contribute to seasonal fluctuations in plasma PRL concentration.

The objective of this Chapter is to present information from two studies examining methods of manipulating plasma PRL concentration. The first study determined T½ of oPRL from 3 different sources in sheep and goats following infusion or injection. oPRL obtained from these 2 suppliers were used in subsequent studies in this programme. The second study measured the duration of effectiveness of Parlodel LA in long day-treated sheep and the effects on plasma PRL concentration and clearance

rate, of two dose rates of PRL in short day-treated Romney sheep.

5.3 METHOD

In the first study two Romney ewes and two Cashmere does all mature and non-pregnant were maintained in metabolism crates, under natural light, from 1 May 1992 at the Flock House Agricultural Centre in Bulls, New Zealand. The animals were fed a diet comprising 15% concentrate pellets (70% lucerne, 30% cereal) and 85% lucerne pellets, at a rate of 1.1 times maintenance. Maintenance requirement was calculated as 0.7 MJME/day/$kg^{0.75}$ and pellets and lucerne were assumed to contain 11 and 10 MJME/kg DM respectively. Water was on offer *ad libitum* and, on alternate days, 2 g (sheep) and 1 g (goat) of a mineral supplement (94% sodium chloride, 6% sodium molybdate w/w) was fed with the pellets to counteract possible copper toxicity.

One ewe (42 kg) and one Cashmere doe (23 kg) each received a single intravenous injection of oPRL obtained from one of three sources at a rate of 0.18 mg/kg LW: source 1 (oPRL-19, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Baltimore, Maryland, USA) obtained in 1991; source 2 (oPRL-19, NIDDK, Baltimore, Maryland, USA) obtained in 1989; Source 3 (Catalog No., L6520, Sigma Chemical Company, St Louis, Mo, USA). Intravenous injections of oPRL were given to the sheep on 16 April, 7 May and 14 May and to the goat on 7 May, 14 May and 24 May 1992. The animals were fitted with venous jugular catheters under local anaesthetic (lignocaine).

An additional doe (25 kg) and ewe (49 kg) were infused with oPRL (Catalog No., L6520, Sigma Chemical Company) at a rate of 0.18 mg/day/kg for two days. The animals were fitted with venous jugular catheters under local anaesthetic (lignocaine). Fixed speed peristaltic pumps (Technicon) and pump tubes (Catalogue No. LK116-0532-040, Elkay Products, Shrewsbury, MA, USA) delivered the infusate to the

venous jugular catheter at a mean calculated rate of 5.4 ml/hr. The infusion started at 1000 h on 7 May and ceased at 1500 h on 9 May 1992.

Blood samples were drawn from venous jugular catheters into EDTA vacutainers from injected and infused sheep and goats at 60, 30 minutes and immediately prior to the start of the PRL treatment. Thereafter the injected animals were sampled at 15, 30, 45, 60, 120, 180, 240, and 600 minutes post-injection while the infused animals were blood sampled at 1000 h and 1500 daily h on each infusion day. Plasma was removed after centrifugation and stored at -15°C. PRL concentration in all samples were determined by radioimmunoassay.

In the second study, twenty non-pregnant Romney ewes were housed in individual metabolism crates and fed as in the first study. On 4 March 1992, the ewes were balanced for liveweight and fleece weight and randomly allocated within blocks to four treatment groups. Six of the sheep (N group) were housed under natural light and temperature at Flock House Agricultural Centre, Bulls, New Zealand. The remainder of the sheep were housed 50 km away under artificial short photoperiod at Massey University, Palmerston North, NZ. The photoperiod treatment was achieved using fluorescent lights with supplementary 150 watt light bulbs on automatic time switch control.

The 14 sheep were treated with short photoperiod (8L:16D, lights on 0800 to 1600 h) and 15°C ambient temperature until 16 June 1992. On that date four sheep (BR group) were given an intramuscular injection of 100 mg of long-acting bromocryptine (Parlodel LA, Sandoz Pharmaceuticals Ltd, Auckland, NZ). The BR sheep and a further four sheep (LD group) were then subjected to long photoperiod (16L:8D, lights on 0400 to 2000 hrs) and 20 °C ambient temperature for 34 days. An additional six sheep continued under the short day photoperiod and were infused with saline only, or with 0.1 (P1) or 0.4 (P2) mg/kg LW of oPRL (Catalog No., L6520, Sigma Chemical Company). In the 3x3 Latin square design replicated time for these

6 sheep each sheep received each treatment commencing on 16 June, 30 June or 14 July 1992. Infusates were administered into the jugular vein of each sheep for three days using fixed speed peristaltic pumps (Technicon) and pump tubes as described previously.

The sheep were weighed two weekly for the first six weeks of the experiment and thereafter at monthly intervals.

Blood samples were collected from N, BR, LD and PRL-infused sheep by jugular vein puncture at 1300 h at two-weekly intervals from 1 April to 20 July 1992. In addition BR, LD and PRL-infused sheep were sampled weekly at 1300 h from 16 June to 20 July 1992. Further blood samples were taken from BR, LD and PRL-infused sheep on 17 June, 2 July and 15 July. Blood samples were collected from these sheep at 1300, 1500, 2300 h and at 0500, 1000 and 1300 h on the following day. Additional blood samples were collected at 1300 h on the day preceding (not on 17 June 1992) and proceeding the extended sampling regime during infusion in the PRL group. Plasma was removed after centrifugation and stored at -15 °C. All samples were assayed for plasma PRL concentrations.

Prolactin and Parlodel Preparation: PRL infusates were prepared in autoclaved, siliconized glassware, using millipored reagents in a laminar flow cabinet to maintain sterility. On the day preceding administration, PRL was dissolved in 0.03 M $NaHCO_3$ (2.5 mg/ml) at pH 10.8. The PRL was stored at 4°C and, immediately prior to administration, PRL volumes were made up to infusion and injection concentrations by dilution with sterile physiological saline.

The long-acting formulation of bromocryptine (Parlodel LA) consisted of microspheres of bromocryptine mesylate coated with polylactic acid. The microspheres and vehicle (6% dextran 70 in 0.9% saline) were stored separately in a double chamber syringe.

They were mixed immediately prior to the administration of the intramuscular injection.

Hormone Assays: In study 1, the plasma PRL concentrations were determined by radioimmunoassay at Massey University (Section 2.2.3.4). The radioimmunoassay of plasma PRL concentration in study 2 was conducted at AgResearch, Ruakura (Section 2.2.3.3).

Statistical analysis: In animals injected with oPRL, the data were fitted to a single-pool exponential decay model (equation 1) using iterative line fitting procedures (SAS, 1987) and k calculated. C_o was determined by back extrapolation to t=0 (equation 1) and the distribution volume of PRL expressed as a percentage of body weight (V%), was then calculated (equation 2). The T½ was calculated for the injected animals using equation 3. MCR was calculated following infusion of PRL to steady state (equation 4). Steady state plasma PRL was assumed to be the mean plasma PRL concentration over the infusion period. T½ was calculated in infused animals using equation 5 and the V% determined from injected animals.

$C_t=C_b+C_oe^{-kt}$ (Shipley and Clark, 1972) [Equation 1]

Where, C_t = Plasma PRL concentration at time t (ng/ml)
C_b = Base plasma PRL concentration determined from the mean of pretreatment values (ng/ml)
C_o = Initial plasma PRL concentration t=0 determined by back extrapolation (ng/ml)
k = Rate constant (min^{-1})

$V_{\%}= ((D_i/C_o)/LW)*100$ (Shipley and Clark, 1972) [Equation 2]

Where, $V_{\%}$ = Dispersion volume expressed on a percentage basis of LW
D_i = Dose of PRL injected (ng)
LW = Liveweight (kg)

$t½=0.693/k$ (Shipley and Clark, 1972) [Equation 3]

Where, t½ = Circulating half-life determined by injection (min).

$MCR = D_f/C_i$ (Akers *et al.*, 1980) [Equation 4]

Where, MCR= Metabolic clearance rate (ml/min)
D_f = Infusion dose rate of prolactin (ng/min)
C_i = Mean increase in plasma PRL concentration at steady state (ng/ml).

$T½ = LW*V\%*0.693$ (Akers *et al.*, 1980) [Equation 5]

Where, T½= Circulating half-life determined by infusion to steady state (min)

In injected animals, data were analysed to determine whether the fit of the exponential model (Equation 1) was improved by fitting separate models for species or source of PRL (SAS, 1987). Since separating these effects did not improve the fit of the model, the data were pooled across source of PRL and species. Separate models were then derived by separating fast and slow rate constants at 0 to 2 hrs and more than 2 hours post injection. The fit of data was compared in these two separate models with the parsimonious pooled model.

In the second study, due to unequal variances in plasma PRL concentrations, the data were $\log_{10}$ transformed. Daily and sequential 24 hour plasma PRL concentrations were analysed using the GLM procedure of SAS (SAS, 1987) repeated measures in time using the Wilks' Lambda test of significance. The mean treatment period plasma PRL concentrations were analysed using general linear models procedures (SAS, 1987) with animal, infusion date and treatment fitted in the model. The effects of infusion dose rate, infusion date and animal on T½ of PRL were determined using general linear model procedures (SAS, 1987). Data in the text are presented as non-transformed least squares means and least squares standard errors. Raw means and standard errors are presented graphically unless otherwise indicated.

5.4 RESULTS

Study 1

In sheep and goats administered with a bolus treatment of PRL, there was no effect of species or source of PRL on k (Figure 5.1). The fit of the pooled data was not improved by fitting fast and slow rate constants. In animals injected with PRL, k was 1.0±0.1 and t½ was 42±6 minutes. The T½ of the PRL administered by infusion was longer at 105 and 104 minutes for the sheep and goat respectively (Figure 5.2). MCR of PRL following infusion of 0.18 mg oPRL/kg LW/day was 1.98 and 1.97 ml/min/kg in the sheep and goat respectively. The mean dispersion volume (V_d) calculated following injection of PRL was 14.5 (11.3-19.7) litres and 7.2 (6.5-8.3) litres for sheep and goats respectively. When V_d was expressed on a liveweight basis the V% was similar in sheep (32%) and goats (29%).

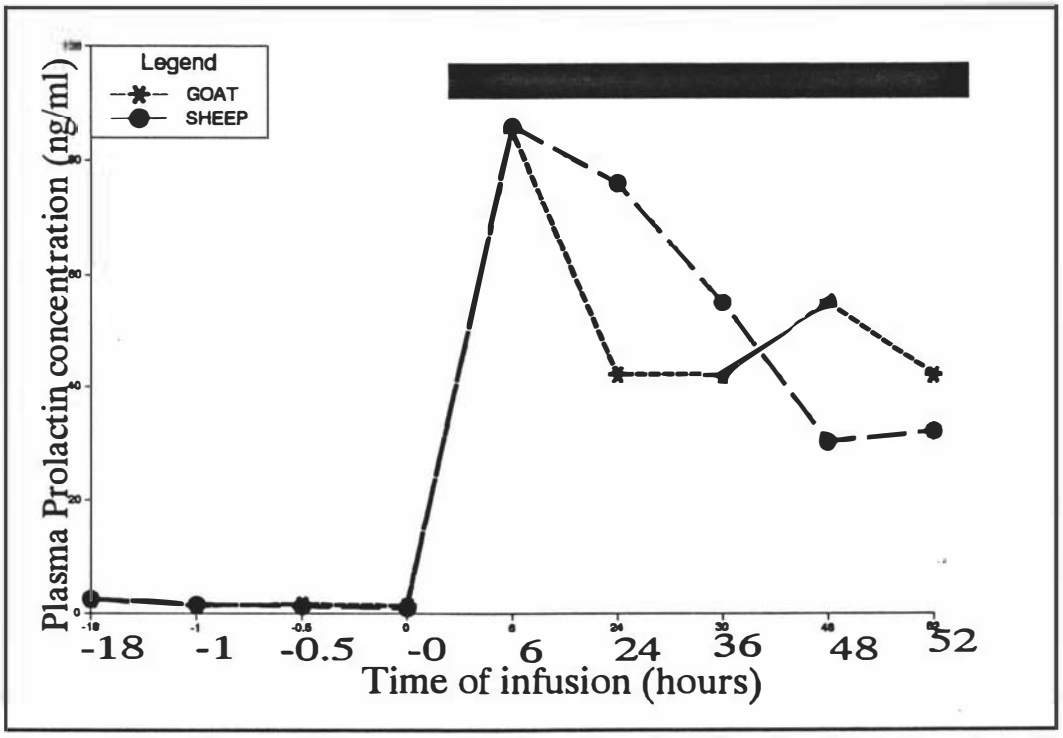

Figure 5.2: Plasma PRL concentration (ng/ml) following infusion of 0.18 mg/kgLW/day of oPRL to a Romney ewe and Cashmere doe.

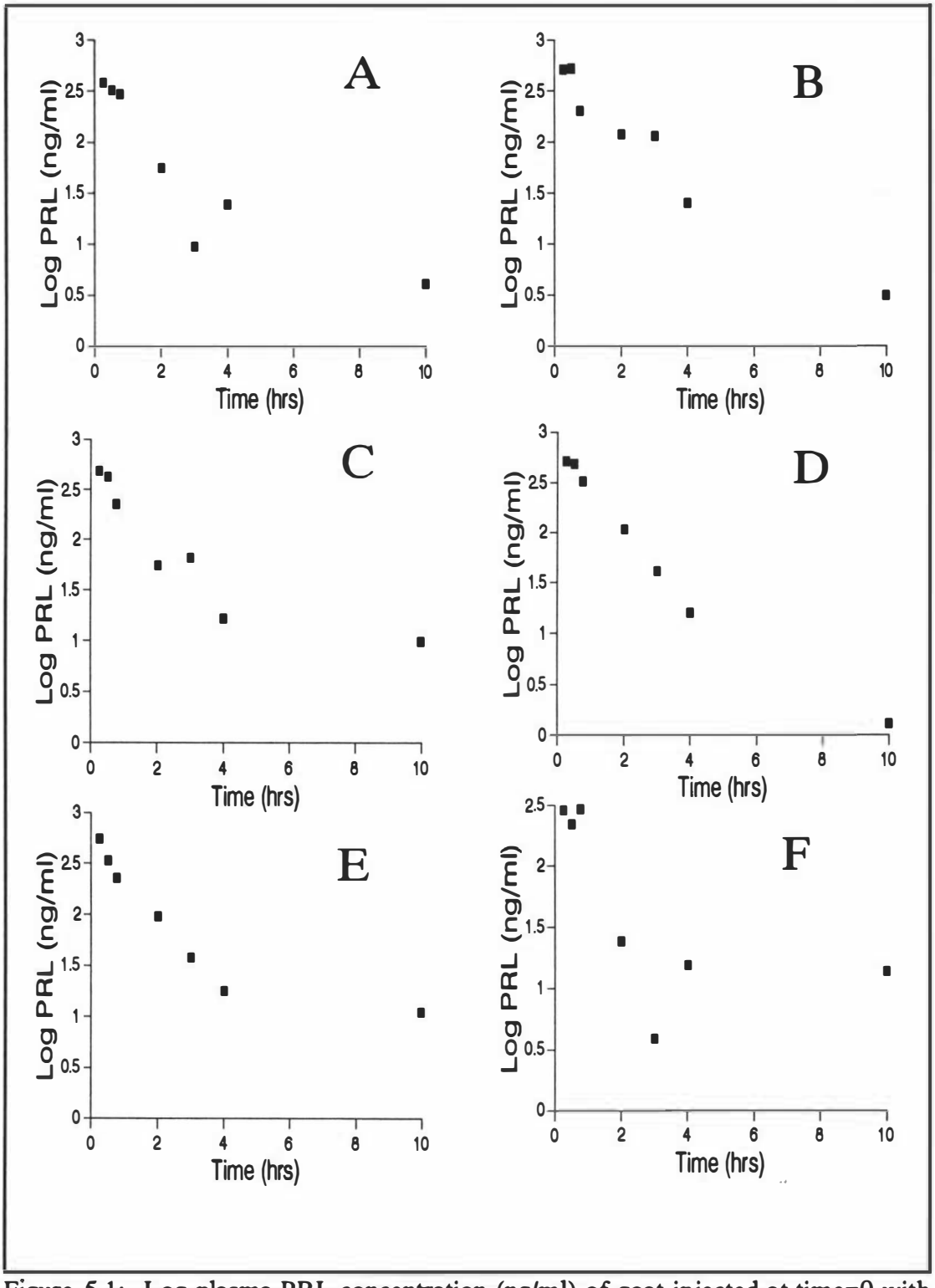

Figure 5.1: Log plasma PRL concentration (ng/ml) of goat injected at time=0 with PRL from source 1 (A), source 2 (B) and source 3 (C) and sheep injected at time=0 with PRL from source 1(D), source 2(E) and source 3(F).

Study 2

Prolactin Infusions: In PRL-infused sheep, the plasma PRL concentrations of blood samples collected 3 hours and immediately prior to the start of the infusions were higher (43±30 ng/ml mean±se) than those of blood samples determined 168 hours (12± 5 ng/ml) (Figure 5.3). The catheterisation of the animals was carried out progressivley over a five hour period immediately prior to the start of the infusions. Stress may have caused an increase in plasma PRL concentration in the blood samples collected during this time. In order to caluclate MCR and T½ values independently of this effect, baseline plasma PRL concentration was assumed to be that determined 168 hours before infusion. This resulted in estimations of mean MCR over the 3 infusion periods of 2.3 and 3.0 ml/min/kgLW for P1 and P2 infused sheep respectively. T½ was longer ($P<0.01$) in P1 (144±15 minutes) than in P2 (55±13 minutes) infused sheep. However, these results are not reliable due to the variability of the data in the pre-treatment period and the lack of consistent elevations in plasma PRL concentrations.

Mean treatment plasma PRL concentration was higher in P2 sheep (99±17 ng/ml), relative to P1 (60±17 ng/ml) and C (59±17 ng/ml) sheep ($P<0.05$). There was no significant effect of infusion date on plasma PRL concentration.

In infusion period 2 (Figure 5.3), plasma PRL concentrations were elevated in P2 (181±57 ng/ml) and P1 (79±57 ng/ml) compared with C (34±57 ng/ml) groups ($P<0.05$). A similar effect was seen during the infusion period 3 (Figure 5.3) when mean treatment plasma PRL concentrations of P2 (68±12 ng/ml) sheep were greater ($P<0.05$) than those of C sheep (41±12 ng/ml) with P1 (53±12 ng/ml) sheep having intermediate values($P<0.05$). However, a different pattern was observed in the first infusion period (Figure 5.3), when C sheep (103±8 ng/ml) had higher mean treatment plasma PRL concentrations than either the P1 (48±8 ng/ml) or the P2 (60±8 ng/ml) groups ($P<0.05$).

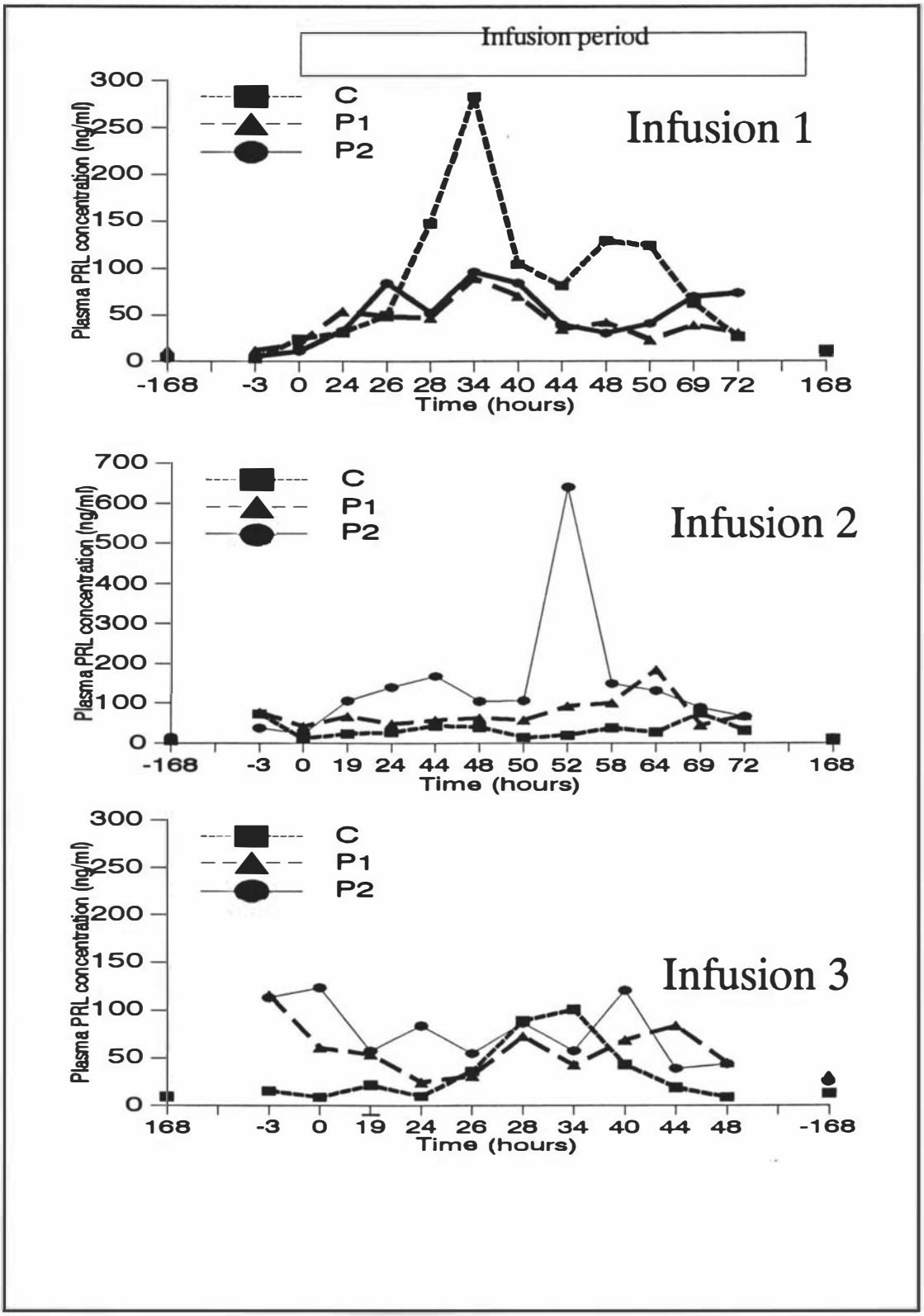

Figure 5.3: Plasma PRL concentration in sheep infused with 0 (C), 0.1 (P1) and 0.4 (P2) mg oPRL/kgLW/day at three infusion dates.

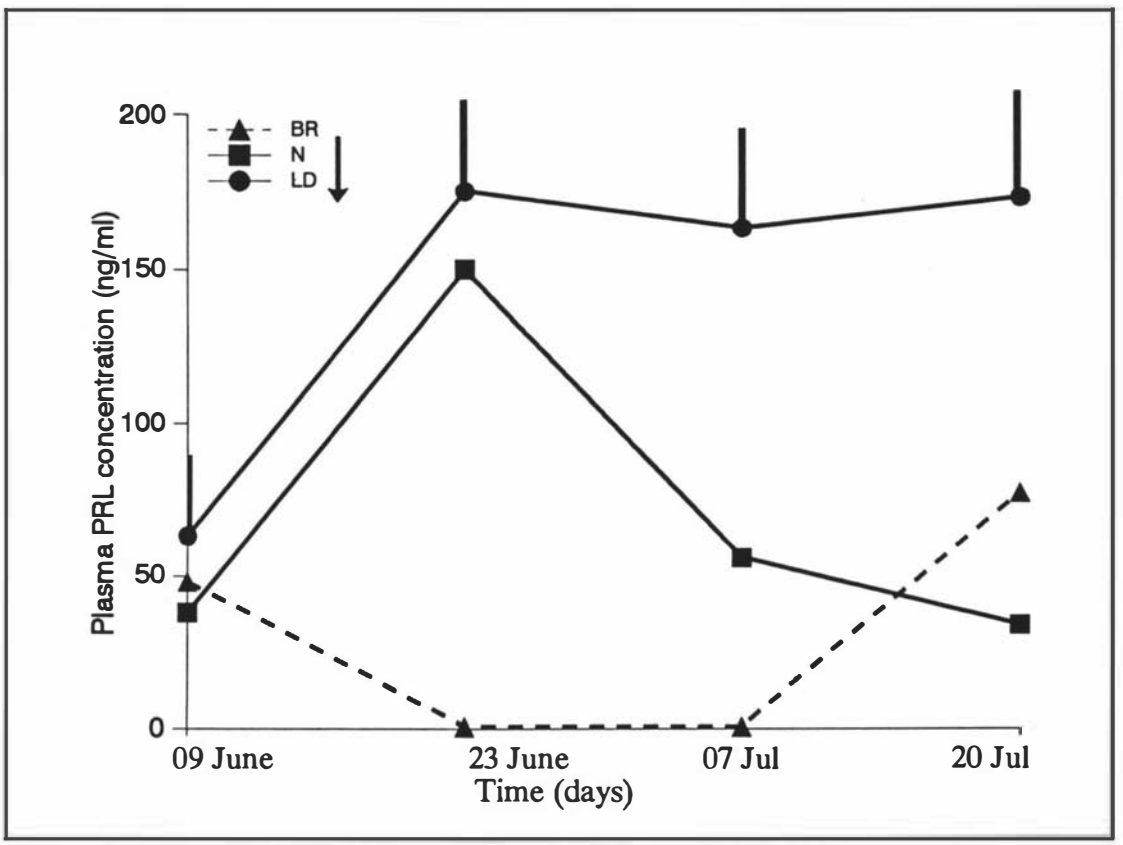

Figure 5.4: Plasma PRL concentrations (ng/ml) at 1300 h in Romney sheep treated with long days (LD), natural light (N) and bromocryptine (BR, Parlodel LA, 100 mg/sheep. Vertical bars are least significant standard errors. Arrow indicates start of bromocryptine and long day treatments.

Bromocryptine treatment: The mean plasma PRL concentrations in blood samples collected at two weekly intervals during the 34 day treatment period were higher in LD (170±33 ng/ml) than in N (80±27 ng/ml, $P<0.05$) and BR (26±32 ng/ml, $P<0.001$) sheep. BR and N animals had similar mean treatment period plasma PRL concentrations at 9 June. However, over the treatment period plasma PRL concentration decreased in N sheep and increased with time in BR sheep ($P<0.05$) (Figure 5.4). However, this was largely associated with an atypical increase in plasma PRC concentration on 23 June in N sleep.

BR treatment commencing on 16 June suppressed plasma PRL concentrations relative

to LD for 29 days ($P<0.05$). Plasma PRL concentrations began to increase 21 days after the Parlodel LA injection. There were insufficient degrees of freedom to analyse the time by treatment interaction over all the 14 blood samples collected at 1000 h in BR and LD sheep. Therefore data from consecutive sampling dates were averaged to reduce the number of data points. Suprisingly, no interaction with time and treatment was identified.

In comparison to LD sheep, mean 24 hour PRL concentrations in BR sheep were suppressed in all three 24 hour sampling periods ($P<0.01$). BR sheep had lower plasma PRL concentrations than LD sheep at every sampling time in the detailed sampling on 17 June and 30 June (Figure 5.6, 5.7). However, by 14 July (Figure 5.8) the Parlodel LA injection was less effective. Plasma PRL concentration in BR sheep was different to that of LD sheep only at 1700 h and 0500 h when LD sheep had elevated plasma PRL concentrations ($P<0.05$).

The mean 24 hour plasma PRL concentrations in the LD sheep tended to increase over the three consecutive dates (Figure 5.6-5.8). There was a treatment by time interaction ($P<0.01$) in plasma PRL concentrations in samples collected at 1700 h, 2300 h and 0500 h. Plasma PRL concentration at these times had the greatest increase associated with long days.

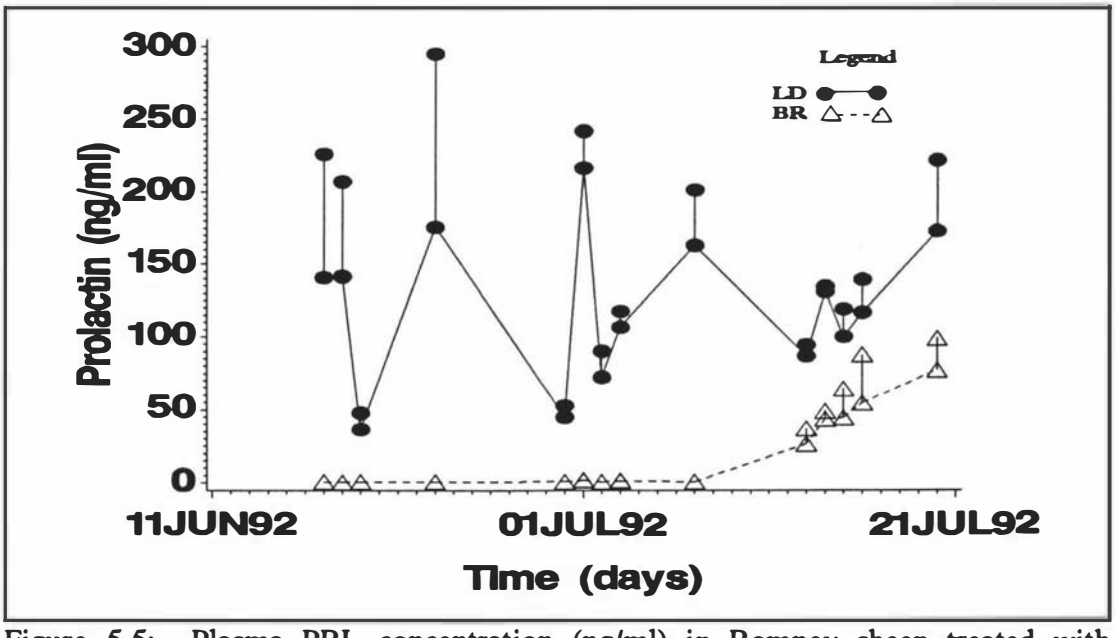

Figure 5.5: Plasma PRL concentration (ng/ml) in Romney sheep treated with bromocryptine (BR, Parlodel LA, 100 mg/sheep) and long days (LD) on 16 June 1992. Vertical bars represent standard errors.

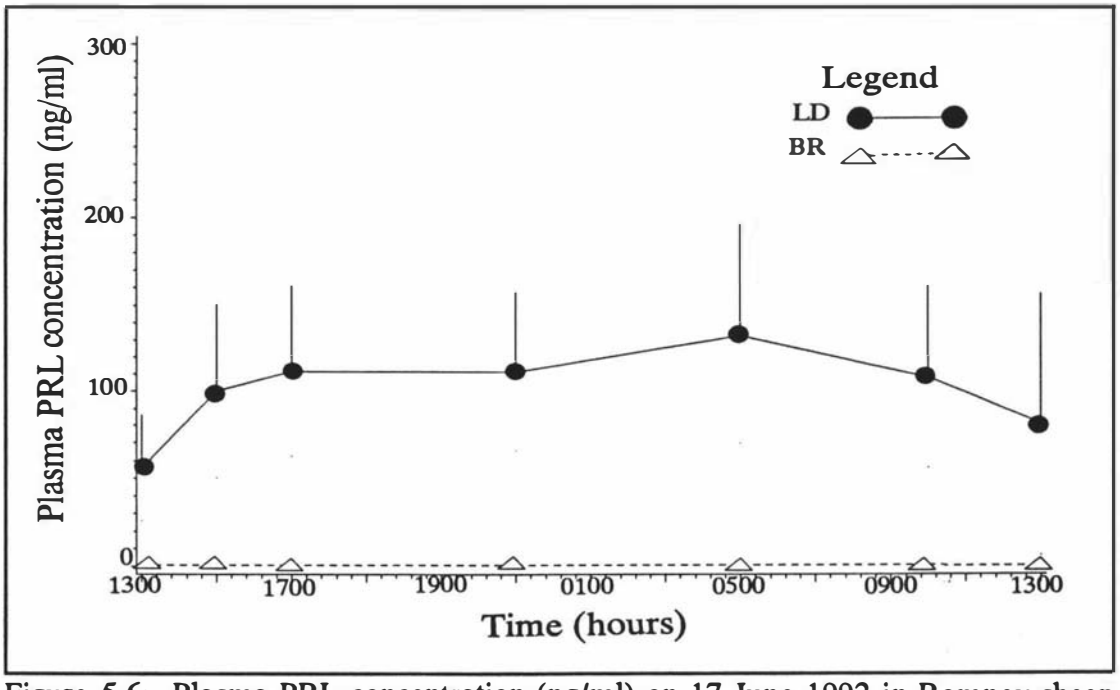

Figure 5.6: Plasma PRL concentration (ng/ml) on 17 June 1992 in Romney sheep treated with bromocryptine (BR, Parlodel LA, 100 mg/sheep) and long days (LD) from 16 June 1992. Vertical bars represent standard errors.

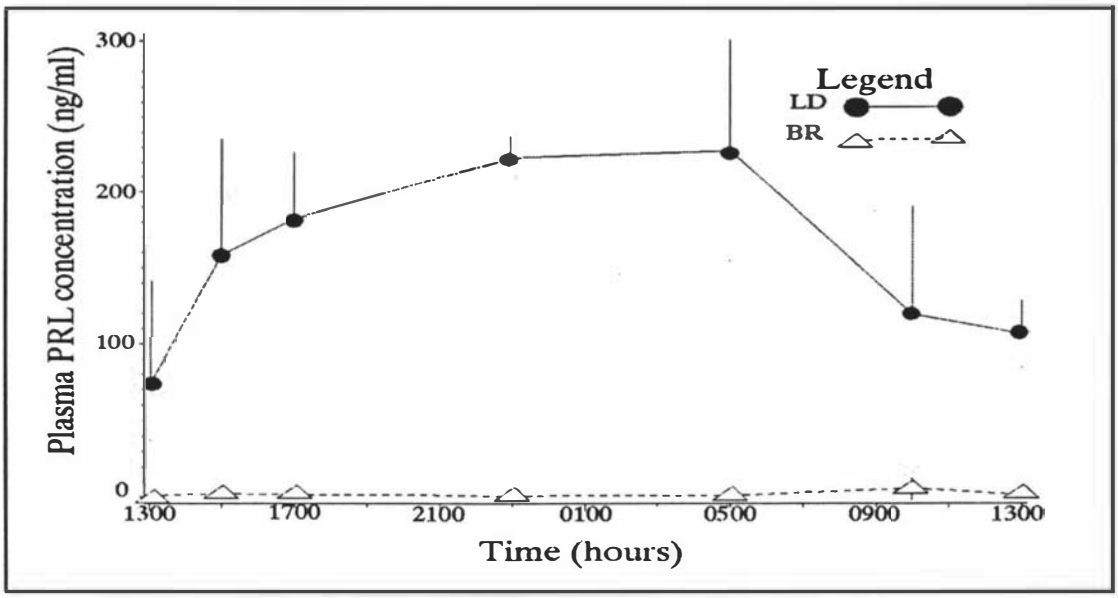

Figure 5.7: Plasma PRL concentration (ng/ml) on 30 June 1992 in Romney sheep treated with bromocryptine (BR, Parlodel LA, 100 mg/sheep) and long days (LD) from 16 June 1992. Vertical bars represent standard errors.

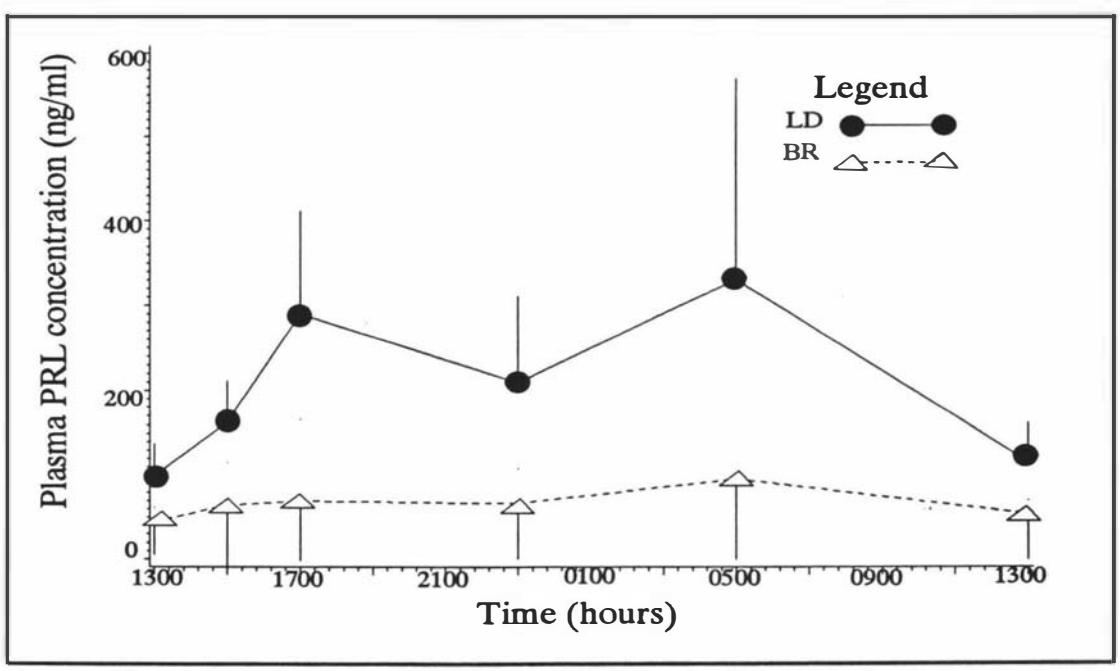

Figure 5.8: Plasma PRL concentration (ng/ml) on 14 July 1992 in Romney sheep treated with bromocryptine (BR, Parlodel LA, 100 mg/sheep) and long days (LD) from 16 June 1992. Vertical bars represent standard errors.

5.5. DISCUSSION

When exogenous PRL enters the circulatory system it disperses into the plasma, extracellular and intracellular body fluids. In study 1, it was calculated that exogenous PRL disperses into fluid equivalent to 30% by weight of the sheep and goats. This is equivalent to the dispersal volume of an intravenous injection of creatinine in Romney sheep (Sun *et al.*, 1992). This indicates that exogenous PRL disperses not only in plasma and extracellular fluid, but it also enters cells and mixes with some but not all of the intracellular fluid. This is expected as PRL crosses cell membrane barriers when it is internalised following binding to cell-surface PRL receptors (Rillema *et al.*, 1988). In mammary cells, internalised PRL is also retransported into the extracellular fluids and excreted in milk (Onstrom, 1990).

It is highly likely that there is more than one pool for PRL in the body. The analysis of the exponential decay in plasma PRL resulting from a bolus injection of PRL failed to identify multiple dispersion pools. However, the frequency of blood samples collected during the time series makes the identification of multiple pools unlikely.

In this study, the clearance of PRL from plasma was calculated following either a bolus injection or a constant infusion of PRL. The mathematical equations used to calculate clearance of PRL from plasma are based on three assumptions: that exogenous PRL is in equilibrium with all PRL pools in the body; that exogenous PRL is cleared from these pools in a manner identical to endogenous PRL; and that PRL secretion rate from the pituitary remains constant during the PRL treatment period. In study two, the plasma PRL concentrations collected in the preinfusion period and in control sheep during the infusion were 4 to 10 fold higher than expected. Stress can elevate plasma PRL concentrations (Greef, 1985; Thomas et al., 1988). In study two, the catheterisation procedure and repeated jugular puncture probably induced a stress-related elevation in endogenous PRL secretion. In future, catheterisation should be undertaken 24 hours before the start of treatment and regular blood samples should

be drawn from contralateral jugular catheters. It is also of concern that there was considerable variation in post-infusion PRL concentration in both experiment 1 and 2. To counteract this problem, steady state PRL concentration was taken as the mean of PRL concentration over the infusion period. In other studies, infused PRL achieved equilibrium after 150 minutes (Akers *et al.*, 1990). Therefore it is assumed that equilibrium with the body pools had occurred by 24 hours when the first blood sample was collected. Clearance figures calculated from the infusion studies should therefore be treated with some caution.

The T½ of PRL measured by infusion in this study ranged from 55 to 144 minutes. In cattle infused with PRL, reported T½ of PRL ranged from 28 to 36 minutes (Akers et al., 1990), while from data in PRL-infused lactating goats, T½ was calculated to range from 76 to 108 minutes (Jacquemet and Prigge, 1991). Other published studies report MCR of PRL rather than T½. In this study, dose rates of 0.10 and 0.18 mg oPRL/kg LW/day produced MCR's ranging from 1.2 to 2 ml/min/kg LW. In sheep treated with the higher dose of 0.4 mg/PRL/kg LW, MCR was higher at 3 ml/min/kg LW. Published figures for MCR of PRL from plasma for sheep range from 1 ml/min/kg LW in ovariectomized sheep up to 2.15 ml/min/kg LW in lactating sheep (Blake *et al.* 1973; Davis and Borger, 1973). In cattle, reported figures range from 2 to 4.2 ml/min/kg LW for dry and lactating cattle respectively (Akers *et al.*, 1980). In this study, the MCR for PRL infused at 0.4 mg/kg LW/day was higher than other reported values for sheep. However, a straight line can be plotted (Figure 5.9) between PRL infusion rate in this study and the MCR rates. Increasing infusion rate by 4 fold increased MCR by 2.6 fold. An increase in MCR following an increase in PRL infusion rate has been reported in rats (Grosevenor *et al.*, 1977; Bont *et al.*, 1983) and goats (Jacquemet and Prigge, 1991). In rats, increasing infusion rate of PRL by 4 fold increased MCR by 3 fold. However MCR ceased to be dose-responsive when infusion dose rates increased beyond 2.2 mg/kg LW/day. In lactating goats, MCR increased by 30% when PRL infusion dose rate increased from

2 mg/kg LW/day to 4 mg/kg LW/day (Jacquemet and Prigge, 1991), and, in sheep infused with I^{125} labelled PRL, MCR was shown to be higher when endogenous PRL secretion was higher (Davis and Borger, 1973). However, in contrast, MCR in cattle did not respond to two-fold changes in PRL infusion rate (Akers, 1980).

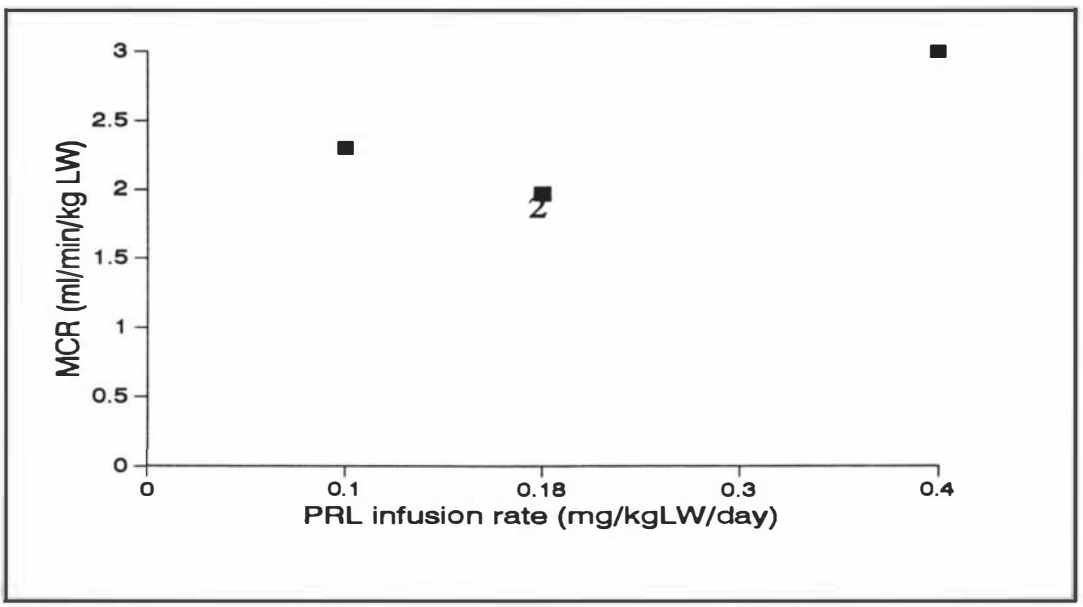

Figure 5.9: Metabolic clearance rate (ml/min/kgLW) of ovine prolactin infused at 0.1, 0.18, and 0.4 mg/kgLW/day in animals from experiment 1 and 2. Number indicates number of multiple data points at this position.

The mechanism by which MCR is increased by PRL dose rate is open to conjecture. The ovaries, liver, kidneys and mammary gland are the principal organs involved in clearing PRL and it was hypothesised by Grosvenor *et al.* (1977) that an elevation of plasma PRL concentration could increase blood flow to these organs. PRL is also cleared from plasma when it binds to receptors, becomes internalised and is broken down by lysosomes within the cell (Rillema *et al.*,1992). An increase in PRL receptor number would result in a greater clearance of PRL from plasma. Following

an increase in plasma PRL concentration, PRL receptors are up-regulated in the mammary gland (Onstrom, 1990; Rillema *et al.*, 1988) and skin (Choy pers. comm.). In rats, changes in MCR were detectable within 30-35 minutes following a change in PRL infusion rate. It is not likely that 3 fold increases in PRL receptor number could occur within 30 minutes. Therefore it is unlikely that PRL receptor up-regulation entirely accounts for the effect of PRL infusion rate on MCR. It can be hypothesised that the body has the capacity to clear PRL plasma concentrations up to normal physiological concentrations. In this study, individual plasma PRL concentrations reached 600 ng/ml. Therefore charges in MCR with investing exogenous PRL loads may simply reflect a PRL clearance mechanism which is operating below its full capacity. Possibly endgenous PRL secretion is decreased by exogenous PRL infusion.

The t½ of PRL calculated from a bolus injection in this study was 42 minutes. This was considerably shorter than that of infused PRL though the infusion data is somewhat dubious. The increase in clearance rate of PRL following a bolus injection may be a further expression of the dose rate response in PRL clearance. However it is also possible that, compared with a long term infusion, PRL from a bolus injection reaches equilibrium with different extracellular and intracellular PRL pools. In addition other physiological processes may extend the half-life of the infused PRL.

The half-life of LH and FSH was doubled during infusion compared to bolus injection (Robertson *et al.*, 1991). It was hypothesised that the extension of the half-lives of the FSH during infusion was due to the accumulation of variants with longer half-life. FSH variants which differ in overall molecular charge and glycosylation differ greatly in half-life (Wilde, 1986).

Various PRL isomers have been identified (Ben-Jonathan, 1985; Lamberts and MacCleod, 1990) and these variants differ in biological activity (Onstrom, 1990) and possibly half-life. The specifications for the exogenous PRL used in this study indicated that it was comprised more than 95% monomeric PRL. There would be

little scope for the accumulation of dimer and oligomer PRL from this exogenous source and subsequent modification of the monomer PRL would have to occur within the plasma. The extended duration of contact of PRL with serum could result in structural modifications such as: binding to plasma proteins; the glycosylation of the PRL molecule; the altering of the net charge of PRL molecule; or isoform modification of the PRL molecule. PRL variants are secreted into the plasma intact, directly from lactotrophs (Stroud *et al.*, 1992). Therefore there is no precedent for serum- induced interconversion of PRL variants. Glycosylated PRL and variations in the charge of PRL molecule do occur in sheep (Ostrom, 1990). To date, it is not known whether these modifications occur within plasma or in the lactotroph. The mechanism by which infused PRL has an extended circulating half-life relative to bolus injected PRL therefore remains a matter of conjecture. But binding could still occur in plasma - though one might expect this to occur fairly quickly.

In study 2, the elevation in plasma PRL concentrations achieved in P1 and P2, when compared to C, sheep was disappointing. However, relative to baseline plasma PRL concentrations collected in the preceding week, the elevation in plasma PRL concentration was similar to that in study 1 and other published results (Brinklow and Forbes, 1984; Spoon and Hallford, 1989; Jacquement and Progge, 1991; Ciereszko and Dusza, 1992).

The long-day photoperiod in this study was highly successful at elevating plasma PRL concentrations. This was not surprising as many studies have shown that plasma PRL concentration follows photoperiodic changes (Pelletier, 1973; Thimonier *et al.*, 1978; Brown *et al.*, 1980; Howles *et al.*, 1980; Munro *et al.*, 1980; Kennaway *et al.*, 1983; Poulton and Robinson, 1987; Leshin and Jackson 1987; Lincoln 1990). The mean peak plasma PRL concentration of 170 ng/ml in LD sheep was higher than reported for other Romney sheep in summer (Poulton and Robinson, 1987; McCloghry, 1992). However this may just reflect differences in radioimmunoassays. In this study, when short photoperiod was switched to long, temperature was also increased from 10°C

to 20°C. High ambient temperatures elevate plasma PRL concentration (Wettemann *et al.*, 1982; Howland *et al.*, 1983) and probably increased plasma PRL concentration over that achieved by long photoperiod.

In comparison to N sheep, LD treated animals had significantly elevated plasma PRL concentrations at 1000 h, 21 days after the switch from short to long photoperiod. It has been reported that increases in plasma PRL concentrations, following a switch from short to long photoperiod, occur within one week but that full expression of the long day signal takes 35 to 60 days (Lincoln, 1979; Howland *et al.*, 1983; Ebling and Lincoln, 1987). In this study, progressive expression of the long day signal was most easily observed in the 24 hour samples. Little diurnal rhythm in plasma PRL concentration could be discerned immediately after the switch from short days to long days. However, by 14 days, a nocturnal peak of plasma PRL concentration was evident. The plasma PRL concentrations during and bounding the period of dark at 1700, 2300 and 0500 h were particular responsive to long day treatment. The enhancement of the diurnal cycle in plasma PRL concentration in sheep during long photoperiods has been reported previously (Lincoln *et al.*, 1978; Lincoln, 1979; Brown *et al.*, 1980; Kennaway *et al.*, 1983). This study shows similar trends but the sampling regimen used was not sufficiently intensive to warrant a detailed discussion of diurnal fluctuations in plasma PRL concentrations.

During a switch from short to long photoperiod, the injection of 100 mg of Parlodel LA successfully suppressed plasma PRL concentrations in Romney ewes. Plasma PRL concentrations were suppressed for 21 days and then increased in linear fashion. In humans, following a Parlodel LA injection, plasma BR concentration declines progressively after 5 days (Lengy *et al.*, 1993) and increases in plasma PRL concentration occur 7 days post-injection (Haase *et al.*, 1993). However, in humans, the increase in plasma PRL concentration after 28 days is only 10% of baseline plasma PRL concentration (Haase *et al.*, 1993) whereas, in the sheep in this study, plasma PRL concentration had returned completely to baseline by 30 days post-

injection. In sheep, Parlodel may be more rapidly metabolised, or the pituitary may be less responsive to BR than in humans.

There is some evidence that higher dose rates of BR are required to suppress plasma PRL concentrations during long compared to short photoperiods (Loudon and Brinklow, 1990; Curlewis *et al.*, 1991). It is possible that the rapid elevation in plasma PRL concentration 21 days after the Parlodel injection was the compounding effect of both a decrease in plasma BR concentration and a decline in lactotroph sensitivity to BR associated with the developing long day signal. To prevent increases in plasma PRL concentration in sheep under long photoperiods, Parlodel injections should be repeated at 2 or 3 weekly intervals.

In conclusion, this study established that the half-life of PRL was similar in sheep and goats and may have been affected by both mode of administration and dose rate of PRL. In sheep in long photoperiods, Parlodel proved to be effective at suppressing plasma PRL concentration for 21 days.

CHAPTER 6

THE EFFECTS OF BROMOCRYPTINE AND LONG DAYS ON SHEDDING AND FIBRE GROWTH IN DOWN-PRODUCING CASTRATE MALE GOATS DURING SPRING.

6.1 ABSTRACT

The effect on fibre growth of down goats of either delaying or advancing the spring rise plasma PRL concentration increase was studied. Goats were maintained under natural spring photoperiod and temperature and either received no further treatment (n=10, C) or were injected with long-acting bromocryptine (n=10, BR) at approximately 3 weekly intervals commencing on 18 July and ceasing on 10 October 1991. In addition, 10 goats were treated with long photoperiod (16L:8D) and 20°C from 2 August until 10 October 1991 and either received no further treatment (n=5, LD) or were injected with long-acting bromocryptine (n=5, BRLD) at three weekly intervals commencing on 2 August and ceasing on 13 September 1991. In the analysis of the data 'treatment period' was defined as being from 18 July to 24 October 1991 in BR goats and from 2 August until 10 October 1991 in LD and BRLD goats.

Mean overall plasma PRL concentration, in comparison to C goats (27 (23-31) ng/ml), was higher in LD goats (87 (69-109) ng/ml, $P<0.001$) and lower in both BR (4.2 (3.5-5.0) ng/ml, $P<0.001$) and BRLD (9 (7-12) ng/ml, $P<0.01$) goats. Plasma PRL concentration in C goats increased from 8 ng/ml to 63 ng/ml over the treatment period. BRLD goats had low (<10 ng/ml) plasma PRL concentrations up to 25 September after which concentrations increased to 52±15 ng/ml on 9 October. Mean plasma PRL concentration was maintained below 8 ng/ml and above 60 ng/ml throughout the treatment period in BR and LD goats respectively.

Mean PA was similar in both the treatment and post-treatment periods in C and BR goats. Mean PA of LD goats had increased to 76±9% by 10 October (C goats 31±5%, $P<0.0.01$; BRLD goats, 51±9%, $P<0.10$). In the post-treatment period, PA increased more rapidly in BRLD than in C goats ($P<0.01$) and as a result the mean date when all primary follicles became active in C goats was 26 December±8 days, which was similar to that of BR (16 January±8 days) goats but later than the 1 November±11 ($P<0.001$) and the 14 November±11 days ($P<0.02$) when maximum activity was achieved in LD and BRLD goats.

In comparison to C goats (15±4%), overall mean SA during the treatment period was higher in BR goats (25±4%, $P<0.05$) but which had similar values to LD (22±6%) and BRLD (29±6%) goats. Mean SA in LD goats increased to 42±9% by 12 September (C goats, 12±6% $P<0.01$; BRLD goats 9±9% $P<0.05$) and then

fell to 19±10% by 10 October. SA increased in BRLD goats throughout the treatment period reaching levels of 59±11% by 10 October (C goats 23±8%, P<0.01). On 24 October mean SA had diverged to be 77±7% in BR goats and only 19±6% in C goats (P<0.001). However by 20 December, SA in BR goats had subsequently decreased to 48±12% while SA in C goats had continued to increase to 85±11% (P<0.01). In BR goats, the early activation of secondary follicles was not associated with early summer down fleece emergence.

It is concluded that increased plasma PRL concentration provided anagenic signals to telogen secondary follicles and catagenic signals to anagen secondary follicles. The only effect of plasma PRL concentration on primary follicles, was an anagenic effect in telogen follicles.

6.2 INTRODUCTION

The fleece of the down-producing goat grows and sheds in a circannual growth cycle governed by photoperiod (Section 1.2.2.1). During short photoperiods, the 'winter' fleece of the down-producing goat has both long outer-coat guard hair and long inner-coat down fibres. In long photoperiods, the guard hair fleece is shorter and down has been assumed to be absent. However, recently it was discovered that the 'summer' fleece of down-producing goats comprises very short down fibres as well as short guard hairs (Nixon, 1991a; Chapter 2). The winter fleece sheds and summer fleece grows after the spring equinox. In spring, exposing down-producing goats to long photoperiods or continuous light induces the early shedding of the 'old' and regrowth of 'new' winter fleece but summer fleece growth was not studied (McDonald and Hoey, 1987; Kloren, 1991). In addition to changes in down growth (see Chapter 3), photoperiod also governs seasonal fluctations in plasma PRL concentration. As in many species, plasma PRL concentrations in goats are low during short, and high during long, photoperiods (Buttle, 1973; Mori *et al.*, 1985, Maeda *et al.*, 1988; Chapter 3). Intervals of rapid change in plasma PRL concentrations occur during the seasonal equinoxes (Kloren, 1991, Chapter 3). High plasma PRL concentrations in goats, which occurs at the summer solstice, are associated with both long photoperiod and high temperature (Tamininí, 1983). In goats treated with constant long

photoperiods, falling temperatures can lead to falling plasma PRL concentrations and the growth of 'winter' down (Gebbie, 1994).

In down-producing goats, the increase in plasma PRL concentration during the spring equinox is associated with shedding of the winter fleece and growth of the summer fleece (Chapter 3, Lynch and Russel, 1990; Kloren, 1991). The association between increase in plasma PRL concentration during spring and pelage transition also occur in animals such as mink and hamsters (Badura and Goldman, 1992; Martinet , 1992), wild sheep breeds (Lincoln, 1990), and deer (Loudon , 1989).

Release of PRL from the caprine *pars distalis* is primarily controlled by the PRL-inhibiting hypothalamic hormone, dopamine (Section 1.3.5). D2 dopamine receptor agonists, such as bromocryptine, suppress plasma PRL concentration in ruminants (Section 4.2) and modify fibre growth. Treatment of hamsters, foxes (Smith, 1987) and deer (Curlewis, 1988) with bromocryptine during spring delays the spring moult and subsequent development of the summer pelage. Following a switch from short to long photoperiod, the follicles of Wiltshire sheep undergo a synchronised period of catagen followed by anagen which may be prevented by bromocryptine treatment (Pearson *et al.*, 1993). However wool growth of domesticated sheep is unaffected by spring bromocryptine treatment (Curlewis, 1991).

In down-producing goats treated with bromocryptine from early spring to the summer solstice, down fibres continue to lengthen (Kloren, 1991) and shedding of the old winter fleece is delayed (Lynch and Russel, 1990a; Kloren and Norton, 1993d). Following cessation of the bromocryptine treatment, the 'winter' down fleece rapidly sheds concurrently with the initiation of a new winter down fleece (Kloren, 1991). The effects on summer fleece growth are not reported and therefore it is not clear whether the duration of summer fleece growth is shortened or absent. In Saanen goats treated with long days (20L:4D) for 8 weeks in spring, a subsequent 8 week treatment with melatonin stimulates winter fleece growth while bromocryptine

treatment is ineffective (Gebbie, 1992a). Therefore the simple relationship that exists between fibre growth and plasma PRL concentration in small mammals (Section 1.4.2) may not hold in goats, particularly under long photoperiods. It is possible that a decline in plasma PRL concentration acts in synergy with other melatonin-dependent hormones, to stimulate winter fleece growth.

Successful suppression of plasma PRL concentrations during increasing spring photoperiods and long days is essential for valid treatment interpretation. In many studies, bromocryptine treatment regimes fail to prevent increases in plasma PRL concentrations associated with long photoperiod (Curlewis, 1991; McCloughry, 1991; Parry *et al.*, 1992). The bromocryptine treatment regimes discussed below are based on the outcome of previous experiments (Chapter 4,5).

In some goats bromocryptine was used to negate the normal spring increase in plasma PRL concentration that occurs with increasing natural photoperiod. Other goats were placed under long photoperiod and high temperature in spring in order to advance spring increases in plasma PRL concentration. Half of the long photoperiod goats were treated with bromocryptine to prevent the long-day induced increase in plasma PRL concentrations.

6.3 METHOD

6.3.1 Animals

Thirty mixed-aged wether down-producing goats (mean live weight 31±1 (se) kg) were housed indoors from 5 July until 31 October 1991 and then released onto pasture at the Flock House Agricultural Centre, Bulls, New Zealand (40° 11′ S 175° 23′ E). While housed indoors, the goats were fed 250 g per goat of maize between 0800 and 0900 h daily with meadow hay and water *ad libitum*.

Following a two week acclimatization period, the goats were balanced for down length (DL) and liveweight, and randomly allocated to treatment groups. The goats were penned within treatments in groups of five goats. Goats undergoing long-photoperiod treatment were transferred to a separate building on 27 July 1991.

6.3.2 Treatment regime

Goats were maintained under natural spring photoperiod and temperature and either received no further treatment (n=10, C) or were injected with long-acting bromocryptine (Parlodel LA, Sandoz Pharmaceuticals Ltd, Auckland, New Zealand (n=10, BR) at approximately 3 weekly intervals commencing on 18 July and ceasing on 24 October 1991. In addition, goats were treated with long photoperiod (16L:8D, lights on 0400 am) and 20°C from 2 August until 10 October 1991 and either received no further treatment (n=5, LD) or were injected with long-acting bromocryptine (Parlodel LA) (n=5, BRLD), at three weekly intervals. In BR goats, long-acting bromocryptine injections were given on 18 July 1991, 7 August 1991, 28 August, 18 September and 10 October 1991 and in BRLD goats on 2 August 1991, 23 August 1991, and 13 September.

All goats were exposed to natural light but during long photoperiod treatment goats they received additional illumination using 150 watt bulbs regulated by a time switch. During long photoperiod treatment, temperature was elevated using four thermostatically controlled fan heaters.

6.3.3 Measurements

The goats were weighed on 8 July 1991, 20 August 1991, 25 October 1991, 20 February 1992 and 25 May 1992.

Fibre growth measurements were conducted at two weekly intervals from 18 July

until 22 November 1991 and thereafter at monthly intervals until 24 October 1992. Over this period stretched down and guard hair length were measured on the neck, front shoulder, midside and hind shoulder (Section 2.2.2.1) and mean individual down (DL) and guard hair length (GL) were calculated.

The amount of shedding was determined by visual scoring at each fibre measurement date. The amount of plucked fibre was scored in a shedding score (SS) from 1 (no shedding) up to 5 (large amount of shedding). The weight of fleece lost due to shedding was assessed by hand combing (Section 4.3.2). The fibre from the initial combing was discarded and goats were then combed at two weekly intervals until 22 November 1991. The fibre captured at each combing was weighed and converted to a percentage weight of fibre being shed relative to the cumulative weight shed over the whole treatment period.

Fibre growth rates (FGR) were measured at approximately monthly intervals (dependent on growth) by clipping the fleece, with Oster animal clippers, from within a 100 cm^2 measured square patch on the right midside of the goats. The fibre was weighed and the amount of fibre growing in an area of skin was calculated.

At each fibre measurement date, skin snip biopsy samples (Section 2.2.2.4) were collected and fixed in phosphate buffered 10% formalin. The samples collected up to 20 February 1992 were viewed under a dissecting microscope (Section 2.2.3.2) and scored for the presence of the intact down fibre tips as follows: 1=newly erupted down fibres (NEDF) (down fibres of less than 3 mm and characterised by an arrow-like tip) and long down (fibre in which the tip exceeds 5 mm and hence was cut); 2=NEDF only; 3=NEDF and short down (fibres 3 to 5 mm with arrow-like tip); 4=short down only; 5=short and long down; 6=long down only.

All skin samples collected up to 20 February 1992 (except those collected on 29 August, and in LD and BRLD goats on 26 September), were processed and

embedded, epidermal surface uppermost, in paraffin-polymer wax (Tissue Tek Ltd, Auckland, New Zealand). Each wax block was cut into serial 8 μm transverse sections and stained using the adapted Sacpic method (Section 2.2.3.3). Approximately 10 follicle groups containing both guard hair-producing (primary) and down-producing (secondary) follicles were scored according to the characteristics of the inner-root sheath (Nixon, 1993). The follicles were categorised into three stages of the fibre growth cycle; anagen (all follicles with actively growing fibres), telogen (fibre not growing); and active+brush (follicles containing both actively growing and inactive fibres). Primary follicle activity (PA), active+brush primary follicles (PAc+brush), secondary follicle activity (SA), and active+brush secondary follicles (SAc+brush) were calculated as a percentage of total number of either primary or secondary follicle types.

Blood samples were collected by jugular venipuncture at 1100 h at two weekly intervals (on a day adjacent to fibre measurements) until 22 November 1991 and thereafter at monthly intervals until 13 March 1992. The 5 ml blood samples were collected on ice into EDTA vacutainers. Plasma was removed by centrifugation at 3000 g at 4° C and stored at -8°C pending radioimmunoassay for PRL concentration. The radioimmunoassay of plasma PRL concentration was conducted at AgResearch Ruakura using the protocol outlined in Section 4.3.3.

6.3.4 Statistical Analysis

In the first analysis data were analysed using the GLM procedures of SAS (SAS, 1987) with repeated measures in time using the Wilks' Lambda test of significance. Pre-treatment data values were fitted as covariates into the model when appropriate (DL, GL, SS, PA, SA) and no covariates when it was inappropriate (FGR, percentage weight of shed fibre, PAc+brush, SAc+brush). Due to differences in the timing and durations of treatments, BR and C goats were compared in one analysis and C goats with BRLD and LD goats in another. In the latter analysis, treatment

contrasts were fitted to the model. Due to unequal variances, plasma prolactin concentrations were $\log_{10}$ transformed prior to analysis. Plasma prolactin concentration data presented in the text are back transformed pooled means with the standard error presented as a back transformed range. The data were analysed separately for the treatment and post-treatment periods with the exception of FGR data which were analysed as one period. The treatment period in BR goats was from 18 July to 24 October 1991 and in BRLD and LD goats from 2 August until 10 October 1991 inclusively.

Data are occasionally presented as an overall mean for a treatment group for the treatment period with attached probability values from the repeated measures analysis. Raw data are presented graphically.

In the second analysis the dates and magnitudes of minimum and maximum fibre events were determined for individual animals. The effect of treatment on these parameters was determined using general linear model procedures and in goats treatments were fitted as contrasts (SAS, 1987).

6.4 RESULTS

6.4.1 Liveweights and losses.

There was no effect of treatment on mean liveweight with treatment group mean liveweights ranging from 32 to 38 kg. On 18 September 1991, one goat from the BR group died from septicemia and was excluded from the analysis.

6.4.2 Plasma PRL concentration

In comparison to C goats (27 (23-31) ng/ml) overall mean treatment period plasma PRL concentration was higher in LD goats (87 (69-109) ng/ml, $P<0.001$) and lower

in both BR (4.2 (3.5-5.0) ng/ml, P<0.001) and BRLD (9 (7-12) ng/ml, P<0.01) goats (Figure 6.1, 6.2).

Fortnightly mean plasma PRL concentration (Figure 6.1) remained below 8 ng/ml in BR goats throughout the treatment period but in C goats it increased from 3 ng/ml on 1 August 1991 up to concentrations of more than 40 ng/ml from 11 September 1991 onwards (Trt*time, P<0.001).

LD goats had high mean plasma PRL concentrations (63-100 ng/ml) throughout the treatment period. BRLD goats had low (<10 ng/ml) plasma PRL concentrations up to 25 September increased subsequently to 52±15 ng/ml on 9 October (Figure 6.2).

In BR goats, mean plasma PRL concentration was 5 ng/ml higher (P<0.05) when samples were collected 21 days, compared to 7 and 14 days, after the Parlodel LA injections.

On 6 November 1991 post-treatment period plasma PRL concentration was suppressed (P<0.001) in BR (37 (32-43) ng/ml) compared to C goats (66 (61-70) ng/ml) but subsequently, plasma PRL concentration tended to be higher (significantly so on 19 December 1991, P<0.05) in BR goats compared to C goats (Figure 6.1). As a consequence, the pattern of post-treatment plasma PRL concentration did differ in BR compared to C goats (P<0.001). However, treatment had no effect on plasma PRL concentration in the post-treatment period in LD and BRLD goats.

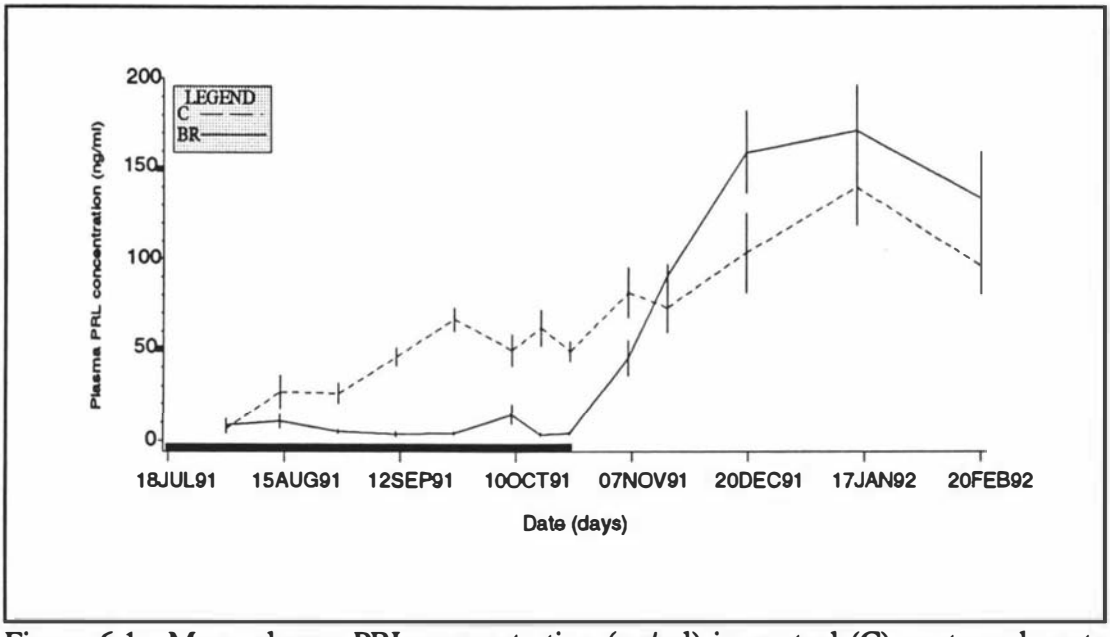

Figure 6.1: Mean plasma PRL concentration (ng/ml) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly igoatntervals. Horizontal bar is period of treatment and vertical bars are standard errors.

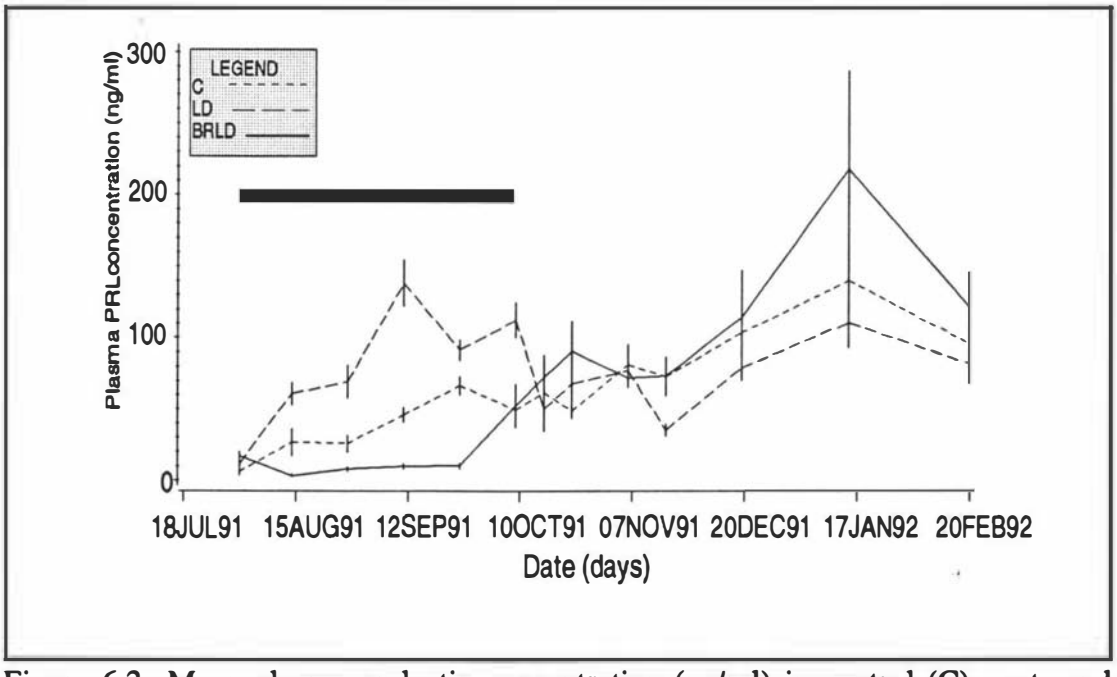

Figure 6.2 Mean plasma prolactin concentration (ng/ml) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

6.4.3 Primary Follicle Activity

Overall mean PA of BR goats was similar to that of C goats in both the treatment and post-treatment periods (Figure 6.3). However on 20 December 1991, PA was 30% lower ($P<0.05$) in BR goats than in C goats. The mean dates of achieving maximum PA in individual BR (16 January±8 days) and C (26 December±8 days) goats were similar.

During the treatment period, PA was higher ($P<0.01$) and increased more rapidly (Figure 6.4) in LD goats than C goats ($P<0.01$). By the end of the treatment period (10 October) PA of LD goats had reached 76±9% while activity levels were only 31±5% in C ($P<0.01$) and 51±9% in BRLD goats. Overall mean PA of BRLD goats (27±6%) was lower ($P<0.05$) than that of LD goats (43±6%) over the treatment period and similar to that of C goats (20±4%).

In the post-treatment period, mean PA of LD goats was 25-50% higher than that of C goats ($P<0.05$) during October and November (Figure 6.4). BRLD goats had intermediate PA values which were similar to both C and LD goats during October and early November. However by late November nearly all the primary follicles of BRLD goats were active compared with 75% in C goats ($P<0.05$). In fact the mean date when all primary follicles became active, in C goats, was 16 January±8 days, 56 ($P<0.001$) and 42 ($P<0.02$) days after maximum activity was achieved in LD and BRLD goats.

In the treatment and post-treatment periods there were similar percentages of follicles containing PAc+brush fibres in both C and BR goats (Figure 6.5) and C, LD and BRLD goats (Figure 6.6).

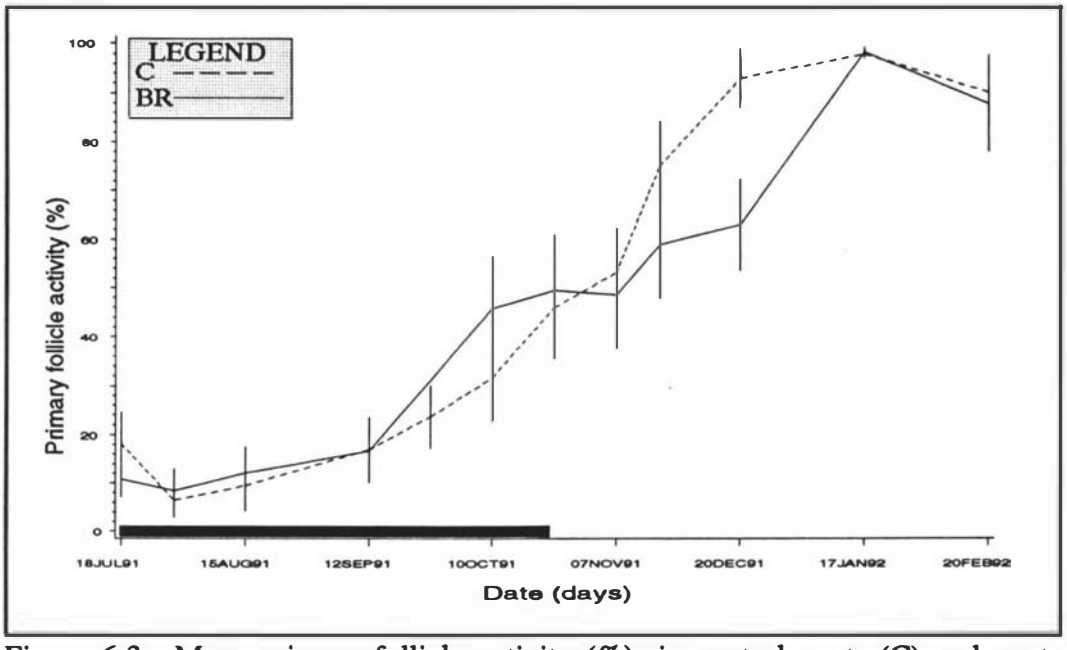

Figure 6.3: Mean primary follicle activity (%) in control goats (C) and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

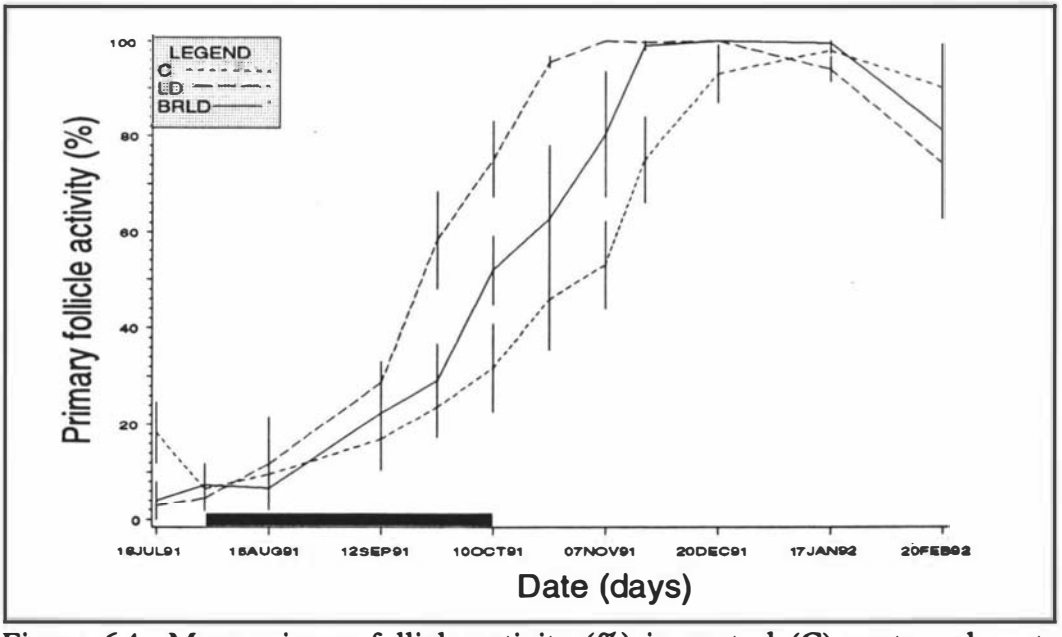

Figure 6.4 Mean primary follicle activity (%) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

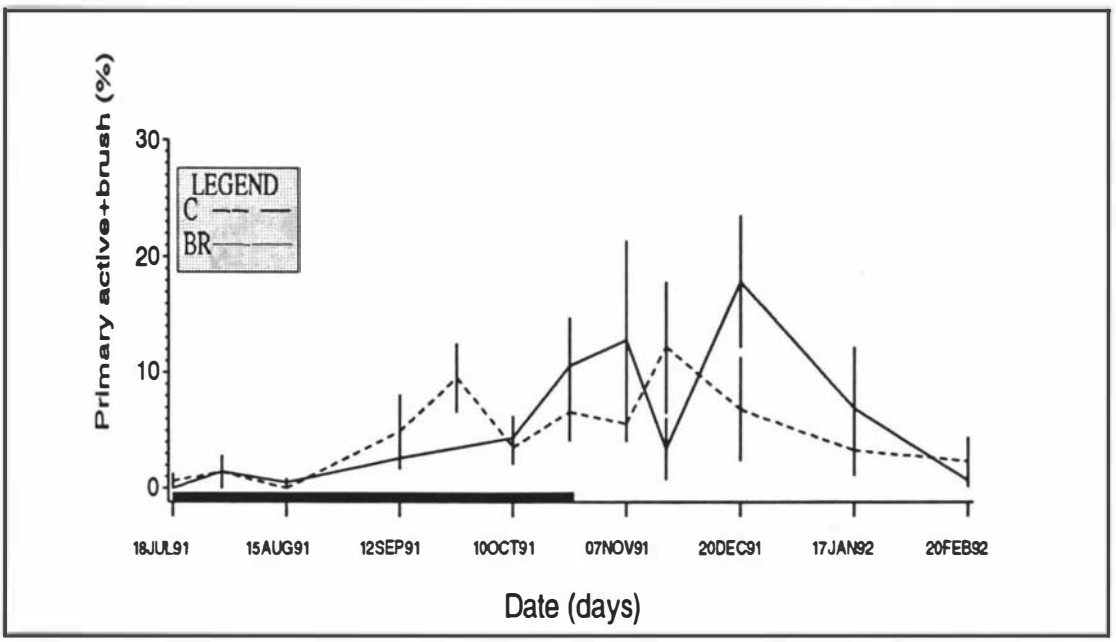

Figure 6.5: Mean proportion of active+brush primary follicles (%) in control (C) and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

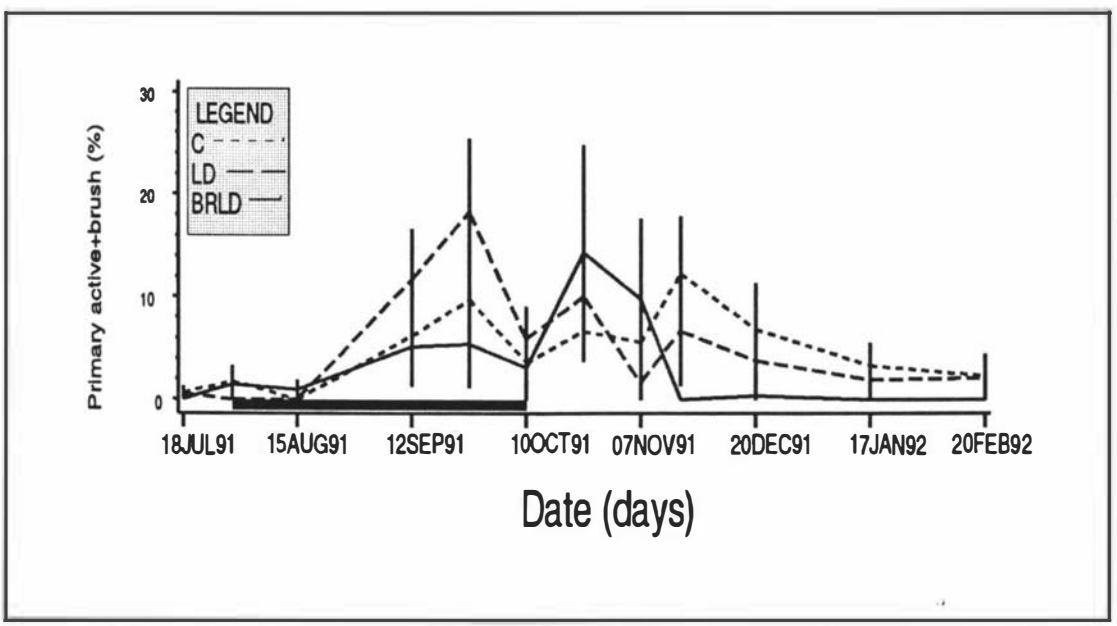

Figure 6.6 Mean proportion of active+brush primary follicles (%) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

6.4.4 Secondary Follicle Activity

In comparison to C goats (15±4%), overall mean SA during the treatment period was higher in BR goats (25±4%, P<0.05) but similar to that of LD (22±6%) and BRLD (29±6%) goats. During the treatment period, the pattern of SA differed in LD goats in comparison to C goats (P<0.05) and BRLD goats (P<0.01).

During the treatment period, mean SA in LD goats increased quickly to 42±9% by 12 September (C goats, 12±6 P<0.01; BRLD goats 9±9%, P<0.05) and then fell to 19±10% on 10 October. By 10 October, in comparison to C goats (23±8%), mean SA had increased more rapidly in BRLD (59±11%, P<0.01) and BR (48±9%, P<0.05) goats (Figure 6.7, 6.8). SA continued to increase in BR goats, in comparison to C goats, up to 7 November when mean SA had reached 71±7% and 25±6% in BR and C goats respectively (P<0.001).

By 24 October (Figure 6.8) mean SA had increased in LD (43±14%) goats, remained static in C (29±10%) goats and decreased in BRLD (30±14%) goats (Figure 6.8). Mean SA also declined after the cessation of treatment in BR goats reaching 41±12% on 20 December (C goats 85±11%, P<0.01).

During November, SA was 25-47% higher (P<0.01) in LD and BRLD goats than in C goats.

Overall, the date of attaining full activity in secondary follicles of individual goats, in comparison to C goats (26 December±6 days), was earlier in LD (4 November±6 days, P<0.01) and BRLD (17 November±9 days, P<0.01) but delayed in BR goats (22 January±7 days, P<0.01).

There was no effect of treatment on SAc+brush follicles in either the treatment or post-treatment periods (Figure 6.9, 6.10). In individual goats, multiple peaks in the

percentage of SAc+brush follicles (Figure A6.3) could be identified. BR goats had a mean of 2.4±0.1 peaks compared to C goats with 1.9±0.1 peaks (P<0.01). Peaks in mean SA occurred in conjunction with the emergence of NEDF's and these peaks averaged 79±9%, 75±9% and 63±9% in BR, BRLD and LD goats respectively but only 38±7% in C goats (P<0.001).

6.4.5 Fibre measurements

6.4.5.1 Guard hair length: Treatment had no effect on mean GL in either the treatment or post-treatment periods (Figure 6.11, 6.12). On individual days of measurement, in comparison to C goats, mean GL of LD was more than 10 mm less (P<0.05) from 24 October until 22 November (Figure 6.12). Mean GL of both LD and C goats was 8 mm less (P<0.05) than BRLD goats from 7 November 1991 until 17 January 1992.

6.4.5.2 Down length: During the treatment period overall mean DL was unaffected by treatment (Figure 6.13). In comparison to C goats, mean DL was 7 mm less in BR goats on 30 July 1991 (P<0.05) and 24 mm less in LD goats on 26 September (P<0.05). During the post-treatment period, overall mean DL was unaffected by treatment though the pattern of change in DL differed (Figure 6.14) in LD and BRLD goats in comparison to C goats (P<0.05). Mean DL of BRLD goats was more than 30 mm greater than that of LD during October (P<0.05). However, during November, DL of BRLD goats reduced rapidly and for the remainder of the post-treatment period was similar to that of LD goats. In comparison to C goats, mean DL was more than 20 mm greater in LD and BRLD goats from January to April 1992 (P<0.01) and 10 mm less in BR goats from April 1992 to 12 September 1992(P<0.01).

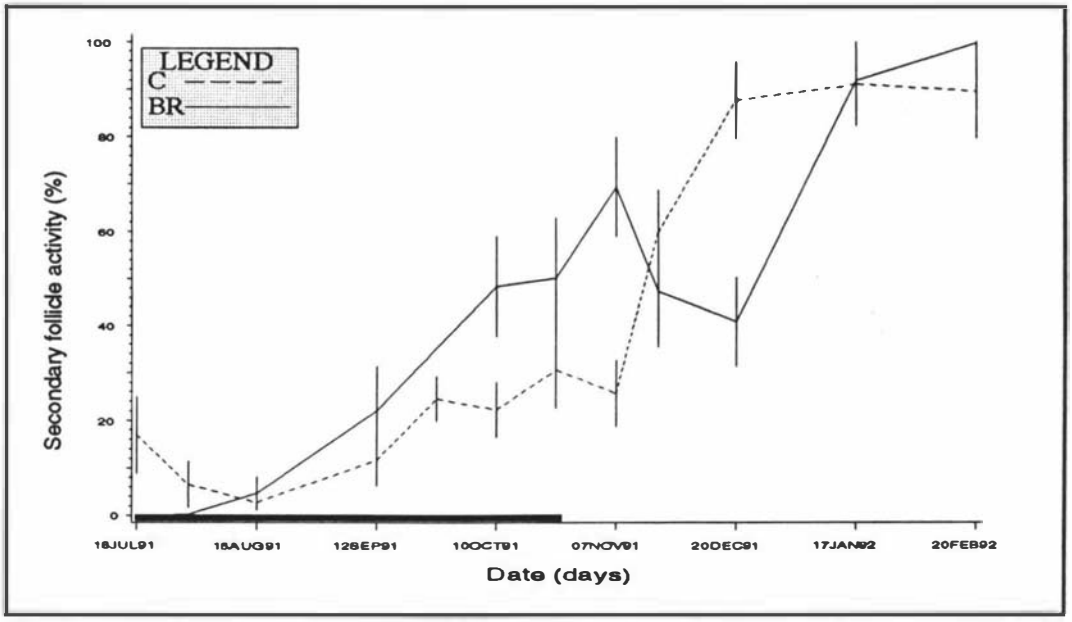

Figure 6.7: Mean secondary follicle activity (%) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

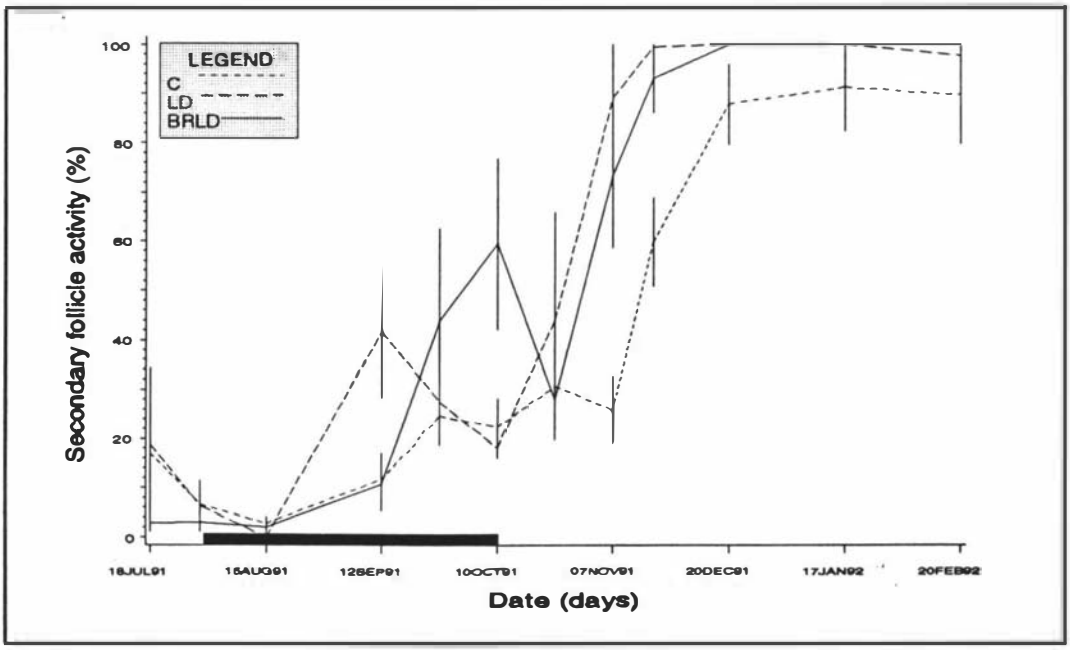

Figure 6.8 Mean secondary follicle activity (%) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

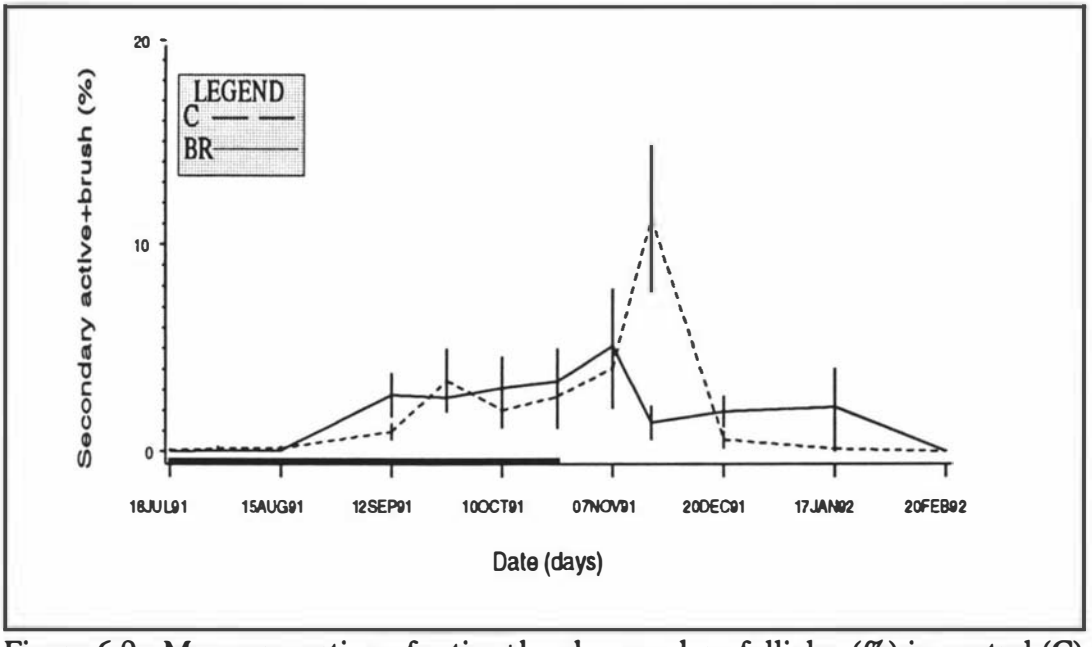

Figure 6.9: Mean proportion of active+brush secondary follicles (%) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

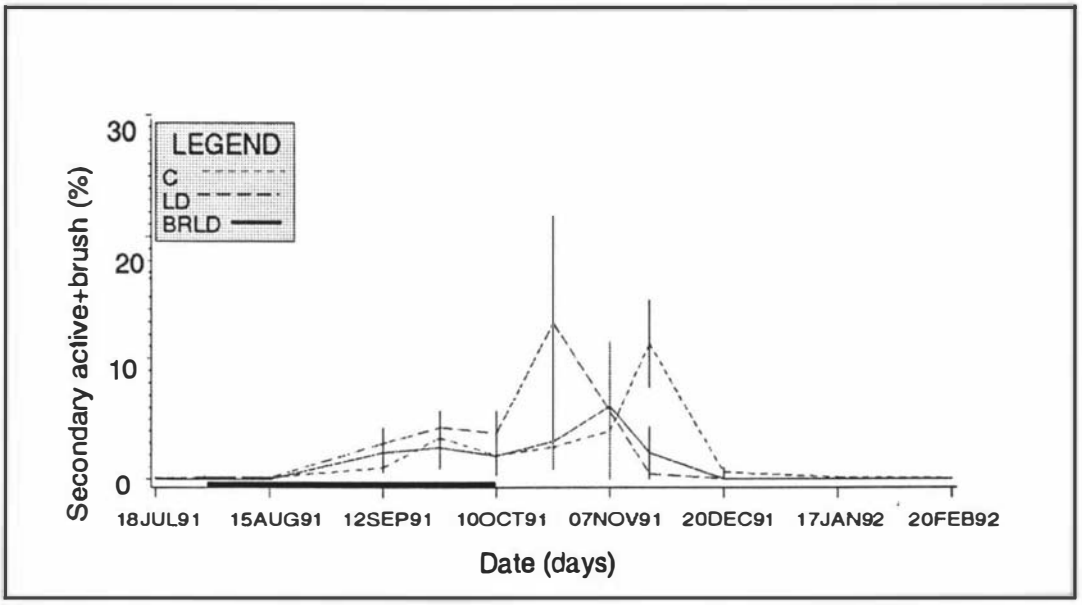

Figure 6.10 Mean proportion of active+brush secondary follicles (%) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

In individual animals, DL fell to minimum levels at a similar date in both BR (4 December±10 d) and C (21 November±10 days) goats and BRLD goats (13 November±10 days), but minimum DL was reached earlier in LD goats (28 September±10 days, ($P<0.001$). However, DL began to increase earlier in individual LD and BRLD goats (14 December±10 days, ($P<0.01$) than in C or BR goats (16 January±7 days).

***6.4.5.3 Fibre growth rate*:** Overall mean FGR was similar in C (0.21±mg/cm^2/day), BR (0.23 mg/cm^2/day), LD (0.23 mg/cm^2/day) and LDBR (0.23 mg/cm^2/day) goats (Figures 6.15, 6.16). During December 1991 ($P<0.01$) and January 1992 ($P<0.05$) FGR was reduced by more than 58% in BR compared to C goats. In comparison to C goats (0.11±0.02 mg/cm^2/day) overall mean FGR from July to December was higher in LD (0.25±0.02 mg/cm^2/day) and BRLD (0.20±0.03 mg/cm^2/day) goats ($P<0.05$). Over this period FGR increased more rapidly ($P<0.05$) in LD and BRLD goats compared to the C goats (Figure 6.16).

6.4.5.4 Shedding: Both SS (Figure 6.17, 6.18) and weight of the combed fibre (Figure 6.19, 6.20) were similar during the treatment period in C, BR, LD and BRLD goats. On 12 September SS tended to be higher ($P<0.10$) in LD compared to C goats while the weight of combed fibre was higher in LD (29±7%) than C (11±5%, $P<0.05$) and BRLD goats (4±7%, $P<0.05$). In the post-treatment period, overall mean SS was similar in C (1.49±0.06), BR (1.4±0.1) and BRLD (1.8±0.1) goats but was lower in LD (1.13±0.08) goats. The pattern in weight of combed fibre over the post-treatment period differed in BRLD goats in comparison to LD and C goats.

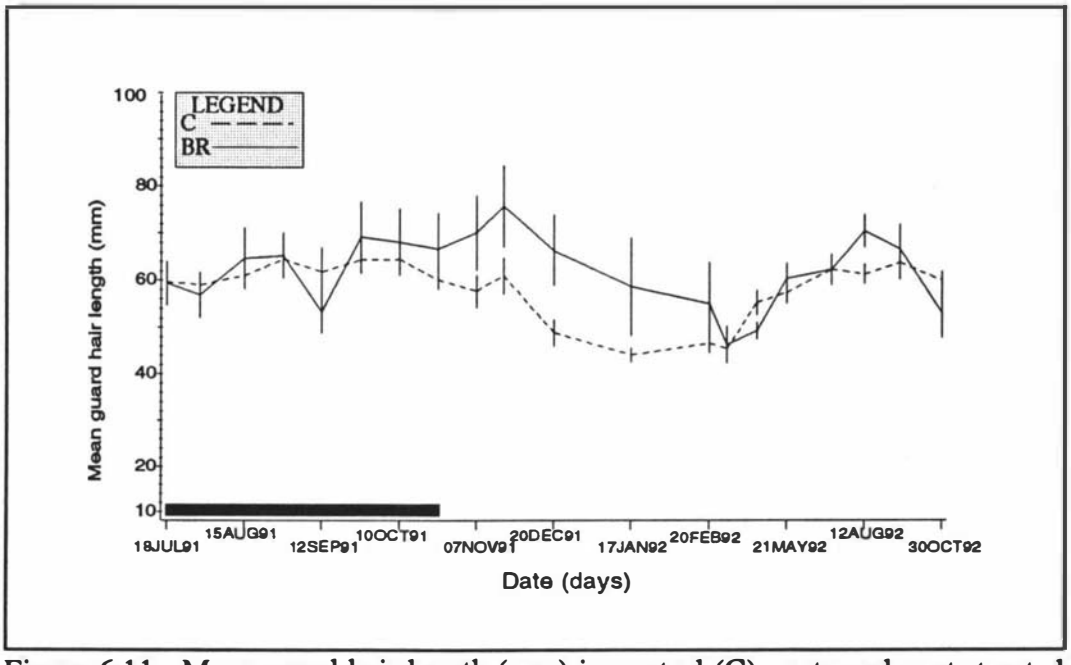

Figure 6.11: Mean guard hair length (mm) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

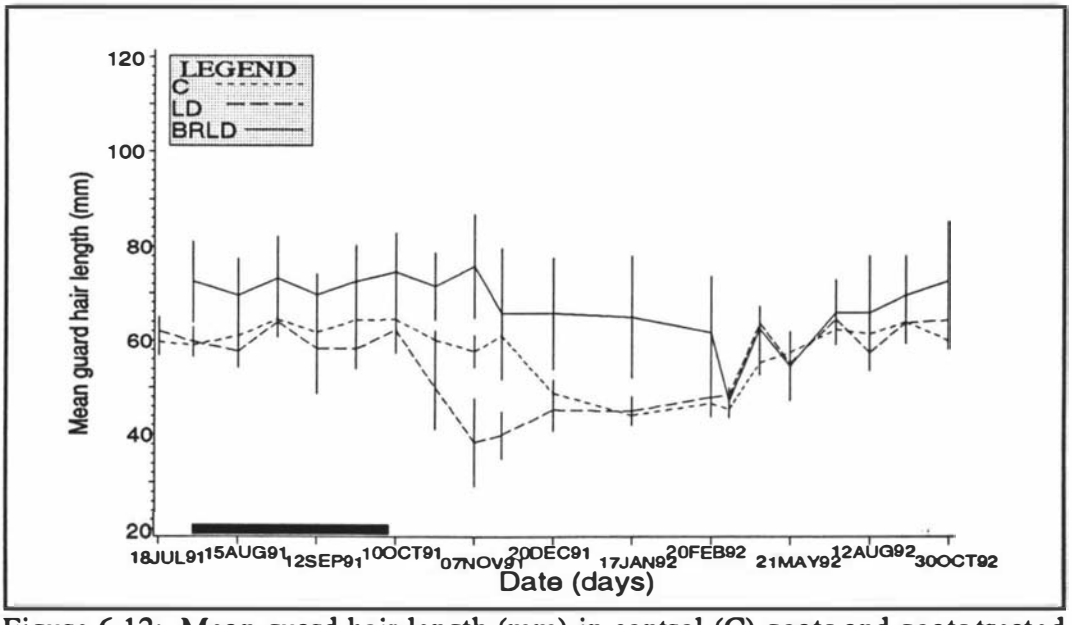

Figure 6.12: Mean guard hair length (mm) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

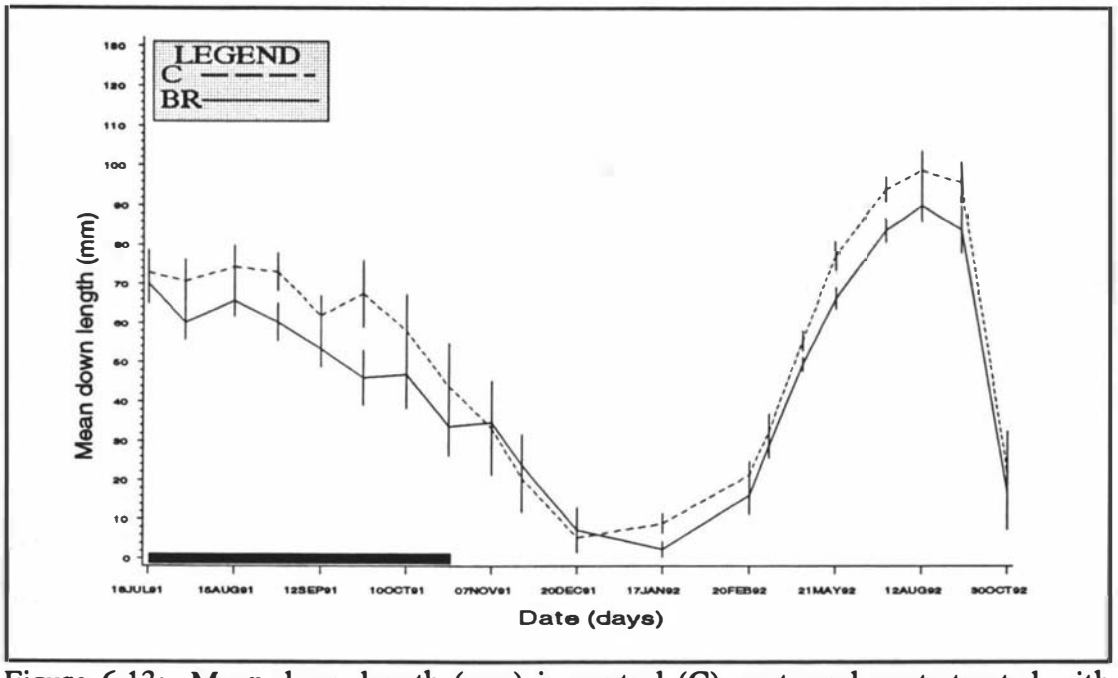

Figure 6.13: Mean down length (mm) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

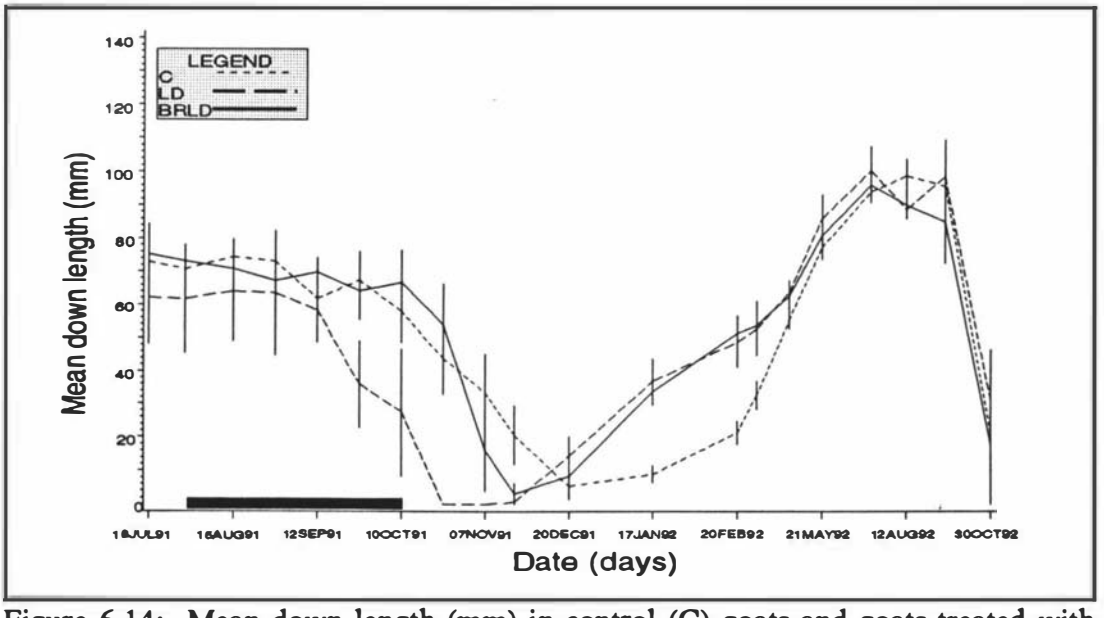

Figure 6.14: Mean down length (mm) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

In BRLD goats most shedding was detected at the 7 November measurement when the percentage weight of shed fibre and SS respectively were higher in BRLD (33±5%, 3.5±0.6) than in C (11±4, 2.0±0.3, both P<0.05) or LD (10±6, 1.0±0.4 both P<0.01) goats. In LD goats shedding had largely ceased by 24 October and therefore the overall mean SS from 24 October until 21 May 1992, was lower in LD (1.1±0.1) than in either BRLD (1.8±0.2, P<0.01) or C (1.56±0.09, P<0.001) goats.
In individual goats the date of maximum percentage weight of combed fibre of C goats (21 September±9 days) was similar to that of BRLD gpats (27 October±10 days) goats but later than that of both BR (28 August±10 days, P<0.10) and LD (14 August±10 days, P<0.01) goats.

6.4.5.5 Newly emerged down fibres:: Overall, goat treatment groups had similar NEDF scores during the treatment period (Figure 6.21. 6.22). However by 26 September 1991, there was a trend for a greater incidence of NEDF's in LD (2±1) goats compared to C (4.6±0.7, P<0.10) and BRLD (5±1, P<0.10) goats. During October, NEDF score continued to remain 2-3 units lower in LD goats compared to BRLD goats (P<0.05).

In the post-treatment period, the pattern of NEDF score was divergent in BRLD and C goats (P<0.01) and overall NEDF score of C goats was lower than that of BR (P<0.10) and BRLD goats (P<0.05). In BR goats fewer samples contained NEDF's on 24 October and 7 November when mean NEDF score of C goats was 2.0 (P<0.05) and 2.1 (P<0.05) units lower than in BR goats while on 20 December, more NEDF's were present in C (4.8±0.3) goats than in either LD or BRLD (both 6.0±0.4, P<0.05) goats.

In individual goats, the mean date of appearance of NEDF's in BR (13 November ±14 days) and BRLD goats (21 October±16 days) days was similar to that of C goats (18 October±12 days) but it tended to be earlier in LD goats (17 September±16 days, P<0.10).

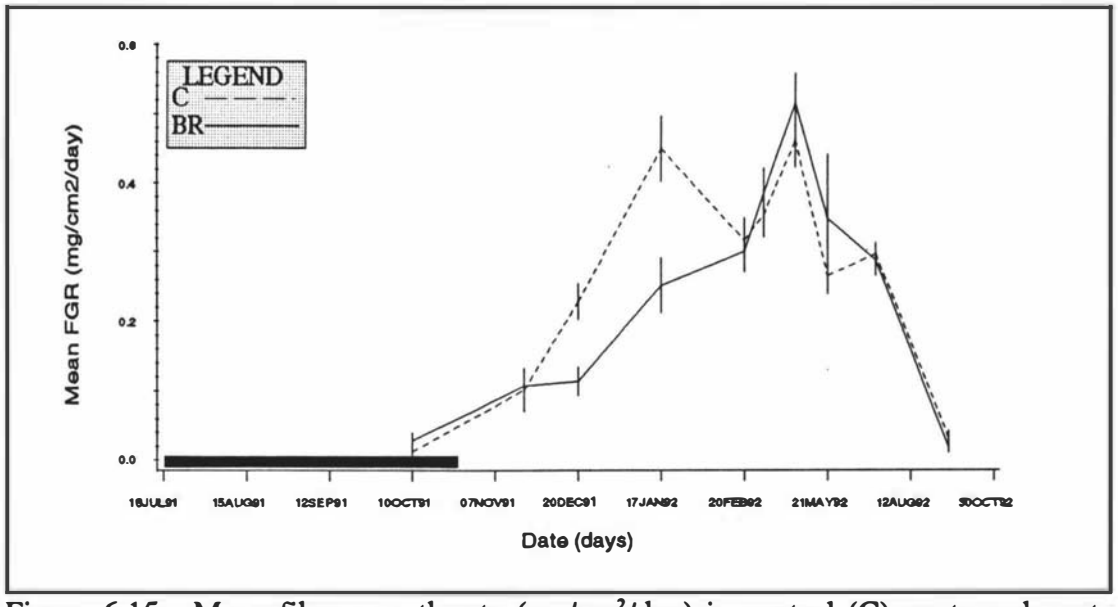

Figure 6.15: Mean fibre growth rate (mg/cm^2/day) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

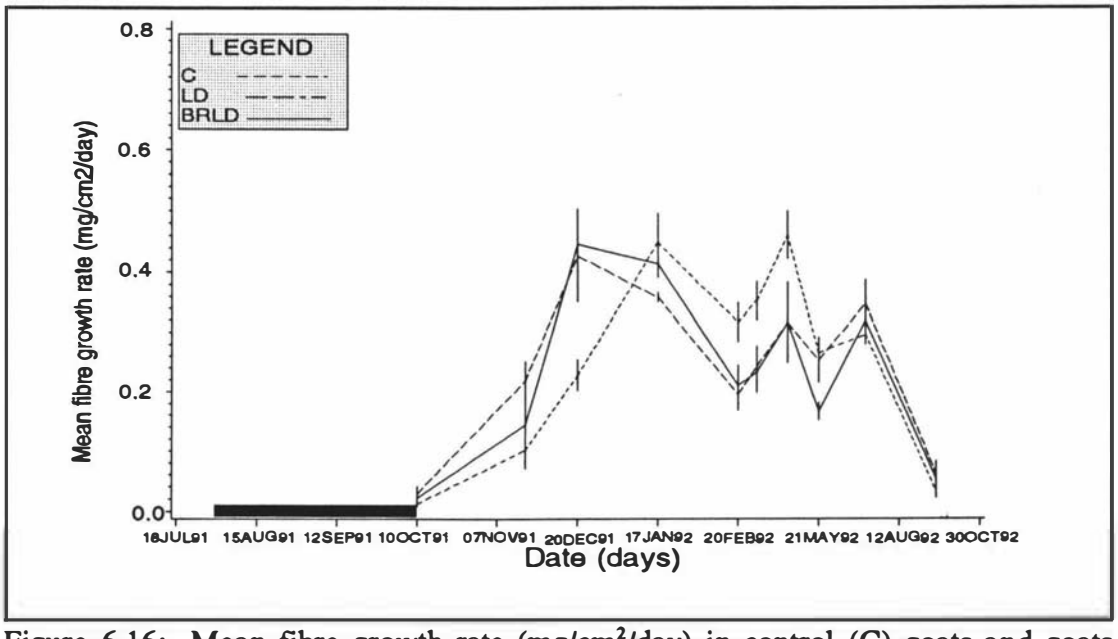

Figure 6.16: Mean fibre growth rate (mg/cm^2/day) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

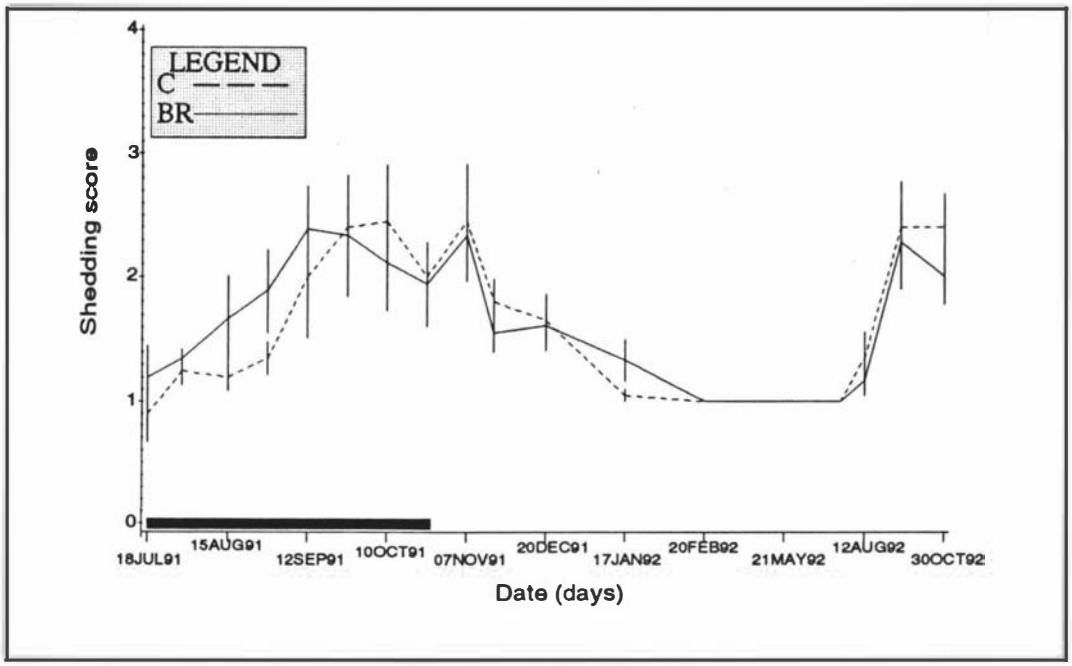

Figure 6.17: Mean shedding score (1=no shedding) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

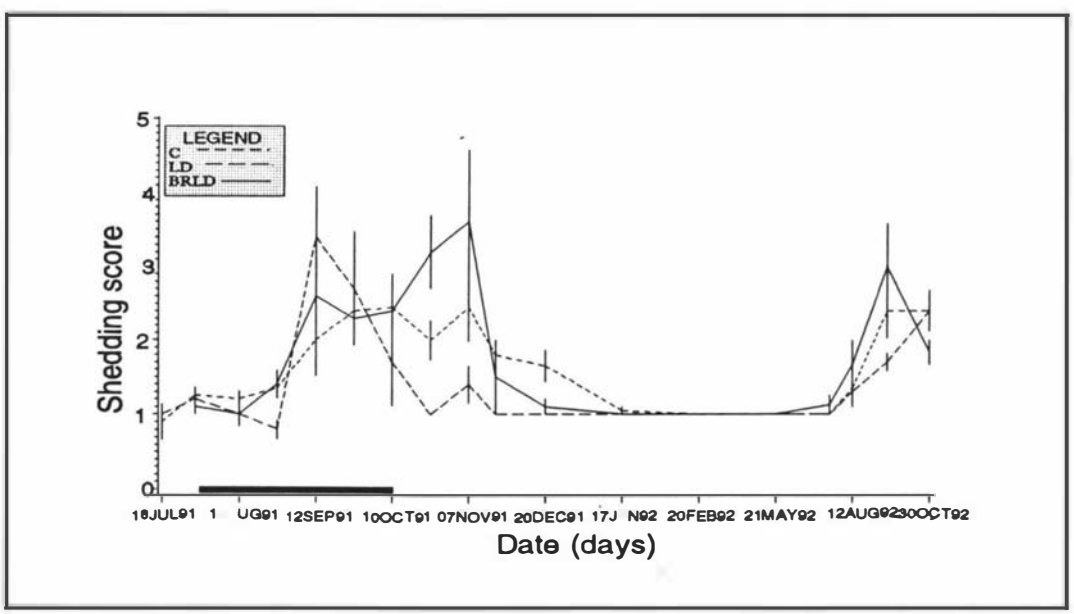

Figure 6.18: Mean shedding score in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

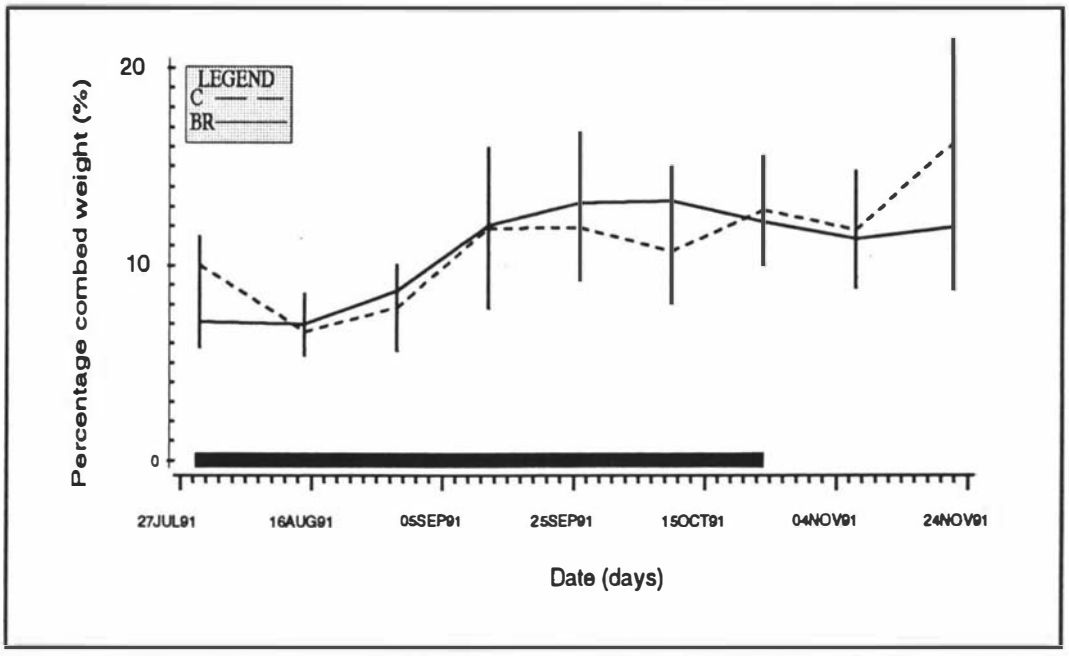

Figure 6.19 Percentage weight of combed fibre (%) in control (C) goats and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

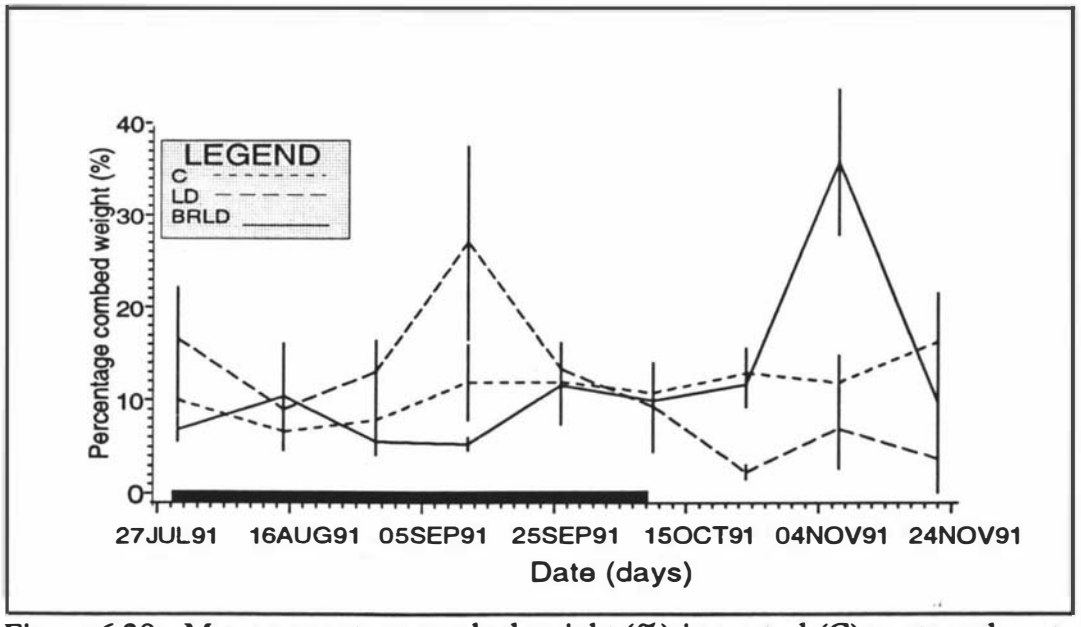

Figure 6.20: Mean percentage combed weight (%) in control (C) goats and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

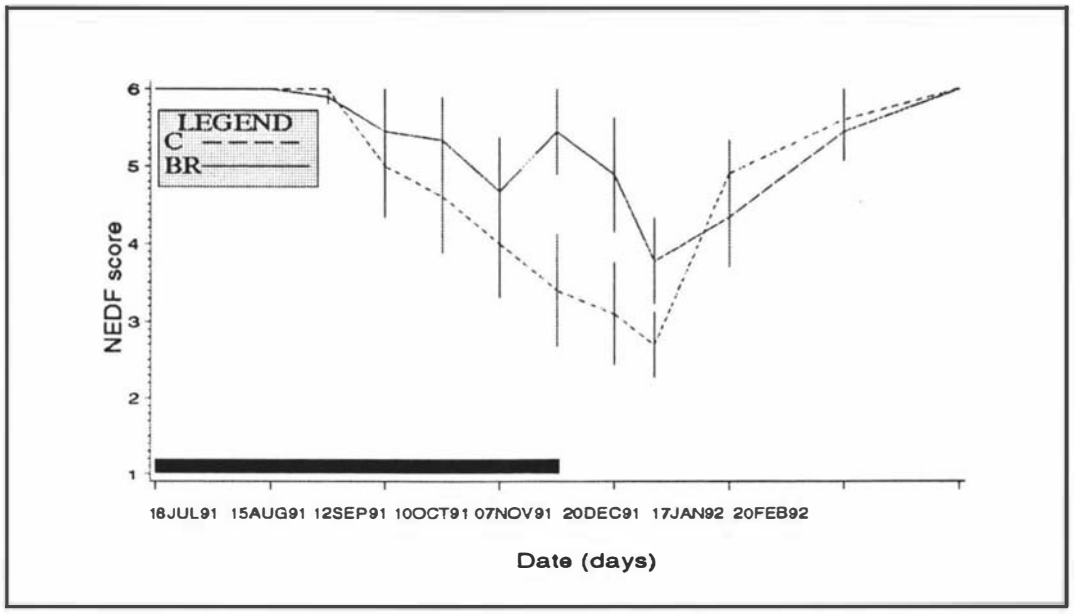

Figure 6.21: Mean guard hair length (mm) in control goats (C) and goats treated with long-acting bromocryptine (BR) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

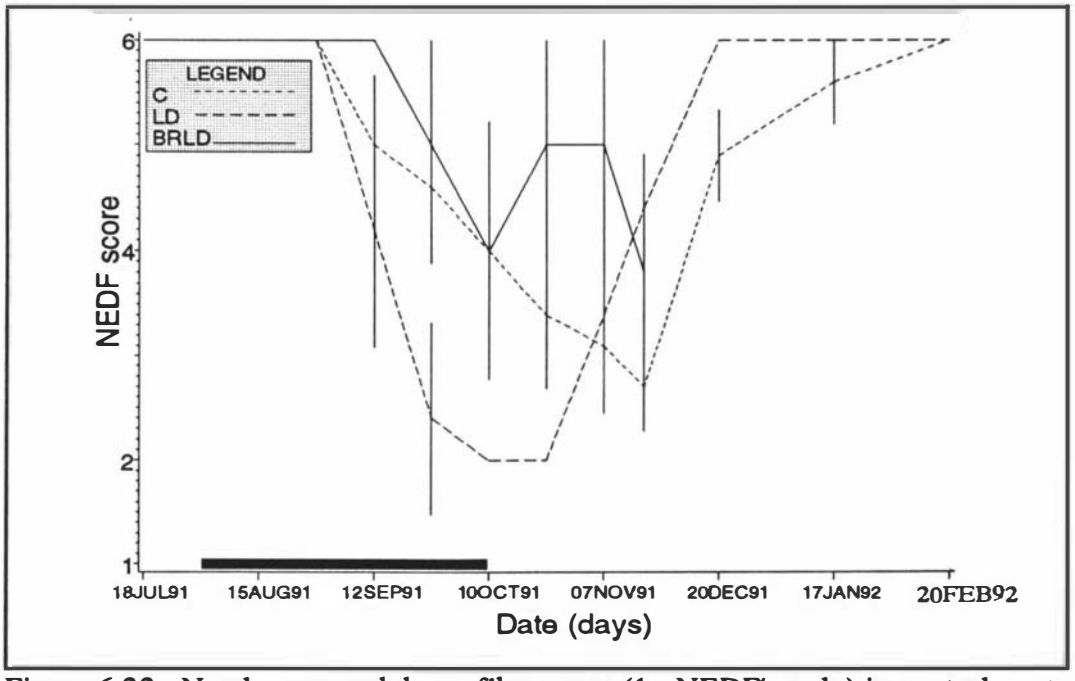

Figure 6.22: Newly emerged down fibre score (1= NEDF's only) in control goats (C) and goats treated with long photoperiod only (LD) or long photoperiod and long-acting bromocryptine (BRLD) at three weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

The mean date of disappearance of NEDF's in individual goats was similar in both BR and C (23 December±8 days) but earlier in LD goats (21 November±11 days, $P<0.05$) and BRLD goats (30 November±12 days, $P<0.05$). The net effect on the duration of presence of NEDF's was a reduction in duration in BR (39±7 days, $P<0.05$) and BRLD (40±12 days, $P<0.10$) goats in comparison to C goats (66±7 days, $P<0.05$) which had a similar duration to that of LD goats (65±10 days).

6.5 DISCUSSION

6.5.1 Plasma PRL concentrations

In this and other studies, down-producing goats in winter photoperiod and low temperatures had immunoactive plasma PRL concentrations below 25 ng/ml. Concentrations of plasma PRL increase as the spring equinox approached (Buttle, 1973; Mudulli, 1979; Mori, 1985; Prandi, 1988; Tamanini, 1988; Kloren, 1991; Grasselli, 1992; Dicks, 1994; Chapter 3). In this experiment, treatment with long-acting bromocryptine during spring successfully suppressed plasma PRL concentration to less than 8 ng/ml for 14 weeks until 6 November.

During the summer solstice, plasma PRL concentrations in goats climb to values in high levels (Buttle, 1973; Maeda, 1986; Tamanini, 1988; Prandi, 1988; Kloren, 1991; Grasselli, 1992; Dicks, 1994). In this experiment, after two weeks of long photoperiod (16L:8D) and 20°C treatment, plasma PRL concentrations had increased above 50 ng/ml. Following 6 weeks of treatment, plasma PRL concentrations were greater than 80 ng/ml and remained high until the end of the 10 week treatment period. Long-acting bromocryptine suppressed the increase in plasma PRL concentration induced by long photoperiod and high temperature to less than 10±2 ng/ml for 8 weeks. However by the tenth week, plasma PRL concentration in BRLD goats had increased to 52±15 ng/ml. An interval of 27 days elapsed between the last Parlodel injection and cessation of the long photoperiod treatment.

In sheep treated with long days, plasma PRL concentration began to increase 21 days after a Parlodel LA injection (Chapter 5). The rise in plasma PRL concentration in BRLD goats, at the end of the experiment, was significant and complicates the interpretation of post-treatment effects on fibre growth. Plasma PRL concentration was only suppressed below 10 ng/ml for the whole 10 weeks in BRLD goat number 14.

The goats were released on to pasture and natural photoperiod on 31 October. This introduced a confounding factor in the analysis of the post-treatment period. Greater illumination levels (Sugawara *et al.*, 1989), higher temperature (Tamanini, 1988) and improved nutrition (Kloren, 1991) can all increase plasma PRL concentrations and may have affected fibre growth. Liveweight increased from 34 to 37 kg from 24 October until 25 May and this above maintenance feeding level may have increased the growth of guard hair. However down growth in low-producing goats is less responsive than guard hair growth to improved nutrition (Ash, 1986; McCall and Fitzgerald, 1987).

6.5.3. Primary follicle activity

In agreement with Kloren (1991) the ablation of the normal spring increase in plasma PRL concentration had little effect on fibre growth from primary follicles. There was a tendency in BR goats, for the guard hair fleece to be longer during November indicating that bromocryptine treatment may have increased length growth rate or delayed the acquisition of the summer guard hair fleece. A similar trend was observed by Kloren (1991) but the effects of bromocryptine treatment on primary follicle growth, in comparison to secondary follicle growth, were small. In contrast, in the study of Dicks (1994), delaying the normal spring increase in plasma PRL concentration using bromocryptine delayed equally the activation of both secondary and primary telogen follicles (Dicks, 1994). In addition, telogen primary follicles were activated by spring melatonin treatments which also suppressed plasma PRL

concentrations (Nixon *et al.*, 1991).

In the post-treatment period, observation of the individual PA changes (Figure A6.4), showed that only 5 out of 9 BR goats had a reduction in PA following the cessation of bromocryptine treatment. However, unlike SA, this decline did not achieve significance as it was not common to all goats or synchronised in time. The catagen phase may also be shorter in the large primary follicles and therefore not sampled effectively.

Primary follicles were activated at a faster rate under long photoperiod and activation was delayed by bromocryptine treatment. However, unlike secondary follicles, the activation of primary follicles was not followed by a period of declining activity. A similar uninterrupted early activation of primary follicles of down goats was observed following a systemic infusion of PRL (Dicks, 1994). Wiltshire sheep were treated with long photoperiod in the same facilities as goats in the present study from 25 July to 22 October 1991 (Craven *et al.*, 1994). In these sheep follicle activation was suppressed by long photoperiod. However as in this study, follicles were rapidly activated following the cessation of long photoperiod treatment. Shedding species appear to differ in their response to photoperiodic treatments and changes in plasma PRL concentration.

Following the cessation of the bromocryptine and long day photoperiod treatment, the PA increased at a greater rate in BRLD goats than in C goats, and both LD and BRLD goats reached maximum PA levels 1 month earlier than in C goats. The early attainment of maximal PA in LD and BRLD, in comparison to C, goats contributed to the doubling of the fleece growth rate (of which guard hair forms the greater proportion by weight) in the post-treatment period. However, this early activation in BRLD goats may have been in response to the increase in plasma PRL concentrations which occurred during the last two weeks of the treatment period. Some support is given to this theory because the only goat not to show this increase

in plasma PRL concentration also did not show early activation of primary follicles.

6.5.3 Secondary follicle growth

A 'subsidiary' cycle of SA during spring was first identified 24 years ago in Scottish feral goats (Ryder, 1970) but the conclusion that this cycle produced a 'vellus' cycle of down growth was only made recently (Nixon, 1991). Summer down growth in natural photoperiods was found to be associated with mean peaks in SA of approximately 30% (Nixon, 1991; Chapter 2, Chapter 3). This low level of peak SA suggested either that only some follicles undergo this cycle (hence the called 'subsidiary') or that all follicles undergo a short anagen period. Manipulation of plasma PRL concentration with either bromocryptine or long photoperiod synchronised this 'subsidiary' down growth cycle. Spring peaks in mean SA which were associated with NEDF emergence were in excess of 60% in LD, BRLD and BR goats compared with 38±7% in C goats. It is highly likely therefore that most secondary follicles (if not all) underwent a short period of down growth in spring which produced a down fleece with characteristics distinct from that of the winter fleece. Small mammals also produce summer and winter fleeces with different characteristics (Smale *et al.*, 1988). This down fleece should be termed a 'summer' down fleece. It is a deficiency of many endocrinological studies on fibre growth in down-producing goats, that the summer down fleece was not studied (Kloren, 1991; Dicks, 1994). However it is not known whether the summer down fleece exists in down goats outside Australasia.

The suppression of the spring rise in plasma PRL concentration in BR goats did not prevent fleece shedding and, at the follicle level, SA in fact increased during bromocryptine treatment. This increase in SA was observed in goats treated with bromocryptine both in natural and long photoperiods but was not associated with early summer fleece emergence.

It can be assumed that periods of new growth in the follicle are associated with an increase in the proportion follicles containing both active and brush-end fibres. In C goats, two peaks in SAc+brush (Figure A6.3) could be identified in 9 out of 10 goats (1 goat had 1 peak). The mean date of the first peak was 12 September ± 10 days and was associated with summer down emergance and the second was on 9 November ± 9 days and was associated with the onset of winter down growth. In BR goats which possessed 3 peaks in SAc+brush follicles the mean date of the initial peak was 21 August and bore no relationship to the date of summer fleece emergence while the remaining peaks occurred on 27 September ± 12 days and 5 December ± 10 days and were related to summer and winter down growth respectively.

In Chapter 3, increases in levels of SAct+brush and mean SA during spring were normally associated with summer down emergence. However, the early activation of secondary follicles following bromocryptine treatment failed to advance the development of the summer down fleece. Summer fleece emergence was similar in C and BRLD goats and even had a tendency to be delayed in BR goats. It can be speculated that the down grown during bromocryptine treatment grew longer than 5 mm and therefore was not recognised. Early growth of 'long' down has been universally observed following spring-treatment of down goats with continuous melatonin implants (Section 1.2.2.1). Interestingly, a melatonin treatment of down goats in spring was also associated with small decreases in plasma PRL concentration (Nixon *et al.*, 1993). It has been postulated in goats (Nixon *et al.*, 1993) and Wiltshires (Pearson *et al.*, 1993) that small natural increases in plasma PRL concentrations serve to inhibit follicle activation and as bromocryptine removes this inhibition secondary follicles become activated. It is also possible that induced in plasma PRL concentration simulate naturally declining plasma PRL concentrations characteristic of the post-summer solstice period. This is less likely as winter down growth in this experiment and others (Kloren 1991; Chapter 2, Chapter 3) commenced in down goats during times of high plasma PRL concentrations.

It is proposed that the new down in bromocryptine-treated goats did not emerge beyond the old 'winter' down fleece before secondary follicles entered catagen. Catagen (as measured by declining SA) occurred following the cessation of the bromocryptine treatment when plasma PRL concentration increased rapidly. In melatonin-treated goats, the exhaustion of the melatonin treatment was also associated with increasing plasma PRL concentrations (Parry *et al.*, 1992) and eventual shedding of 'long' down and followed by a rapid initiation of new 'long' down growth (Litherland *et al.*, 1990; Parry *et al.*, 1990). The association between large increases in plasma PRL concentrations and the onset of catagen in previously active Wiltshire follicles has been well documented following photoperiod and bromocryptine manipulation (Parry *et al.*, 1992; Pearson *et al.*, 1993; Pearson *et al.*, 1994). A rapid onset of shedding in December and simultaneous new down growth was also observed in down-producing goats following cessation of four months of bromocryptine treatment (Kloren, 1991). It can be speculated that the short (< 5 mm) DL of the summer down fleece resulted from the catagen-inducing rise in plasma PRL concentration which occurred shortly after spring down growth was initiated. This theory is supported by the 20 day reduction in the duration of summer down growth in bromocryptine-treated goats. However, Dicks (1994) failed to observe a catagenic effect of high plasma PRL concentration on secondary follicles when PRL was infused into the systemic circulation of goats.

Various studies have involved treatment of down goats with bromocryptine during spring. In these experiments responses during the period of treatment are unlike those in this experiment. A study in which down goats were treated with bromocryptine from 5 July to 26 October (the Southern hemisphere equivalent), delayed the shedding of the winter down coat (Lynch-Dicks, 1994) and delayed spring increases of both SA and PA. However, plasma PRL concentrations were not suppressed for the whole 17 weeks of treatment (Lynch-Dicks,1994). In fact, bromocryptine treatment served only to suppress the magnitude of the spring increase in plasma PRL concentration. Therefore it was not surprising that loss of the winter down coat and summer follicle

activation were delayed rather than initiating the early activation of secondary follicles as observed in this experiment.

In the Australian study of Kloren (1991), plasma prolactin concentration was lowered by bromocryptine treatment from 4 August to 21 December. DL in these bromocryptine-treated goats increased for the duration of the treatment period. This is contrary to results from this present study, where mean DL declined in both BR and C goats. However the goats in the Australian study and in this experiment were at different stages of secondary growth. In this experiment, SA was very low and DL static while in the Australian down goats of Kloren (1991) mean DL was increasing at the start of the treatment and continued to increase in control goats until September. Down lengths in the majority of Australian and New Zealand cashmere-producing goats decline from August (McDonald *et al.,* 1987). In the present experiment, the increase in the secondary follicle growth cycle of C goats occurred approximately one month later than that previously reported for New Zealand goats of similar genetic background but grazing outdoors on pasture (Chapter 2). It has been reported that lighting regimes and temperature fluctuations (Tamanini *et al.*, 1988; Grasselli *et al.*, 1992) fluctuations can affect plasma PRL concentrations and these may differ in housed compared to outdoor goats. One male goat in the study of Kloren (1992) had reached static DL at the start of treatment and in this goat DL remained constant until the cessation of the treatment.

To date there has been very little published evidence of high plasma PRL concentrations affecting anagen. However, the incorporation of PRL into the medium of cultured follicles of goats (Ibraheem, 1993; Galbraith, 1994) and deer (Thomas, 1993) did increase fibre length growth rates. In this experiment, the elevated plasma PRL concentration, induced by long photoperiod, advanced summer fleece emergence and shedding of the old winter fleece by a month. This was negated by bromocryptine treatment. During treatment with long-photoperiod, telogen secondary follicles were initially activated and then subsequently underwent a period of catagen. In another study when PRL was infused into the systemic circulation of down goats

from 5 July to 24 August (Southern hemisphere equivalent dates) there was a rapid loss of winter down from the fleece while SA was increased in previously inactive follicles (Dicks, 1994).

In the post-treatment period, the summer fleece disappeared and new winter 'down' growth was initiated one month earlier in both LD and BRLD goats in comparison to C goats. It is possible that an as yet unidentified factor associated with long photoperiod, and unaffected by bromocryptine treatment, resulted in the early initiation of winter down growth. In down goats, the relationship between down growth and plasma concentrations of thyroxine, growth hormone and insulin has been studied but to date no association has been found (Kloren, 1991). However, plasma PRL concentration in BRLD goats was not successfully controlled for the last two weeks of treatment when concentrations increased from 11±2 to 52±15 ng/ml goats. Only goat 14 of LDBR treatment group had plasma PRL concentrations suppressed below 5.5 ng/ml for the duration of the treatment period. Secondary fibre growth of goat 14 behaved differently to that of its companions in that secondary follicles remained in telogen and DL was static during the treatment period. In goat 14, a summer down growth phase was not identified, and winter down growth reached maximum levels on 20 December (compared to 26 December±6 in C goats, 14 November for other 4 BRLD goats). Therefore it appears likely that the 40 ng/ml increase in plasma PRL concentration, over a 2 week period, at the end of the treatment period in 4 out of 5 BRLD goats was sufficient to stimulate summer fleece growth and early winter fleece regrowth. In Chapter 4 it was shown that, during spring, a 2 week period of manipulation of plasma PRL concentration, using domperidone, was sufficient to perturb down growth.

6.6 CONCLUSIONS

The studies in this Chapter showed that primary and secondary follicles responded differently to the prevention and advancement of elevations in spring plasma PRL concentrations. Increased plasma PRL concentration provided anagenic signals to telogen secondary follicles and catagenic signals to anagen secondary follicles. The only effect of changing plasma PRL concentration in primary follicles, was an anagenic effect in telogen follicles.

CHAPTER 7

THE EFFECT OF BROMOCRYPTINE TREATMENT FOLLOWING A SWITCH FROM SHORT TO LONG PHOTOPERIOD ON FIBRE GROWTH IN WILTSHIRE SHEEP AND DOWN-PRODUCING GOATS

7.1 ABSTRACT

Mixed-aged, non-pregnant Wiltshire ewes (n=12) and down-producing does (n=18) were pre-treated with short photoperiod (8L:16D) and low temperature (10-15°C) from 15 June 1992 until 6 January 1993. From 7 January until 31 March 1993 one group of goats continued under short-photoperiod and low temperature (SD goats, n=6). Other sheep and goats were treated with long photoperiod (16L:8D) and 20°C with either no further treatment (LD goats n=6, LD sheep n=6), or with long-acting bromocryptine (BRLD goats n=6, BRLD sheep n=6) at two weekly intervals. On 31 March 1993 the animals were released onto pasture under natural photoperiod (sunrise 0630 h, sunset 1818 h).

Mean plasma PRL concentration during the treatment period was 6.0 (5.3-6.8) ng/ml, 8.5 (7.6-9.5) ng/ml, and 97 (89-109) ng/ml in SD, BRLD and LD goats respectively (cf LD $P<0.001$) and 0.67 (0.74-0.87) ng/ml and 135 (123-148) ng/ml in BRLD and LD sheep respectively ($P<0.001$). In sheep, in comparison to goats, mean plasma PRL concentration was 8 ng/ml lower during bromocryptine treatment ($P<0.0001$) and 38 ng/ml higher during long photoperiod treatment ($P<0.05$).

During the treatment period mean PA and SA of SD goats remained high (PA 49-73%, SA >90%). However after the reversal from short to long photoperiod, in LD sheep and goats, SA and PA fell to below 40% on 3 March 1993 before returning to be in excess of 73% on 31 March 1993. In BRLD sheep, mean PA and SA remained above 86% throughout the treatment period, but in BRLD goats, mean PA and SA declined to be less than 40% at the end of the treatment period. Unlike LD goats, follicles in BRLD goats failed to reactivate until after cessation of treatment. Following the cessation of the treatment, mean SA and PA progressively returned to full activity in LD and BRLD goats and LD sheep, and was unaffected by treatment but mean post-treatment PA of SD goats was 20% lower ($P<0.05$) than either BRLD or LD goats.

Shedding progressed at maximum rates in LD sheep and goats in late March and mid April after the photoperiod reversal was completely prevented in BRLD sheep but only delayed by approximately one month in both BRLD and SD goats. The pattern in FGR was similar in both LD sheep and goats while i0n BRLD goats the

pattern was similar but lagged that of LD animals ($P<0.001$). Fluctuations in FGR were greatly diminished in BRLD sheep and SD goats. FGR declined earlier, approaching the subsequent post-treatment spring in LD animals compared to the other treatment groups ($P<0.05$).

In conclusion, bromocryptine treatment prevented the catagenic effect of long photoperiod on anagen Wiltshire sheep follicles. In goats, bromocryptine treatment during long-photoperiod did not prevent follicles entering telogen but may have delayed the transition from telogen to anagen.

7.2 INTRODUCTION

The down-producing goat grows and sheds its fleece in a circannual cycle which is governed by photoperiod (Section 1.2.2.1). It is thought that the increasing spring photoperiods, which induce increases in plasma PRL concentration, are prerequisites for the shedding of the winter fleece (Lynch and Russell, 1989; Kloren, 1991; Chapter 3). However, it is highly probable that shedding of the winter fleece is linked to summer fleece growth and hence follicle reactivation (Nixon *et al.*, 1991a; Chapter 3 and 6). Therefore, it has been assumed that increases in plasma PRL concentration have an anagenic effect on quiescent follicles (Kloren, 1991). However, recent information on the effect of increasing photoperiod and plasma PRL concentration on the fibre growth of down-producing goats does not totally support this simplistic hypothesis. Certainly, telogen follicles of down-producing goats, in natural spring photoperiod, were activated and underwent summer down growth when the animals were treated with either long photoperiod (Chapter 6) or systemic PRL (Dicks, 1994). But, while the follicles of systemically PRL-infused goats remained active (Dicks, 1994), the follicles of long photoperiod-treated animals subsequently entered telogen (Chapter 6). In addition, the effects of long photoperiod on follicles could only be partially prevented by bromocryptine treatment which suppressed plasma PRL concentration (Chapter 6). Conflicting results showed that bromocryptine treatment in spring photoperiod activated telogen follicles with no effect on winter fleece shedding (Chapter 6) or delayed winter fleece shedding (Kloren, 1991; Lynch-Dicks,

1994). However, large increases in plasma PRL concentration, which occur as a consequence of both long photoperiod treatment (Chapter 6) and the cessation of bromocryptine treatment (Kloren, 1991; Chapter 6), subsequently stimulated catagen which was followed by follicle reactivation and shedding. Therefore it appears that changes in plasma PRL concentration may have both anagenic and catagenic effects on the follicle, dependent on its activity state and that plasma PRL concentration may only partially explain the effects of long photoperiod on follicle activity in down goats.

In small mammals which annually produce two separate pelages, in contrast to down goats, declining plasma PRL concentration regulates the transition from summer to winter pelage and an increase in plasma PRL concentration stimulates the converse pelage transition (Duncan and Goldman, 1984; Smith *et al.*, 1987; Badura and Goldman, 1992). In Wiltshire sheep, large increases in plasma PRL concentration expedited the entry of anagen follicles into telogen (Parry *et al.*, 1993; Pearson *et al.*, 1993). Some Wiltshire telogen follicles became activated prior to the spring increase in plasma PRL concentration. Therefore, in contrast to telogen goat follicles, plasma PRL concentration are not an essential prequisite for the activation of Wiltshire follicles (Pearson *et al.*, 1995).

Studies which are conducted to compare the effect of plasma PRL concentration on seasonal fibre growth cycles in different species must ensure that follicles of the different species are in a similar activity state at the time of treatment application. In natural photoperiod the follicle activity in New Zealand (NZ) Wiltshire sheep, as in down-producing goats, is maximal shortly after the summer solstice and remains high until the winter solstice at which time the follicles progressively enter telogen. The NZ Wiltshire sheep, in comparison to the feral goat, has a shortened period of follicle inactivity during winter and, in some sheep, the follicle population does not fall to complete inactivity (Parry *et al.*, 1991). The abatement of seasonality in Wiltshire follicles mimics that observed differences in goats with increasing Angora

ancestry (Chapter 2). It is not surprising to learn, therefore, that the NZ Wiltshire genetic pool contains some genes from non-shedding Poll Dorset sheep (Parry *et al.*, 1991).

The follicles of Wiltshire sheep activate in August, 1 month earlier than those of down-producing goats (Parry *et al.*, 1991; Chapter 2 and 3). As in down-producing goats, many NZ Wiltshire sheep have an additional spring follicle cycle in October and November which is synchronous with fleece moulting (Parry *et al.*, 1991). Approximately one third of NZ Wiltshires have a characteristic decline in follicle activity during spring which occurs as plasma PRL concentrations peak prior to the longest day (Pearson *et al.*, 1995). The exact nature of this subsidiary cycle has yet to be fully explored as previous histological sampling regimes have been too infrequent to provide a detailed description (Parry *et al.*, 1991; Craven *et al.*, 1994). Fleece shedding in the Wiltshire progresses upwards and inwards from the neck, belly and tail regions and may not progress to completion, leaving a ridge of fleece along the backbone (Parry *et al.*, 1991).

In both down goats and Wiltshire sheep, the period bounded by the winter and summer solstices sees the shedding of the winter fleece, possibly in association with the subsidiary spring growth cycle (Chapter 3), and the initiation of new winter fleece growth (Nixon *et al.*, 1991; Parry *et al.*, 1991; Chapter 2, 3). In both species there is considerable variation in the timing of follicle reactivation both between and within follicle populations (Parry *et al.*, 1991; Chapter 3). In Wiltshire sheep, six months of constant short photoperiod (8L:16D), applied from 2 weeks after the winter solstice until release into natural long photoperiod 2 weeks after the summer solstice, synchronised follicle changes (Pearson *et al.*, 1995). After photoperiod reversal, anagen follicles progressed through a period of catagen and telogen which was rapidly followed by follicle reactivation and eventual complete denuding of the sheep eight weeks after the photoperiod reversal (Parry *et al.*, 1993; Pearson *et al.*, 1995). In common with Wiltshire sheep, shedding was induced in Soay sheep by a switch from

short to long photoperiod (Lincoln *et al.*, 1980). In down-producing goats the growth cycle has also been shown to be responsive to photoperiodic manipulations applied in spring (McDonald and Hoey, 1987; Chapter 6). If, as it appears, the fibre growth of Wiltshire sheep and down goats is similarly responsive to short-long photoperiod reversals, then this could be an appropriate method for over-riding the small, between- and within-species, differences in the timing of natural fibre growth cycles. In addition to synchronising follicle changes, this large photoperiod reversal has been shown to reduce the time taken to progress through the complete fibre growth cycle (Craven *et al.*, 1995).

Long-acting bromocryptine (Parlodel LA) has been used to suppress plasma PRL concentration in a wide number of species (see Chapter 4, 5, 6). In previous Chapters it was established that Parlodel LA treatments of 50 mg/goat (mean LW 35 kg) (Chapter 6) and 100 mg/sheep (mean 55 kg) in Romney sheep (Chapter 5) effectively reduced plasma PRL concentrations to below winter solstice concentrations. Plasma PRL concentration increased in both sheep and goats twenty one days after they received the Parlodel LA injection (Chapter 5, 6). In this experiment, in order to minimise species differences in liveweight, injections were administered at a rate of 3.9 mg/kg$LW^{0.75}$ at fortnightly intervals.

In brief, both Wiltshire sheep and down-producing goats were treated indoors with six months of short days, followed by three months of long photoperiod, followed by release onto pasture to post-autumn equinox photoperiods. During treatment with long photoperiod, a sheep and goat treatment group also received Parlodel LA to suppress long photoperiod increases in plasma PRL concentration. One goat group was maintained under short day treatment for the entire 9 months of indoor housing. Fibre growth was described in generalised terms during the pre-treatment period, in detail for the 3 month treatment and 2.5 month post-treatment periods, and in less detail for the remaining 6 month period leading up to the natural spring moult.

7.3 MATERIALS AND METHODS

7.3.1 Animals and management

On 3 June 1992, mixed-aged, non-pregnant Wiltshire ewes (n=12) and down-producing does (n=18) were placed indoors. Six animals of each species and similar liveweight were accommodated in each pen and placed under natural light at the Flock House Agricultural Centre, Bulls (40° 11'S 175° 23' E). On 15 June 1992, short day photoperiod treatment (8L:16D, lights on 0800 h) commenced using natural light regulated by exterior window shutters with supplementary lighting from 100 watt light bulbs on a automatic time switch. The animals were exposed to natural temperature fluctuations.

On 4 August 1992, the animals were transported 50 km to the controlled environment rooms at Massey University, Palmerston North. Until 6 January 1993 the animals continued under the same short photoperiod regime (8L:16D) but with ambient temperature maintained between 10 and 15°C. All natural light was excluded from the rooms and illumination was by fluorescent tubing with supplementary 150 watt light bulbs. Light intensity measurements were not taken. During the pre-treatment period the animals were contained in two rooms and penned in groups of similar liveweight with either 3 sheep or 4-5 goats in each pen.

In the initial stages of the experiment the goats and sheep were fed diets previously shown to ease adaption to concentrate feeding for the respective species following transition from pasture. Throughout the experiment the goats were fed energy equivalent portions of chaffed red clover hay, maize and pelleted feed (70% lucerne, 30% cereal mixture) at a rate of 1.1 times maintenance. Up to 4 August 1992, sheep were fed energy equivalent portions of pellets (70% lucerne, 30% cereal mix) and chaffed red clover hay fed at 1.1 times maintenance. From 4 August 1992, the sheep's diet was identical to that of the goats. Water was available *ad libitum*.

7.3.2 Treatment regime

Animals were allocated to treatment groups (n=6) on 6 January 1993 by blocking to remove room and pen bias and balanced for liveweight (5 January 1993) and PA and SA follicle activity (19 November 1992). From 7 January until 31 March 1993 a goat treatment group (SD goats) continued under the same short-photoperiod and low ambient temperature regime. In addition, sheep and goats were treated with long photoperiod (16L:8D, lights on 0400 h) and 20°C with either no further treatment (LD goats, LD sheep), or treatment with Parlodel LA (BRLD goats, BRLD sheep). The animals treated with long photoperiod were housed in two rooms in treatment groups of 3 animals per pen with each room balanced for species and treatment. Short day-treated goats were housed in a separate room in a single pen containing 6 goats.

Long-acting bromocryptine (Parlodel LA, Sandoz Pharmaceuticals Ltd, Auckland, NZ) was administered as an intramuscular injection in the anterior region of the neck at a rate of 3.9 mg/kg $LW^{0.75}$ (for formulation see Section 5.3) at two weekly intervals with the last injection given on 17 March 1993. Injections were administered in the afternoon following the collection of fibre measurements and blood samples.

At the cessation of treatments on 31 March 1993 (sunrise 0630 h, sunset 1818 h) the animals were released onto pasture at Flock House Agricultural Centre.

7.3.3 Measurements

From 27 July 1992 until 6 January 1993 fibre measurements were taken from both sheep and goats at 6 to 7 weekly intervals. During the treatment period and up to 15 June 1993, fibre measurements were taken at two weekly intervals and thereafter at monthly intervals until 10 December 1993. In goats, stretched down and guard hair length were measured on the left side of the neck, front shoulder, midside and hind shoulder (Section 2.2.2.1) from which mean down (DL) and guard hair length (GL)

were calculated. The amount of fleece shedding was determined by both combing the right side of the goat and visual scoring of the amount of fibre plucked from the left side of the goat. Shedding scores (SS) of the plucked fibre ranged from 1 (no shedding) up to 5 (large amount of shedding). From 19 January 1993, separate SS were collected for both the down and guard hair fleeces using the same scale. In addition, the goats were combed (Section 6.3.4) to obtain a quantitative estimate of fleece shedding. The fibre from the first combing was discarded and thereafter the fibre captured at each combing was weighed.

In sheep, shedding was recorded by sketching the position of long unshed wool on standard sheep outlines and the percentage area of shed fleece estimated. Following three consecutive measurement dates where shedding area did not increase it was assumed that shedding had progressed to completion and the shedding area was then reset to 0.

In both sheep and goats, fibre growth rate (FGR) was determined on the midside at each measurement date during the pre-treatment period and at monthly intervals thereafter. During patch collection, the sheep were laid flat on their side while goats were restrained by their horns and sampled in an upright standing position. A square quadrat was drawn in the region of the midside and the fibre within the quadrat was clipped to skin level using Oster animal clippers. The dimensions (sides A, B, C, D, plus diagonal E) of the patch were measured using calipers and the patch site was then enlarged by clipping. All fibre samples over 2 g in weight were scoured using the standard wool scour at Massey University and the yield of clean fibre was calculated. A mean yield for sheep and goats respectively was used to estimate clean weight of fibre in samples less than 2 g. Greasy and clean FGR were then calculated using the following equations:

GWT=	Greasy patch weight (g)
CWT=	Greasy patch weight*Yield (g)
days=	Number of days between patch collection dates (days)
S_1=	(A+B+E) (cm)

S_2= (C+D+E) (cm)
Area= sqrt $S_1(S_1-A)(S_1-B)(S_1-E)$+sqrt $S_2(S_2-C)(S_2-D)(S_2-E)$
Greasy FGR= (GWT/Area)/days*1000 (mg/cm^2/day)
Clean FGR= (CWT/Area)/days*1000 (mg/cm^2/day)

Mid-side skin snip biopsy samples (Section 2.2.2.4) were collected and fixed in formalin in both sheep and goats at each fibre measurement date. All skin samples were viewed under a dissecting microscope (Section 2.2.3.2) and scored for the presence of newly emerged fibres (Section 2.2.3.2). In goats, newly emerged down fibre (NEDF) scores ranged from 1 to 6 as in previous Chapters. In sheep, newly emerged wool fibres (NEWF's) were either absent (Score=6) or occurred in the conjunction with long wool (Score=1).

Skin samples of both sheep and goats were embedded, epidermal surface uppermost, in paraffin wax. Samples collected on 7 October 1992, 19 November 1992, 7 January, 3 February, 15 February, 3 March, 31 March, 22 April, 20 May, and 15 June 1993 were cut into serial 8 μm transverse sections and stained using the adapted Sacpic method (Section 2.2.3.3). Approximately 10 follicle groups containing both primary and secondary follicles were scored according to the characteristics of the inner-root sheath (Nixon, 1993). The follicles were scored as being active (anagen), inactive (telogen) or active+brush (Section 2.2.3.3). Primary follicle activity (PA), secondary follicle activity (SA), active+brush primary (PAc+brush) and active+brush secondary (SAc+brush) levels were calculated as a percentage of total number of either primary or secondary follicles.

Blood samples were collected at 6-7 week intervals during the pre-treatment period and then at weekly intervals during the treatment period from 20 January to 24 March inclusive and thereafter at two weekly intervals until 15 June 1993. Blood samples were collected at 1300 h on the same day as fibre measurements were collected following a minimum 1 hour period during which the animals were left in seclusion. Samples were collected into EDTA tubes on ice by venous jugular puncture.

Plasma was separated by centrifugation at 3000 g at 4°C for 20 minutes and plasma samples were frozen at -8°C pending assay for PRL concentration. Due to the accidental disposal of many stored plasma samples, only pre-treatment samples collected on 19 November 1992 and 6 January 1993 and post-treatment samples collected on 19 and 24 April 1993 could be assayed for PRL concentration. The radioimmunoassay of plasma PRL concentration was conducted at AgResearch Ruakura using the protocol outlined in Section 2.3.2.3.

7.3.4 Statistical Analysis

Two types of statistical analysis were undertaken. In the first analysis, data collected over time were analysed using the GLM procedures of SAS (SAS, 1987) with repeated measures in time using the Wilks' Lambda test of significance. Treatment effects were determined from analysis within common species groups and effects of species were determined from analysis within common treatment groups. Due to unequal variances, plasma PRL concentrations were $\log_{10}$ transformed prior to analysis. Plasma PRL concentration data presented in the text are back transformed pooled means with the standard error presented as a back transformed range. The data were analysed separately over four time periods: pre-treatment (up to and including 6 January, 1993); treatment period (7 January to 31 March 1993 inclusive); post-treatment period (22 April to 15 June 1993 inclusive); and next cycle (20 May to 10 December 1993).

In the second analysis the dates and magnitudes of minimum and maximum fibre events were determined for individual animals. The effect of treatment on these parameters was determined using general linear model procedures and in goats treatments were fitted as contrasts (SAS, 1987). Unless otherwise stated, data presented in the text are least squares means and standard errors. Raw means and standard errors are presented graphically.

7.4 RESULTS

7.4.1 Pre-treatment period

7.4.1.1. Plasma PRL concentration: Mean plasma PRL concentrations on 19 November 1992 and 6 January 1993 were 25±8 and 94±4 ng/ml respectively in goats (Figure 7.1) and 31±8 ng/ml and 27±9 ng/ml respectively in sheep (Figure 7.2). The high plasma PRL concentration on 6 January in goats was because of values of 522 and 579 ng/ml in two individual goats (Figure A7.1). When the data from these two goats were removed, mean plasma PRL concentration for goats on 6 January 1993 was 38±11 ng/ml.

7.4.1.2 Follicle activity: Mean follicle activities, measured only over the last 3 measurement dates of the pre-treatment period, were in excess of 60% in sheep and goats (Table 7.1). On 7 January 1993, PA was higher in sheep (96±4%) than in goats (69±8%, $P<0.01$). In goats, SA rose from 75±11% on 7 October 1992 to reach 95±3% on 7 January while in sheep SA was in excess of 90% over this period.

7.4.1.3 Fibre Measurements: Gross fibre measurements showed (Table 7.1) that a proportion of both sheep and goats underwent partial or complete shedding during September and October 1992. Fleece SS rose above a score of 1 in 16 of the 18 goats. The extremities of all sheep showed evidence of shedding and NEWF's were detected on midside skin in 50% of sheep. Over the pre-treatment period mean DL decreased by more than 10 mm in 75% of the goats and NEDF's were observed on the skin surface of 40% of the goats.

In goats, greasy FGR was less than 0.2 mg/cm^2/day during September and October but had reached 0.52±0.05 mg/cm^2/day by 7 January. Mean DL decreased from 72±4 mm on 2 September to 28±6 mm on 19 November before increasing to 53±5 mm on 7 January 1993.

There were no differences in subsequent responses to treatments between animals which underwent a period of shedding and those which did not. Fleece growth was high at the start of the treatment period in all animals.

7.4.2 Treatment period

7.4.2.1 Liveweights and S/P ratio: Mean liveweights increased from 23 to 28 kg for goats and from 50 to 62 kg in sheep over the pre-treatment period. During treatment, mean liveweights ranged between 28 and 29 kg in goats and 64 and 67 kg in sheep. During the treatment period and subsequently, treatment had no effect on liveweight in either species.

The ratio of secondary to primary follicles was 6.0±0.1 in goats and 4.8±0.1 in sheep.

7.4.2.2 Plasma PRL concentrations

7.4.2.2.1 Goats: In comparison to SD and BRLD goats, LD goats had higher ($P<0.001$) mean plasma PRL concentration at every measurement date during the treatment period (Figure 7.1). Mean plasma PRL concentration during the treatment period in LD goats was 97 (89-109) ng/ml compared to 8.5 (7.6-9.5) ng/ml, and 6.0 (5.3-6.8) ng/ml in BRLD and SD goats respectively (both $P<0.001$). Overall, there was a tendency ($P<0.10$) for plasma PRL concentration to be lower in SD than in BRLD goats.

On individual dates, plasma PRL concentration was more than 5 ng/ml higher ($P<0.05$) in BRLD compared to SD goats on 3, 10 and 24 February 1993 (all $P<0.05$). This elevation was due to the failure of Parlodel to adequately control plasma PRL concentration in one bromocryptine-treated goat (Tag #20). During the treatment period, plasma PRL concentration of goat #20 was more than 20 ng/ml on 60% of the measurement dates (Figure A7.1).

Table 7.1: Mean±se fibre growth measurements at various dates (day.month.yr) in non-pregnant down-producing does and Wiltshire ewes during short-day (8L:16D) and low temperature (10°C) treatment from 15 June 1992 until 6 January 1993.

Fibre Measurements	Dates				
	27.07.92	02.09.92	07.10.92	19.11.92	07.01.93
GOATS					
Primary follicle activity (%)			69±10	78±5	69±8
Secondary follicle activity (%)			75±11	98.0±0.8	95±3
Guard hair length (mm)	51±2	53±3	51±2	50±2	44±2
Greasy fleece growth rate (mg/cm^2/day)		0.13±0.03	0.19±0.05	0.38±0.04	0.52±0.05
Fleece shedding score	2.0±0.2	1.9±0.3	2.2±0.3	1.4±0.2	1.05±0.05
Down length (mm)	73±4	72±4	43±8	28±6	53±5
Newly emerged down fibre score	5.2±0.4	5.2±0.4	5.1±0.4	5.7±0.3	5.7±0.3
SHEEP					
Primary follicle activity (%)			75±11	97±1	96±4
Secondary follicle activity (%)			93±2	99.0±0.5	99.0±0.5
Greasy fleece growth rate (mg/cm^2/day)		0.52±0.06	0.72±0.03	0.66±0.05	0.51±0.05
Area shed (%)	0±0	6±6	21±10	45±10	21±10
Newly emerged wool fibre score	6±0	4.8±0.6	4.3±0.7	5.6±0.4	6±0

In BRLD goats, plasma PRL concentration remained constant over the treatment period but in SD goats concentrations increased from 5 to 15 ng/ml (Figure 7.1) over the last 5 weeks of the treatment period (Trt*time P<0.05). Plasma PRL concentration in BRLD goats was unaffected by date of Parlodel injection.
When this animal was removed from the analysis the overall mean difference between BRLD and SD goats declined to only 1 ng/ml and was not significant.

Following the cessation of treatment, mean plasma PRL concentration on 19 and 29 April 1993 (Figure 7.1) was similar at 1.5-6 ng/ml for both BRLD and LD goats but it was higher in SD goats (32 (20-50) ng/ml, P<0.001).

7.4.2.2.2 Sheep: Mean plasma PRL concentration during the treatment period (Figure 7.2) was 0.67 (0.74-0.87) ng/ml and 135 (123-148 ng/ml) in BRLD and LD sheep respectively (P<0.001). In the post-treatment period, mean plasma PRL concentration in LD sheep was 25 (18-35) ng/ml compared to 2.9 (2.3-3.7) ng/ml for BRLD sheep (P<0.001). But over this period, plasma PRL concentration was falling in LD sheep and rising slightly in BRLD sheep (Trt*time P<0.01).

7.4.2.2.3 Species comparison: Over the treatment period, the overall mean plasma PRL concentration in LD sheep was 38 ng/ml higher than in LD goats (P<0.05) and this difference was significant (P<0.05) on 6 out of 10 individual measurement dates. After the cessation of treatments mean plasma PRL concentrations over the two measurement dates in LD goats (3 (2-4) ng/ml) was lower than in LD sheep (30 (23-40) ng/ml) largely due to an earlier decline in LD goats compared to LD sheep.

In individual animals, following the switch from short to long photoperiod, plasma PRL concentration in LD animals progressively increased up to a maximum on 6 February ±9 days and 3 February ±9 days in sheep and goats respectively (Table 7.2, 7.3).

Plasma PRL concentration was 8 ng/ml higher in BRLD goats than in BRLD sheep ($P<0.05$). On individual treatment measurement dates, the mean difference to BRLD goats ranged from 4 to 15 ng/ml and was significant at every date ($P<0.05$). In the post-treatment period, plasma PRL concentrations were similar in both groups.

7.4.2.3 Primary follicle activity

7.4.2.3.1 Goats: The mean activity of primary follicles of goats at the start of the treatment period was only 68±12% but PA ranged in individual goats from 0 to 100% (Figure A7.3). Mean PA in LD goats was more than 25% lower (both $P<0.01$) than in either BRLD or SD goats at the start of the treatment period (Figure 7.3).

During the treatment period, though mean PA remained relatively constant (PA 49-70%) in individuals goats fluctuated PA asynchronously between 0 and 100% (Figure A7.3). However in BRLD and LD goats the follicles of individuals responded similarly. In BRLD goats mean PA declined progressively to be less than 25% over the treatment period (Figure 7.3). Mean PA in LD goats fell to 18±7% on 3 March 1993 (BRLD goats, 18±7% NS; SD goats 49±7%, $P<0.01$) but then increased to 73±14% on 31 March (BRLD goats, 24±14, $P<0.05$; SD goats 58±14%, NS). The pattern of PA in LD goats over the treatment period differed to that of both SD and BRLD goats (Trt*time, $P<0.01$).

Following the cessation of treatment on 31 March 1993, PA in BRLD and LD goats had returned to full activity by 20 May 1993. Over the post-treatment period PA was more than 20% lower (both $P<0.05$) in SD goats than in the other treatment groups and this was due to asynchronous increases and falls in PA by individual SD goats (Figure A7.3).

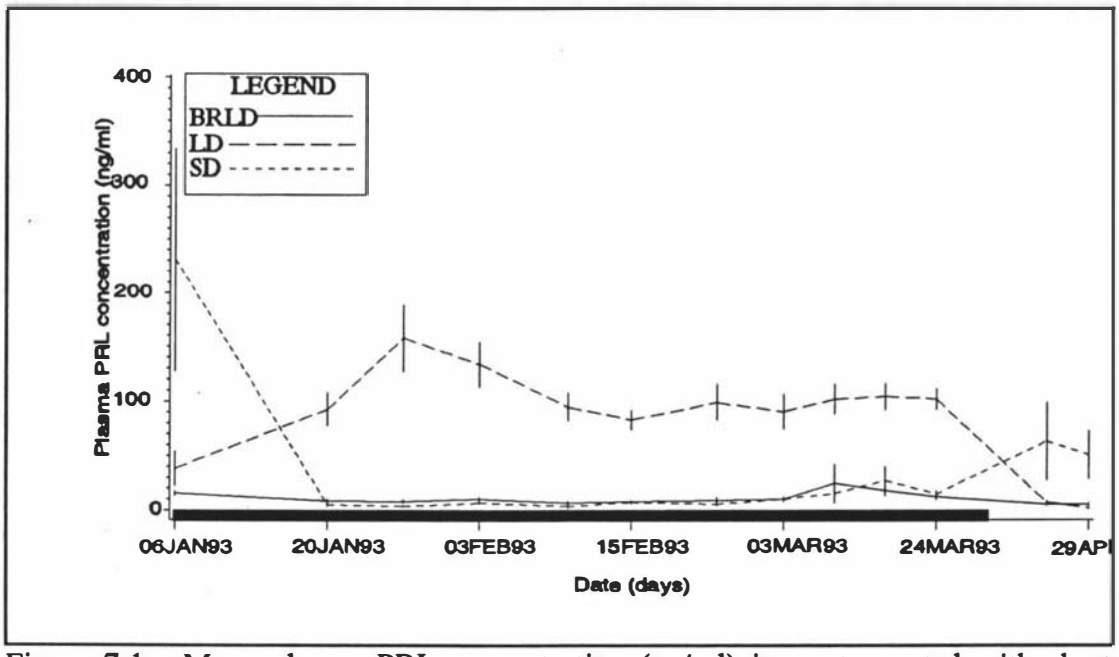

Figure 7.1: Mean plasma PRL concentration (ng/ml) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

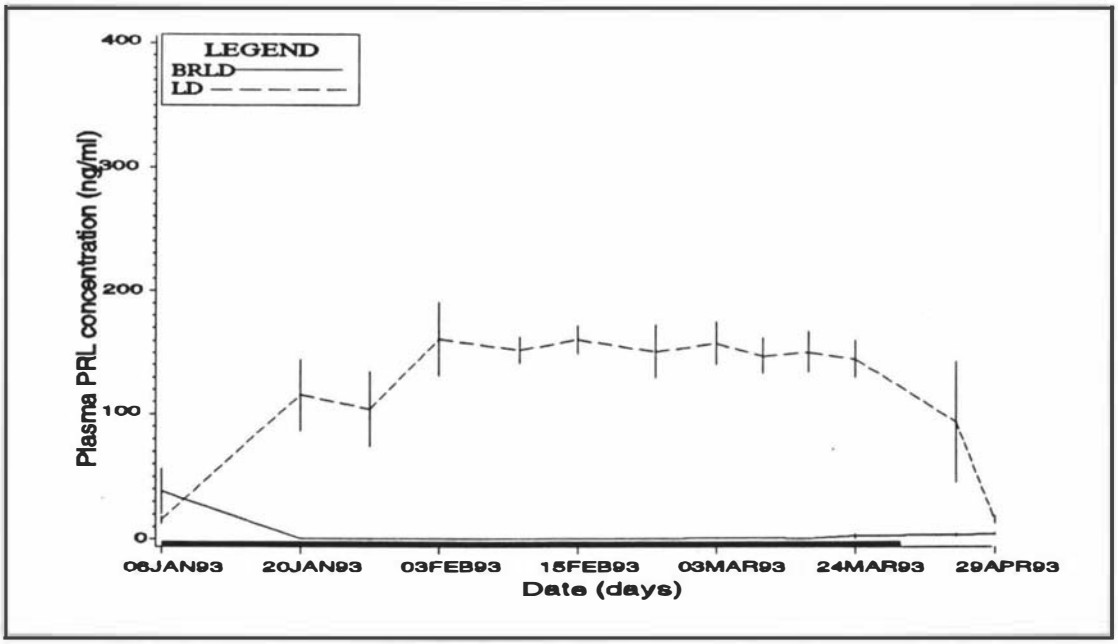

Figure 7.2: Mean plasma PRL concentration (ng/ml) in sheep treated with long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BR) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

Table 7.2: Dates of fibre growth events (for Abbreviations section) in non-pregnant down-producing does treated with short photoperiod (8L: 16D) (SD goats) or long photoperiod (16L:8D) with either no further treatment (LD goats) or treated with long-acting bromocryptine (BRLD goats) from 7 January 1993 until 31 March 1993.

Fibre Growth Event	Date of Fibre growth event (days)			Significance Level
	LD goats	BRLD goats	SD goats	
PRL_{max}	3 February ±9	na	na	
DL_{max}	3 February ± 10^a	11 February ± 10^a	7 April ± 10^b	***
SA_{min}	7 February ± 9^a	6 March ± 10^b	23 April ± 9^c	***
PA_{min}	16 February ± 13	23 March ± 13	5 March ± 13	NS
$NEDF_e$	16 March ± 11	16 March ± 10	26 March ± 18	NS
FGR_{min}	16 March ± 7^a	9 April ± 7^b	12 May ± 7^c	***
SS $down_{max}$	25 March ± 6^a	9 May ± 6^b	3 May ± 6^b	***
SS $guard_{max}$	31 March ± 8^a	1 May ± 8^b	12 May ± 8^b	***
CMB_{max}	4 April ± 10^a	19 May ± 10^b	27 May ± 10^b	**
DL_{min}	18 April ± 5^a	21 May ± 5^b	25 May ± 5^b	***
$NEDF_d$	24 April ± 23	26 April ± 22	6 June ± 37	NS
SA_{max}	2 May ± 7^a	20 May ± 7^{ab}	5 June ± 7^b	*
PA_{max}	2 May ± 10	10 May ± 10	15 May ± 10	NS
$gFGR_{max}$	31 May ± 9^a	2 July ± 9^b	27 June ± 9^b	*

* P>0.05, ** P<0.01, *** P<0.001, na=not applicable

Table 7.3: Dates of fibre growth events (for abbreviations see Abbreviation section) in non-pregnant Wiltshire sheep treated with long photoperiod (16L:8D) with either no further treatment (LD sheep) and treated with long-acting bromocryptine (BRLD sheep) from 7 January 1993 until 31 March 1993.

Fibre Growth Event	Date of Fibre growth event (days)		Significance Level
	LD sheep	BRLD sheep	
PRL_{max}	6 February±9	na	
SA_{min}	28 February±7	22 March±7	*
PA_{min}	28 February±13	27 March±13	*
$NEWF_e$	11 March±3	na	
greasy FGR_{min}	21 March±13	28 April±13	NS
SS $area_{max}$	11 April±11	na	
$NEWF_d$	16 April±8	na	
SA_{max}	2 May±11	2 May±11	NS
PA_{max}	28 April±13	16 May±13	NS
greasy FGR_{max}	9 June±8	9 June±8	NS

* $P>0.05$, ** $P<0.01$, *** $P<0.001$
na=non applicable

There was no effect of treatment on mean date of reaching minimal PA (16 February-23 March 1993), mean minimum PA (11 and 14%) and date of return to full PA (2-15 May 1993) (Table 7.2).

Treatments had no overall effect on the proportion of PAc+brush follicles nor their pattern over time. However on 31 March 1993 there were 31±7% PAc+brush follicles in LD goats compared to only 5±7% ($P<0.05$) and 0±7% ($P<0.01$) in BRLD and SD goats respectively (Figure 7.4). BRLD goats had a peak (31±11%) in PAc+brush follicles on 22 April 1993 and in SD goats an equivalent peak (33±10%) occurred on 20 May 1993. During the post-treatment period there was a tendency ($P<0.10$) for the proportion of follicles in PAc+brush to be lower in LD goats than in BRLD goats.

7.4.2.3.2 Sheep: The primary follicles of all sheep were approaching full activity (PA>90%) at the start of the treatment period and PA remained in excess of 86% during treatment in BRLD sheep (Figure 7.5). However in LD sheep, PA levels fell to a minimum of 16±6% on 3 March (BRLD sheep 87±6%, $P<0.0001$) before rising again to 83±8% (BRLD sheep 94±8%, NS) on 31 March 1993. Both the pattern over time ($P<0.01$) and magnitude ($P<0.0001$) of PA differed in these two sheep treatment groups (Figure 7.5). In the post-treatment period mean PA was more than 90% in both treatment groups and was unaffected by the preceding treatment. In individual LD sheep, minimum PA was 14±4% and it was achieved at a mean date of 28 February±13 days. In LD sheep, primary follicles had returned to full activity levels by 28 April±7 days.

During the treatment period in LD sheep, increasing proportions of PAc+brush follicles were present, rising from 0.6±0.4% on 15 February 1993 to reach levels of 4.1±2% by 31 March 1993 (Figure 7.6). In contrast, PAc+brush follicles were absent during the treatment period in BRLD sheep (Trt $P<0.01$; Trt*time, $P<0.01$). Treatment had no effect on the proportion of PAc+brush follicles in sheep in the post-

treatment period though levels of PAc+brush follicles ranged between 4 and 15% in LD sheep and were only between 0.6 and 1.5% in BRLD sheep.

7.4.2.3.3 Species comparison: PA was similar in both BRLD sheep and goats at the start of the treatment period (goats, 92±2%; sheep, 100±2%). PA levels in BRLD goats then decreased by 70% during the treatment period while in BRLD sheep activity levels remained in excess of 80% (Species effect $P<0.0001$, Species*time $P<0.01$) (Figures 7.3 and 7.5). The levels of PAc+brush follicles were similar in BRLD sheep and goats.

PA differed between LD sheep (93±13%) and goats (44±13%, Species $P<0.05$) at the beginning of treatment period. However both species subsequently responded similarly to long day treatment. In individual animals, PA declined to a common minimum level of 14±5% at similar dates in both LD goats (16 February±5 days) and sheep (28 February±5 days) (Table 7.2, 7.3). Full activity returned to primary follicles in early May in both LD sheep and goats. The mean proportion of primary follicles in the PAc+brush stage over the treatment period in LD goats was 7±2% compared to 1.2±0.6% in LD sheep ($P<0.05$).

7.4.2.4 Secondary follicle activity

7.4.2.4.1 Goats: At the onset of treatment application, mean SA of goats was in excess of 90% and in SD goats it remained at this level throughout the treatment period (Figure 7.7, Figure A7.5). In contrast, SA had fallen to 18±10% in LD goats by 3 Februaryand remained at this low level until 31 March it when SA had increased up to 79±9%. SA also declined in BRLD goats, but mean SA had decreased to only about 40% by the later half of the treatment period. In contrast to those of LD goats, the follicles of most BRLD goats had failed to reactivate prior to the cessation of the treatment on 31 March 1993. Over the treatment period, mean SA in all three treatments followed a different pattern (Trt*time $P<0.001$) with mean SA levels being

similar in LD and BRLD goats but higher in SD goats (P<0.01).

There was no overall effect of treatment on SA in the post-treatment period. However on 20 May there was a tendency for SA to be lower in SD goats (84±5%) than in BRLD (98±5%, P<0.10) and LD (99±5%, P<0.05) goats. In individual goats, there was a tendency for SA to fall to minimum levels earlier in LD (7 February±9 days) than in BRLD (6 March±10 days, P<0.06) goats and both achieved minimum SA earlier than SD goats (23 April±9 days, P<0.01).

Mean minimum SA in LD (8±9%) was lower than in BRLD (34±9%, P<0.10), and SD (69±9%, P<0.001) goats (Table 7.2). The date at which SA began to increase differed by approximately 1 month (P<0.05) in all three treatment groups (LD goats 13 March±9 days; BRLD goats 19 April±9 days; SD goats 19 May±9 days). Secondary follicles had returned to full activity on similar dates (Table 7.2) in both LD (2 May±7 days) and BRLD (20 May±8 days) goats but the return was later by more than 15 days in SD goats (5 June±7 days, P<0.01).

There was no effect of treatment on the proportion of SAc+brush follicles during the treatment or post-treatment periods (Figure 7.8).

***7.4.2.4.2 Sheep*:** In sheep, the secondary follicles were almost fully active at the start of the treatment period and mean SA remained in excess of 90% throughout the treatment period in BRLD sheep (Figure 7.9). However in LD sheep mean SA fell to reach 48±7% (BRLD sheep, 98±7%, P<0.001) and 36±9% (BRLD sheep, 97±9%, P<0.0001) on 15 February and 3 March respectively. By 31 March, secondary follicles had reactivated and SA levels were similar in LD (90±5%) and BRLD (92±8%) sheep. During the post-treatment period, mean SA was in excess of 94% in both previously treated LD and BRLD sheep (Figure 7.9).

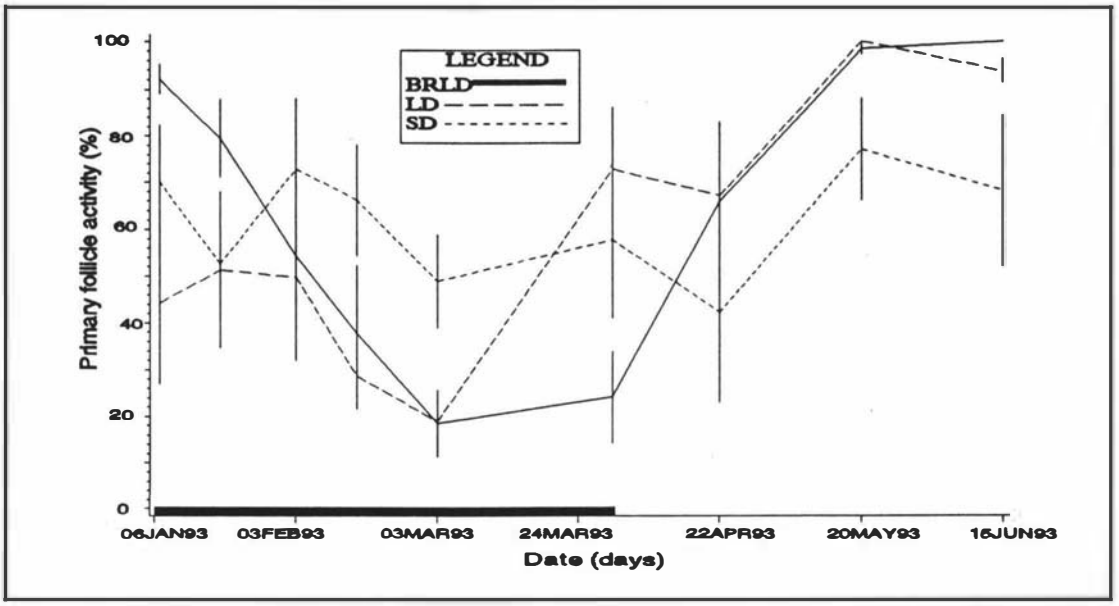

Figure 7.3: Mean primary follicle activity (%) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

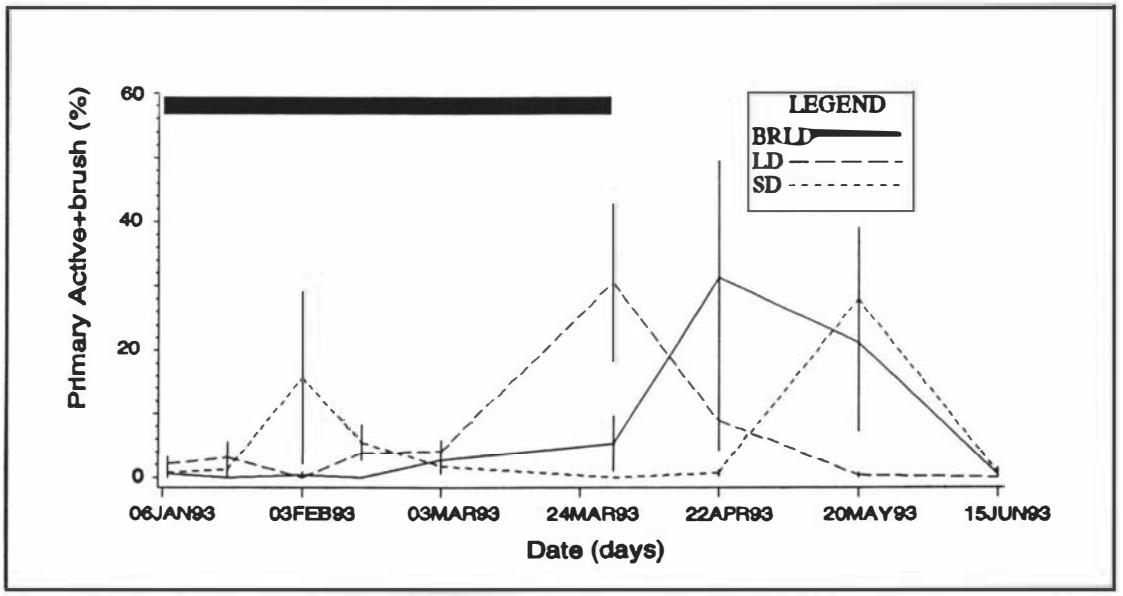

Figure 7.4: Mean proportion of active+brush primary follicles (%) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

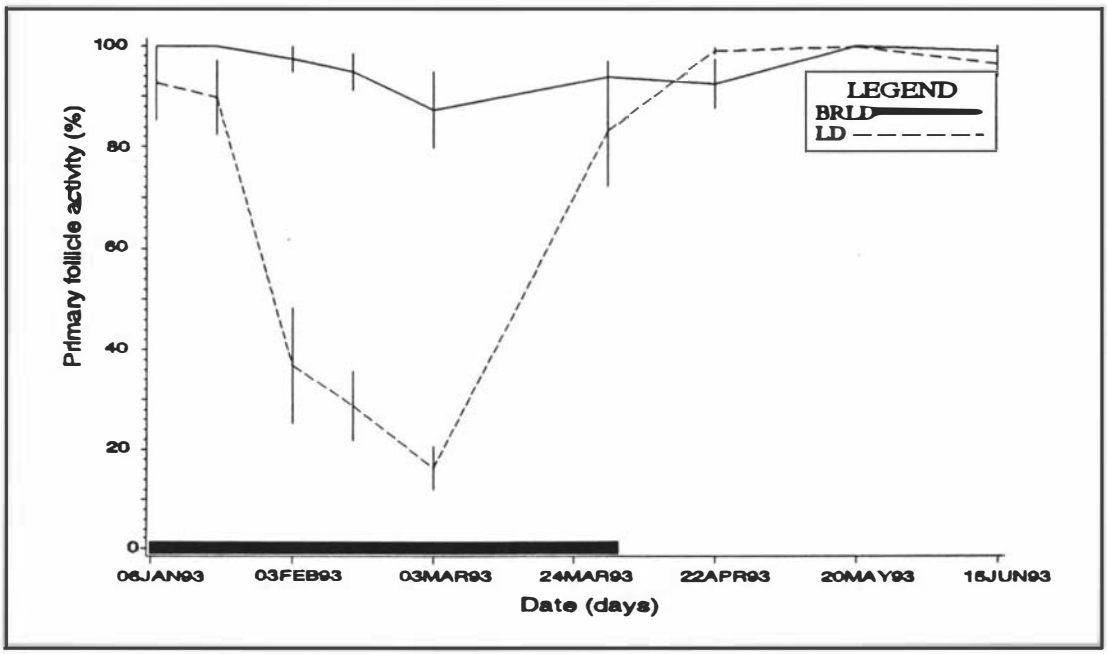

Figure 7.5: Mean primary follicle activity (%) in sheep treated with long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

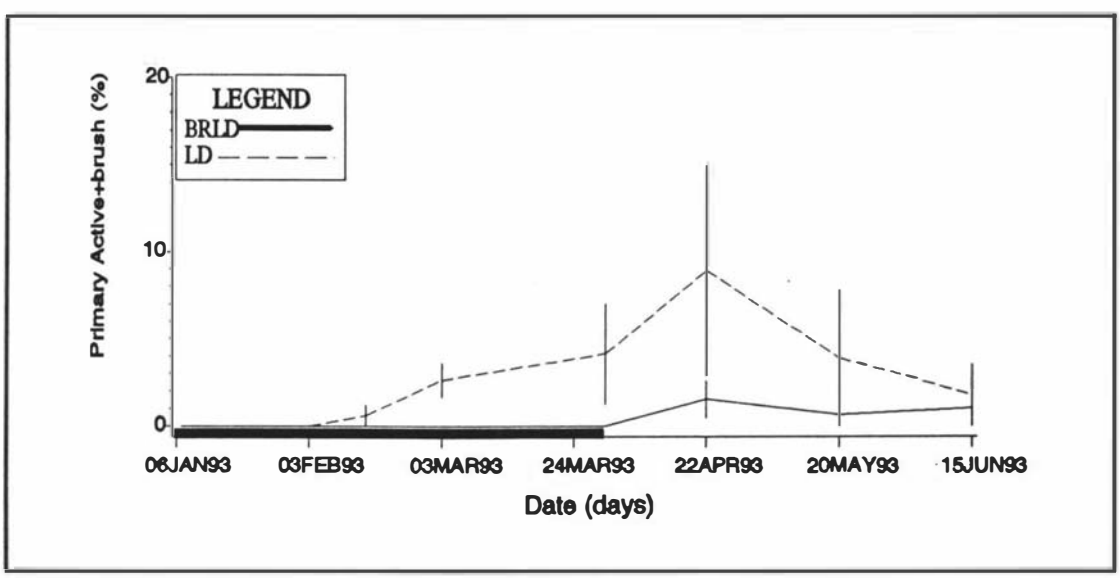

Figure 7.6: Mean proportion of active+brush primary follicles (%) in sheep treated with long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

In individual LD sheep, SA fell to a mean minimum level of 34±9% on 28 February±7 days (Table 7.3) with only 50% of the sheep having minimum SA levels of less than 20% (Figure A7.6). Full activity had returned to LD sheep secondary follicles by 2 May±11 days (Table 7.3).

During the treatment period, the proportion of SAc+brush follicles was less than 0.3% in BRLD sheep while in LD sheep proportions ranged from 0.2% up to 5% (P<0.10) (Figure 7.10). In the post-treatment period, prior treatment had no effect on the proportion of SAc+brush follicles.

7.4.2.4.3 Species comparison: In BRLD sheep SA remained high during the treatment period while in BRLD goats it had declined steadily to reach approximately 40% by the end of the treatment period (species effect P<0.001). Minimum SA in individual animals was lower in LD goats (8±9%) than in LD sheep (34±9%, P<0.05). This minimum was also achieved 21 days earlier (P<0.01) in goats than in sheep. However SA had returned to full activity, in both LD sheep and goats, by 2 May±8 days. There was no difference in the proportion of SAc+brush follicles in either sheep or goats in their common treatment groups.

7.4.2.5 Fibre Measurements in Goats

7.4.2.5.1 Guard hair length: During the treatment period, mean GL was similar in all treatments (Figure 7.11). On 22 April 1993, however, mean GL had dipped in LD goats (29±3 mm) in comparison to BRLD goats (49±3 mm, P<0.001) or SD goats (48±3 mm, P<0.001). Over the remainder of the post-treatment period mean GL in LD goats increased, while it was comparatively static in SD goats and dipped temporarily in BRLD goats on 20 May 1993 (Trt*time, P<0.001). In the next cycle, mean GL was shorter (P<0.05) and the pattern of GL differed (Figure 7.11) in SD goats compared to LD and BRLD goats (Trt*time, P<0.01).

7.4.2.5.2 Down Length: During the treatment period, mean DL continued to increase in SD goats, while it remained static in BRLD goats and was initially static before declining over the last 2 weeks of treatment in LD goats (Figure 7.12). Therefore, during the treatment period, the pattern of mean DL of LD goats differed to that of BRLD and in SD goats (Trt*time, P<0.01) and mean DL overall was shorter in LD than SD goats (P<0.01).

In the post-treatment period, the overall mean DL was lower in LD (20±6 mm) than in BRLD (41±6 mm, P<0.01) goats which in turn had lower DL than in SD goats (74±9 mm, P<0.001). Both SD and BRLD goats had a similar pattern in mean DL over the post-treatment period with both undergoing a dip in mean DL, indicative of shedding, in late May. In contrast, in LD goats the down fleece had shed during the treatment period and during the post-treatment period increased steadily in length (Trt*time, P<0.01). In the next cycle, the down fleeces of all treatment groups increased in length until 24 August 1993. However, the increase in DL from 15 June to 24 August 1993 was 12 mm greater in LD compared to SD and BRLD goats (P<0.05). Over the next cycle period, due to previously accumulated down, the fleece of SD goats was longer than that of BRLD and LD goats (P<0.05). During the spring of 1993 (Figure 7.12) mean DL declined earlier in BRLD goats than in the other two treatments (Trt*time P<0.05).

In individual goats, down fleeces reached a similar mean maximum length at a similar date in LD goats (64±7 mm, 3 February±10 days) and BRLD goats (64±7 mm, 11 February±10 days) (Table7.2). In comparison, the down fleeces of SD goats continued to increase in length until 7 April±10 days (P<0.01) and therefore achieved a greater maximum length (96±7 mm, P<0.01). Minimum DL in individual LD goats occurred on 18 April±5 days which was earlier than for both BRLD (21 May±5 days, P<0.001) and SD (25 May±5 days, P<0.001) goats (Table 7.2). Minimum DL of LD (6±7 mm) and BRLD (22±7 mm) goats was similar and lower than in SD (53±7 mm, P<0.001) goats.

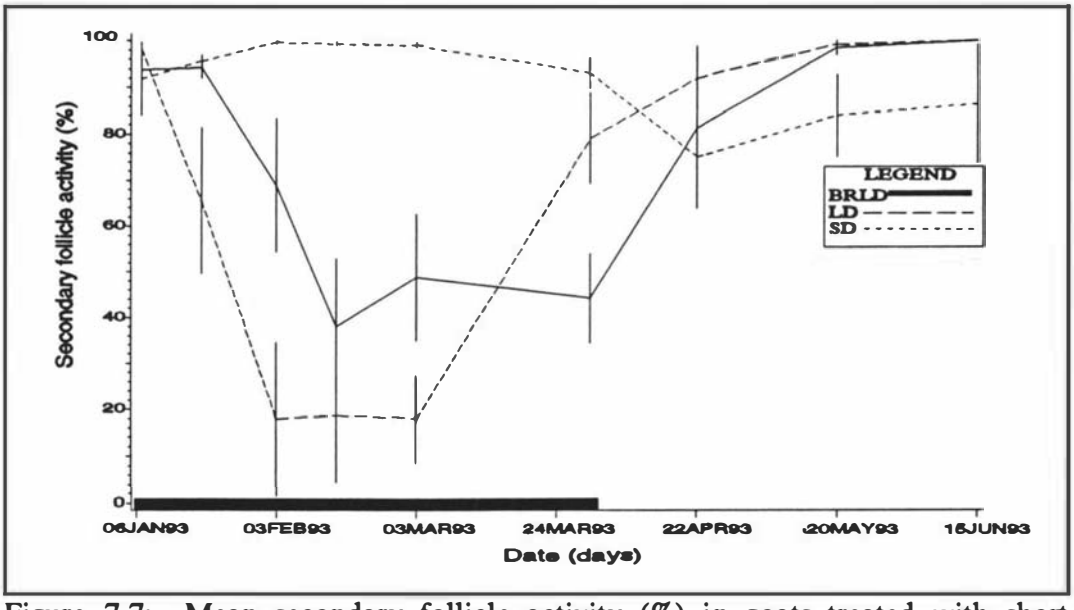

Figure 7.7: Mean secondary follicle activity (%) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

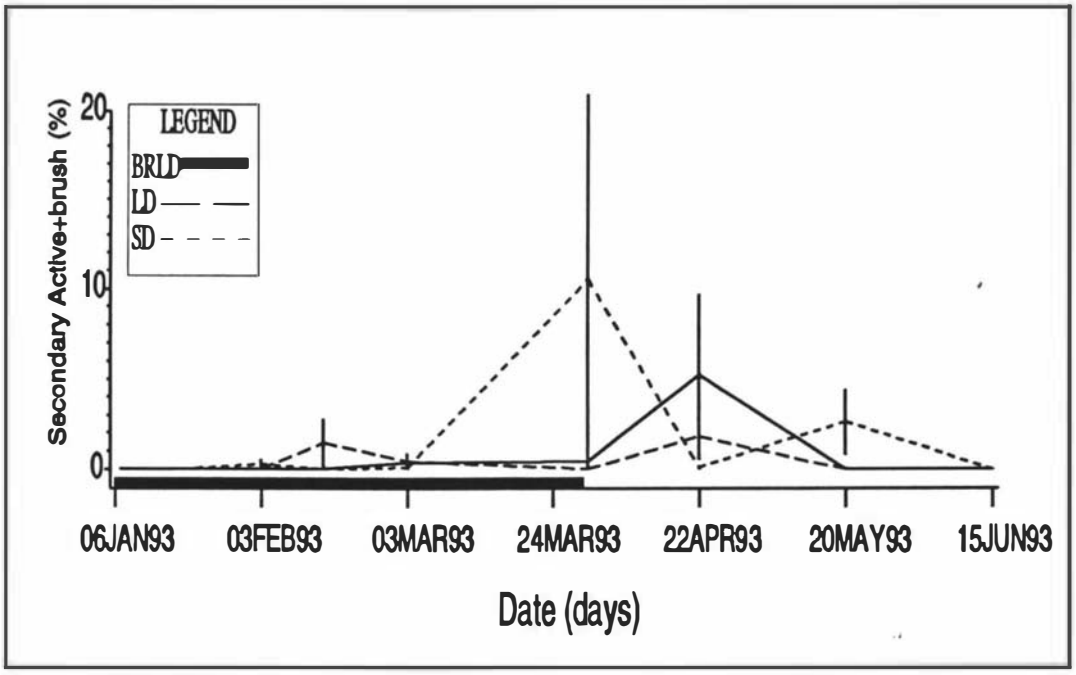

Figure 7.8: Mean proportion of active+brush secondary follicles (%) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

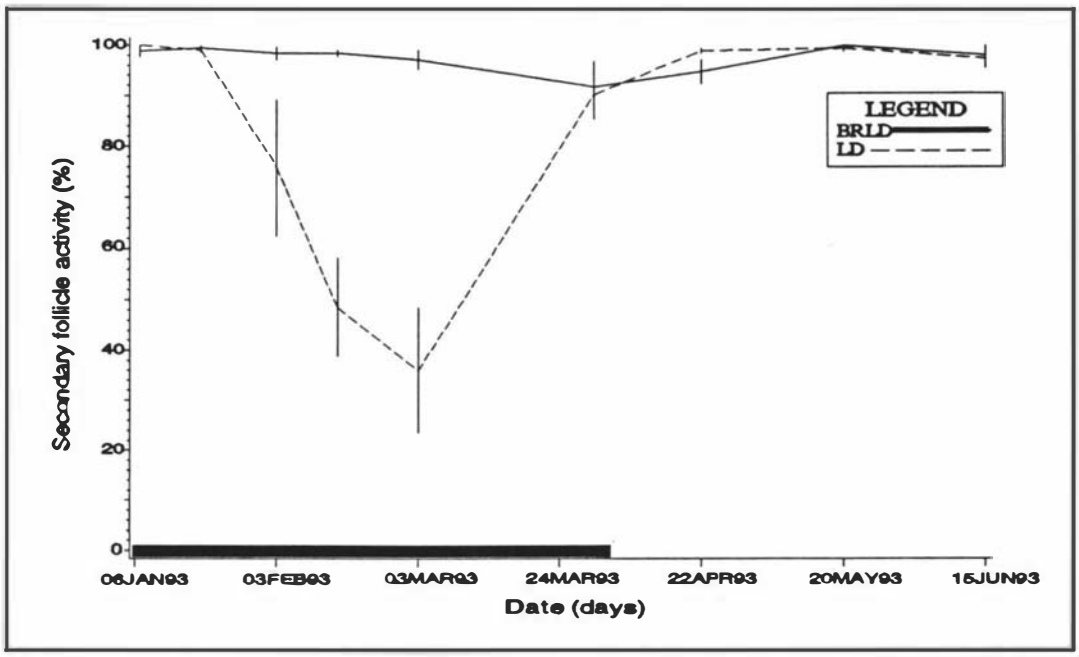

Figure 7.9: Mean secondary follicle activity (%) in sheep treated with long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

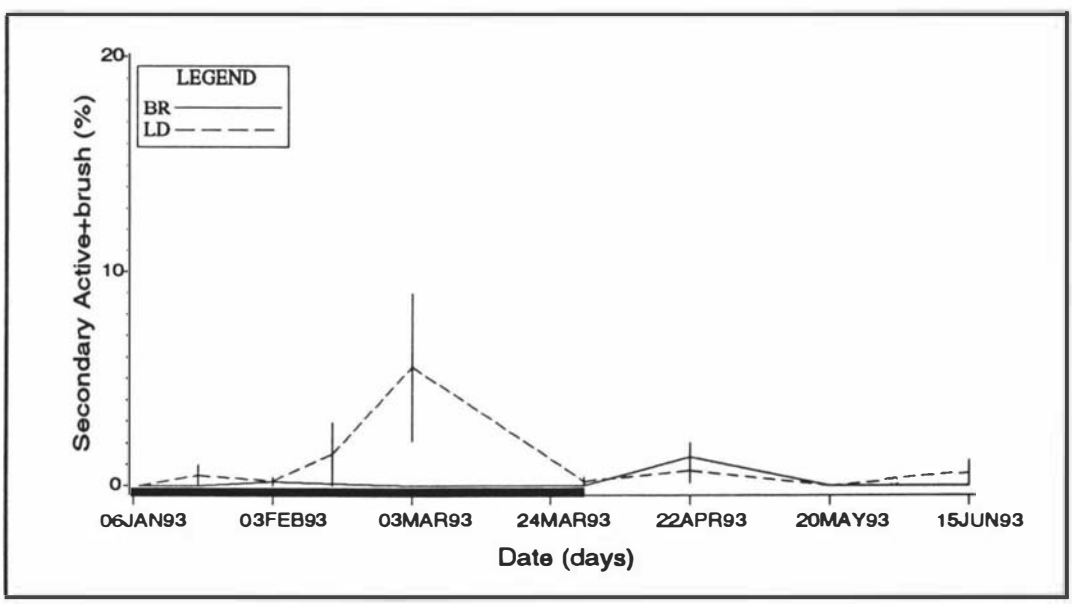

Figure 7.10: Mean proportion of active+brush secondary follicles (%) in sheep treated with long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

7.4.2.5.3 Fleece growth rate: The trends expressed were similar for both greasy and clean FGR (Figure 7.13) and only greasy FGR is discussed. The fleece of SD goats continued to grow at slowly declining rates of 0.48 to 0.37 mg/cm^2/day over the treatment period (Figure 7.13). In contrast, mean greasy FGR of both BRLD and LD goats decreased sharply during the treatment period and were minimal on 31 March 1993. As a consequence both the magnitude of FGR ($P<0.0001$) and pattern ($P<0.05$) of growth differed in SD goats in comparison to the other two treatment groups which were similar. During the post-treatment period, mean greasy FGR followed a different pattern in all treatment groups (Trt*time, $P<0.001$). Changes in mean FGR in BRLD goats, were equivalent but lagged behind those of LD goats (Figure 7.13). In the next cycle, greasy FGR was lower ($P<0.05$) in LD goats than in BRLD and SD goats.

In individual LD goats, greasy FGR reached a mean minimal rate of 0.04±0.05 mg/cm^2/day on a mean date of 16 March±7 days. Minimal FGR was similar in BRLD goats (0.03±0.05 mg/cm^2/day) but it occurred 24 days later ($P<0.01$) (Table 7.2). Minimum greasy FGR in SD goats, in comparison to LD and BRLD goats, was higher (0.22±0.05 mg/cm^2/day, $P<0.05$) and also occurred later, on 12 May±7 days ($P<0.001$). Greasy FGR's reached maximum rates in LD goats on 31 May±9 days which was earlier (Table 7.2) than in either BRLD goats (2 July±9 days, $P<0.05$) or SD goats (27 June±9 days, $P<0.05$). Both BRLD and LD goats had a similar peak greasy FGR of 0.84±0.05 mg/cm/day which was higher than the 0.63±0.05 mg/cm^2/day peak of SD goats ($P<0.01$).

7.4.2.5.4 Shedding:

7.4.2.5.4.1 Weight of combed fibre: During the treatment period, the amount of fleece removed by combing was unaffected by treatment up to 15 February (Figure 7.14) but in the last month of treatment the weight of combed fibre was more than 2.5 times higher ($P<0.10$, 3 March, $P<0.01$ 31 March) in LD goats than in either SD or BRLD goats.

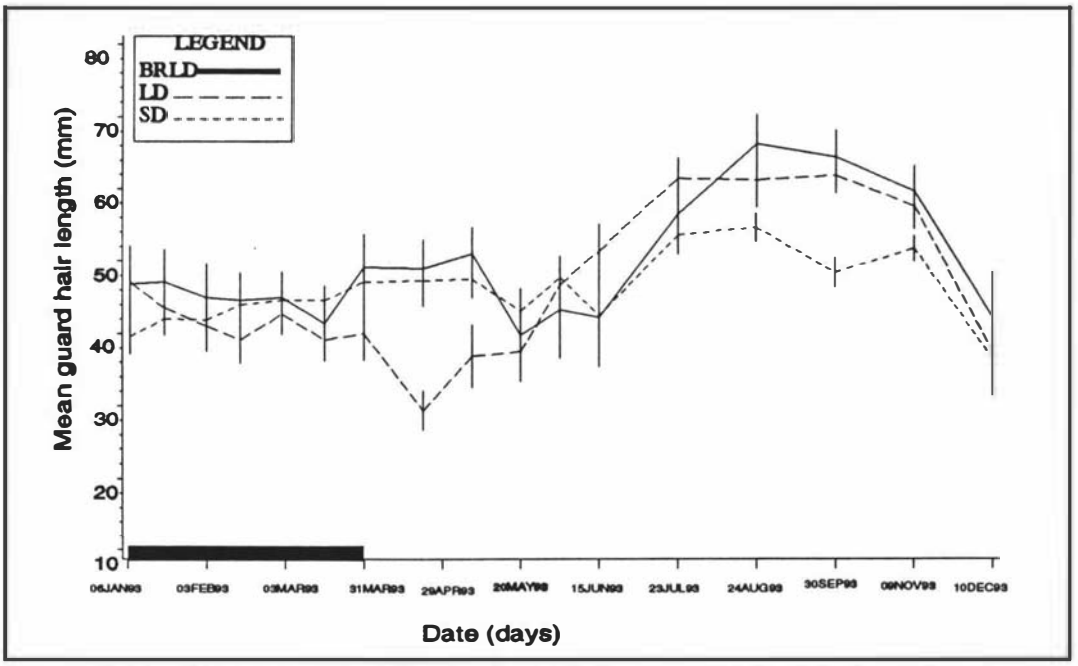

Figure 7.11: Mean guard hair length (mm) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

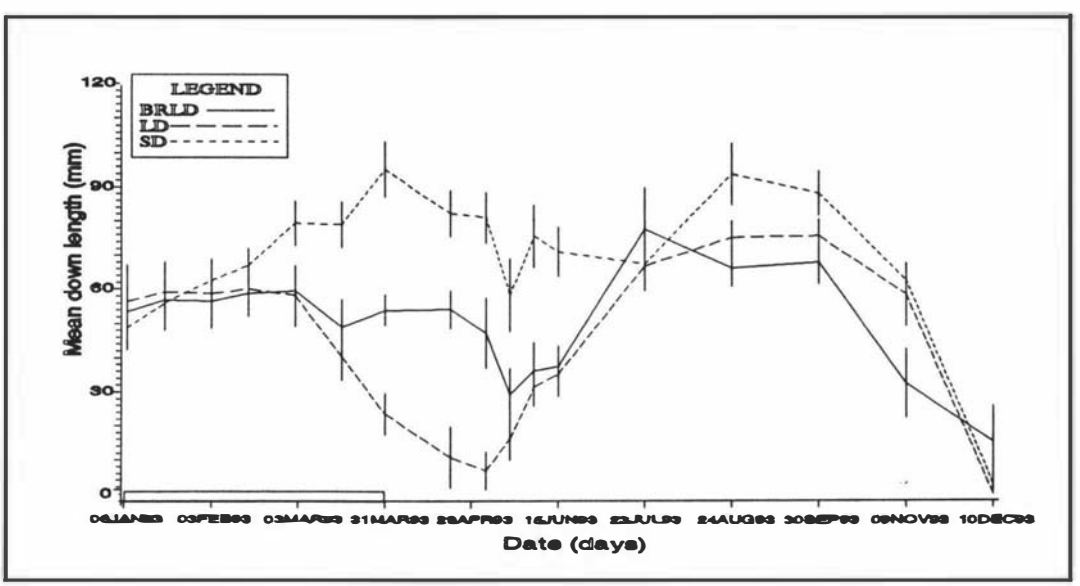

Figure 7.12: Mean down length (mm) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

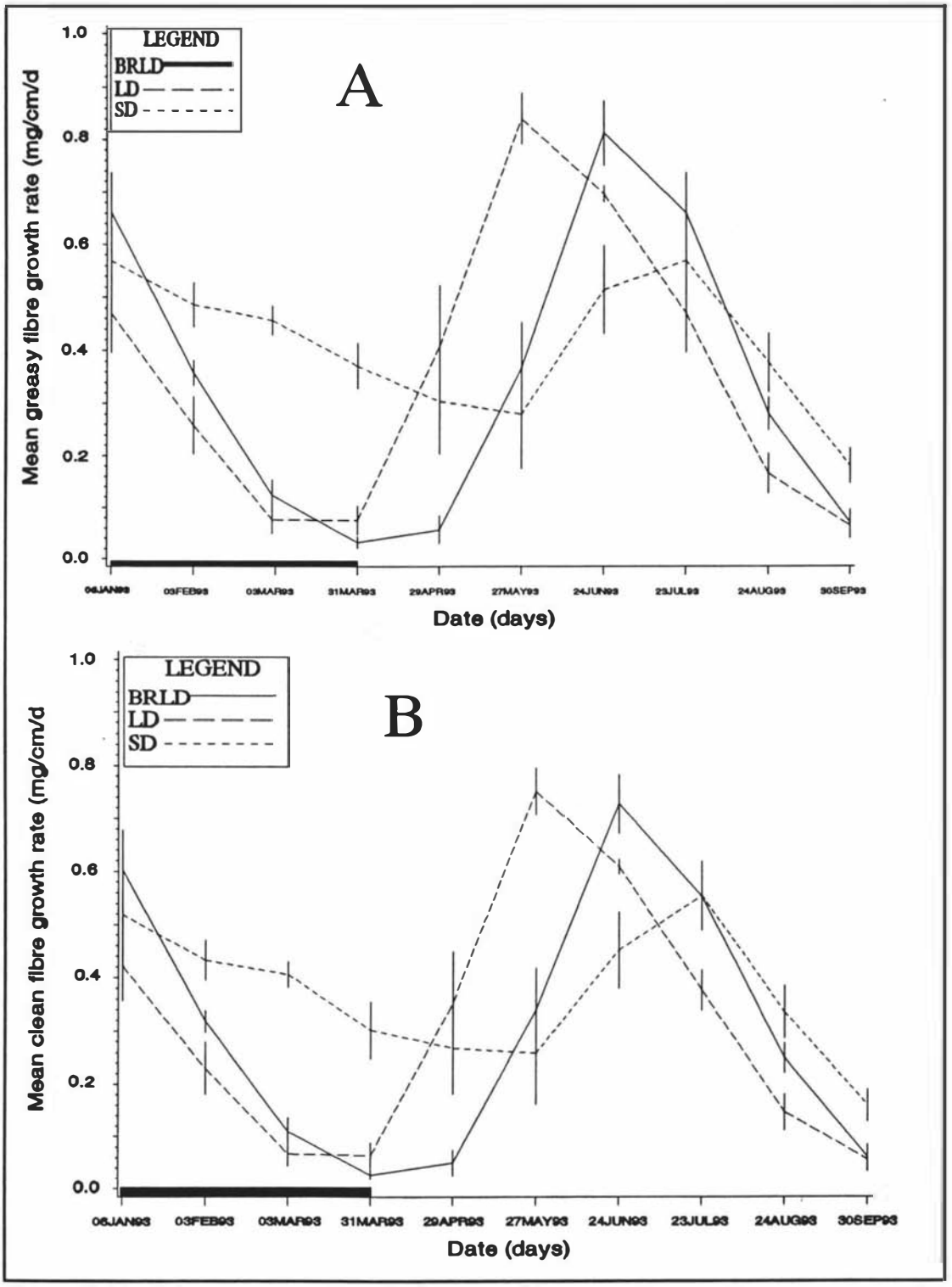

Figure 7.13: Mean greasy (A) and clean (B) fibre growth rate of goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

The weight of fleece removed by combing was virtually identical in BRLD and SD goats during the treatment period.

In the post-treatment period, the amount of fleece captured by combing in LD goats had fallen to low levels while BRLD and SD goats underwent a period of shedding which was similar in pattern and magnitude. On 22 April 1993, the mean weight of combed fibre was greater ($P<0.05$) in SD (1.25±0.2 g) than in BRLD goats (0.58±0.2 g).

The date of attaining maximum combed weight was earlier in LD goats (4 April±10 days, $P<0.05$) than in the other two groups (Table 7.2). The apparent earlier increase in weight of combed fibre was not reflected in an earlier date of attainment of maximum combed weight in SD (27 May±10 days) compared to BRLD (19 May±10 days) goats. There were no differences between treatment groups in weight of combed fibre in the next cycle period (Figure 7.14).

7.4.2.5.4.2 Shedding score: Over the complete monitoring period guard hair SS was similar in pattern and magnitude in BRLD and SD goats (Figure 7.15). However, in comparison to these two groups, LD goats had higher guard hair SS during the treatment period ($P<0.05$) and a different interaction with time in the post-treatment period ($P<0.05$). All three treatment groups had similar patterns of guard hair SS over the next cycle period.

The magnitude of the down SS was lower ($P<0.05$) in the treatment period and higher ($P<0.01$) in the post-treatment period in SD compared to LD goats (Figure 7.13). Mean SS was similar in the post-treatment period in BRLD and SD goats and lower in LD goats ($P<0.01$). Overall mean down SS during the next cycle period was unaffected by treatment. However on 9 November, down SS of LD (3.0±0.2) and SD (2.8±0.2) goats was higher than that of BRLD goats (1.3±0.4, $P<0.05$).

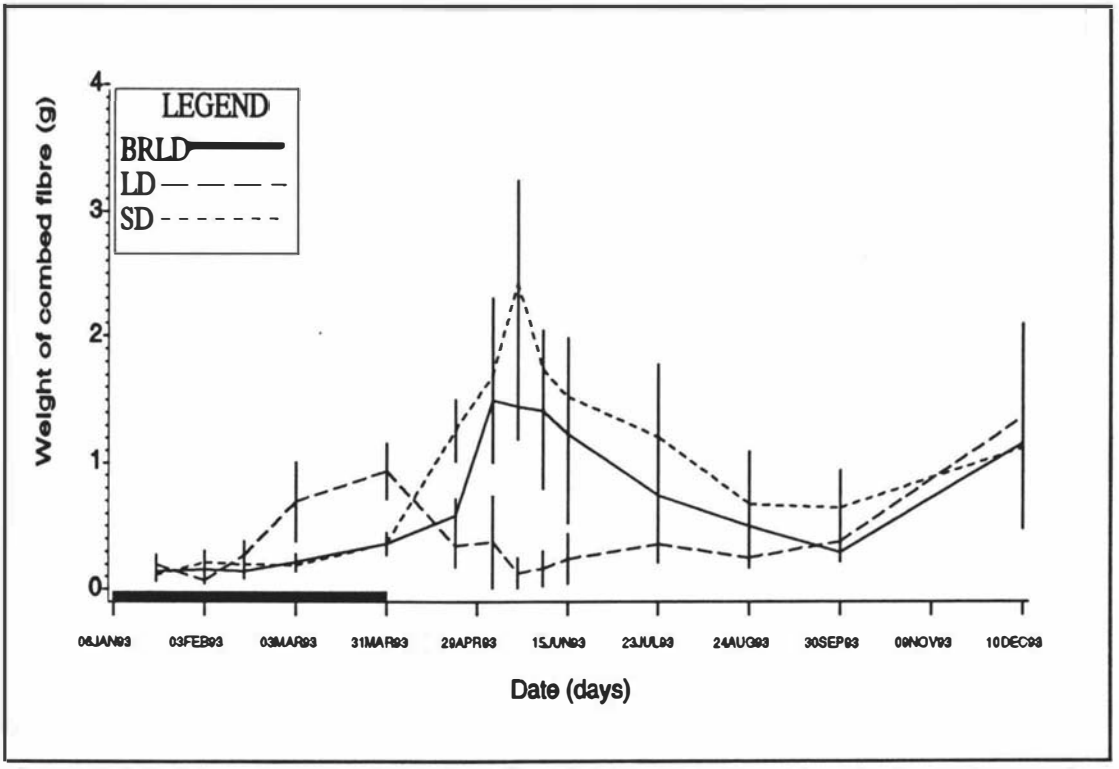

Figure 7.14: Mean weight of combed fibre (g) in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

In individual goats, the dates of maximum SS of guard and down fleeces were 30 to 40 days earlier in LD compared to BRLD and SD goats ($P<0.01$) (Table 7.2).

7.4.2.5.5 Newly emerged down fibres: The pattern and magnitude of the mean NEDF score was similar in BRLD and LD goats in both the treatment and post-treatment periods (Figure 7.16). In BRLD and LD goats, mean NEDF's score began to decrease, indicating emergence of NEDF's in some goats 8 weeks after the start of treatment application. However NEDF's were largely absent in SD goats and therefore, in comparison to LD and BRLD goats, NEDF score in SD goats differed in both pattern ($P<0.05$) and magnitude ($P<0.05$) over the treatment period. All treatments had similar NEDF scores in both the post-treatment and next cycle period.

NEDF's were identified in 83%, 67% and 33% of BRLD, LD and SD goats respectively over the combined treatment and post-treatment periods. In the goats in which NEDF's were identified, the mean dates of emergence were unaffected by treatment and ranged between 16 and 26 March 1993 (Table 7.2). The duration of presence of NEDF's was 39±15 days, 43±13 days and 73±23 days in BRLD, LD and SD goats respectively. Due to the small number of SD goats with NEDF's the differences in duration were not significant.

In BRLD and SD goats NEDF's were identified predominantly in association with long down (Table 7.4). However in LD goats NEDF's were found in equal proportion with long down, by themselves and in association with short down.

Table 7.4: Mean percentage of occurrence of newly emerged fibre scores pooled over 14 measurement dates from 6 January to 24 July 1993 in goats treated with short photoperiod (8L:16D) (SD goats), and sheep and goats treated with long photoperiod (16L:8D) which either received no further treatment (LD goats, LD sheep) or were treated with long-acting bromocryptine (BRLD goats, BRLD sheep) from 6 January to 31 March 1993.

Treatment	Percentage of occurrence of newly emerged fibres (%)					
	1	2	3	4	5	6
BRLD goats	15	1	1	0	2	81
LD goats	7	6	6	0	1	80
SD goats	7	0	0	0	0	93
BRLD sheep	17	0	0	0	0	83
LD sheep	1	0	0	0	0	99

Score 1: long down + NEDF's; Score 2: NEDF only; Score 3: NEDF + short down; Score 4: short down only; Score 5: short down + long down; Score 6: long down only. (for sheep read "wool" for "down" ie NEWF for NEDF)

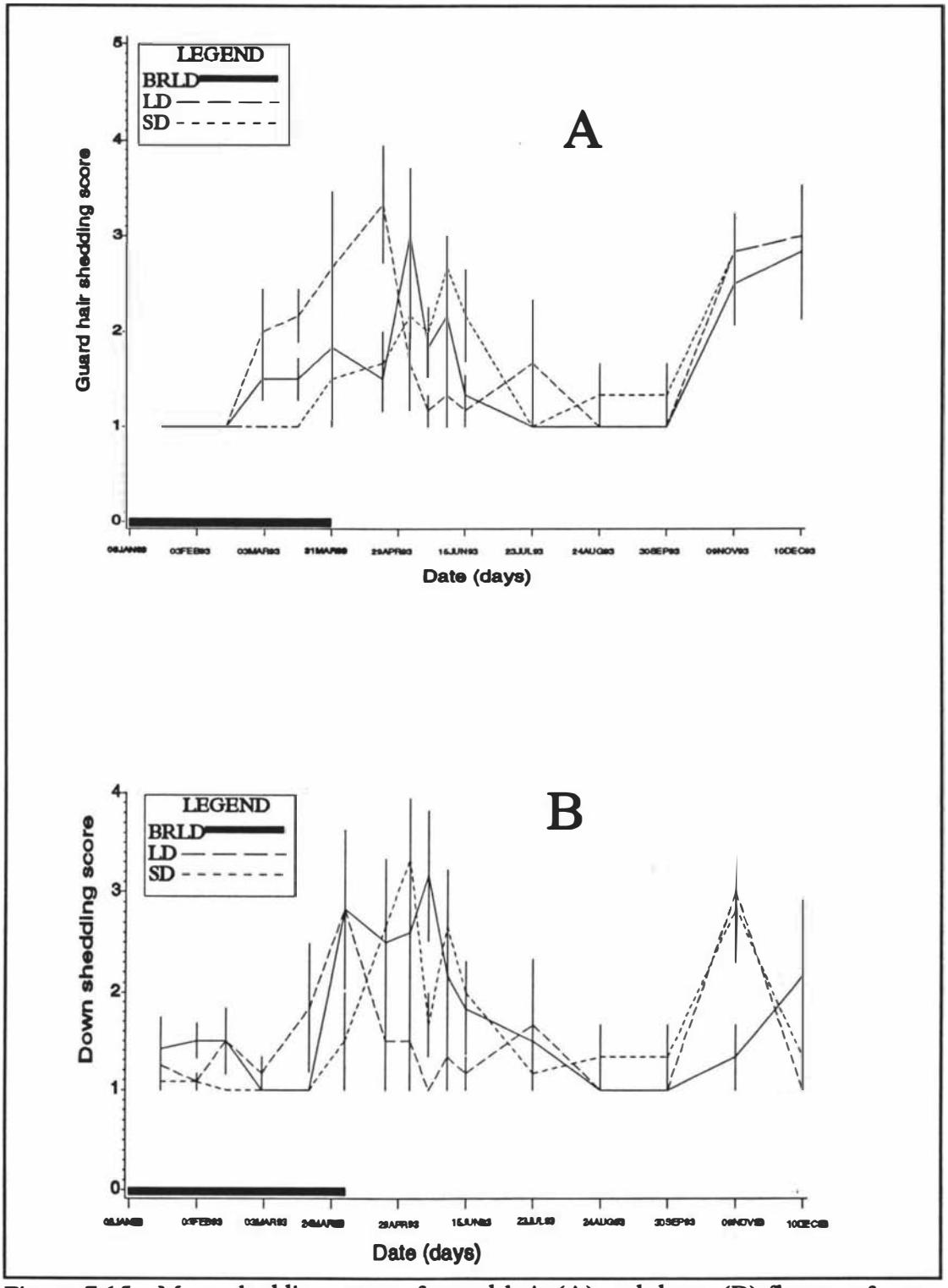

Figure 7.15: Mean shedding score of guard hair (A) and down (B) fleeces of goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

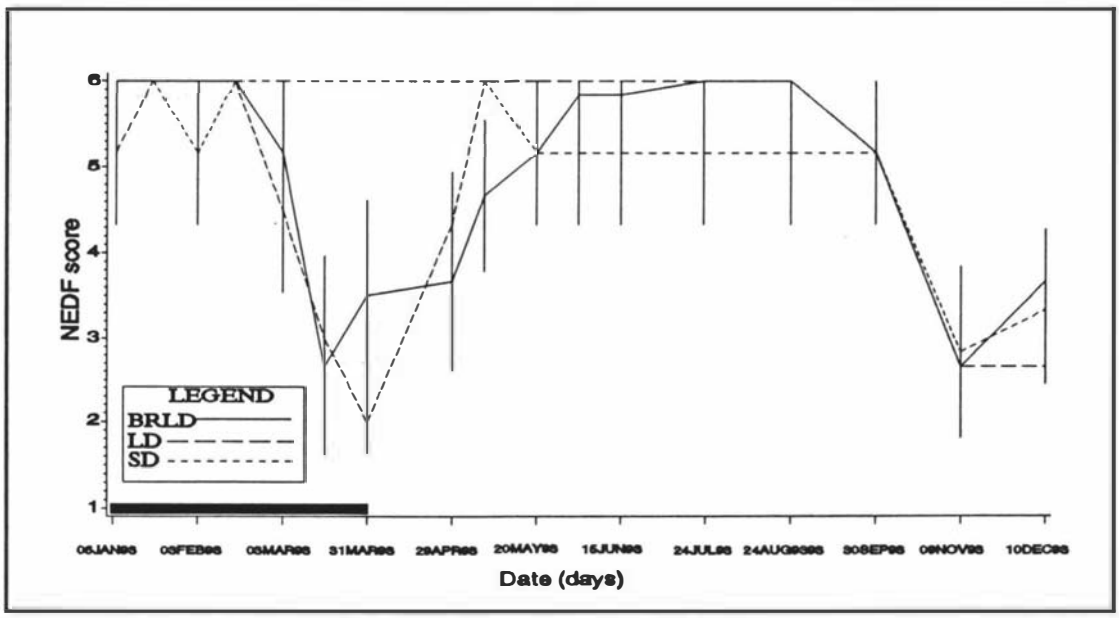

Figure 7.16: Mean newly emerged down fibre score in goats treated with short photoperiod (SD), long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

7.4.2.6 Fibre measurements in sheep

7.4.2.6.1 Fibre growth rate: In sheep, greasy FGR (Figure 7.17) was relatively constant during the treatment period varying between rates of 0.54 to 0.67 mg/cm^2/day in BRLD sheep while in LD sheep greasy FGR had declined from equivalent levels down to 0.11 mg/cm^2/day on 31 March (Trt*Time $P<0.001$, Trt $P<0.001$).

Greasy FGR was similar in both LD and BRLD sheep during the post-treatment period but, in the next cycle, greasy FGR was lower in LD sheep than in BRLD sheep. This was most apparent in August when greasy FGR in LD sheep (0.12±0.05 mg/cm^2/day) was less than a third of greasy FGR in BRLD sheep (0.38±0.05 mg/cm^2/day, $P<0.001$).

In individual sheep, the mean dates of minimum and maximum greasy FGR were

In individual sheep, the mean dates of minimum and maximum greasy FGR were similar in both LD and BRLD sheep (Table 7.3). However the level of minimum greasy FGR was lower in LD sheep (0.08±0.04 mg/cm^2/day) compared to BRLD sheep (0.57±0.04 mg/cm^2/day, P<0.01). Maximum greasy FGR was similar at 0.74 and 0.78 mg/cm^2/day in LD and BRLD sheep respectively.

7.4.2.6.2 Shedding Area: Fleece casting began on 17 March and was maximal in individual sheep on 11 April±11 days in LD sheep but was completely absent during the treatment and post-treatment periods in BRLD sheep (P<0.001) (Figure 7.18). Mean maximal shedding area in individual LD sheep was 91±4%. Overall mean shedding area during the next cycle was unaffected by the preceding treatments. However, shedding had progressed to a greater extent in LD compared to BRLD sheep on 30 September (LD 10±3%, BRLD 2±3%, P<0.10) and 9 November (LD 34±6%, BRLD 15±3%, P<0.05).

7.4.2.6.3. Newly emerged wool fibres: In the treatment period, NEWF were absent in BRLD sheep but were observed in LD sheep from 3 March 1993 onwards (P<0.001). During the post-treatment and next cycle periods the levels of NEWF were similar in both treatments (Figure 7.19).. The mean duration of presence of NEWF in LD sheep was 36±5 days. NEWF were only detected in conjunction with long wool fibres (Table 7.4).

7.4.2.7 Sheep and goat comparison

The mean yield of clean fleece was 89±2% and 66±3% in goats and sheep respectively. Yield was determined on 75% of sheep samples and 19% of goats samples.

7.4.2.7.1 Long day treatment: During the treatment period greasy FGR showed a similar decline in both sheep and goats.

However the magnitude of FGR was lower ($P<0.05$) on 3 February in goats (0.26 ± 0.06 mg/cm^2/day) than in sheep (0.48 ± 0.06 mg/cm^2/day).

In individual LD sheep the magnitude and date of attaining minimum greasy FGR, and the date of maximum FGR, were similar to that of LD goats. However the magnitude of the maximum growth rate was higher ($P<0.05$) in LD goats (0.85 ± 0.03 mg/cm^2/day) than in LD sheep (0.74 ± 0.03 mg/cm^2/day). Newly emerged fibres appeared at the same time in LD sheep and goats and were present for a similar period.

7.4.2.7.2 Bromocryptine treatment: BRLD sheep continued to grow fleece at near maximal rates during the treatment period while in goats greasy FGR decreased to be near minimal levels by the end of the treatment period (Species, $P<0.001$; Species*time $P<0.001$).

While the date of reaching minimum greasy FGR was similar in BRLD goats and sheep, the magnitude of minimal greasy FGR was greater ($P<0.0001$) in BRLD sheep (0.50 ± 0.02 mg/cm^2/day) than in BRLD goats (0.03 ± 0.02 mg/cm^2/day). Greasy FGR subsequently increased to maximal rates on 9 June $\pm$ 8 days in BRLD sheep which was earlier than that of BRLD goats (2 July $\pm$ 9 days, $P<0.05$). The rate of maximal greasy FGR was higher in BRLD goats (0.84 ± 0.05 mg/cm^2/day) than in BRLD sheep (0.78 ± 0.05 mg/cm^2/day).

During the treatment period newly emerged fibres were not identified on the skin of BRLD sheep but were identified in 83% of BRLD goats (Species, $P<0.05$). Similar levels were found in both species during the post-treatment period.

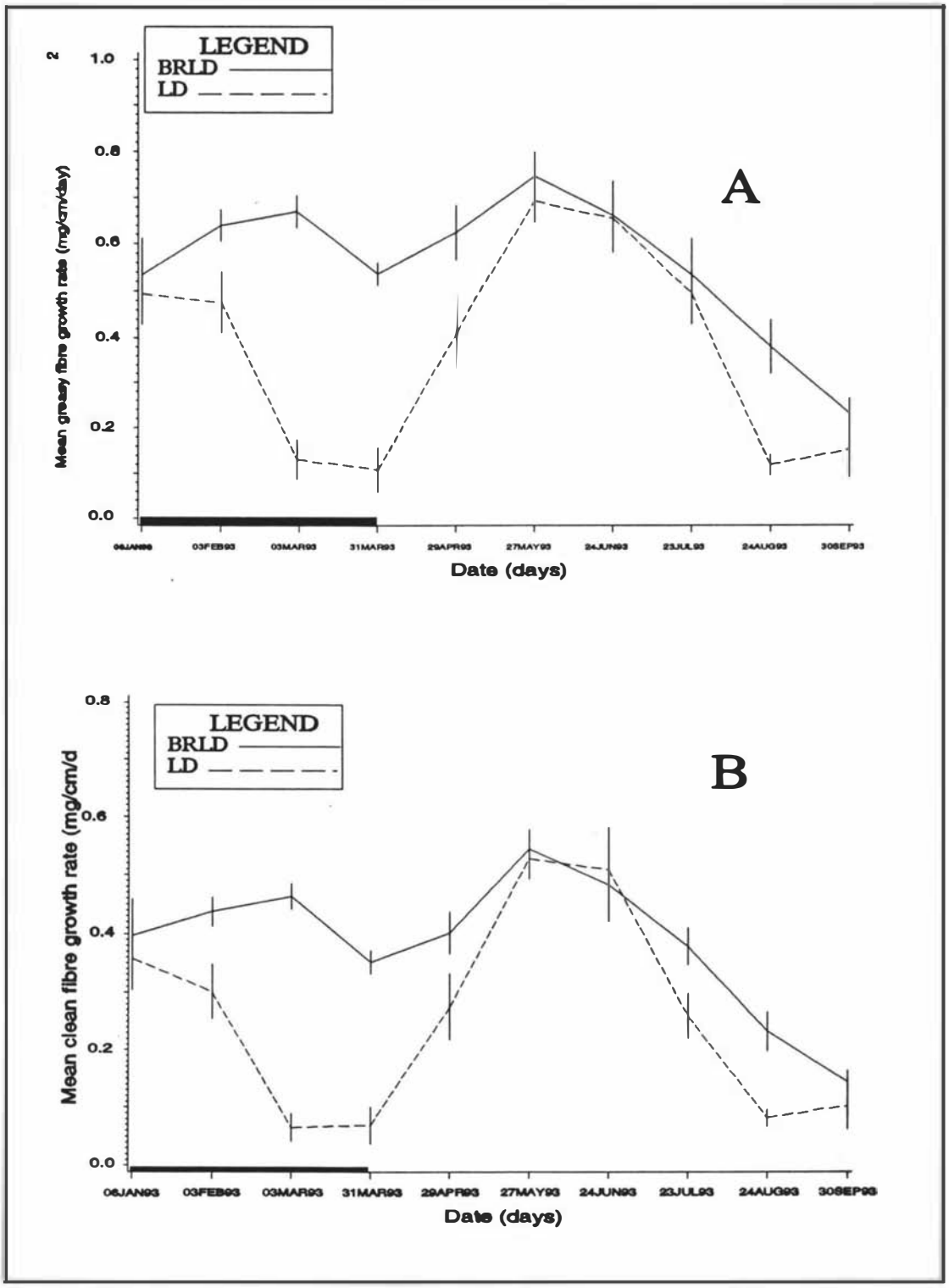

Figure 7.17: Mean greasy (A) and clean (B) fibre growth rate of sheep treated with long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD sheep) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

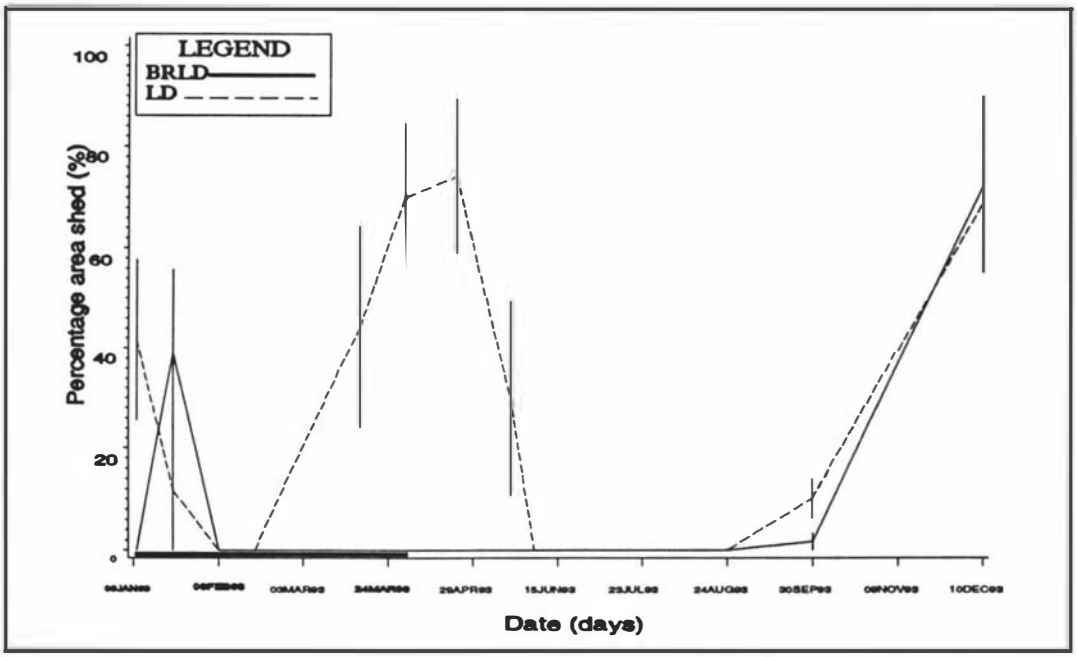

Figure 7.18: Mean percentage area of fleece shed in sheep treated with long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BR) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

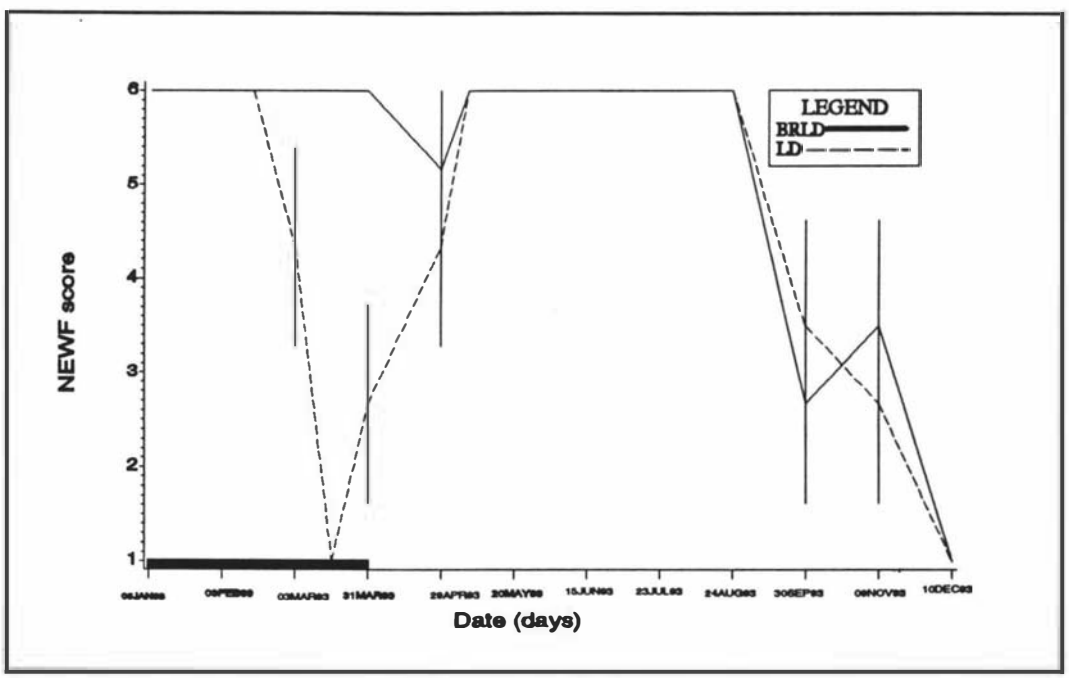

Figure 7.19: Mean newly emerged wool fibre score in sheep treated long photoperiod (LD) or long photoperiod and long-acting bromocryptine (BRLD) at two weekly intervals. Horizontal bar is period of treatment and vertical bars are standard errors.

7.5 DISCUSSION

7.5.1 Pre-treatment period

Constant short photoperiod treatment of Wiltshire sheep and down-producing goats, from the winter solstice until shortly after the summer solstice, failed to prevent fleece shedding during spring. Despite the short day treatment most goats had a characteristic decline in DL indicative of fleece shedding and all sheep showed some fleece casting at the body extremities. However, shedding did not progress to completion in most animals before new growth began. By the end of the pre-treatment period, primary and secondary follicles of sheep and secondary follicles of goats were largely fully active. However, PA of goats was more variable at the start of the treatment period. Asynchronous primary follicle cycling was also apparent in Wiltshires during short day pre-conditioning (Pearson *et al.*, 1995). At the start of the treatment period the down fleece of goats had reached 50 mm in length. In natural photoperiods the down fleece would be typically less than 10 mm shortly after the summer solstice (McDonald *et al.*, 1987; Betteridge *et al.*, 1988; Chapter 2, 3). During the short day pre-treatment period, plasma PRL concentration (20-40 ng/ml) was also higher than normally expected (<20 ng/ml) in short photoperiod for goats (Maeda *et al.*, ; Kloren, 1991) and Wiltshire sheep (Lincoln, 1990; Pearson *et al.*, 1995). However, assay differences may accound for this variation. Within this experiment, plasma PRL concentrations in goats on 6 January were high, particularly in two individuals, and this may have been associated with stress because the goats had been randomised in new pen groups on the day of sample collection.

It is not known why the preconditioning short photoperiod was partially ineffective in both preventing fleece shedding and suppressing plasma PRL concentrations. Plasma PRL concentration can become photorefractory to both constant short and long photoperiod treatment in a variety of animal species (Mori *et al.*, 1985; Karsh *et al.*, 1989; Badura and Goldman, 1992; Martinet *et al.*, 1992). The time taken for

the PRL photorefractory condition to develop has been reported to be 4-5 months in goats (Mori *et* al., 1985, Maeda *et al.*, 1986) and 3-12 months in sheep (Howles *et al.*, 1980; Karsh *et* al., 1986; Malpaux *et al.*, 1988; Peticlerc *et al.*, 1991). Nonetheless Wiltshire sheep have, on three previous occasions, treated with constant short days for six months (Pearson *et al.*, 1993, 1994) without plasma PRL concentration becoming photorefractory, though in one experiment there was a 10 ng/ml increase in plamsa PRL concentration at the same time as control sheep reached maximum PRL concentrations (Pearson *et al.*, 1995). If the goats had become photorefractory to constant short photoperiod then it would be expected that this condition would continue during the extension of short photoperiod treatment in SD goats. This certainly was not the case for the first 2 months of additional short day treatment.

It is possible that the switch of animals between facilities in August may have triggered catagen in some follicles. In addition, there were several week-long breakdowns in the air conditioning units of the second facility which affected the temperature control. Temperature (Tamanini *et al.*, 1988) and lighting intensity (Sugawara *et al.*, 1989) can modify plasma PRL concentration and fibre growth independent of the prevailing photoperiod (Gebbie *et al.*, 1994). If a proportion of follicles entered telogen then physical shedding would have ensued when follicles were reactivated by the continuing short day signal. Or alternatively, plasma PRL concentration was effectively controlled and plasma PRL concentration is not required for follicle reactivation and associated shedding (Pearson *et al.*, 1995).

The consequence of follicle cycling during the short day pre-treatment photoperiod was that activity levels in primary (but not secondary) follicles differed in the two species at the start of the treatment period. Primary follicles continued to randomly cycle in goats, and to a lesser extent in BRLD sheep, during the extended short-day treatment. Random follicle cycling may lead to differences in the duration of anagen between the species which could affect their responsiveness to photoperiod and other

hormonal manipulation (Pearson *et al.*, 1994).

7.5.2 Treatment period

Following a switch from short to long photoperiod, plasma PRL concentration increased and anagen primary and secondary follicles, of both sheep and goats, entered telogen between 4 to 6 weeks after the photoperiodic reversal. An associated inhibition of fibre growth was detected after 10 weeks. As has been shown in previous Chapters (Chapter 3,6) shedding occurred in close association with follicle re-entry into anagen and was progressing at maximum rates 12 weeks after the photoperiod reversal. By 16 weeks, follicles were fully active and FGR reached maximum levels 21 weeks after the photoperiod reversal.

It has been previously reported that a switch from short to long photoperiod induced moulting and new fleece growth in Soay sheep (Ryder, 1976; Lincoln *et al.*, 1980) and, in the absence of histological measurement, it was assumed that long photoperiod activated quiescent follicles. Certainly, telogen follicles of down-producing goats were initially activated and then deactivated by long photoperiod treatment during spring (Chapter 6). However, in down-producing goats and Wiltshire sheep with predominantly active follicles, in this and other experiments (Pearson *et al.*, 1995), the preeminent outcome of a switch from short to long photoperiod is the induction of catagen and subsequent entry into telogen. A switch from short to long photoperiod was also inhibitory to anagen follicles of Limousine sheep (Rouget, 1961; Allain *et al.*, 1986).

In natural photoperiods down goat follicles are largely inactive during increasing spring equinox photoperiods and follicles become quiescent after the autumn equinox during declining photoperiods (Chapter 2, 3). If long photoperiods are to have a role in the induction of catagen under natural photoperiods in down goats follicles, then the terminatation of summer down growth and subsequent activation of follicles for

winter down growth are the likely key fibre growth events. This may be also the case in Wiltshire sheep but, in contrast to goats, follicles in many Wiltshires activate in spring prior to spring equinocal increases in plasma PRL concentration (Pearson *et al.*, 1995). These follicles may subsequently be induced by increasing spring photoperiods to undergo a follicle cycle, thereby synchronising follicle activation with the spring equinox (Pearson *et al.*, 1995).

The maximum FGRs attained in LD goats were almost double those measured in untreated goats for late May photoperiods (Chapter 6). It appears that the switch from short to long photoperiod triggered a cycle of fibre growth which was less responsive to the prevailing photoperiod. In Soay sheep, skin sections collected in autumn and grafted on sheep in winter remained active independent of the surrounding quiescent follicles which suggested, that once growth had been initiated, it was unimpeded by declining photoperiod (Ryder and Priestley, 1977). A switch from short to long photoperiod in early January also entrained the wool growth cycle and resulted in higher wool growth rates during the winter in comparison to untreated control sheep of both shedding and non-shedding sheep breeds (Pearson *et al.*, 1994). It appears that seasonal FGR patterns may be the consequence of follicle synchronisation in response to a past photoperiodic signal rather than a continuing response to the prevailing photoperiod.

The follicles of both sheep and goats responded similarly to the short-long photoperiod reversal but some minor differences were detected. Following the photoperiod reversal, the fall to minimal PA and SA levels was delayed by two weeks in Wiltshires compared to goats. Minimal PA and SA of goats and PA of sheep were between 8 and 14%, however SA of sheep only reached minimal levels of 34%. The apparent failure of a switch from short to long days to induce entry to telogen in approximately a third of the Wiltshire sheep secondary follicle population, and the delay in entry to telogen of the remainder of the sheep follicles, suggested that in the Wiltshire sheep, compared to the down goat, follicle population was less responsive

to photoperiod manipulation. However, observation of Wiltshire follicles undergoing a similar photoperiod reversal has revealed that all follicles enter catagen and then telogen when viewed in longitudinal section but, due to imperfect synchronisation and limitations in the transverse section system, it appears that some folicles are in anagen at each date (Parry *et al.*, 1995).

The magnitude and pattern of follicle activity changes in this experiment were similar to those observed for other NZ Wiltshires treated with similar photoperiod reversal (Pearson *et al.*, 1993; Craven *et al.*, 1994). However in individual animals in this experiment it took 16 weeks before full activity was reinstated following the photoperiod reversal and, in others, this occurred after only 5 to 7 weeks (Craven *et al.*, 1994). The 16 week follicle cycling period was more symptomatic of a 3 compared to a 6 month short day pre-treatment period (Craven *et al.*, 1994).

Long photoperiod is thought to regulate fibre follicles via increases in plasma PRL concentration (Lynch and Russell, 1989; Lincoln, 1990; Kloren, 1991; Pearson *et al.*, 1993; Dicks, 1994, Chapter 3, Chapter 6). In goats, the long photoperiod treatment elevated plasma PRL concentration to levels previously reported for both constant long and summer solstice photoperiods (Buttle, 1974, Chapter 6). However, in Wiltshire sheep, peak plasma PRL concentration (208 ng/ml) in this experiment, was lower (240-328 ng/ml) than that recorded for other NZ Wiltshire sheep undergoing similar photoperiod reversals (Craven *et al.*, 1994). The lower peak in this experiment may have contributed to the extended duration for follicle cycling. It is not likely that assay variability accounted for the differences in peak plasma PRL concentration as all PRL assays in NZ animals were conducted through a common laboratory. Rather it is probable that the higher reported peak plasma PRL concentrations of other experiments ensued when long photoperiod treatment was applied by release outdoors. In this experiment the animals were retained indoors for long photoperiod treatment in artificial lighting, and constant high temperature.

As in many species (ferrets'; Ryan and Robinson, 1989; hamsters'; Steger *et al.*, 1991) plasma PRL concentration reached a maximum 4 weeks after a switch from short to long photoperiod in both goats (Chapter 6) and Wiltshire sheep (Craven *et al.*, 1994). However, peak plasma PRL concentrations were approximately 40 ng/ml higher in sheep compared to goats. Therefore the diminished and delayed response to long photoperiod by the Wiltshire sheep compared to the goat follicle, was not due to either lower plasma PRL concentration or a delay in attainment of peak plasma PRL concentration. In support of this argument, peak plasma PRL concentration has found to vary in shedding and non shedding sheep breeds but differences in peak values were not associated with any aspects of the seasonal wool growth cycle (Lincoln, 1990). It is possible that plasma PRL concentration increased more quickly in goats than in sheep during the first two weeks following the switch to long photoperiod. However, blood samples were not collected over this time. Certainly the decline in plasma PRL concentrations following the cessation of the long day treatment was slower in LD sheep than in LD goats. It is also possible that the Wiltshire sheep follicle is inherently less responsive to photoperiod change than the goat follicle.

During long photoperiod treatment, the close association between increases in plasma PRL concentration and catagen induction in follicles suggested that plasma PRL concentration may be the endocrinological transmitter of the inhibitory long photoperiod signal. In this experiment, bromocryptine treatment unequivocally prevented entry into telogen by anagen follicles of Wiltshire sheep treated with long photoperiod. In Wiltshire sheep, bromocryptine treatment suppressed plasma PRL concentrations to less than 1 ng/ml and follicle activities remained at high levels despite the prevailing long photoperiod treatment. In addition, following the cessation of the treatment and release into declining post-autumn equinocal photoperiods (12L:12D), plasma PRL concentration failed to increase, follicles remained active and shedding was not observed until the following spring. Pearson *et al.*, (1993) also suppressed plasma PRL concentration in Wiltshire sheep, using bromocryptine, following a switch from short to long photoperiod in early January and found that

entry into catagen was delayed. In that experiment, bromocryptine treatment was applied for 1 month compared to the 3 months in this experiment, and on cessation of bromocryptine treatment, sheep were released into pre-autumn equinocal photoperiods (14L:10D). Under these post-treatment photoperiods, plasma PRL concentrations rose to 125 ng/ml and follicles entered telogen (Pearson *et al.*, 1993).

In goats, the effect of bromocryptine treatment was not as straightforward as in sheep. In BRLD goats, both secondary and primary follicles underwent a sustained decline in follicle activity which was of slightly reduced magnitude and it was delayed by one month compared to LD goats. In association with the decline in follicle activity in LD and BRLD goats, FGR declined and the down fleece stopped elongating. In contrast, during short day treatment follicles remained active, FGRs were high, and the down fleece continued to increase in length during the treatment period.

During long photoperiod treatment follicles reactivated, FGR increased, mean DLs decreased and the fleece was shed. However, follicle reactivation in the BRLD group did not occur in the majority of the goats until after the cessation of treatment with a concomitant delay in DL decline and shedding. Primary follicle reactivation was apparent in BRLD goat #16, and in both #16 and #20 secondary follicles had reactivated prior to the cessation of treatment. It is therefore possible that bromocryptine treatment only delayed the normal passage of events associated with long photoperiod. The cessation of treatment in SD goats triggered a small decline in follicle activity and the fleeces of SD and BRLD both shed at comparable times.

In goats, bromocryptine treatment in long photoperiods neither simulated the effects of short photoperiods nor completely prevented the expression of long photoperiod in follicle fibre growth (Chapter 6 and 7). In this Chapter, anagen follicles of BRLD goats entered catagen, but unlike LD goats, reactivation was delayed. In the previous Chapter, telogen goat follicles treated with both long photoperiod and bromocryptine

during spring were activated but failed to enter catagen as did LD goats until after the cessation of treatment. Given the current level of knowledge it is difficult to reconcile these two results. There are no other published data which have shown that bromocryptine treatment activated telogen follicles and therefore did not impede shedding (Chapter 6). In all other experiments in goats, the effect of suppressing plasma PRL concentration with bromocryptine was one of delaying follicle reactivation and thereby delaying shedding (Kloren,1991; Lynch-Dicks, 1994; Chapter 7). Shedding was also averted in BRLD sheep but this was due to a failure of follicles to enter telogen rather than a delay in reactivation.

Plasma PRL concentrations engendered by bromocryptine treatment did differ between the sheep and goats. It is assumed that this differences represent true differences and are not associated with assay differences in recovery of PRL in the assay between the two species. If this is a true effect then In sheep, long-acting bromocryptine applied on a liveweight basis reduced plasma PRL by an additional 8 ng/ml compared to concentrations in goats. Bromocryptine administration has consistently reduced plasma PRL concentrations to lower levels in sheep than in goats throughout this research programme (Chapter 4, 5, 7). Bromocryptine is a dopamine receptor agonist (Javis *et al.*, 1988) and the dopamine pathway may be of more importence in regulating plasma PRL concentration during long days in sheep than in goats. Species differences in the dopamine pathway are supported by the observation that reductions in dopamine concentrations in the hypothalamus occur in the ram but not in the bull during long compared to short days (Ssewannyana and Lincoln, 1990; Zinn *et al.*, 1991).

It is not known whether the 8 ng/ml difference in plasma PRL concentration between the two species is physiologically significant and if it accounts for the species differences in fibre growth during bromocryptine treatment. Sheep breeds with higher winter wool growth rates also have higher winter plasma PRL concentrations (Lincoln, 1990; Pearson *et al.*, 1994). The addition of PRL to the media of cultured

follicles of goats (Ibraheem *et al.*, 1993; Galbraith *et al.*, 1994) and deer (Thomas, 1993) increases linear fibre growth rates. Yet in BRLD goats plasma PRL concentration was higher than in sheep, but FGR's were lower.

Follicle activity during treatment remained high in SD goats but declined in BRLD goats. There was a tendency for plasma PRL concentration to be higher during bromocryptine than during short day treatment. However, this difference was largely attributable to the inexplicable failure to control plasma PRL concentration in bromocryptine-treated goat #20. This goat had the same pattern of follicle activity as LD goats (A7.3) but its inclusion in the BRLD fibre data analysis did not alter the statistical parameters of the BRLD group. The difference in mean plasma PRL concentration between SD and BRLD goats, following the removal of goat #20, was only 1 ng/ml which failed to achieve significance and is unlikely to account for the differences in follicle activity between BRLD and SD goats.

Despite generating comparable plasma PRL concentrations, bromocryptine treatment has also been shown to be less effective than either short days or melatonin treatment in provoking autumn moult in minks (Allain *et al.*, 1994) or winter fleece growth in deer (Milne *et al.*, 1990). It is possible that bromocryptine influences other seasonal hormones which may be involved in seasonal fibre growth cycles. In deer, for instance bromocryptine treatment, in addition to suppressing plasma PRL concentration, also decreased thyroxine concentrations (Bubenik *et al.*, 1985; Milne *et al.*, 1989). However, thyroxine and growth hormone concentrations were unaffected by bromocryptine treatment in goats (Kloren, 1991; Chapter 4).

While immunoactive plasma PRL concentrations were similar in both SD and BRLD goats, it is possible that bioactive concentrations of plasma PRL differed. Within the pituitary of sheep, the PRL molecule exists in different molecular forms in different seasons (Farkoldh *et al.*, 1979; Stroud *et al.*, 1992). PRL molecules differ in molecular weight (Ben-Jonathan, 1985; Lamberts and MacCleod, 1990; Mana *et al.*,

1992), and net charge (Takahashi, 1992) and some PRL molecules can be glycosylated (Ostrom, 1990). These forms have different immunoactive and biological activities (Wallis, 1988; Stroud *et al.*, 1992). However, higher molecular weight and glycosylated PRL variants were equally prevalent in winter (Stroud *et al.*, 1992) and under the inhibiting action of dopamine (Shin *et al.*, 1987). 'Winter PRL', in comparison to 'summer' PRL, was found to be more biologically active in NB_2 cell binding assays (Stroud *et al.*, 1992). Conversely, mammary gland binding assays have shown that monomeric and nonglycosylated PRL are more biologically active than 'big' PRL variants (Lamberts and MacCleod, 1990; Onstrom, 1990). The reviewed data collected to date are inconclusive regarding the likelihood of PRL biological activity differing under photoperiod and bromocryptine treatment. In sheep immunoactive concentrations of plasma PRL were so low (often <0.6 ng/ml) that it is unlikely that any changes in PRL biological activity induced in this treatment would have any physiological impact. However, this may not be the case in goats.

The failure to achieve equivalent plasma PRL concentrations (if this is not an assay effect) in the two species constrains the interpretation of the data in this experiment. For instance, it has been assumed in this discussion that the cessation of bromocryptine treatment in BRLD goats triggered follicle changes but it is possible that bromocryptine treatment only delayed follicle response to long photoperiod. Despite effective plasma PRL concentration suppression in goat #16, follicle reactivation did occur prior to treatment cessation. However the timing of follicle reactivation events was similar in SD and BRLD goats and this indicated that cessation of treatment was the likely stimulus for change in some goats in both these treatment groups. Follicle reactivation events such as date of maximum shedding, minimal DL, maximum greasy FGR, and maximum follicle activities were similar in SD and BRLD goats. However, fewer follicles were involved in these events in SD compared to BRLD goats.

There was an advance in the onset of the next cycle of fibre growth in the subsequent

spring in LD-treated sheep and goats. In LD animals FGR had fallen to a greater extent in August than in BRLD animals. Down SS was also greater in November in LD compared to BRLD-treated goats. In comparison to patterns of FGR in natural photoperiod, it appears that LD-treated goats and sheep reflect the normal pattern of growth (Parry *et al.*, 1991, Chapter 6) and the decline in growth as spring approached was delayed in BRLD and SD goats. In BRLD sheep and goats, and SD goats, between 12 and 60% of the follicles entered catagen and were reactivated in late May. These newly activated follicles may have been initially unresponsive to 'switch-off' photoperiod signals. In an analogous situation, goats treated with melatonin for 8 weeks in spring grew two down fleeces, the second of which was initiated in autumn and shed one month later than that of non-treated fleeces (Betteridge *et al.*, 1987; O'Neill *et al.*, 1992). An alternative hypothesis is that the responsiveness of the pituitary may have been altered by the previous treatments with LD animals being subsequently more responsive, and SD and BRLD animals less responsive, to prevailing photoperiod. Similarily, in ferrets long-term shifts in follicle cycles occur in ferrets treated with melatonin in autumn (Nixon *et al.*, 1995).

There is ample evidence for the duration of daylength relative to a melatonin sensitive phase being the basic system for photoperiod interpretation (Kennaway *et al.*, 1987; Pelletier and Thimonier, 1987). However, this is a simplification of the photoperiod sensing system as the interpretation of daylength signal is also dependent on the previous photoperiodic history (Deveson *et al.*, 1992). This was demonstrated in SD goats which on release from short (8L:16D) into natural (12L:12D) photoperiods had a mean plasma PRL concentration of 30 ng/ml while in LD goats (16L:8D) plasma PRL concentration was only 3 ng/ml. In SD goats, this post-treatment rise in plasma PRL concentration induced catagen in approximately 30% of SD follicles. In addition there was a small rise (5 to 15 ng/ml) in plasma PRL concentration late in the treatment period which may been due to the development of photorefractoriness (Mori *et al.*, 1985; Maeda *et al.*, 1986). The relative small increase in plasma PRL concentration and the relatively long period over which they occurred may have

contributed to the more asynchronous onset of catagen in secondary follicles in SD goats (Figure A7.3). Over the two dates measured in the post-treatment period there was no evidence that increases in plasma PRL concentrations preceded follicle activation in BRLD goats.

The suppression of plasma PRL concentration using bromocryptine completely prevented the inhibitory effect of long photoperiod on anagen Wiltshire sheep follicles. However, bromocryptine treatment could only partially mask the inhibitory influence of long photoperiod on goat follicles. Unfortunately, interpretation of species differences was compromised by poor plasma PRL concentration control both during the short-day pre-treatment period and in the two species during bromocryptine treatment. It is difficult to postulate an unifying theory for the role of plasma PRL concentration in regulating fibre growth in down goats and Wiltshire sheep. If, as it appears in Wiltshires, plasma PRL concentration is largely inhibitory to fibre growth then in goats either some as yet unknown changes in PRL bioactivity transpire in BRLD goats or additional hormones have inhibitory or stimulatory effects during long or short photoperiods respectively. Alternatively, if the cessation of bromocryptine treatment does not trigger follicle change then bromocryptine treatment may only delay the expression of long photoperiod in goats. Finally, it cannot be ruled out that bromocryptine may act on the follicle independently of its action on plasma PRL concentration. In order to rule out this possibility further studies should involve treatment with PRL directly.

7.6 CONCLUSIONS

Four to six weeks following a switch from short to long photoperiod anagen follicles of both goats and Wiltshire sheep entered telogen and then reentered the anagen phase. Bromocryptine treatment unequivocally precluded the catagenic effect of long photoperiod on anagen Wiltshire sheep follicles. In goats, bromocryptine treatment during long photoperiod did not prevent follicles entering telogen but may have

delayed telogen-anagen follicle transitions characteristic of long photoperiod. In contrast short-day treated goat follicles remained active. Plasma PRL concentration, which probably mediated the action of long photoperiod in Wiltshire follicles, may have a role in mediating the anagenic effect of long photoperiod on telogen goat follicles.

CHAPTER 8

THE EFFECTS ON FIBRE GROWTH OF WHOLE BODY AND LOCAL SKIN INFUSIONS OF PROLACTIN IN WILTSHIRE SHEEP AND DOWN-PRODUCING GOATS.

8.1 ABSTRACT

Two experiments determined the effects of local skin and whole body PRL infusions on follicle and fibre growth in Wiltshire sheep and down-producing goats treated with short days. On 15 July 1993, 27 non-pregnant, mixed-age, down-producing does and 12 six and seven year old Wiltshire ewes were placed indoors and pre-conditioned with short photoperiod (8L:16D, lights on 0900 to 1700 hours) and low temperature (10-15°C) treatment.

In experiment 1, from 02 February 1994 to 3 March 1994, two treatment groups of goats continued under short photoperiod and low temperature (10-15°C) with one group receiving no additional treatment (SD, n=5) while the other received a systemic infusion of PRL (0.5 mg/kg$^{0.75}$/day) (P, n=5). The third group was treated with long photoperiod (16L:8D, lights on 0400 h) and low temperature (10-15°C) (LD, n=5).

Mean log transformed plasma PRL concentration during the treatment period in SD (10 (8-13) ng/ml) goats was lower than in either LD (52 (42-72) ng/ml, $P<0.01$) or P (29 (23-36) ng/ml, $P<0.05$) goats. In the post-treatment period, mean log transformed plasma PRL concentrations were similar in all treatment groups.

PA declined earlier during the treatment period in LD goats compared to that of SD goats (Trt*time, $P<0.05$). During the post-treatment period, mean PA reached minimal levels (21-30%) on 20 March in LD goats but not until 8 April in P and SD goats, with PA returning to levels in excess of 94% by 6 May in all treatments.

On 3 March 1993, mean SA in LD goats had declined to be 10±6% (P 98±6%; SD 90±6%, $P<0.01$). On 17 April SA of LD goats reached its lowest mean level (2±17%) and mean SA began to decline in P and SD goats (P goats 47±19, SD goats 48±17%). Mean SA in P and SD goats reached a minima (12-14%) on 8 April at which time SA in LD goats had returned to high levels (99±7%, $P<0.001$). By 6 May 1994, mean SA in all three treatment groups was in excess of 99%. The fibre growth (DL, GL, FGR, SS, NEDF) decline was 2 to 6 weeks earlier in LD goats compared to P and SD goats which were similar.

In experiment 2, down goats (n=8) and Wiltshire sheep (n=8) were from 6 February until 12 March 1994 progressively fitted with catheters to both sides of the descending lateral branches of the deep circumflex iliac veins and arteries which supply blood to an area of skin on the flank of the animal. Ovine PRL (1.87-5.6 ug/hr) or saline were infused locally to the skin and 12 animals received intramuscular injections of long-acting bromocryptine (Parlodel LA).

Mean plasma PRL concentration was 9 (7-81) ng/ml, 110 (83-145) ng/ml and 43 (35-57) ng/ml in blood collected from the venous jugular, PRL- and saline-infused iliac catheters respectively ($P<0.01$). The mean blood flow through the skin patch was 12±4 ml/min and 6±1 ml/ml in goats and sheep respectively ($P<0.05$).

Mean PA and SA was in excess of 83% at the start of the local PRL infusion. Mean PA was 70±10% and 53±15% and mean SA was 76±10% and 56±9% at the end of the infusion in saline-infused and PRL-infused sides respectively and was unaffected by treatment. The overall decline in SA was greater in sheep (56±8%) compared to goats (10±7%) ($P<0.05$). Mean patch fibre growth rate was unaffected by either treatment or species. The direct infusion to the skin of PRL (from two different sources) resulted in the visible swelling of the proximal hind-leg 6.5±0.8 days after the start of the infusion. This effect was independent of species of animals, PRL source, parlodel LA treatment. Bacterial culture of the PRL infusates established infusate sterility.

In conclusion, follicle and fibre growth were unaffected by either systemic or local infusions of PRL in down-producing goats and Wiltshire sheep. The infusion of PRL directly to the skin caused an extreme local tissue reaction.

8.2 INTRODUCTION

In temperate mammals seasonal changes in fibre growth and plasma PRL concentration are governed by photoperiod (Leshin and Jackson 1987; Lincoln 1990). Plasma PRL concentration has an established causal role in the photoperiodically linked moulting and regrowth of pelage in mustalids (Martinet *et al.*, 1984). In the preceding Chapter, it was demonstrated that, following a switch from short to long photoperiod, the suppression of plasma PRL concentration by bromocryptine prevented the normal follicle sequence of telogen followed by anagen in Wiltshire sheep follicles. In down-producing goats, bromocryptine treatment did not prevent follicles entering telogen but did delay follicle reentry into anagen. Bromocryptine

is widely used as a suppressor of plasma PRL concentration, but has side effects independent of its effects on pituitary PRL secretion (see Chapter 4). The manipulation of plasma PRL concentration using bromocryptine does not, therefore, provide unequivocal evidence of a role for plasma PRL concentration in inducing catagen in anagen follicles. Only treatment with biologically active-PRL can establish a causative link. In this experiment, whole body infusions of PRL were used to simulate long photoperiod plasma PRL concentrations in down-producing goats. The large body size of the Wiltshire sheep prohibits the whole body treatment with PRL.

While there is strong evidence for a role of plasma PRL concentration in seasonally shedding animals (Kloren and Norton, 1993; Pearson *et al.*, 1993; Dicks, 1994) it has not been established whether PRL acts either directly on the hair follicle or via an intermediatory pathway to affect fibre growth. PRL does bind directly to PRL receptors in a large number of target organs (Forsyth *et al.* 1985; Johnson and Hart, 1985) including the follicle (Choy *et al.*, 1994). In this experiment, the effects of PRL infusions on follicle activity of Wiltshire sheep and goats were compared.

8.3 METHODS

8.3.1 Animals and management: From 15 July 1993 until 8 January 1994, 27 non-pregnant, mixed-age, down-producing does and 12 six and seven year old Wiltshire ewes were contained in three rooms at the Animal Physiology Unit, Massey University, Palmerston North. The animals were penned in species groups of similar liveweight (3 sheep or 4-5 goats/pen). From 8 January 1994 the animals were contained in individual sheep metabolism crates and either released on to pasture on 3 March 1994 (Experiment 1) or progressively removed to alternate controlled environment facilities at AgResearch, Palmerston North from 6 February (Experiment 2). While indoors the animals were fed energy equivalent portions of chaffed meadow hay, maize and pelleted concentrate feed (70% lucerne, 30% barley)

at 1.1 times maintenance with water on offer *ad libitum.*

From 15 July 1993 and until treatment application, all animals were treated with short photoperiod (8L:16D, lights on 0900 to 1700 hours) and low temperature (10-15°C) indoors. All natural light was excluded from the rooms and illumination was by fluorescent tubing with additional supplementary 200 watt bulbs on a time switch.

On 7 January 1994 the animals were blocked on room number and allocated to treatments and experiments after balancing for mean primary and secondary follicle activity (measured on 9 December 1993 and 5 January 1994), mean down length (5 January 1994) and liveweight (5 January 1994).

8.3.2 Experiment One

8.3.2.1 Experimental details: From 02 February 1994 to 3 March 1994 two treatment groups of goats continued under short photoperiod and low temperature (10-15°C) with one group receiving no additional treatment (SD, n=5) and the other a systemic infusion of PRL (P, n=5). The third treatment group of goats was treated with long photoperiod (16L:8D, lights on 0400 h) and low temperature (10-15°C) (LD, n=5). From 3 March 1994 goats, were grazed on pasture out-of-doors.

On 29 January 1994, catheters were fitted to one (LD and SD) or both (P) external jugular veins of the goats. The width of the metabolism crates was then reduced by 30 cm for P goats and all goats were double tethered by the neck. The PRL-infusing catheters were checked daily for patency by pump reversal and non-patent catheters were routinely replaced.

PRL was administered as a continuous infusion of 0.5 mg/kg$^{0.75}$/day of ovine PRL (L6520, Sigma Chemical Company, St Louis, Mo, USA) via a variable speed perstaltic pump (Watson Marlow Ltd, Falmouth, Cornwall, TR1 14RU, England) and

pump tubes (LK116-0532-040, Elkay Products, Shrewsbury, MA, USA) at a rate of 7.5 ml/hr. PRL infusates were prepared at 5 day intervals in autoclaved, siliconized glassware, using millipored reagents in a laminar flow cabinet to maintain sterility. The PRL was dissolved in 0.03 M $NaHCO_3$ (2.5 mg/ml) at pH 10.8. The PRL was stored at 4°C and, immediately prior to administration, PRL volumes were made up to infusion concentrations by dilution with sterile isotonic saline (9 g NaCl/l).

8.3.2.2 Measurements: During the pre-treatment period, gross fibre growth was measured at six weekly intervals, at the beginning and end of the treatment period and thereafter in the post-treatment period at two to three weekly intervals until 10 June 1994. Stretched down and guard hair length were measured on the left side of the goat and DL and GL were calculated (Section 2.2.2.1). Fibre growth rate (FGR) was determined from within a defined measured 12x12 cm square on the right midside of the goat and greasy FGR was calculated (Section 7.3.3).

Skin snip biopsies were collected at 6 weekly, 5-6 days and 2-3 weekly intervals during the pre-treatment, treatment and post-treatment periods respectively. Skin samples were fixed in Bouins solution (v:v 70% saturated aqueous picric acid, 25% formalin, 5% glacial acetic acid) for eight hours and transferred to 70% alcohol for storage. Skin samples collected on 11 and 31 January 1994, on 9, 16, 21 February and on 3 March 1994, and post-treatment samples, were scored (Section 2.2.3.2) for the presence of newly emerged down fibres (NEDF's). Skin samples collected on 31 January, 16 and 21 February and on 3, 20 March, 8 April and 6 May 1994 were processed through gradient alcohols and embedded, serial sectioned at 8 μm in the transverse plane and stained using the adapted Sacpic method (Section 2.2.3.3). Approximately 10 follicle groups were scored for activity state (Section 2.2.3.3) and mean proportions of follicles which were active primary (PA), active secondary (SA), active+brush primary (PAc+brush) and active+brush secondary (SAc+brush) were calculated.

Blood samples were collected at six weekly intervals during the pre-treatment period, daily during the treatment period, and at two-three weekly intervals in the post-treatment period. Blood samples were collected at 1300 h by jugular venipuncture during the pre- and post-treatment periods. During the PRL infusion, blood samples were drawn daily from the contralateral venous jugular catheter. The 5 ml blood samples were collected on ice into EDTA vacutainers. Plasma was removed by centrifugation at 3000 g at 4° C and stored at -8°C pending radioimmunoassay for plasma PRL concentration which was conducted at AgResearch Ruakura using the protocol outlined in Section 2.3.2.3.

Blood samples were collected by jugular venipuncture into EDTA tubes on 2 March (the penultimate treatment day), and 9 March 1994 for determination of blood haematology (Section 8.3.3.3).

8.3.3 Experiment two

8.3.3.1 Animals and surgical modifications: From 6 February until 12 March 1994, sheep and goats were relocated to the controlled environment rooms, in groups of 2-4, at AgResearch, Grasslands, Palmerston North.

The animals were fitted with fine-bore polyvinylchloride catheters, under fluorothane anaesthesia, into both sides of the descending lateral branches of the deep circumflex iliac vein (V) and artery (A) (Harris *et al.*, 1989). A small cut was made in the iliac vessel and the tapered catheter was advanced 2-6 cm proximally to the region of the sub-iliac lymph node and secured in place. The catheter tubing was passed under the skin to emerge from the central back of the animal in the region of the ninth thoracic vertebra. Inert dyes (Patent Blue V (2 ml) or (from 2 March 1994) Evans Blue were injected into the arterial catheters and flushed through with saline. The appearance of colour on the skin surface confirmed preparation patency and patch dimensions. At weekly intervals, the infusion zone was reconfirmed with inert dyes. All animals were given a broad-spectrum antibiotic injection (Streptopen, Pitman Moore NZ Ltd,

Upper Hutt, New Zealand) on each of 3 days following surgery. Patency of all catheters was maintained by continuous infusion of sterile, heparinized (30 i.u./ml) saline (9g NaCl) via a peristaltic pump (A 60 ml/d, V 150 ml/d). An external jugular vein catheter was fitted and patency was maintained by daily flushing with heparinized (30 i.u./ml) saline.

To minimise the confounding effect of potential whole-body increases in plasma PRL concentration associated with surgical stress and PRL movement from the skin to the whole body, on the day preceding the infusion, animals (with the exception of Tag # 674, 208, 502, 607; Table 8.1) were given a deep intramuscular injection of long-acting bromocryptine (Parlodel LA, Sandoz Pharmaceuticals Ltd, Auckland, NZ) at a rate of 3.9 $mg/kg^{0.75}$ and 5.0 $mg/kg^{0.75}$ for sheep and goats respectively (for formulation see Section 5.3).

Three to four days after surgery the A catheters received a continuous infusion of *para*-amino hippuric acid (PAH, 0.1 grams/500 ml infused at 60 ml/d) in heparinised (1000 iu/ml) sterile saline (9g NaCl) for 10 minutes. The animals were primed (sheep, 10 ml; goats 5 ml) with heparinised (1000 iu/ml) saline and after the 10 minute PAH infusion period, 1 ml duplicate blood samples were taken simultaneously from V and A catheters. Samples were collected by peristaltic pump at 0.36 g/min via PVC tubing into tubes on ice containing 10 μl heparinized (1000 i.u./ml) saline.

A 5 ml blood sample was also drawn from the jugular of non-treated animals of equivalent species. The concentration of PAH in the venous blood, determined by laboratory assay, was used to both calculate blood flow rate (Section 8.3.3.4) through the skin preparation and also to indicate physiological connection between A and V catheters.

At the termination of the PRL-infusion period, each animal was injected with a lethal dose of barbiturate (Sagatal; May and Baker, Dagenham, Essex). At the same time,

coloured latex was infused rapidly into functional A's and its presence within blood vessels of the infusion area was noted.

8.3.3.2 Experimental details: The infusion of PRL directly into the skin resulted in an extreme and unexpected tissue reaction. In order to overcome this reaction, as the experiment progressed, changes were made to the PRL source, infusion rate and infusion duration and to the use of Parlodel LA. The generalised description of the treatment protocol is given below but specific details on individuals are provided in Table 8.2.

A continuous infusion (1.87-5.6 μg/hr, Table 8.1) of ovine PRL (Source 1: L6520, Sigma Chemical Company, Source 2: oPRL-19, NIDDK, Baltimore, Maryland, USA, Source 2 only Tag # 540, 535, 479, 555) was administer into a single lateral iliac artery at a rate of 8 ml/hr via a peristaltic pump. Where opposing A catheters were available (13 out of 16 animals) these were infused with heparanized (30 i.u./ml) saline. The side for the PRL infusion was selected on best blood flow and infusion zone size.

Initially, the PRL infusion rate was calculated to increase plasma PRL concentration of measured blood flowing through the skin site by 50 ng/ml. Later, lower PRL infusion rates were attempted (Table 8.2).

8.3.3.3 Measurements: Fibre growth measurements were collected, from within the zone defined by the Patent blue patch, at the beginning and end of the infusion period from both PRL- and saline-infused sides. Snip skin biopsy samples were collected (Section 2.2.2.4) and fixed in Bouins solution (v:v 70% saturated aqueous picric acid, 25% formalin, 5% glacial acetic acid) for eight hours and transferred to 70% alcohol for storage. Skin samples were processed throught gradient alcohols and embedded, serial sectioned at 8 μm in the transverse plane and stained using the adapted Sacpic method (Section 2.2.3.3).

Table 8.1: Description of experimental protocol in sheep and goats infused locally with PRL into the skin. Within species, surgery sequence was as per table sequence.

TAG	SPECIES	PRL Infusion rate (mg/hr)	Duration of Infusion (days)	Injected with Parlodel
20	Ovine	0.002	25	Yes
166	Ovine	0.0044	9	Yes
440	Ovine	0.003	14/8[1]	Yes
535	Ovine	0.002	22	Yes
555	Ovine	0.002	23	Yes
674	Ovine	0.002	15	No
208	Ovine	0.002	6	No
102	Ovine	0.008	8	Yes
641	Caprine	0.002	24	Yes
667	Caprine	0.006	22	Yes
600	Caprine	0.009	19	Yes
540	Caprine	0.002	23	Yes
479	Caprine	0.002	21	Yes
502	Caprine	0.002	7	No
607	Caprine	0.002	8	No
604	Caprine	0.008	6	Yes

[1] PRL infusion side was switched after 14 days to confirm tissue reaction

At the beginning and end of the infusion period in animals which had undergone an infusion period in excess of 20 days (7 of 16 animals), the site of the infusion zone, as defined by Patent Blue V staining, was sketched on the animal and fibre regrowth within the zone clipped to skin level using Oster clippers. The patch area was transferred to acetate and area determined by weight. Growth rate of greasy wool (FGR) was then calculated.

Liveweight was measured at monthly intervals from 30 July 1993 until 4 March

1994. From two days preceding the PRL infusion and thereafter daily for the duration of the infusion, blood samples were drawn by syringe from the external jugular vein. In addition, during the PRL infusion (Tag # 102, 604 had additional pre-infusion V samples) blood was drawn from patent V catheters by peristaltic pump (as for blood flow determination) and these samples were analysed for plasma PRL concentration (8 animals, n=26 samples). Blood was collected on ice into EDTA vacutainers and for the determination of PRL concentration, plasma was removed by centrifugation at 3000 g at 4° C and stored at -8°C pending radioimmunoassay. The radioimmunoassay of plasma PRL concentration was conducted at AgResearch Ruakura using the protocol outlined in Section 2.3.2.3.

From 2 March 1994, blood samples were also drawn from the jugular venous catheter (3 goats, 3 sheep) for the determination of blood haematology at days 0, 2 and 6 of the PRL infusion. In addition blood samples were drawn daily from two days prior to the infusion and thereafter daily from the jugular, saline and PRL-infused V catheters (goat #604, sheep #102) for the duration of V catheter patency (3-5 days). Air dried blood smears were made from whole blood within two hours of sample collection and a differential leucocyte count (Segmented neutrophils, lymphocytes, eosinophils, basophils) was determined under oil immersion microscopy (Batchelar Animal Health Laboratory). The blood was left to stand, serum separated from the clot and mean haemoglobin concentration (H), packed cell volume (PCV) and white blood cell (WBC) concentrations were all measured by the Coulter counter S7 modified for the analysis of animal blood (Batchelar Animal Health Laboratory, Palmerston North).

Mean cell haemoglobin concentration (MCHC) was then calculated using the following equation.

$$MCHC = \frac{H}{PCV} * 100$$

8.3.3.4 Blood flow: The rate of blood flow through the patch was determined using PAH dilution in 10 of the 16 animals. In the last 6 animals it was assumed that blood flow through the skin patch was 5 ml/min. PAH concentration was determined by precipitation of proteins in the blood sample following 1:2 (v/v) dilution with 10% trichloroacetic acid, centrifugation and mixing of the supernatant with an equal volume of 1% 4-dimethyl aminobenzaldehye in ethanol. The developed colour was read against standard samples at absorbance 470 nm. Standards were prepared under the same protocol with known amounts of PAH added to control blood samples. Blood flow (ml blood/min) was calculated using the following equation:

$$\frac{\text{Weight of PAH infused/min into catheter A}}{\text{[(wt PAH/ml blood at catheter V)-(wt of PAH/ml blood at catheter A)]}}$$

8.3.4 Statistical analysis

When data were collected over 3 or more time periods they were analysed using general linear model procedures of SAS (SAS, 1987), treatments fitted as contrasts, with repeated measures in time using the Wilks' Lambda test of significance. During the treatment period in experiment one, insufficient degrees of freedom were available to derive treatment by time interactions within treatment contrasts for plasma PRL concentration. Therefore plasma PRL concentrations were averaged over three consecutive days and these data were analysed. When two or fewer sampling periods existed (FGR) then analysis was by general linear model procedures with a split plot in time fitted where appropriate (eg SA, PA). Due to unequal variances, plasma PRL concentrations were log10 transformed prior to analysis.

Plasma PRL concentration data presented in the text are back transformed pooled least squares means with the pooled least squares standard error presented as a back transformed range.

In experiment one, the data were analysed separately for the pre-treatment (20 July 1993 to 1 February 1994), treatment (2 February-3 March 1994) and post-treatment (4 March-10 June 1994) periods. In addition, dates and magnitudes of minimum and maximum fibre events were determined for individual animals. The effects of treatment on these parameters were determined using general linear model procedures with treatments fitted as contrasts (SAS, 1987). Unless otherwise stated data presented in the text are pooled least squares means and standard errors. Raw means and standard errors are presented graphically.

8.4 RESULTS

8.4.1 Experiment one

***8.4.1.1 Pre-treatment period*:** Mean (±se) plasma PRL concentration was 9 (3-30) ng/ml, 19 (18-49) ng/ml, 9 (4-23) ng/ml on 13, 17 and 20 January 1994 respectively.

From August to October during short day treatment, fleece SS rose above 1 in 80% of the goats. In individual goats, declines in mean DL of more than 10 mm were also observed in 14 of the 15 goats. However in the two month period prior to the beginning of treatment mean SA and PA were in excess of 88%, mean DL was increasing at 10 mm per month and FGR was approximately 0.4 mg/cm^2/day (Table 8.2).

8.4.2 Treatment period

8.4.2.1 Liveweight and S/P ratio: Mean liveweight over the pre-treatment period increased from 28±2 kg on 20 July 1993 to be 31±2 kg at the start of treatment and it remained static during treatment administration.

Treatment had no effect on liveweight. The mean ratio of secondary to primary follicles was 5.7±0.4.

8.4.2.2 Plasma PRL concentrations: Mean plasma PRL concentration during the treatment period in SD (10 (8-13) ng/ml) was lower than in either LD (52 (42-72) ng/ml, P<0.01) or P (29 (23-36) ng/ml, P<0.05) goats (Figure 8,1). On the individual triplicate mean day groups (n=7), mean plasma PRL concentration in SD goats was lower (P<0.05) than in LD goats on 6 occasions and in P goats on 3 occasions, while LD plasma PRL concentrations were higher than in P goats on a single occasion (Table 8.3). In comparison to P goats, mean plasma PRL concentration of LD (P<0.05) and SD (P<0.05) goats progressively increased over the treatment period (Figure 8.1). One SD goat (Tag #537) had consistently elevated plasma PRL concentrations in comparison to the remaining four SD goats (overall mean for #537 was 43 vs 7 ng/ml).

In the post-treatment period, mean log transformed plasma PRL concentrations were similar in all treatment groups and rose from 62 (49-78) ng/ml on 9 March 1994 up to 234 (204-269) ng/ml on 8 April 1994 but then declined rapidly to reach 2.0 (1.6-2.5) ng/ml on 10 June 1994.

8.4.2.3 Primary follicle activity: At the start of the treatment period mean PA was approximately 87% and remained at more than 60% in both SD and P goats during the month of treatment. In contrast, after a month of treatment, mean PA in LD goats had declined to be 47±12% thereby displaying a different pattern with time to that of P goats (Trt*time, P<0.05) but not SD goats (Figure 8.2).

During the post-treatment period, mean PA in all treatments followed a similar pattern of decreased and then increased activity but the decline to low activity occurred earlier in LD compared to P and SD goats (Time*Trt P<0.001). Mean PA reached minimal levels (21-30%) on 20 March in LD goats but not until 8 April in P and SD goats (Table 8.3). Follicle activity in all treatments had returned to levels in excess of 94% by 6 May (Figure 8.2).

The mean proportions of PAc+brush follicles during the treatment period were similar in all three treatment groups (Figure 8.3) and ranged from 0 to 14%. In the post-treatment period, a large peak in mean PAc+brush follicles was apparent in LD goats (41±10%) on 8 April and in P goats (35±13%) and SD goats (16±13%) on 6 May.

8.4.2.4 Secondary follicle activity: By the start of the experimental period secondary follicles were approaching full activity (SA>90%). Mean SA continued to be high in SD goats and despite PRL treatment SA also remained high in P goats (Figure 8.3). However in contrast, mean SA in LD goats declined to be 10±6% by 03 March (P goats, 98±6%; SD 90±6%, P<0.01). Therefore during the treatment period, the pattern over time (P<0.0001) and magnitude (P<0.0001) of SA was highly divergent in LD goats compared to SD and P goats (P<0.0001)(Figure 8.4).

Seventeen days after the cessation of treatment, SA of LD goats reached its lowest mean level (2±17%) and mean SA began to decline in P and SD goats (P goats 47±19, SD goats 48±17%). Mean SA in P and SD goats reached a minimum (12-14%) on 8 April at which time SA in LD goats had returned to high levels (99±7%, P<0.001). By 6 May 1994, mean SA in all three treatment groups was in excess of 99%. During the post-treatment period both the pattern and magnitude of mean SA were similar in SD and P goats and both were highly divergent to that of LD goats (P<0.0001).

In individual goats, the mean date of minimum SA in LD goats was 23 March±4 days which was earlier than either P (10 April±4 days, P<0.01) or LD (6 April±4 days, P<0.05) goats. SA also returned to full activity levels 16 days earlier in LD (21 April±4 days) than in P or SD goats (both 6 May±4 days). In LD and P goats, minimum SA fell to be less than 2% but in SD goats levels it was slightly higher at 10±3% (P<0.05). Treatment had no effect on the proportion of SAc+brush follicles in either the treatment or post-treatment periods (Figure 8.5).

Table 8.2: Mean±se fibre growth and plasma PRL concentration measurements at various dates (day.month.year) in down-producing does treated with short photoperiod (8L:16D) and low temperature (10-15°C) from 15 July to 2 February 1994.

Fibre Measurements	Dates					
	20.07.93	26.08.93	14.10.93	09.12.93	05.01.93	01.02.93
Primary follicle activity (%)				89±7		88±3
Secondary follicle activity (%)				94±6		96±2
Guard hair length (mm)	61±2	55±3	55±4	43±3	44±2	45±2
Greasy fleece growth rate (mg/cm^2/day					0.41±0.04	0.47±0.04
Fleece shedding score	1.1±0.1	1.2±0.2	1.9±0.2	1.00±0.04	1.08±0.05	1.13±0.09
Down length (mm)	76±4	61±6	46±7	21±3	32±4	43±5
Newly emerged down fibres					5.6±0.3	5.5±0.4

Table 8.3: Mean least squares and least squares standard error of the mean (lsse) log plasma PRL concentration pooled over three daily intervals in goats treated from 2 February (day 1) to 3 March (day 30) 1994 with either long photoperiod (16L:8D) (LD), or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P).

Treatment	Days								Significance level	
	1-4	5-8	9-12	13-16	17-20	21-24	25-30	TRT	Days	TRT*days
LD	1.6^a	1.3^a	1.8^a	1.9^a	1.8^a	1.8^a	1.8^a	**	NS	**
P	1.5^a	1.6^a	1.3^b	1.4^a	1.6^a	1.4^{ab}	1.5^{ab}			
SD	0.9^b	0.9^a	0.8^c	1.0^b	0.9^b	1.1^b	1.2^b			
LSSE	0.1	0.2	0.1	0.1	0.1	0.1	0.1			

** P<0.01

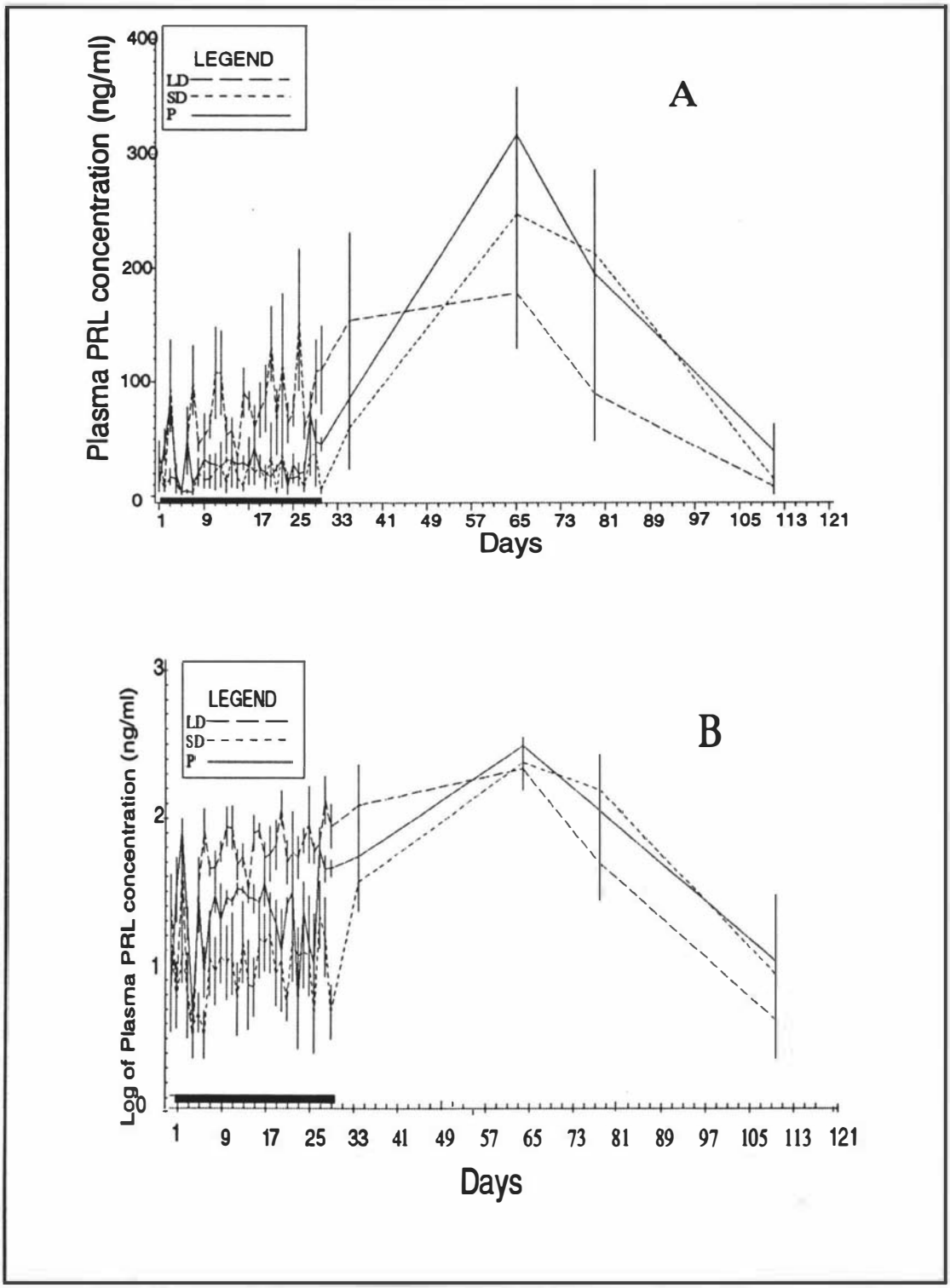

Figure 8.1: Plasma PRL concentration (A=non-transformed, B log10 transformed) in goats treated with long photoperiod (16L:8D) (LD) or short-photoperiod with no further treatment (SD) or treated with a systemic infusion of PRL (P). Horizontal bar represents period of treatment and vertical bars are standard error of the mean.

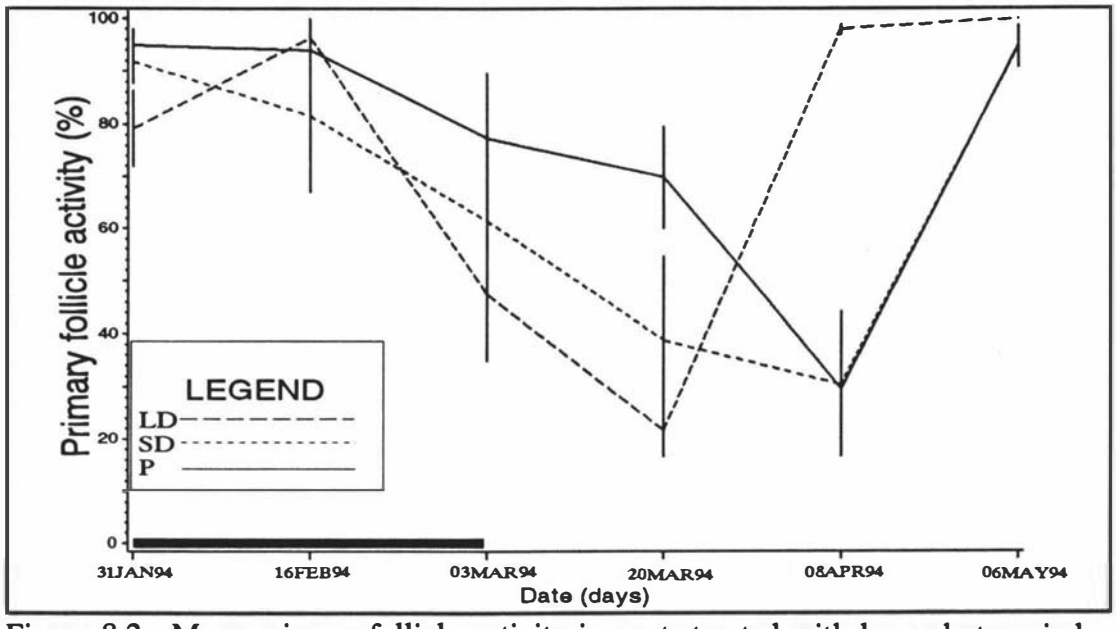

Figure 8.2: Mean primary follicle activity in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) and with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard error of the mean. Horizontal bar is period of treatment.

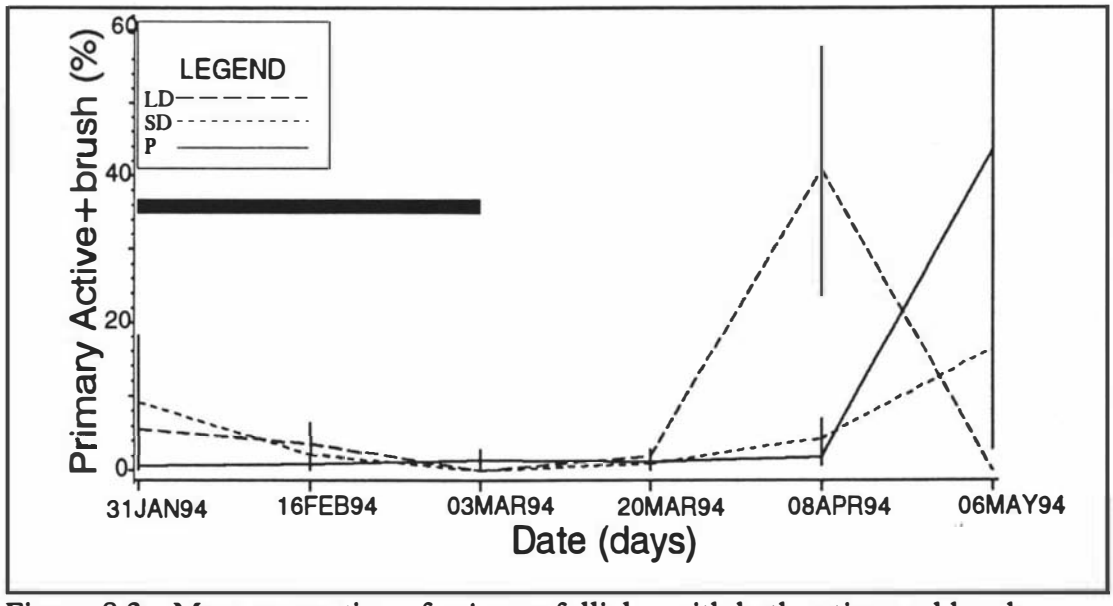

Figure 8.3: Mean proportion of primary follicles with both active and brush fibres (PAc+brush) in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard errors of the mean. Horizontal bar is period of treatment.

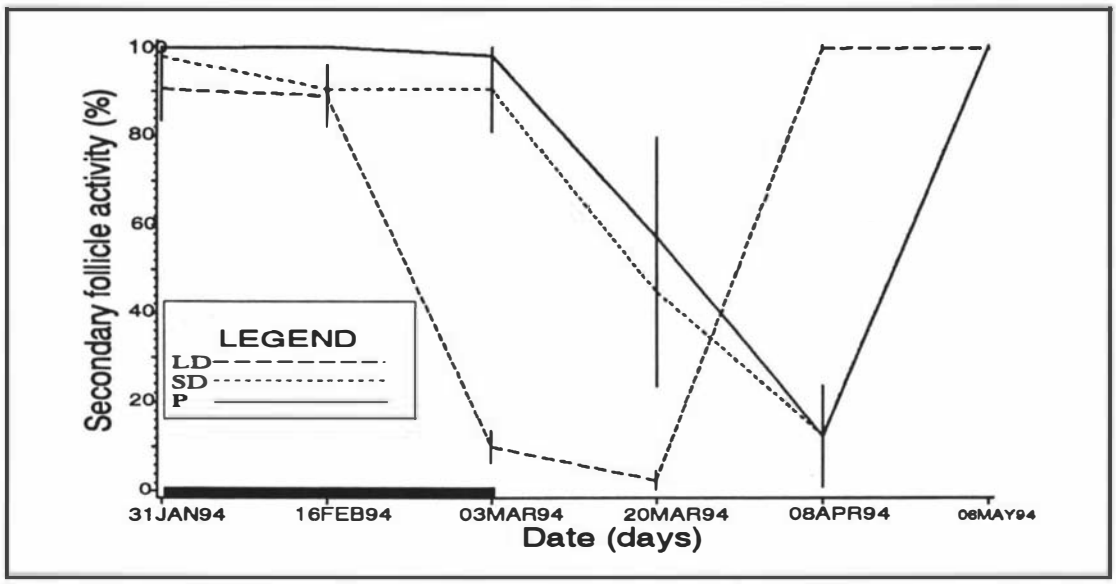

Figure 8.4: Mean secondary follicle activity in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard error of the mean. Horizontal bar is period of treatment.

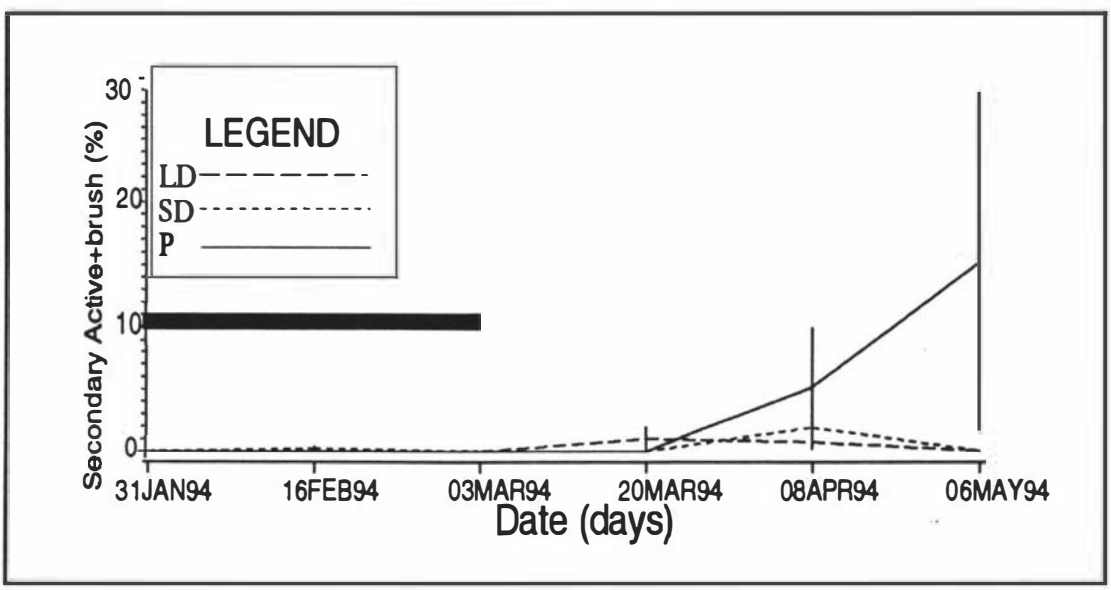

Figure 8.5: Mean proportion of secondary follicles with both active and brush fibres (SAc+brush) in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard error of the mean. Horizontal bar is period of treatment.

8.4.2.5 Fibre measurements

***8.4.2.5.1 Fibre hair length*:** Treatment had no effect on mean GL and DL during the treatment period (Figure 8.6, 8.7). In the post-treatment period, overall mean GL and DL were lower in LD (GL 48±3 mm, DL 26±3 mm) than in SD goats (GL 58±3 mm $P<0.05$; DL 42±6 mm, $P<0.01$). In P goats, mean DL but not GL (GL, 51±4 mm, NS; DL 41±5 mm, $P<0.01$) was higher than those of LD goats ($P<0.05$). The pattern in DL over the post-treatment period in SD ($P<0.01$) and P ($P<0.01$) goats differed to that of LD goats.

In individual goats (Table 8.4), mean DL was minimal on 15 April±7 days in LD goats, approximately one month earlier than in P (13 May±7 days, $P<0.05$) and SD (17 May±7 days, $P<0.05$) goats. The magnitude of minimum DL was lower in LD (0.5±3 mm) compared to either SD or P goats (10±11 mm, $P<0.05$).

***8.4.2.5.2 Fleece growth rate*:** FGR over the treatment period (Figure 8.8) was lower in LD goats (0.19±0.03 mg/cm^2/day) than in P (0.49±0.03 mg/cm^2/day, $P<0.001$) or SD (0.38±0.03 mg/cm^2/day, $P<0.01$) goats. Following the cessation of the treatment FGR declined in P and SD goats before increasing but FGR increased earlier in LD goats (Trt*time, $P<0.001$).

In individual goats, mean minimal FGR was similar at less than 0.09 mg/cm^2/day in all three treatment groups. Minimal FGR occurred in LD goats on 11 April±5 days but not until 26 April±5 days in SD goats ($P<0.05$) and 1 May±5 days in P goats ($P<0.01$) (Table 8.4).

8.4.2.5.3 Shedding: There was no overall effect of treatment on either the magnitude or pattern of mean SS of down and guard fleeces in either the treatment or post-

treatment periods. However in LD goats, mean SS of guard (3.0±0.4) and down (2.6±0.4) fleeces were higher (P<0.05) on 8 April than those of SD and P goats (1.0-1.4±0.4)(Figure 8.9). On 24 May in P and SD goats, mean SS in down and guard fleeces was in excess of 3.3 while fleece shedding had ceased (SS=1) in LD goats (P<0.05).

In individual goats, the date of maximum SS in down and guard fleeces was more than a month later (P<0.05) in SD (down 8 May±11 days; guard 19 May±6 days) and P (down 11 May±11 days; guard 20 May±6 days) goats in comparison to LD (Down 1 April±11 days; guard 7 April±6 days) goats (Table 8.4). The magnitude of maximum SS was between 3.2 and 4.8 for both down and guard fleece and was similar in all treatments.

8.4.2.5.4 Newly emerged down fibres: NEDF score was in excess of 5 in all treatment groups in January prior to the start of the treatment period (Table 8.1). Treatment had no effect on mean NEDF score during the treatment period and overall mean NEDF was 5.8±0.2 indicating that the summer down fleece had not yet emerged (Figure 8.9). In the post-treatment period, overall mean NEDF score was higher in LD goats (5.5±0.1) in comparison to SD and P goats (both, 4.8±0.2, P<0.01). However NEDF score declined and increased earlier in the post-treatment period in LD goats than in either SD (P<0.001) or P (P<0.001) goats.

In individual goats, the mean date of NEDF emergence in LD goats (10 April±4 days) was earlier than in SD and P goats (both 27 April±4 (P<0.001, Table 8.4). In addition, NEDF's disappeared earlier in LD (4 May±3 days) than in SD (29 May±3 days, P<0.001) or P (26 May±5 days; P<0.001) goats. The duration of presence of NEDF's was similar at 24-33 days for all three treatments.

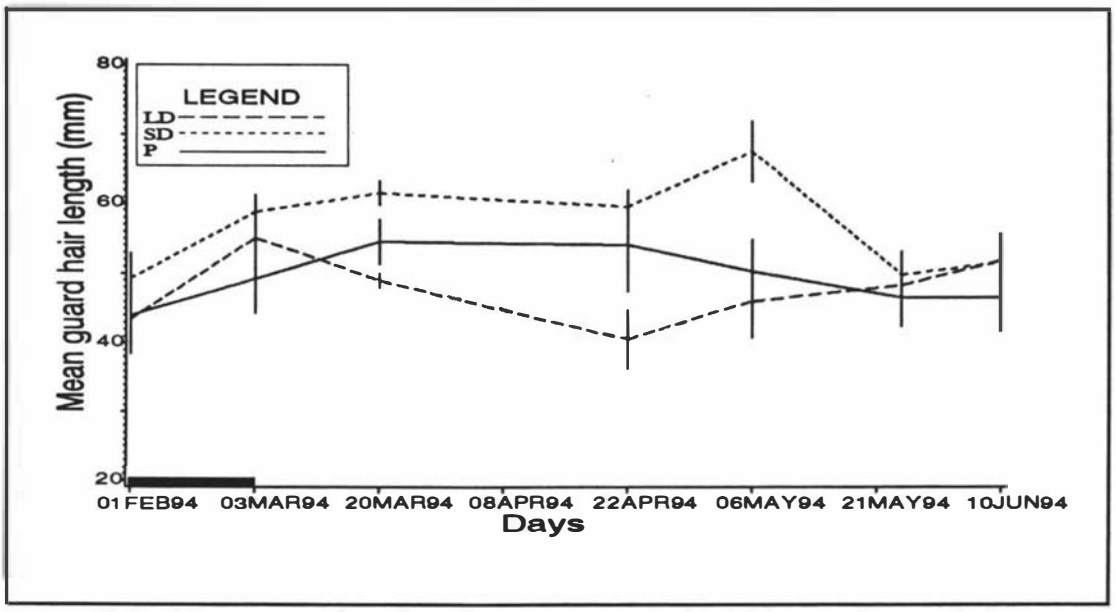

Figure 8.6: Mean guard hair length in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard error of the mean. Horizontal bar is period of treatment.

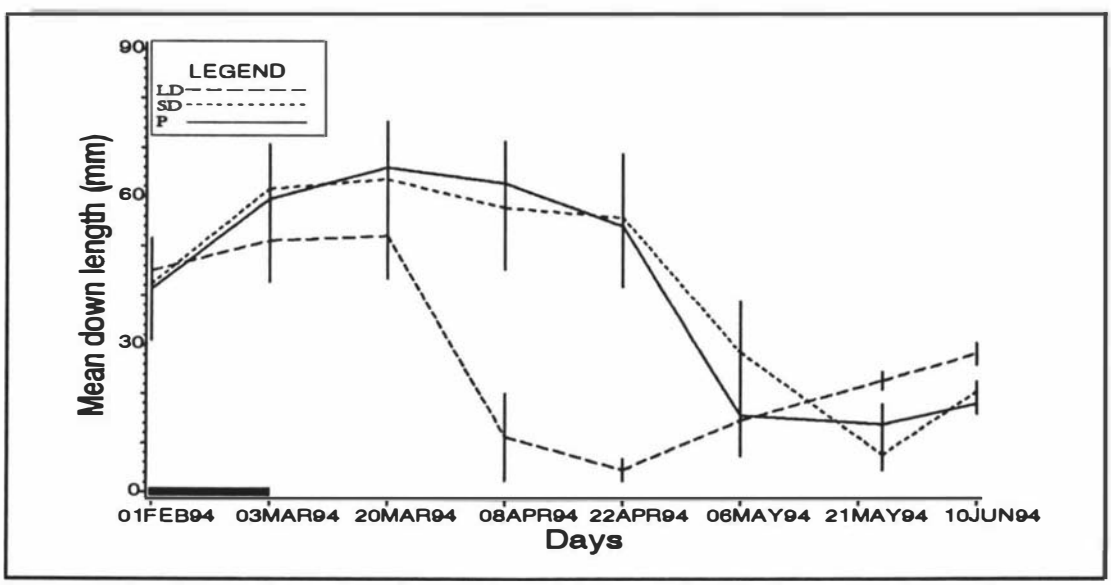

Figure 8.7: Mean down hair length in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard errors of the mean. Horizontal bar is period of treatment.

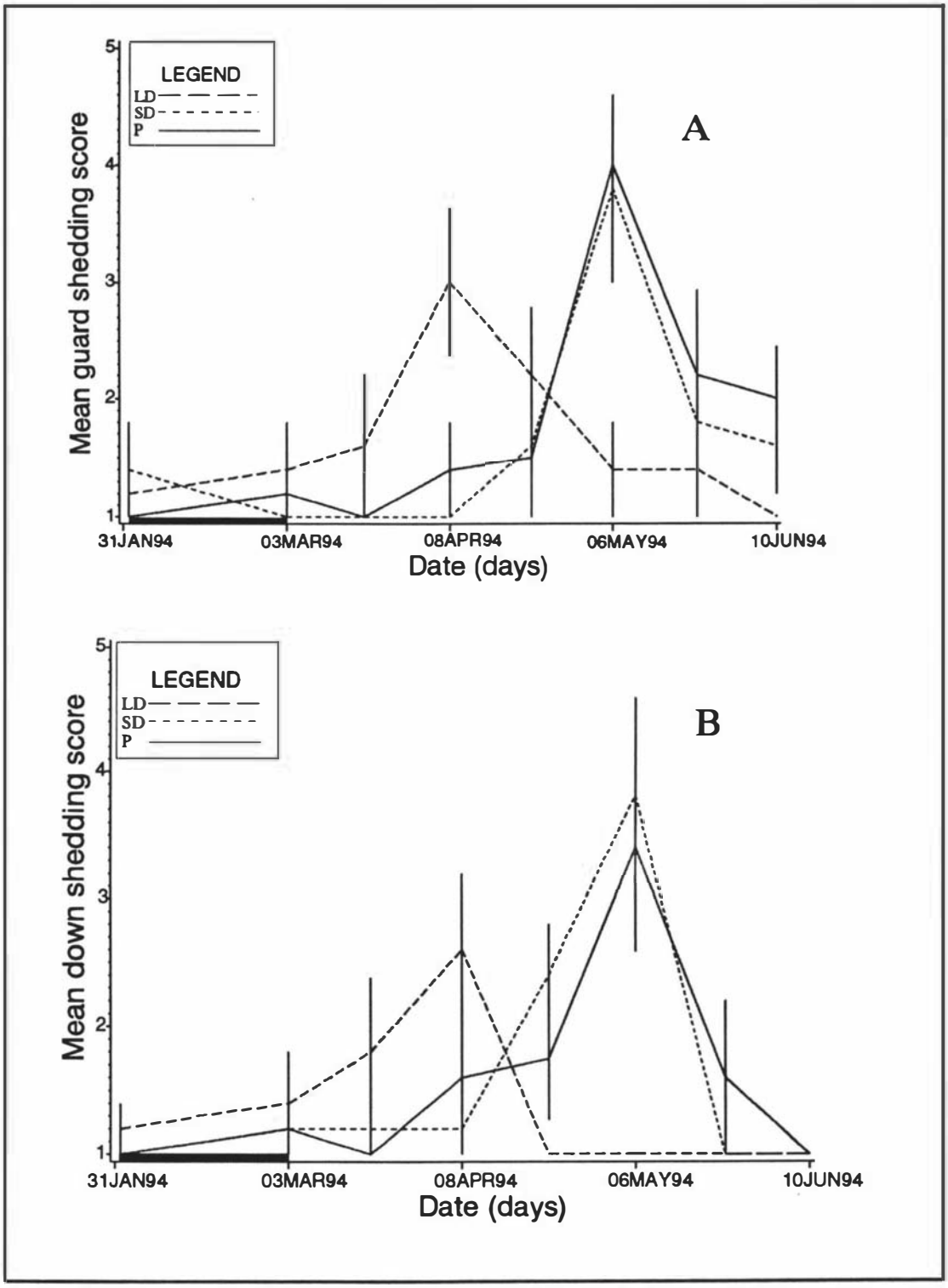

Figure 8.9: Mean guard hair (A) and down (B) fleece shedding score in goats treated with long photoperiod (16L:8D) (LD) or short-photoperiod with no further treatment (SD) or treated with a systemic infusion of PRL (P). Horizontal bar represents period of treatment and vertical bars are standard error of the mean.

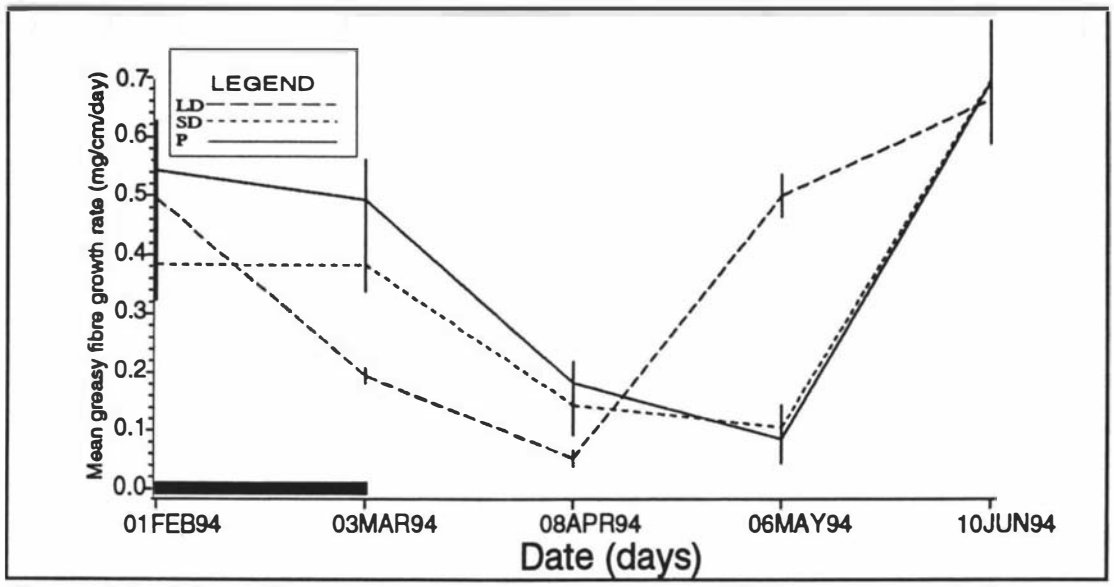

Figure 8.8: Mean fibre growth rate in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard errors of the mean. Horizontal bar is period of treatment.

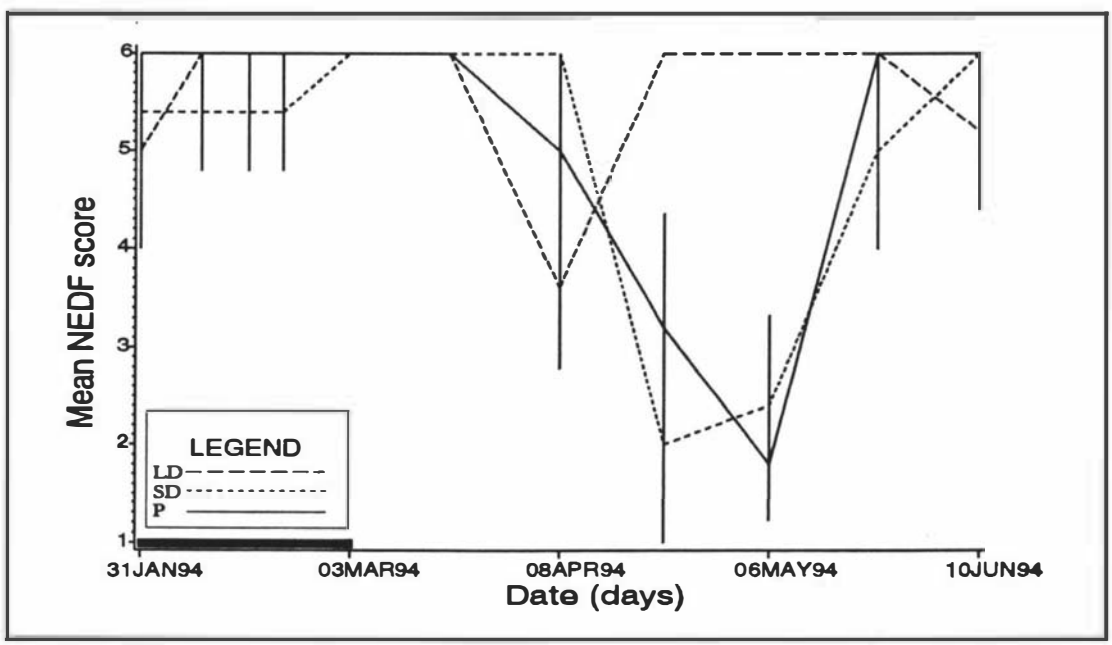

Figure 8.10: Mean newly emerged down fibres (NEDF's) in goats treated with long photoperiod (16L:8D) (LD) or with short photoperiod (8L:16D) with no further treatment (SD) or with a systemic infusion of PRL (P). Vertical bars are standard errors of the mean. Horizontal bar is period of treatment.

8.4.2.6 Haematology

On the penultimate day of treatment (2 March 1994), the concentration of haemoglobin in the blood was higher in LD goats (11.2±0.6 g/dl) than in either P or SD goats (both 9.3±0.6 g/dl, $P<0.05$). One week later, haemoglobin concentrations were similar in all three treatments (Table 8.5) and therefore LD goats had a different pattern in time (Time*trt $P<0.05$) to both SD and P goats. A similar trend was seen in PCV (Table 8.5) with LD goats declining over time relative to the other two treatment groups ($P<0.05$). MCHC in the post-treatment period was higher than in the treatment period in all treatments ($P<0.001$) (Table 8.5).

Overall mean WBC concentration declined after the cessation of treatment ($P<0.05$). On 2 March, lymphocyte concentrations were higher in P goats (9.0±0.5 $x10^9$/l) than in either LD (7.2±0.04 $x10^9$/l $P<0.05$) or SD (7.7±0.5 $x10^9$/l, $P<0.05$) goats. Lymphocyte concentration was unaffected by treatment on 9 March 1994 and treatments behaved similarly over time. Treatment and time did not affect other leucocyte cell populations (Table 8.5).

8.4.3 Experiment two

8.4.3.1 Blood flow and catheter patency

The mean blood flow through the skin patch was 12±4 ml/min and 6±1 ml/min in goats and sheep respectively ($P<0.05$). Of the 29 A catheters, 16 were patent at the end of the infusion period (ranging from 6-25 days in duration).

Table 8.4: Least squares mean (±lsse) of dates of various fibre growth events (see Abbreviation Section) in goats treated from 2 February to 3 March 1994 with either long photoperiod (16L:8D) (LD), or with short photoperiod (8L:16D) and with no additional treatment (SD) or with a systemic infusion of PRL (P).

Fibre Growth Event	Date of Fibre growth event (days)			Significance level
	LD goats	P goats	SD goats	
SA_{min}	23 Mar±4[a]	10 Apr±4[b]	6 Apr±4[b]	*
FGR_{min}	11 Apr±5[a]	1 May±5[b]	26 Apr±5[b]	**
$NEDF_e$	10 Apr±4[a]	27 Apr±4[b]	27 Apr±4[b]	**
SS $down_{max}$	1 Apr±11[a]	11 May±11[b]	8 May±11[b]	*
SS $guard_{max}$	7 Apr±6[a]	20 May±6[b]	19 May±6[b]	**
DL_{min}	15 Apr±7[a]	13 May±7[b]	17 May±7[b]	**
SA_{max}	21 Apr±4[a]	6 May±4[b]	6 May±4[b]	**
$NEDF_d$	4 May±5[a]	26 May±5[b]	29 May±5[b]	***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$.
Values with different superscripts in each row within each date indicate treatments which are significantly different at the 5% level.

Table 8.5: The least squares mean concentrations (±lsse) of haemoglobin, packed red blood cell volume (PCV), mean red blood cell haemoglobin concentration (MCHC), white blood cells (WBC) and various white blood cell types in whole blood of goats treated with either long photoperiod (16L:8D, LD) or short photoperiod (8L:16D, SD) or short photoperiod and a systemic infusion of PRL (P) after 28 days (2 March 1994) of treatment and 6 days after the cessation of treatment (9 March 1993).

	Haemoglobin (g/dl)	PCV (l/l)	MCHC (g/dl)	WBC ($x10^9$/l)	Segmented Neutrophil ($x10^9$/l)	Lymphocyte ($x10^9$/l)	Monocyte ($x10^9$/l)	Eosinophil ($x10^9$/l)	Basophil ($x10^9$/l)
2 March 1994									
LD	11.2±0.8^a	0.35±0.03^a	32±1	12±1	3.5±0.8	7.4±0.6^b	0±0	1.6±0.6	0.07±0.05
P	9.3±0.7^b	0.30±0.03^b	31±1	13±1	3.5±0.7	9.0±0.5^a	0±0	0.6±0.5	0.14±0.05
SD	9.3±0.6^b	0.30±0.03^{ab}	31±1	13±1	3.6±0.6	7.8±0.5^{ab}	0.24±0.05	1.1±0.4	0.08±0.05
9 March 1994									
LD	9.9±0.3	0.29±0.01	34.0±0.6	10±1	2.6±0.8	4.4±0.7	0.06±0.06	3.0±0.9	0.0±0
P	10.4±0.3	0.31±0.02	33.0±0.6	10±1	3.8±0.8	6.0±0.7	0.1±0.05	0.4±0.8	0.10±0.05
SD	11.2±0.8	0.35±0.03	32.0±0.5	12±1	3.5±0.8	7.0±0.6	0.13±0.04	1.4±0.7	0.0±0
Trt	NS	NS	***	*	NS	**	NS	NS	NS
Time	NS	NS	***	*	NS	**	NS	NS	NS
Time*Trt	*	0.08	NS	NS	NS	NS	NS	NS	NS

*P<0.05, ** P<0.01, ***P<0.001

Values with different superscripts in each column within each date indicate treatments which are significantly different at the 5% level.

8.4.3.2 Plasma PRL concentration

Mean plasma PRL concentration on 21 January was 4.4 (4.0-4.7) ng/ml. After the insertion of A and V iliac catheters and prior to the start of the PRL infusions mean systemic plasma PRL concentration was lower in goats (9.1 (7.9-10.4) ng/ml) than in sheep (14.8 (12.6-17.4) ng/ml, $P<0.05$). At all other times plasma PRL concentration was unaffected by species.

Following treatment with bromocryptine and local infusion of PRL, mean plasma PRL concentration was 9 (7-81) ng/ml, 110 (83-145) ng/ml and 43 (35-57) ng/ml in blood collected from the venous jugular, PRL infused and saline infused iliac catheters respectively ($P<0.01$).

Towards the end of the experiment, 2 sheep and 2 goats received no bromocryptine treatment and mean plasma PRL concentration in the systemic circulation was 29 (21-41) ng/ml in these animals compared to 5 (4-6) ng/ml bromocryptine-treated animals.

8.4.3.3 Fibre and follicle growth

For analysis of fibre data two groups of animals were defined: those receiving a long and those receiving a short duration PRL infusion (mean±se 21±1 days vs 7.7±0.8 days).

8.4.3.3.1 Primary follicle activity: In long duration PRL-infused animals, mean PA was 86±8% and 83±7% at the start and 70±10% and 53±15% at the end of the infusion in saline-infused and PRL-infused sides respectively. While in short duration PRL-infused animals, mean PA was 75±14% and 77±10% at the start and 56±14% and 43±16% at the end of the infusion in control and PRL-infused sides respectively. There was no effect of PRL infusion or species on change in PA over either infusion duration groups (Table 8.6).

Table 8.6: Least squares mean (±lsse) decline in PA over the treatment period in isolated skin patches which were continuously infused with either saline or PRL for between 6 to 24 days.

Treatment	Decline in PA (%)	
	Goat	Sheep
Saline-infused	22±13	36±12
PRL-infused	41±10	51±19
TRT	NS	
Species	NS	
TRT*species	NS	

8.4.3.3.2 Secondary follicle activity: Mean SA at the start of the treatment period ranged between 92 and 96%. Mean SA in saline- and PRL-infused sides was 76±10% and 56±9% and 56±14% and 43±16%, in long and short duration infusions respectively. The overall decline in SA was greater in sheep (56±8%) compared to goats (10±7%) during both short ($P<0.05$) and long ($P<0.001$) PRL-infused animals but mean SA was similar in saline and PRL-infused animals.

8.4.3.3.3 Fibre growth rate: Mean patch fibre growth rate was unaffected by either treatment or species following the long duration of PRL directly to the skin (Table 8.8).

8.4.3.4 Haematology

In animals with haematological measurements collected on day 0, 2 and 5 of the PRL infusion, mean MCHC was higher in goats (34.5±0.5 g/dl) than in sheep (31.8±0.5 g/dl, $P<0.01$). There was no other effect of species nor an effect of time on haematological measurements collected on days 0, 2 and 5 (Table 8.9).

Table 8.7: Least squares mean (±lsse) decline in SA % over the treatment period in isolated skin patches which were continuously infused with either saline or PRL for between 6 to 24 days.

Treatment	Decline in SA (%)	
	Goat	Sheep
Saline-infused	5±5	46±13
PRL-infused	15±7	65±19
TRT	NS	
Species	***	
TRT*species	NS	

Table 8.8: Least squares mean (±se) midside patch fibre growth rate following direct continuous infusion of either saline or PRL to the skin for more than 15 days.

Treatment	Fibre growth rate ($mg/cm^2/day$)	
	Goat	Sheep
Saline infused	0.45±0.1	0.54±0.1
PRL infused	0.30±0.1	0.57±0.1
TRT	NS	
Species	NS	
TRT*species	NS	

Serial blood samples were collected in a goat and sheep from 2 days before the start of PRL infusion and daily up to 5 days from patent jugular, C and P venous catheters. Descriptively (data not suitable for statistical analysis), haemoglobulin concentrations decreased, and lymphocyte concentrations increased during the infusion of PRL. There were few consistent differences between samples collected from the jugular, saline or PRL-infused skin (Table 8.10, 8.11).

8.4.3.5 Health

The direct infusion to the skin of PRL (from two different sources) resulted in the visible swelling of the proximal hind-leg which was first noted 6.5±0.8 days after the start of the infusion. Bacterial culture of the PRL infusates established infusate sterility. External examination revealed hind-leg oedema and hyperplasia of the iliac lymph node. Food intake fell markedly. Post-mortems carried out on seven of the animals (3 sheep and 4 goats) revealed that infusions of PRL to the skin, in contrast with saline infusions, were associated in all cases with cellulitis and in 62% of cases with enlarged lymph nodes (Table 8.12). Adrenal and thyroid glands were enlarged relative to normal animals in 50 and 37% of animals respectively.

8.5 DISCUSSION

8.5.1 Pre-treatment period

Constant short photoperiod treatment from the winter solstice until shortly after the summer solstice, as in the previous year (Chapter 7), failed to prevent fleece shedding during spring. In fact, the pattern of fleece shedding and DL growth was virtually identical in the two years with all goats partially shedding their fleece without progression to completion prior to the onset of new winter down growth.

Table 8.9: Least squares mean (±lsse) concentration of haemoglobin, packed red blood cell volume (PCV), mean red blood cell haemoglobin concentration (MCHC), white blood cells (WBC) and various white blood cell types in whole blood collected from the jugular vein in goats (n=3) and sheep (n=3) receiving an infusion of PRL to the skin two hours after the start of the infusion (Day 0) and 2 and 4 days after the start of the infusion.

	Haemoglobin (g/dl)	PCV (l/l)	MCHC (g/dl)	WBC ($x10^9$/l)	Segmented Neutrophil ($x10^9$/l)	Lymphocyte ($x10^9$/l)	Monocyte ($x10^9$/l)	Eosinophil ($x10^9$/l)	Basophil ($x10^9$/l)
Goat									
Day 0	9±1	0.26±0.03	34±1	14±5	8±3	5±1	0.02±0.01	0.3±0.1	0.04±0.02
Day 2	7.5±0.9	0.22±0.03	34.5±0.5	15±3	9±3	6±1	0.08±0.05	0.2±0.1	0±0
Day 5	7.0±0.8	0.20±0.03	35±0.9	16±2	8±2	7±1	0.22±0.09	0.1±0.09	0.22±0.09
Sheep									
Day 0	9±1	0.29±0.03	32±1	13±5	7±3	6±1	0.0±0.01	0.4±0.1	0.0±0.02
Day 2	9±0.9	0.29±0.03	31.6±0.5	14±3	6±3	7±1	0.10±0.04	0.3±0.1	0±0
Day 5	9.6±0.8	0.30±0.03	31.6±0.9	15±2	9±2	6±1	0.0±0.09	0.18±0.09	0.0±0.09
Time	NS	NS	NS	NS	NS	NS	NS	NS	NS
Species	NS	NS	**	NS	NS	NS	NS	NS	NS
Timexspecies	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 8.10: The concentration (mean ±se) of haemoglobin (H), packed red blood cell volume (PCV), mean red blood cell haemoglobin concentration (MCHC), white blood cells (WBC) and various white blood cell types in whole blood either from the venous jugular, from saline (C) or PRL- infused (P) descending iliac veins commencing 2 days prior to the infusion and continuing for 5 days in sheep 102.

Day	TRT	H (g/dl)	PCV (l/l)	MCHC (g/dl)	WBC ($x10^9$/l)	Segmented Neutrophil ($x10^9$/l)	Lymphocyte ($x10^9$/l)	Monocyte ($x10^9$/l)	Eosinophil ($x10^9$/l)	Basophil ($x10^9$/l)
-2	C	10.3	0.30	31.2	15.3	9.1	6.9	0.15	0	0
	J	9.5	0.33	31.7	14.5	8	5.0	0	0.46	0
	P		0.29	33.1				0.58	0	0
-1	C	9.3	0.29	31.1	2.9	1.9	1.0	0	0	0
	J	8.9	0.28	31.8	5.9	1.7	4.25	0	0	0
	P		0.29	33.1		3.4		0	0	0
0	C	9.1	0.27	33.7	8.2	3.6	4.3	0	0.25	0
	J	9.7	0.29	33.4	13.6	5.9	7.3	0	0.41	0
	P	9.6	0.30	32.0	13.0	5.6	6.9	0	0.52	0
1	C	10	0.30	33.3	8.6	3.7	4.9	0	0	0
	J	9.1	0.27	33.7	10.6	4.4	6.0	0	0.21	0
	P	9.9	0.30	33.0	6.4	2.2	3.9	0	0.26	0
2	C	9.2	0.28	32.9	8.0	2.2	5.2	0	0.48	0
	J	9.9	0.32	30.9	10.6	1.6	8.4	0	0.64	0
	P	9.8	0.30	32.7	9.4	3.2	5.4	0	0.85	0
3	C	8.3	0.25	33.2	9.4	1.0	7.9	0	0.38	0
	J	9.3	0.28	33.2	11.4	1.7	9.6	0	0.11	0
	P	8.5	0.25	34.0	7.6	0.6	6.7	0	0.30	0
4	C	8.8	0.27	32.6	10.0	2.5	7.3	0.2	0	0
	J	9.2	0.28	32.9	9.4	2.7	6.5	0.19	0	0
	P									
5	C	5.4	0.28	30.0	6.5	2.9	3.4	0	0.2	0
	J	8.1	0.25	32.4	12.7	4.3	8.0	0	0.38	0
	P									

Table 8.11: The concentration (mean±se) of haemoglobin (H), packed red blood cell volume (PCV), mean red blood cell haemoglobin concentration (MCHC), white blood cells (WBC) and various white blood cell types in whole blood collected from the venous jugular, from saline (C) or PRL-infused (P) descending iliac vein commencing 2 days prior to the infusion and continuing for 5 days in sheep 604.

Day	TRT	H (g/dl)	PCV (l/l)	MCHC (g/dl)	WBC ($x10^9$/l)	Segmented Neutrophil ($x10^9$/l)	Lymphocyte ($x10^9$/l)	Monocyte ($x10^9$/l)	Eosinophil ($x10^9$/l)	Basophil ($x10^9$/l)
-2	C	13.6	0.39	34.9	20.7	13.2	5.38	0	0	2.0
	J	12.7	0.37	34.3	19.6	13.5	3.92	0.78	0	1.4
	P	13.9	0.43	32.3	20.3	12.2	5.08	0	0	3.0
-1	C	12.5	0.35	35.7	11.7	6.3	5.27	0	0.12	0
	J	12.3	0.40	30.8	5.7	1.0	4.56	0	0	0.11
	P	12.9	0.32	40.3	5.6	0.84	4.70	0	0	0.06
0	C	12.1	0.35	34.6	14.0	7.3	6.30	0	0	0.14
	J	11.8	0.33	35.8	6.0	3.1	2.70	0.06	0.06	0
	P	11.5	0.34	33.8	14.1	9.7	3.53	0.56	0.28	0
1	C	9.6	0.29	33.1	9.4	3.8	5.36	0	0.19	0
	J	10.0	0.28	35.7	9.6	4.2	5.28	0	0.10	0
	P	9.7	0.29	33.4	9.2	4.0	5.15	0	0	0
2	C	9.4	0.27	34.8	13.0	6.8	5.98	0.13	0	0.13
	J	9.3	0.26	35.8	11.9	6.0	5.36	0.12	0.24	0.12
	P	9.0	0.26	34.8	11.3	5.9	5.31	0	0.11	0
3	C	-	-	-	-	-	-	-	-	-
	J	9.0	0.27	33.3	14.9	7.9	6.56	0.15	0.30	0
	P	9.2	0.27	34.1	15.2	9.1	5.62	0.30	0	0.15
4	C	-	-	-	-	-	-	-	-	-
	J	8.6	0.23	37.4	17.7	12.2	5.49	0	0	0
	P	8.7	0.25	34.8	18.2	11.5	6.73	0	0	0
5	C	-	-	-	-	-	-	-	-	-
	J	8.2	0.25	32.8	14.3	6.9	7.15	0	0	0.29
	P	7.3	0.23	31.7	14.8	7.6	7.25	0	0	0

Table 8.12. Proportion of animals undergoing post-mortem which possessed specific clinical symptoms following the infusion of PRL directly to the skin.

Symptom	Percentage of animals expressing symptoms (%)
Cellulitis in PRL infused skin	100
Haematoma at site of PRL infusion	85
Hyperplastic iliac or popliteal lymph node	62
Proteinaceous material in mammary gland	62
Large macrophages in iliac lymph node	50
Adrenal hyperplasia	50
Thyroid hyperplasia	37
Pituitary gland cysts	12
Glomerulonephritis	12

In addition, in common with the previous year's experiment, mean day-time plasma PRL concentrations ranged from 15-30 ng/ml during short photoperiod treatment which was higher than that normally found during short photoperiod (<20 ng/ml). During June in an out-of doors environment these goats had mean plasma PRL concentrations of less than 10 ng/ml. It appears that the environmental conditions of the facilities of the previous and current experiments do not adequately simulate short day/low temperature conditions found out-of doors. Only high temperature (>25°C) is known to over-ride the seasonal photoperiod message (Wettemann *et al.*, 1982; Howland *et al.*, 1983; Tamanini *et al.*, 1988). Spectral properties of light have been shown to be unimportant in moderating plasma PRL concentration (Petitclerc and Zinn, 1991). However, either the abrupt transition of light in the artificial dawn/dusk periods (Laakso *et al.*, 1988) or the low levels of illumination (Sugawara *et al.*, 1989) may have weakened the short photoperiod signal thereby resulting in a elevated plasma PRL concentration and partial follicle deactivation. However, as in the previous Chapter, a transient shedding occurred in spring when follicles reactivated despite low plasma PRL concentrations and follicles continued to full activity at the start of the treatment period and consequently responded quickly to changes in photoperiod.

8.5.2 Plasma PRL concentrations

The systemic infusion of PRL, via the jugular vein, elevated mean log transformed plasma PRL concentration in short photoperiod-treated goats by 19 ng/ml however, the mean increase when raw data was averaged was only 10 ng/ml. Following the 0.5 mg/kgLW/day (approximately 6 mg/goat/day) PRL infusion, the elevation in plasma PRL concentration generated in this experiment, was lower than that achieved in earlier experiments in this thesis (Chapter 5). In sheep, the 0.4 mg/kgLW/day infusion of the same source of PRL resulted in an overall 40 ng/ml increase in plasma PRL concentration but the increase was not consistent at every sample time (Chapter 5). Twice daily subcutaneous injections of PRL (4 mg/day) in down goats increased plasma PRL concentrations by 50 ng/ml in goats in spring photoperiods (Dicks, 1994). It is not clear why plasma PRL concentrations in this experiment resulted in approximately half the expected elevation in plasma PRL concentration as PRL clearance rate was 60 minutes faster in PRL-injected compared to infused goats (Chapter 5). However, it is possible that plasma PRL clearance rate may increase with sustained PRL infusion. Plasma PRL concentration, following log transformation, of LD goats did not differ significantly to that of P goats but both were higher than SD goats. However analysis of the data without log transformation failed to identify a significant elevation in plasma PRL concentration in P compared to SD goats. In addition the elevation in plasma PRL concentration achieved by infusion was less consistent than that of long photoperiod due to the difficulty of maintaining catheters in mobile goats.

Unlike previous experiments (Chapter 6, 7) long photoperiod treatment in this experiment was not coupled with high temperature treatment and mean plasma PRL concentration following a month of long-photoperiod treatment only reached 63 ng/ml compared to 130 ng/ml in the same facilities in the previous year. Between assay variation may account for these magnitude differences between years but the large differences in plasma PRL concentration following release into natural photoperiod in the two different years cannot be accounted for by between assay variation. In the previous year, one month after release

to natural photoperiod on 31 March 1993 plasma PRL concentrations were less than 35 ng/ml. However in this experiment, plasma PRL concentrations one month after release on 3 March 1994, were 230 ng/ml. It is not clear whether it was the shorter duration of artificial photoperiod manipulation prior to release, or the earlier and hence longer day length at release which resulted in the greater elevation in plasma PRL concentration. The difference in actual day length between 3 and 31 March is only 74 minutes but plasma PRL concentration changes quickly during equinocal photoperiod changes and this difference may have had physiological significance.

When PRL was infused directly to the skin, the venous plasma from the skin patch contained an 8 fold higher concentrations of PRL than in jugular plasma. However, closer observation of experimental animals later in the experiment also revealed high plasma PRL concentrations in the venous drainage of saline-infused skin. Therefore it is not clear that PRL concentrations were elevated by local infusion of PRL.

Higher plasma PRL concentration found locally in the skin, in comparison to the systemic venous supply, was unexpected and has not been reported for other hormones (Insulin; Pierznowski *et al.*, 1994). While the *pars distalis* region of the pituitary is the major site for PRL synthesis, molecules with a similar structure to PRL are synthesised in the placenta (Yamakawa *et al.*, 1990), lymphocytes (Sabharwal *et al.*, 1992; Swarlo-Santo, 1992; Arkins *et al.*, 1993) and possibly also in the skin (Walker *et al.*, 1989). These molecules may have similar immunoactivity to PRL molecules and therefore be detected by the immunoassay of PRL. It has been hypothesised that PRL acts as an endocrine, paracrine and autocrine regulator in the immune system (Swarlo-Santo, 1992). PRL may act in this manner in the skin. However, it is doubtful that paracrine or autocrine regulators would appear in such high concentrations in the venous blood. The discovery of enhanced plasma PRL concentrations in the skin requires further validation, preferably in the absence of PRL infusions.

8.5.3 Blood flow

Blood flow rates in the skin of 6 and 12 ml/min for Wiltshire sheep and down goats in this experiment were comparable to values of 7 ml/min reported for Romney sheep on low intake (Harris *et al.*, 1994) and within the range of 10-18 ml/ml reported for Angora goats (Pierzynowski *et al.*, 1996). Increased blood flow to the skin has been found to be associated with genetic selection for high wool production in sheep (Hales and Fawcett, 1993). However fibre growth rates in down goats and Wiltshire sheep during the period of PRL infusion were comparable and therefore not likely to account for differences in blood flow rates in the skin. While SA declined to a greater extent during the PRL infusion in Wiltshire sheep compared to down goats blood flow measurements were conducted prior to the start of the PRL infusion when SA was comparable in the two species.

8.5.4 Fibre growth

The systemic infusion of PRL, and subsequent elevation in plasma PRL concentration of 20 ng/ml, during short days had no effect on either follicle or fibre growth from either primary or secondary follicles. Within normal bounds of between animal variation, the patterns and magnitude of fibre growth were identical in both SD and P goats but differed to those of LD goats. In the preceding Chapters, bromocryptine treatment during long days has failed to identify clear evidence for plasma PRL concentration mediating the catagenic action of long days on the anagen goat follicle. In the previous experiments, bromocryptine treatment during long-photoperiod did not prevent follicles entering telogen but did prevent follicle reactivation (Chapter 7). Indeed there is more evidence for an anagenic, rather than catagenic, role of plasma PRL concentration in goat follicles. Anagen goat follicles, in culture, have an improved linear growth rate when PRL is incorporated into the media (Thomas *et al.*, 1993). Telogen down-goat follicles were activated when plasma PRL concentration was elevated by 50 ng/ml following twice daily subcutaneous injections of ovine PRL (4 mg/day) (Dicks, 1994). However this study was conducted during natural

spring photoperiod and the possibility that plasma PRL concentration change acts in synergy with other hormones or growth regulators which are not present during short days cannot be discounted. However, in contrast with this theory, both mink and hamsters maintained in short days, acquired and maintained summer pelage growth with exogenous PRL treatment (Duncan and Goldman, 1984; Badura and Goldman, 1992). The results of the systemic PRL infusion in this experiment are inconclusive. Possibly elevations in plasma PRL concentration do not induce catagen in anagen goat follicles, or the elevations in plasma PRL concentration in this experiment were not large enough to induce a physiological response, or finally that the PRL used in the experiment was not biologically active. Time limitations prevented the determination of the biological activity of the PRL by bioassay.

The local infusion of PRL directly to the skin failed produce significant effects on fibre growth. Similarly, a four day infusion of PRL directly to the skin of Romney sheep had no effect on the follicle bulb replication rate (Kelly *et al.*, 1993). In this experiment, fibre and follicle growth measurements, following local infusion of PRL to the skin, were seriously compromised by the local adverse tissue reaction. Indeed there was a declining trend in follicle activity on both saline- and PRL-infused sides, possibly in response to stress in the animal coupled by a drastic decline in intake engendered by the tissue reaction to PRL and subsequent swelling of the adjoining hind leg. The decline in SA was much greater in sheep compared with goats. In both the systemic and local skin infusion of PRL the outcome of the results was inconclusive.

The short-long photoperiod transition maintained its consistent effect on fibre growth observed in the preceding Chapter. Following a switch from short to long photoperiod (or release to natural long photoperiod for SD and P goats), plasma PRL concentration increased and, in close association, anagen primary and secondary follicles entered telogen between 4 to 7 weeks after the photoperiodic reversal. In this experiment the fibre growth events associated with the photoperiod induced anagen-telogen-anagen follicle cycle (ie SS_{max}, FGR_{max}, $NEDF_{e,}$, DL_{min}, SA_{max}) followed previously reported sequences (Chapter 7)

but these fibre events occurred 2-3 weeks earlier than those reported in Chapter 7. This may have arisen from the relatively larger PRL response following release to natural photoperiod compared to previous years, experiments.

8.5.5 Health

The tissue reaction following PRL infusion was unexpected as other pituitary hormones (Harris *et al.*, 1994; Pierzynowski *et al.*, 1995) have been infused into the skin without eliciting a 'toxic' response. However these hormones have been for infused for periods of less than 4 days and leg swelling was expressed after 6 days. The swelling of the legs and associated tissue reaction was independent of animal species, PRL source and Parlodel LA treatment.

Postmortem examination revealed extensive cellulitis in PRL-infused skin, combined with swelling and enlargement of the iliac lymph node. Enlargement of the lymph node was more prevalent in animals which had only received infusions of PRL for short periods. It proved to be impossible to differentiate a cause and effect between the symptoms based on postmortem examination. Cellulitis may have caused an enlargement of the lymph node but conversely the enlargement of the lymph node may have blocked lymph drainage resulting in the pooling of lymph fluid in the tissue followed by cellulitis.

Towards the end of the experiment, in order to determine whether the tissue reaction was a local response to PRL by the skin immune system, blood from the arterial and venous catheters were collected for measurement of haematological parameters, particularly those of blood leucocyte cell populations. PRL is known to have an immunoregulatory function on both peripheral and central immune tissues (Skwarlo-Sonta, 1992). In fact a reciprocal interdependence exists between PRL secretion and leucocyte cell function. PRL binds to receptors on lymphocytes (and macrophages) and stimulates lymphocyte cell activity and secretion of a substance with PRL-like activity (Skwarlo-Sonta, 1992). It is possible that the infusion of PRL directly to the skin caused an over stimulation of the immune system

in the environs of the catheter tip. The iliac lymph node is located within 2 cm of the catheter tip and the high concentrations of PRL being released at the catheter tip may have provided a powerful mitogenic signal possibly accompanied by an increase in lymphocyte cell population. However, in the two animals in which venous iliac blood was collected from the PRL-infused skin, there was no evidence of a local increase in lymphocyte cell populations associated with PRL treatment in comparison to saline infusion. In addition, in 6 animals, PRL infusion to the skin was not associated with a systemic increase in leucocyte cell populations. However in systemically PRL-infused goats, lymphocyte cell population was greater during the infusion, compared to post-infusion period, when compared to SD and LD goats. But the failure of long photoperiod, with the associated increase in plasma PRL concentration, to also ellicit an increase in lymphocyte cell population, suggests other factors (eg. the additional catheter) in PRL-infused goats may have led to the increase in lymphocyte cell populations.

Systemic bromocryptine treatment impairs antibody production, reduces thymus weight, and decreases macrophage and lymphocyte activity (Morikawa *et al*., 1993; Lopezkarpovitch *et al*., 1994). Exogenous PRL treatment reverses these effects (Skwarlo-Sonta, 1992; Arkins *et al*., 1993) and therefore it was thought that bromocryptine acted on the immune system by its associated effects on the secretion of PRL from the pituitary. However, it has been found *in vitro* that dopamine acts directly on immune cells to inhibit the production of essential autocrine factors, eventually leading to cell death. Cell death was prevented by treatment with PRL (Braesch-Andersen *et al*., 1992). Further, *in vitro* lymphocyte cell proliferation was suppressed in response to the inclusion of bromocryptine (dopamine agonist) in the media (Morikawa *et al*., 1993). In this experiment, the observed swelling of the leg was similar in animals irrespective of bromocryptine treatment. However, the knowledge that bromocryptine acts directly on lymphocyte cells which move readily through the skin provides another pathway by which bromocryptine could modify fibre growth independent of its effects on pituitary PRL secretion.

Follicle cycling is associated with changes in the skin immune system (Ebling and Hale,

1983; Gibson *et al.*, 1991) particularly the catagen phase during which the follicle components below the level of the sebaceous gland are surrounded by macrophages and resorbed (Gibson *et al.*, 1991). In addition, during anagen it is speculated that the follicle is 'protected' from the immune system possibly by the proteoglycan sheath which, on breakdown during catagen, permits the immune system to attack cells not possessing the appropriate antigen markers of self (Gibson *et al.*, 1991; Westgate *et al.*, 1991; Messenger, 1993). It is possible that bromocryptine treatment used in the earlier experiments in this programme (Chapter 6, 7) acted directly to interfere with this process. However, due to the presence of PRL receptors on the cell surface of lymphocytes (Pellegrini *et al.*, 1992; Arkins *et al.*, 1993), macrophages, peripheral lymphoid organs (Gagnerault, 1993) and Wiltshire sheep follicles (Choy *et al.*, 1995) it is likely that changes in plasma PRL concentration may affect these tissues directly. PRL receptors are found in the ovine apocrine sweat and sebaceous glands and the dermal papilla of follicles in all growth stage in the skin of NZ Wiltshire sheep (Choy *et al.*, 1995; Choy pers. comm.). Equivalent studies have yet to be conducted on the skin of down-producing goats.

It has recently been discovered that a PRL-binding protein exists in the serum of humans (Leite *et al.*, 1992) and rats (Cohen *et al.*, 1993). It is possible that the tissue reaction in sheep and goats in this experiment was due to high concentrations of unbound PRL in the skin blood circulation. Blood PRL binds to immunoglobulins (proteins). In humans during drug related elevations in plasma PRL concentration, binding to immunoglobulins was greater than in untreated humans (Walker *et al.*, 1992). Immunoglobulins are secreted from activated B lymphocytes following binding by antigens. PRL clearly has an integral role in immune function in the skin and it is possible that both plasma PRL concentration and the immune system interact to affect follicle growth.

8.6 CONCLUSIONS

Follicle and fibre growth were unaffected by either systemic or local infusions of PRL in down-producing goats and Wiltshire sheep. However it is not clear whether plasma PRL concentration was elevated by infusions. Plasma PRL concentration is higher in the skin than the systemic circulation. The infusion of PRL directly to the skin caused an extreme local tissue reaction.

CHAPTER 9

CONCLUSIONS

The objective of this research programme was to determine whether PRL has a causal role in regulation of the moulting and growth of fleece in Wiltshire sheep and down goats. At the start of this programme of research it was thought that the increase in plasma PRL concentration in spring prompted shedding in cashmere goats and Wiltshire sheep. Shedding in Wiltshire sheep was thought to occur in response to follicle activation but in down goats shedding of down was considered to be a process independent of follicle activation.

The complexity of the secondary growth cycles in down-producing goats was described using a combination of various fibre and follicle measurements. The combination of gross fibre and follicle measurements, after physiological manipulation, revealed subtleties of winter and summer down growth responses which otherwise would have remained concealed. For instance, in Chapter 6 follicles became activated in spring, during bromocryptine treatment, but did not produce the characteristic summer down fleece until after cessation of the bromocryptine treatment. The identification of the short summer down anagen period was made possible by noting the emergence of down fibres above the skin. Following physiological manipulations, the timing and duration of fibre events was often condensed and data interpretation was aided by the definition of 'new follicle growth' as the presence of active+brush fibres within the same follicle. However, when the progression of fibre events were known and predictable (eg summer down growth in natural spring photoperiods), but not synchronised between animals, accurate descriptions were possible by using a combination of 'time saving' gross fibre measurements such as DL decrease, shed fibre and NEDF, after allowing for the delay between follicle and gross fibre changes (Chapter 3). Many previous studies conducted on down-producing goats have been compromised by the

absence of measurements of both summer and down winter growth patterns and have relied on one or two measurements of fibre growth. It is particularly important in physiological experiments with down-producing goats that a combination of both gross fibre and follicle measurements are taken to aid data interpretation.

Down-producing goats, of the genotypes studied had a highly seasonal follicle growth cycle which was closely aligned to natural photoperiod (Chapters 2, 3) and was modified by photoperiod manipulation (Chapters 6, 7). Both primary and secondary follicles of down-producing goats possessed a biannual growth cycle (Chapter 2, 3). During spring the seasonal shedding of the 'winter fleece' was associated with growth of new fibres (Chapter 3). In secondary follicles, the period of follicle growth in spring was short (30-50 days) and all secondary follicles of down goats underwent this transient follicle cycle. As a result the down fleece produced in summer was barely discernible to the naked eye (Chapter 3, 6). The anagen period for winter down growth was longer (100-150 days) than that for summer down growth and the winter down fleece grew to more than 8 cm in length. The winter down fleece commenced growing prior to the summer solstice and growth continued until after the autumn equinox when follicles progressively entered telogen.

The incorporation of genes of the Angora, (a goat with a diminished expression of fibre seasonality) into down genotypes was associated with a switch from biannual to annual anagen growth cycles in secondary follicles, an extension of the duration of the anagen phase in primary follicles, and a reduction in the duration of telogen in secondary follicles (Chapter 2). However, the timing of the onset of primary and secondary growth in spring remained highly associated in down-producing goats of various genotypes. This suggested that the onset of growth in spring occurred in response to a photoperiodically linked signal which was conserved in all studied genotypes.

Plasma PRL concentration was characterised by a seasonal cycle and associations were found between increases in plasma PRL concentration and various secondary and primary growth events in down goats (Chapter 3). However, causal relationships between secondary follicle activation and plasma PRL concentration increase could not be found. In contrast, primary follicle activation in spring was found to be associated with a increasing plasma PRL concentration.

Plasma PRL concentrations were successfully manipulated using photoperiod reversals, treatment with dopamine agonist (bromocryptine) and an antagonist (domperidone) and by the infusion of PRL. In goats, bromocryptine and domperidone treatment decreased and increased plasma PRL concentrations respectively in both summer and spring (Chapter 4). The greater increase in plasma PRL concentration following domperidone treatment in summer suggests that dopamine is more inhibitory to pituitary PRL secretion in summer. A single daily injection of domperidone produced supra-physiological plasma PRL concentrations and the response was transitory. Plasma PRL concentration was not elevated when domperidone was administered via an osmotic pump (Chapter 4) either due to low dose rates reaching the pituitary or precipitation of domperidone in the pump. Consequently, domperidone treatment was discarded as a method for manipulating plasma PRL concentration. Long-acting bromocryptine injections given at two-three weekly intervals were effective at suppressing plasma PRL concentrations (Chapter 5, 6, 7, 8). However, when animals were treated with bromocryptine on a liveweight basis, plasma PRL concentrations were suppressed to a greater extent in sheep than in goats (Chapter 5, 6, 7). The detection of caprine and ovine PRL may have differed within the radioimmunoassay or the role of dopamine in regulating pituitary PRL secretion may differ slightly between the species. It is also possible that bromocryptine may be cleared differentially between the two species. The circulating-half life of PRL was similar in both sheep and goats but it was 60 minutes shorter in PRL-injected compared to PRL-infused animals (Chapter 5). Infusions of PRL failed to give consistent increases in plasma PRL concentrations (Chapter 5, 8).

Clearance rate of PRL may be dose responsive leading to a decline in plasma PRL concentration with sustained infusion or the infused PRL may feed back and reduce endogenous PRL secretion.

Plasma PRL concentration were manipulated using these techniques to determine potential effects on follicle growth in down goats and Wiltshire sheep. In these studies follicle growth was responsive to the onset, termination and duration of the plasma PRL manipulation. However, it was likely that follicle growth was controlled by a combination of endogenous and exogenous factors and this confounded data interpretation. In down goats, the follicle and fibre growth responses to bromocryptine and long photoperiod treatment did not indicate a simple causal relationship between plasma PRL concentration and fibre growth. Follicle responses varied with different methods of manipulation of plasma PRL concentration (photoperiod vs bromocryptine vs PRL infusion), with follicle growth stage at time of treatment (anagen vs telogen) and with the follicle type (primary vs secondary).

The transition from short to long photoperiod in down goats consistently elicited large increases in plasma PRL concentrations (Chapter 5, 6, 7, 8) and profound effects on follicle growth (Chapter 6, 7, 8). However, the effects on fibre growth were dependent on the growth phase of the follicle at the time of manipulation. During natural spring photoperiods, telogen primary and secondary follicles were activated by sudden elevations in photoperiod. Follicle activation advanced summer down growth and, in association, the shedding of the winter down fleece was also advanced. Secondary follicles then subsequently entered telogen characteristic of the summer down growth phase. Initiation of secondary follicles for winter growth occurred after the cessation of the long-day treatment. However, reactivation may have occurred independently of treatment termination. In contrast, primary follicles were activated and progressed to full activity during long photoperiod treatment (Chapter 6).

In later experiments a period of constant, short-day pre-conditioning was used to ensure that follicles of both down goats and Wiltshire sheep were largely active at the time of treatment application. Nonetheless some follicles underwent periods of asynchronous follicle cycling and in many follicles a short synchronised follicle cycle was observed in spring in both goats and sheep despite constant short photoperiod (Chapter 7, 8). However at the termination of the short photoperiod conditioning period, follicles were largely fully active and were highly responsive to the reversal from short to long photoperiod. Four to 6 weeks after photoperiodic reversal, anagen follicles of down goats and Wiltshire sheep entered telogen. A characteristic summer down fleece was expressed but its duration was shortened. Follicles then reactivated prior to the cessation of the long photoperiod treatment. This follicle cycle generated by the short-long photoperiod transition was highly synchronised within animals, within follicle type, repeatable across years and occurred both in goats and sheep (Chapters 7, 8). It is hypothesised that in down goats during natural photoperiods, telogen follicles are activated by increasing photoperiod. However, only secondary, and not primary, follicles deactivate as natural photoperiod lengthens during spring. Therefore the catagenic effect on primary follicles observed following artificial short to long photoperiod reversals may have little significance in normal seasonal follicle cycles in goats.

The role of plasma PRL concentration in mediating the effects of long photoperiod was studied using bromocryptine treatment to suppress plasma PRL concentration during normal spring photoperiods, during long photoperiod applied during spring and lastly, during the reversal from short to long photoperiods. Following pre-treatment with natural winter and spring photoperiods, telogen secondary follicles progressively activated during bromocryptine treatment under both natural and long photoperiods. However, these activated follicles failed to produce the characteristic short summer fleece as follicles failed to deactivate until after the cessation of the treatment. This gave further credence to the theory that increases in plasma PRL concentration may terminate the summer secondary follicle anagen phase, although it gave no support

to the theory that increases in plasma PRL concentration in spring result in the activation of secondary follicles (Chapter 6). However in goats in natural spring photoperiods which were treated with domperidone to increase plasma PRL concentrations, telogen secondary follicles were activated and summer down growth advanced (Chapter 4). Primary follicles were unresponsive to bromocryptine during natural photoperiods and follicle activation was only slightly delayed by bromocryptine treatment during spring-long photoperiod treatment. Bromocryptine treatment cannot negate the effect of increasing photoperiod on telogen primary follicles (Chapter 6).

Following a period of constant, short-day pre-conditioning, the anagen follicles of down goats and Wiltshire sheep had differing responses to bromocryptine treatment during the long photoperiod reversal. In sheep with follicles in anagen, bromocryptine treatment unequivocally prevented follicle deactivation following long photoperiod reversal. In goats, however, follicles entered telogen but reactivation was delayed by bromocryptine treatment. In contrast, during continued short-day treatment goat follicles remained fully active.

These experiments demonstrated that bromocryptine treatment can prevent the catagenic effect of long photoperiod on anagen Wiltshire sheep follicles. However these experiments failed to demonstrate a simple causal relationship between plasma PRL concentration and fibre growth in down goats. The possibility that in down goats, hormones other than PRL also act on the follicle during photoperiod treatment cannot be discounted. In addition it is possible that bromocryptine may act on the follicle independently to of effects on pituitary PRL secretion.

Infusion of PRL locally to the skin and systemically, during continued short-day treatment, had no effect on secondary and primary follicles of down goats and Wiltshire sheep. However, it is not known whether the systemic infusions elevated biologically active plasma PRL concentration. Unexpectedly, plasma PRL concentrations were higher in the circulatory system of the skin compared to the

general circulation. It is possible that significant amounts of PRL are synthesised in the skin and released into the blood supply.

Local infusions of PRL into the skin produced a local tissue reaction which may have been the consequence of hyperplasia of the local lymph node. The presence of high concentrations of PRL in the environs of the lymph node may have triggered the over- stimulation and enlargement of the lymph node, impairment of lymph drainage, and the consequent fluid accumulation in the hind-leg tissues. However, despite lymph node hyperplasia, increases in lymphocyte cells in the skin circulatory system were not detected following PRL infusion.

In many ways this research programme has posed more questions on the role of plasma PRL concentration in regulating seasonal fibre growth in down-producing goats than it has resolved. In fact no clear role has emerged for increasing plasma PRL concentration following short photoperiod treatment in mediating shedding and follicle activation in down goats or Wiltshire sheep. At this stage it would be appropriate to attempt to propose an unifying theory which accounts for all the observed results in this programme. However, this is not possible which suggests other unknown factors interact with plasma PRL concentration in regulating seasonal fibre growth in down goats.

Some alternative explanations may have emerged if time had permitted the testing of the infused-PRL for biological activity. Possible biological assays include the pigeon crop, lactation induction in rabbits, or PRL receptor binding in the skin. However, even if the PRL was shown to be biologically active, the concentrations achieved in these experiments may have been too low to elicit follicle growth responses.

In addition, a small experiment should be conducted to determine whether plasma PRL concentrations in down goats can be suppressed to similar concentrations, using long-acting bromocryptine, as was achieved in Wiltshire sheep. If similar plasma

PRL concentrations could be achieved in both species during long-photoperiod treatment the outcome of this experiment may be different.

The preliminary evidence that plasma PRL concentration was enhanced in the skin circulatory system of both down goats and Wiltshire sheep was unexpected and requires validation. Furthermore, it should be determined whether skin production of PRL is seasonal and responsive to bromocryptine infusion directly to the skin. In order to understand the complexities of the role of plasma PRL concentration within the follicle, interactions with other hormones and follicle PRL receptor regulation should be also examined. Finally, further study is required on the association between plasma PRL concentration increase, immune system activation and induction of catagen in the Wiltshire sheep.

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APPENDIX CHAPTER 2

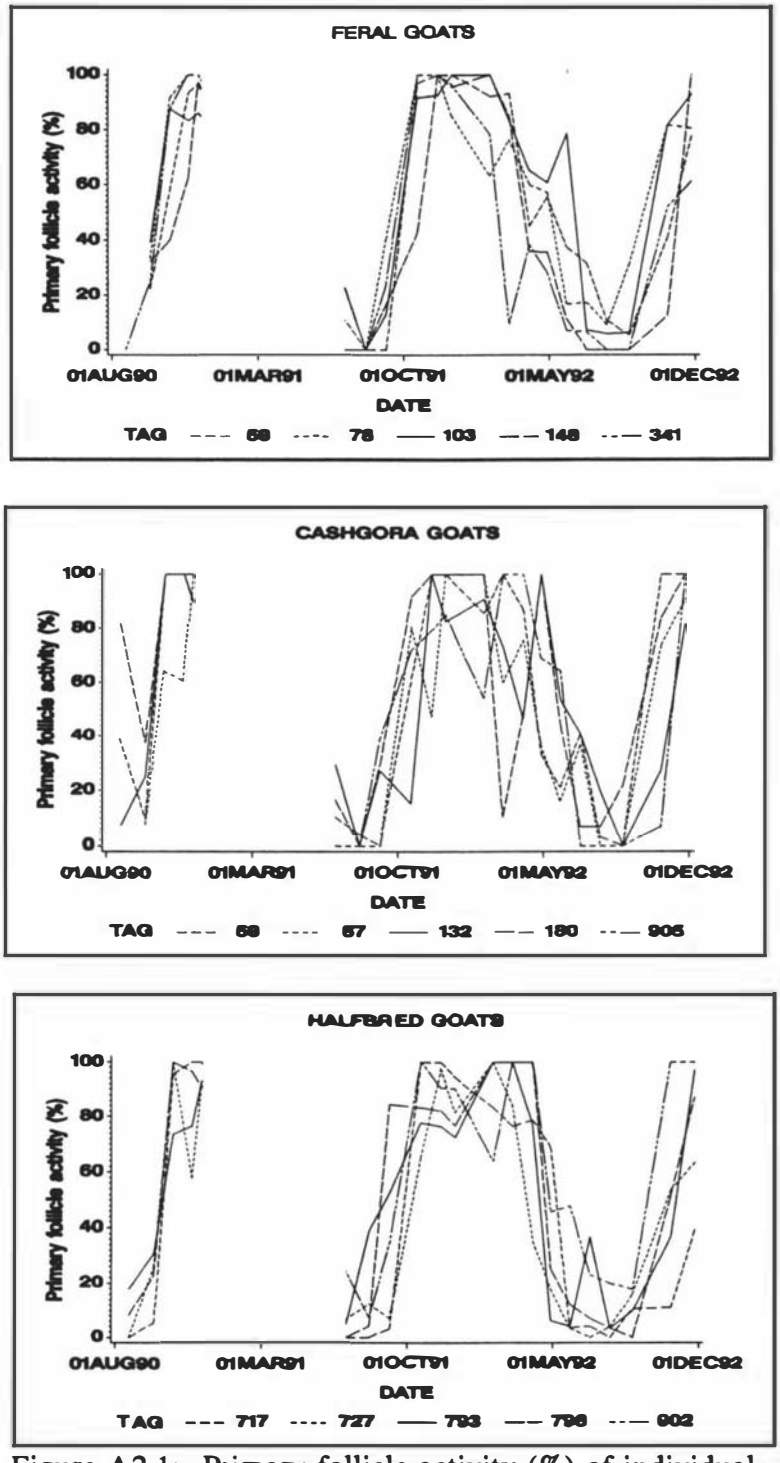


Figure A2.1: Primary follicle activity (%) of individual goats in the feral, cashgora and halfbred Angora x feral goat genotypes.

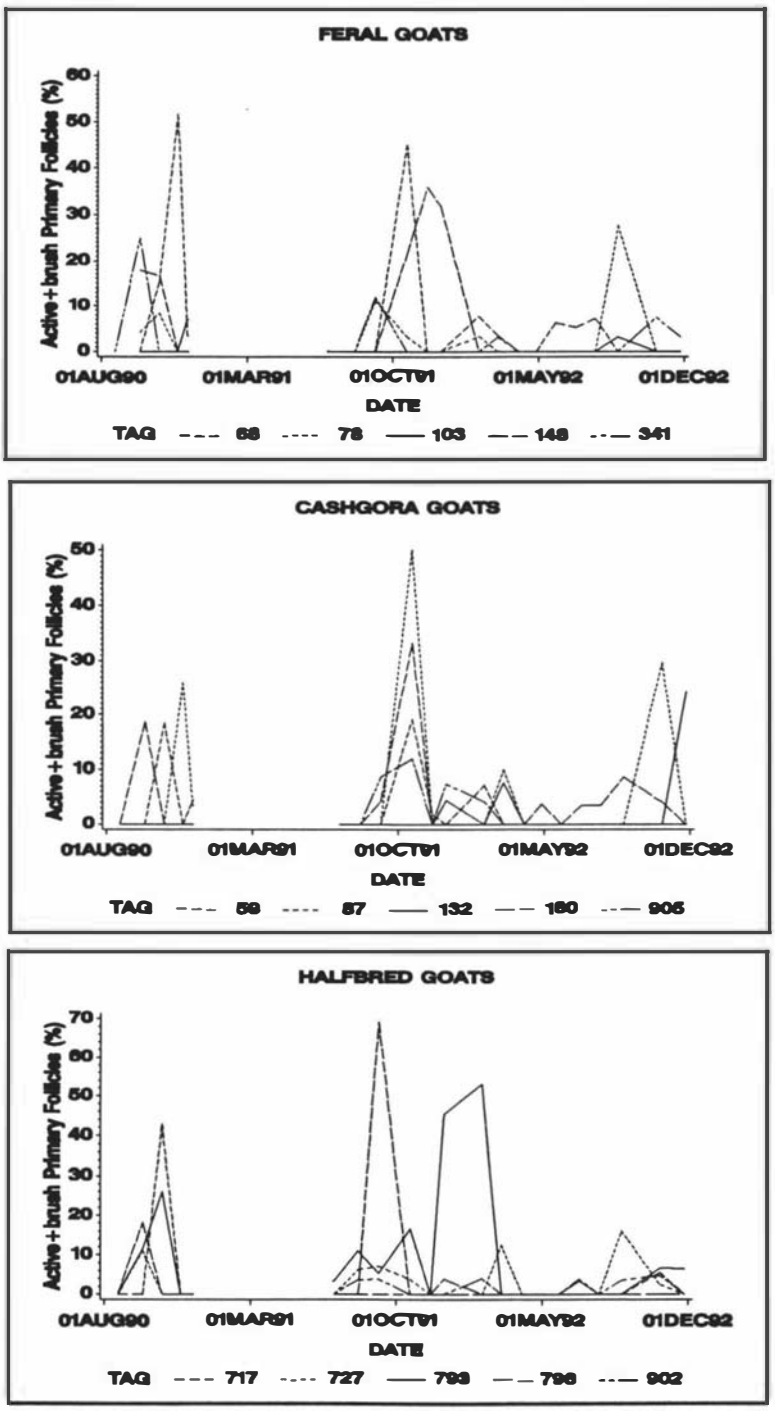
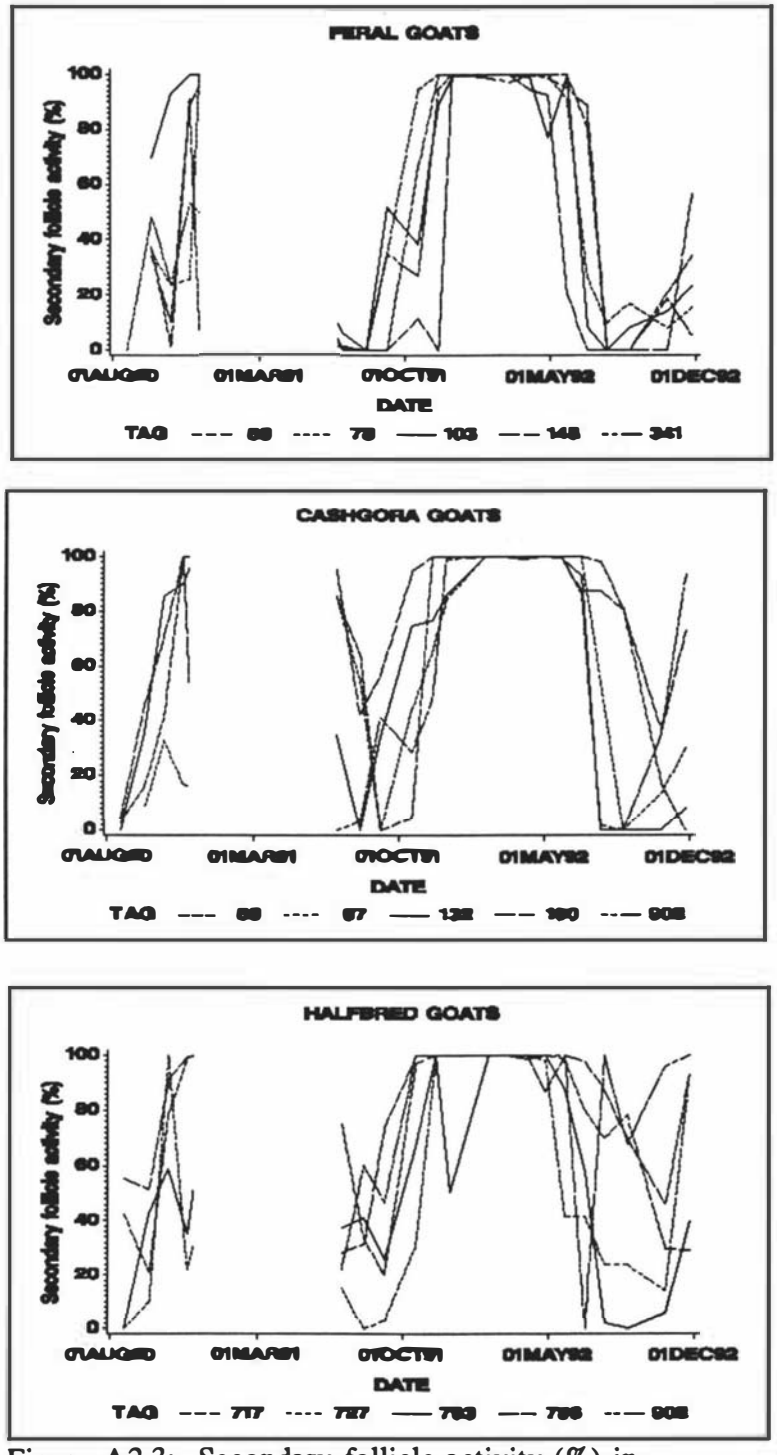


Figure A2.2: Percentage of primary follicles containing both active+brush fibres in individual goats in the feral, cashgora and halfbred Angora x feral goat genotypes.



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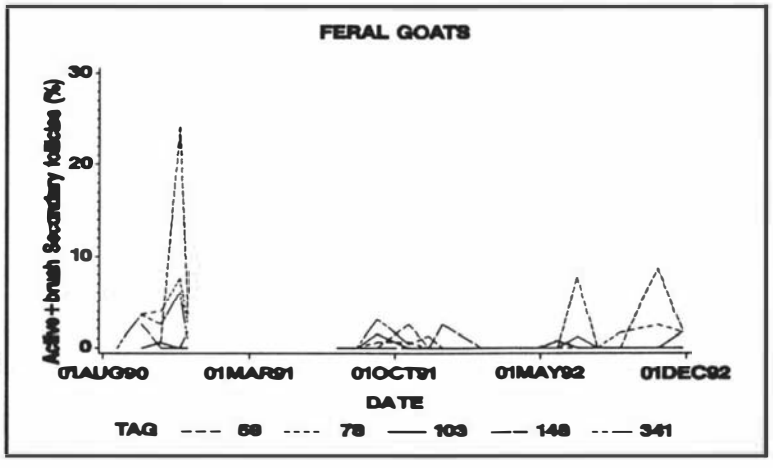

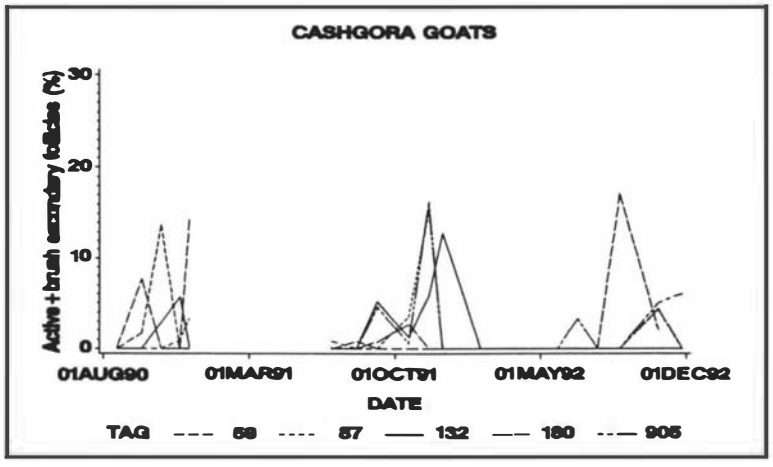

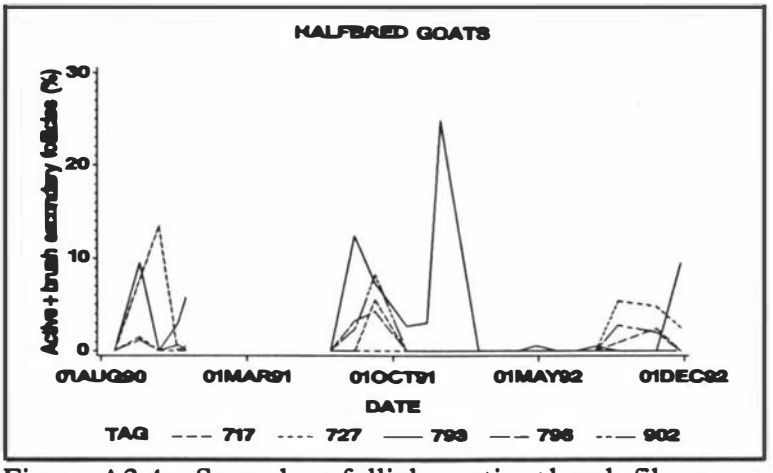

Figure A2.4: Secondary follicles active+brush fibres (%) in individual goats in the feral, cashgora and halfbred Angora x feral goat genotypes.

APPENDIX CHAPTER 3

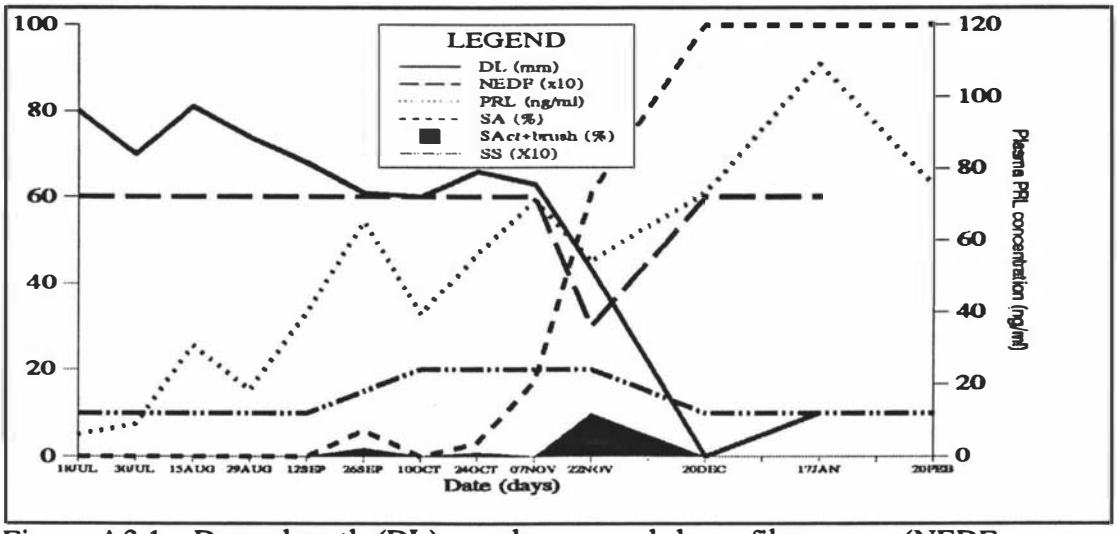

Figure A3.1: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 3.

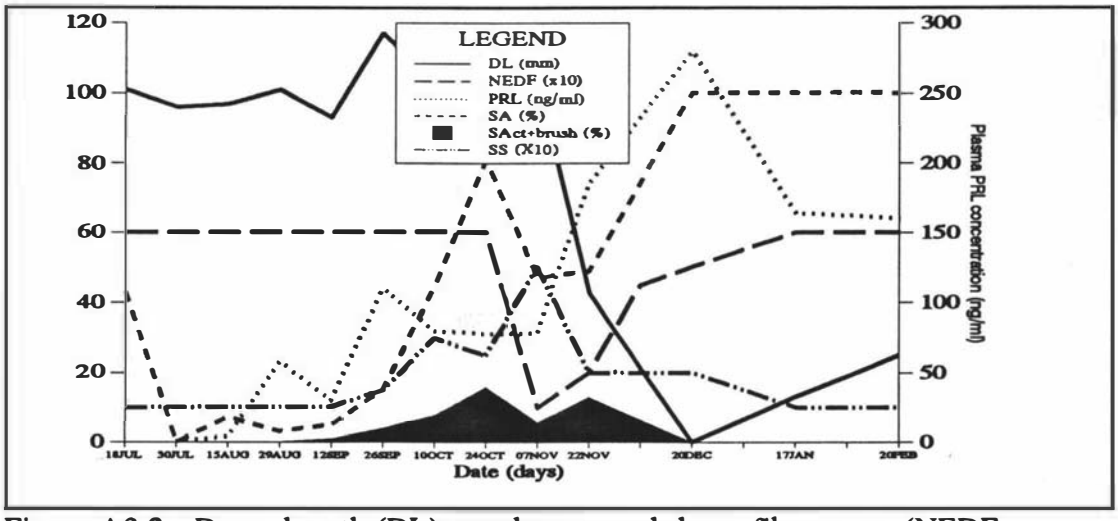

Figure A3.2: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 5.

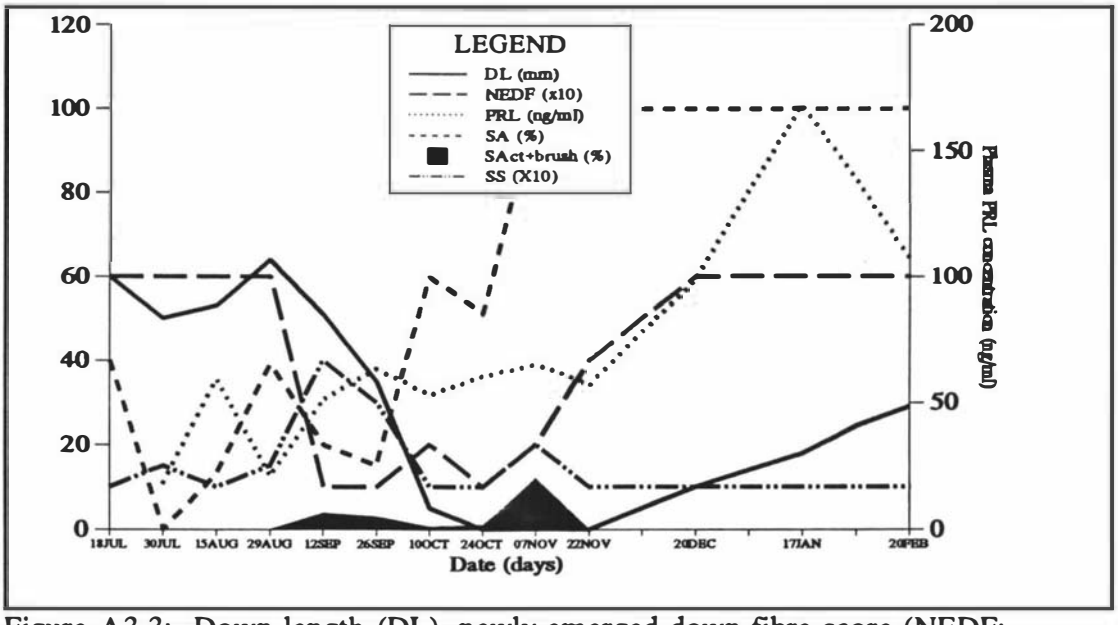

Figure A3.3: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 8.

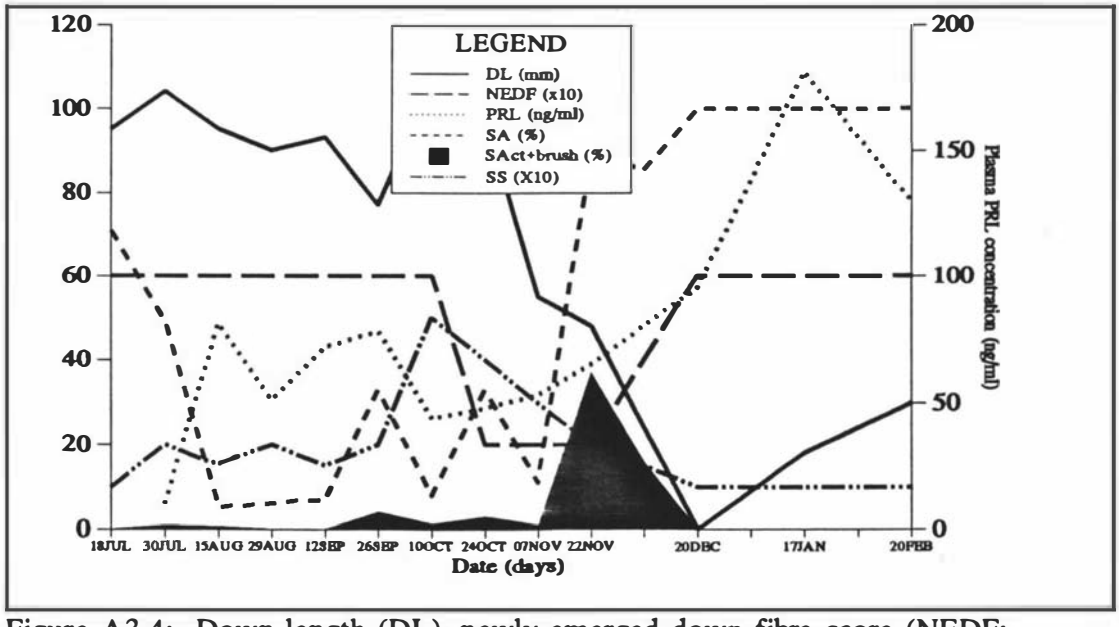

Figure A3.4: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 9.

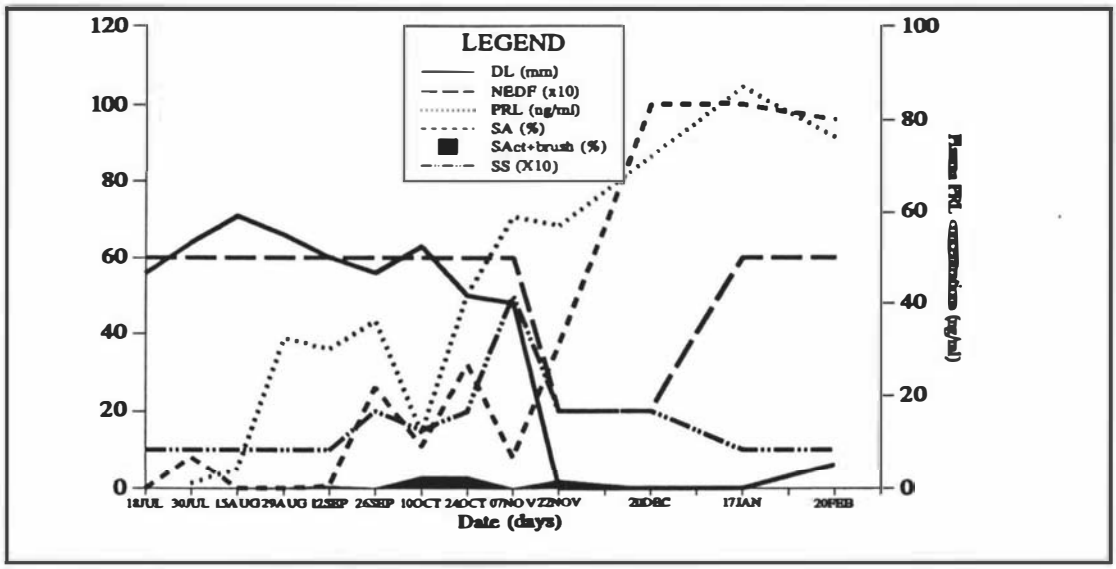

Figure A3.5: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 17.

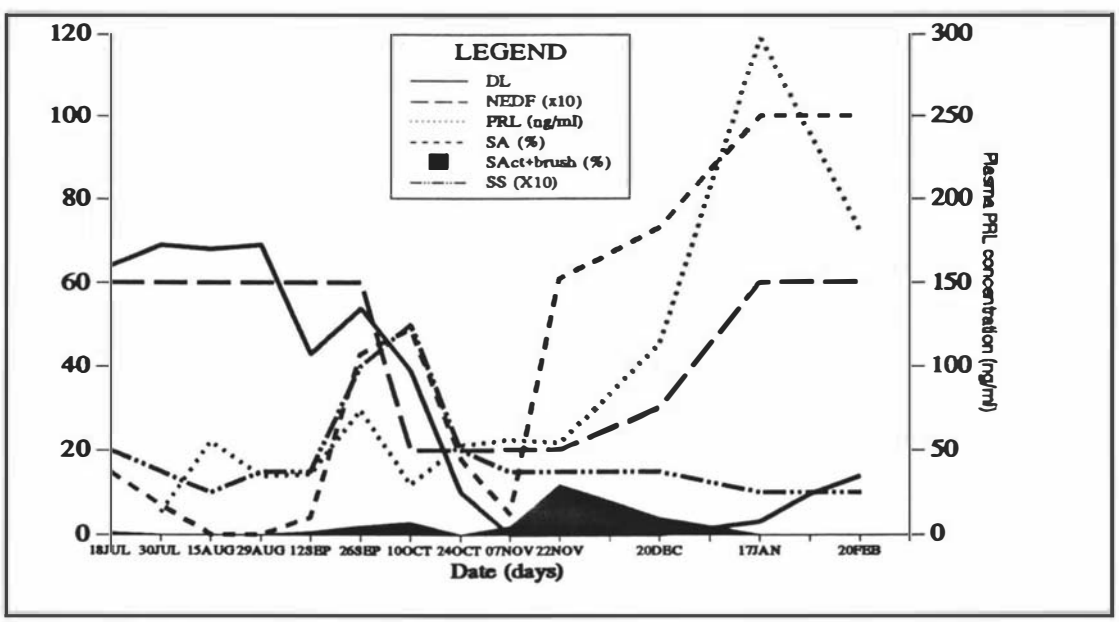

Figure A3.6: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 19.

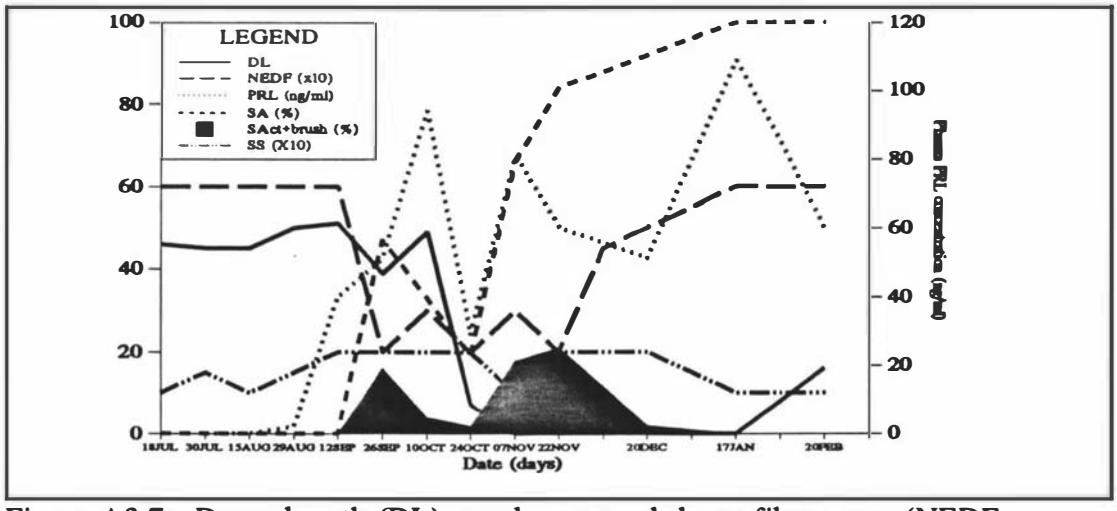

Figure A3.7: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 29.

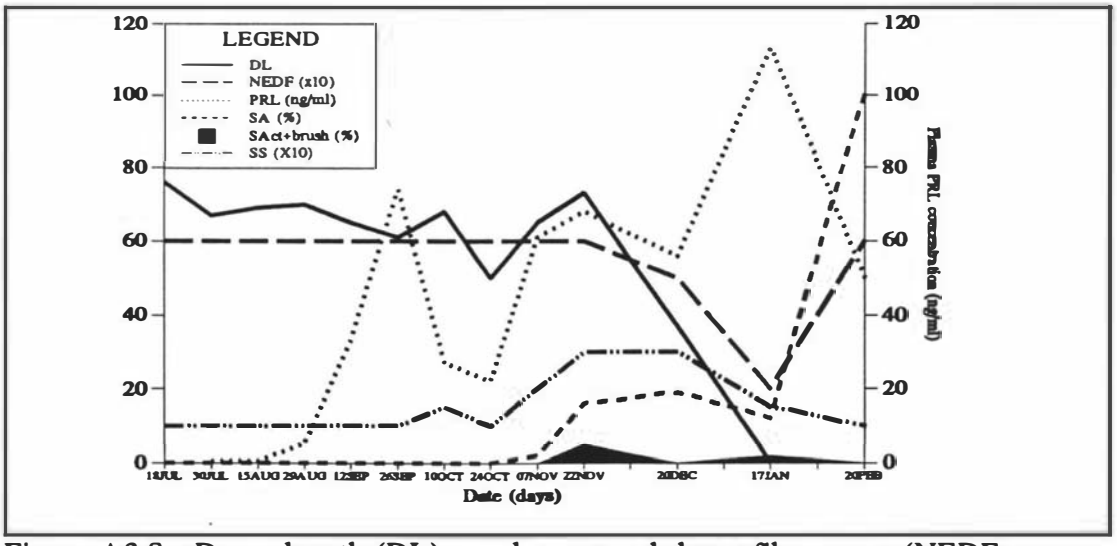

Figure A3.8: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 30.

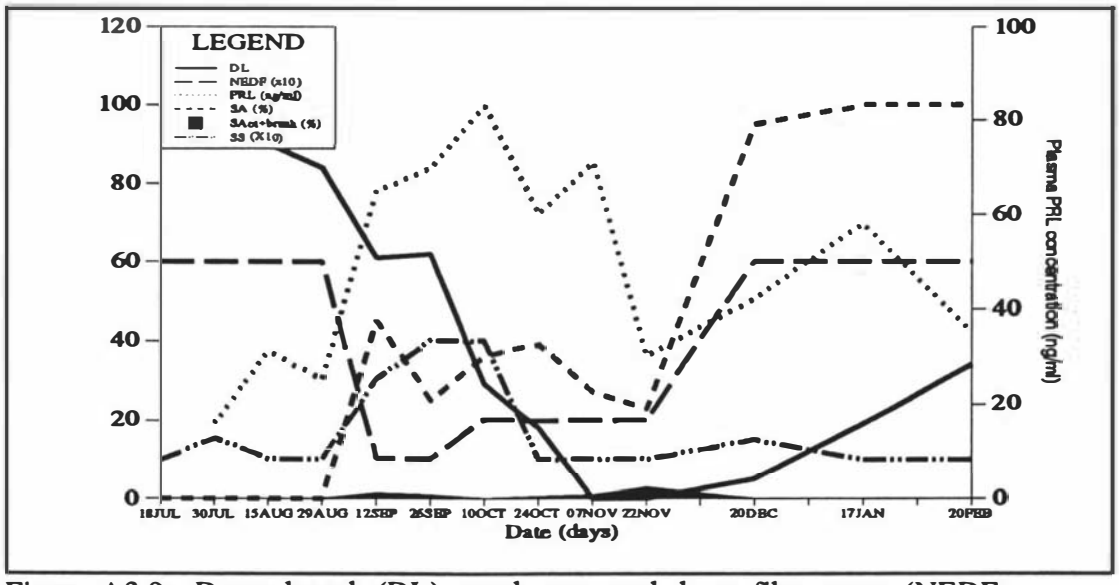

Figure A3.9: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 36.

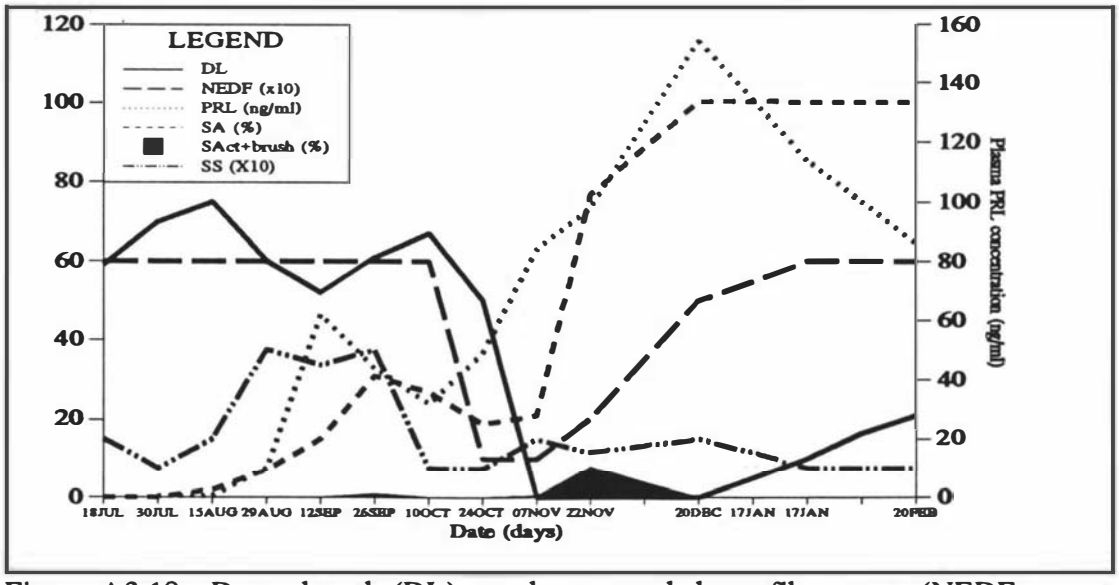

Figure A3.10: Down length (DL), newly emerged down fibre score (NEDF; 1=NEDF's only, 6=long down only), plasma prolactin concentration (PRL), secondary follicle activity (SA), proportion of follicles containing both active and brush fibres (SAct+brush) and shedding score (SS; 1=no shedding) in down-producing goat tag number 39.

APPENDIX CHAPTER 6

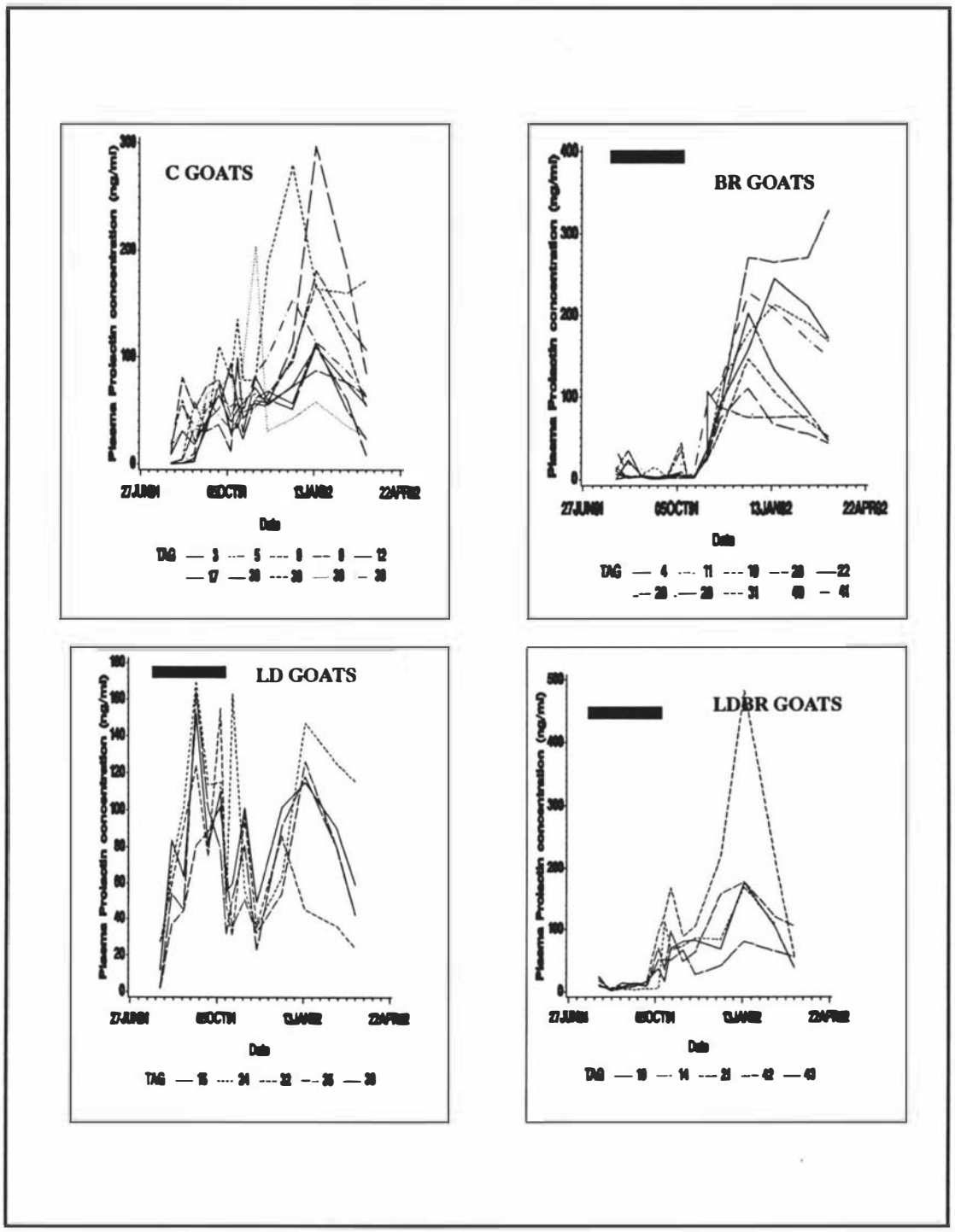

Figure A6.1: Plasma PRL concentration (ng/ml) in untreated control goats and goats treated with long photoperiod (LD)and goats treated with long-acting bromocryptine during natural photoperiod (BR) or long photoperiod (BRLD). Horizontal bars are period of treatments.

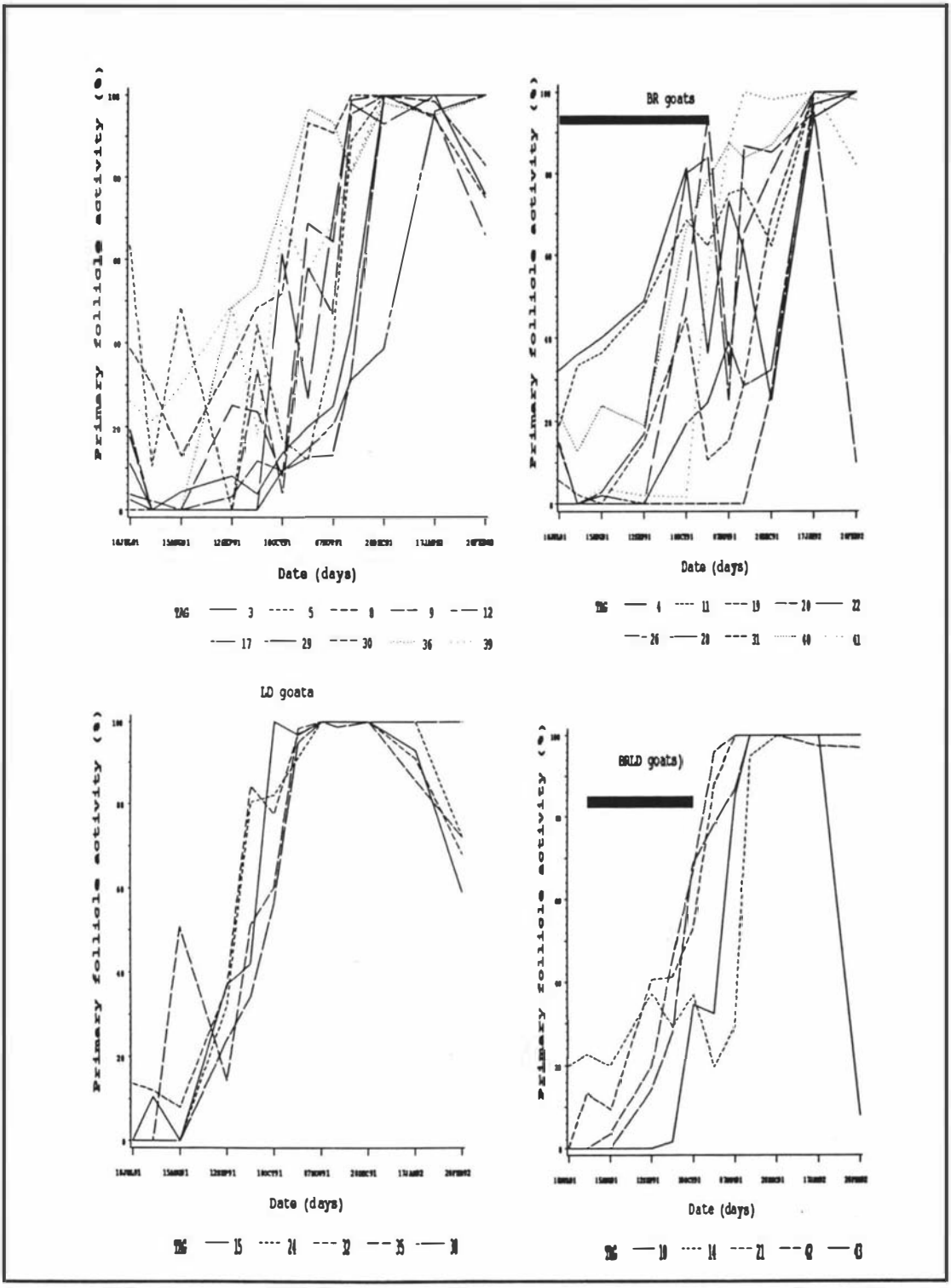

Figure A6.2: Primary follicle activity (%) in untreated control goats (top left), goats treated with long photoperiod (LD) and goats treated with long-acting bromocryptine during natural photoperiod (BR) or during long photoperiod (BRLD). Horizontal bars are period of treatments.

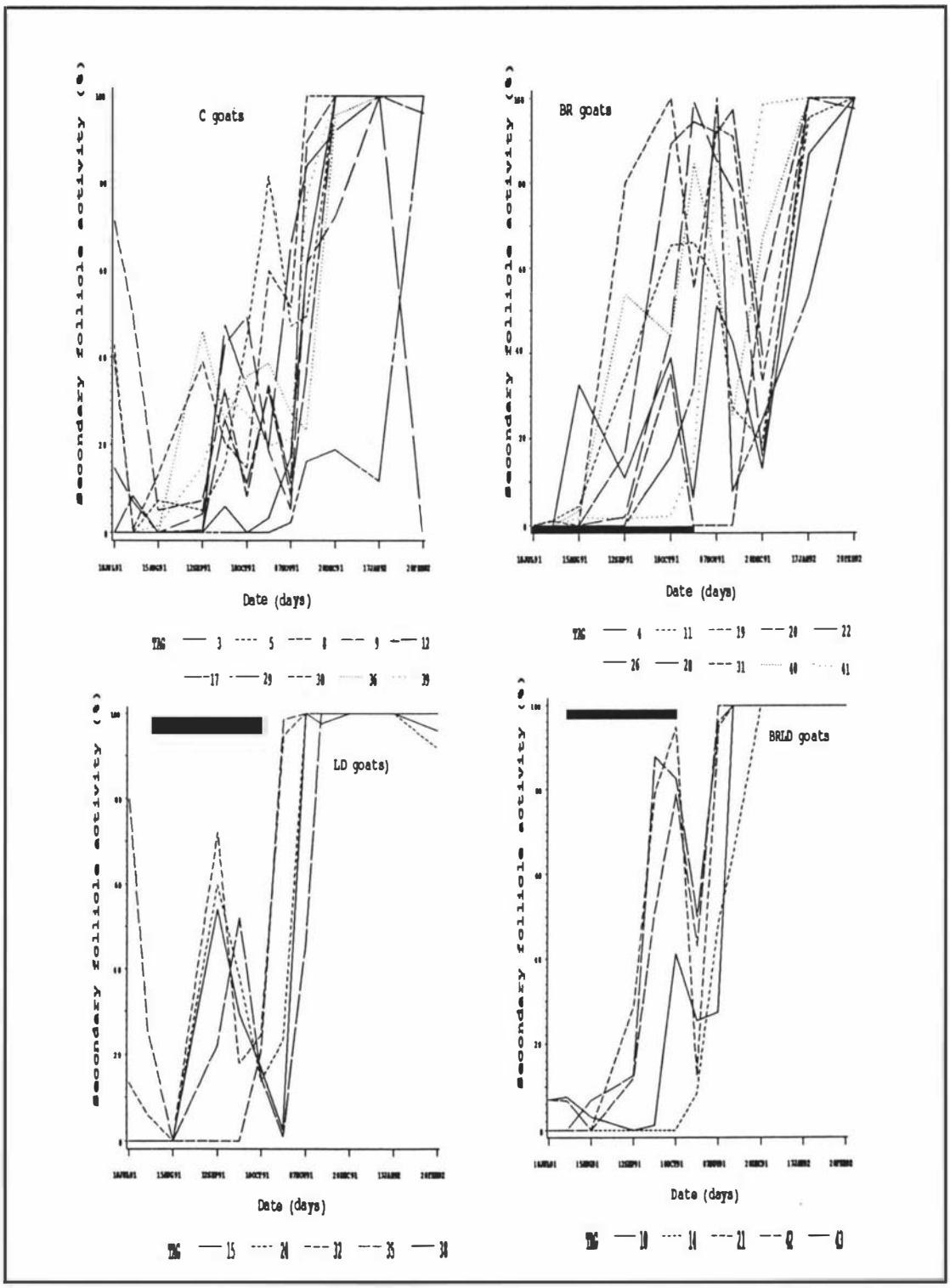

Figure 6.3: Secondary follicle activity (5) in untreated control goats (c), goats treated with long photoperiod (LD) and goats treated with long-acting bromocryptine during natural photoperiod (BR) or during long photoperiod (BRLD). Horizontal bars are period of treatments.

APPENDIX CHAPTER 7

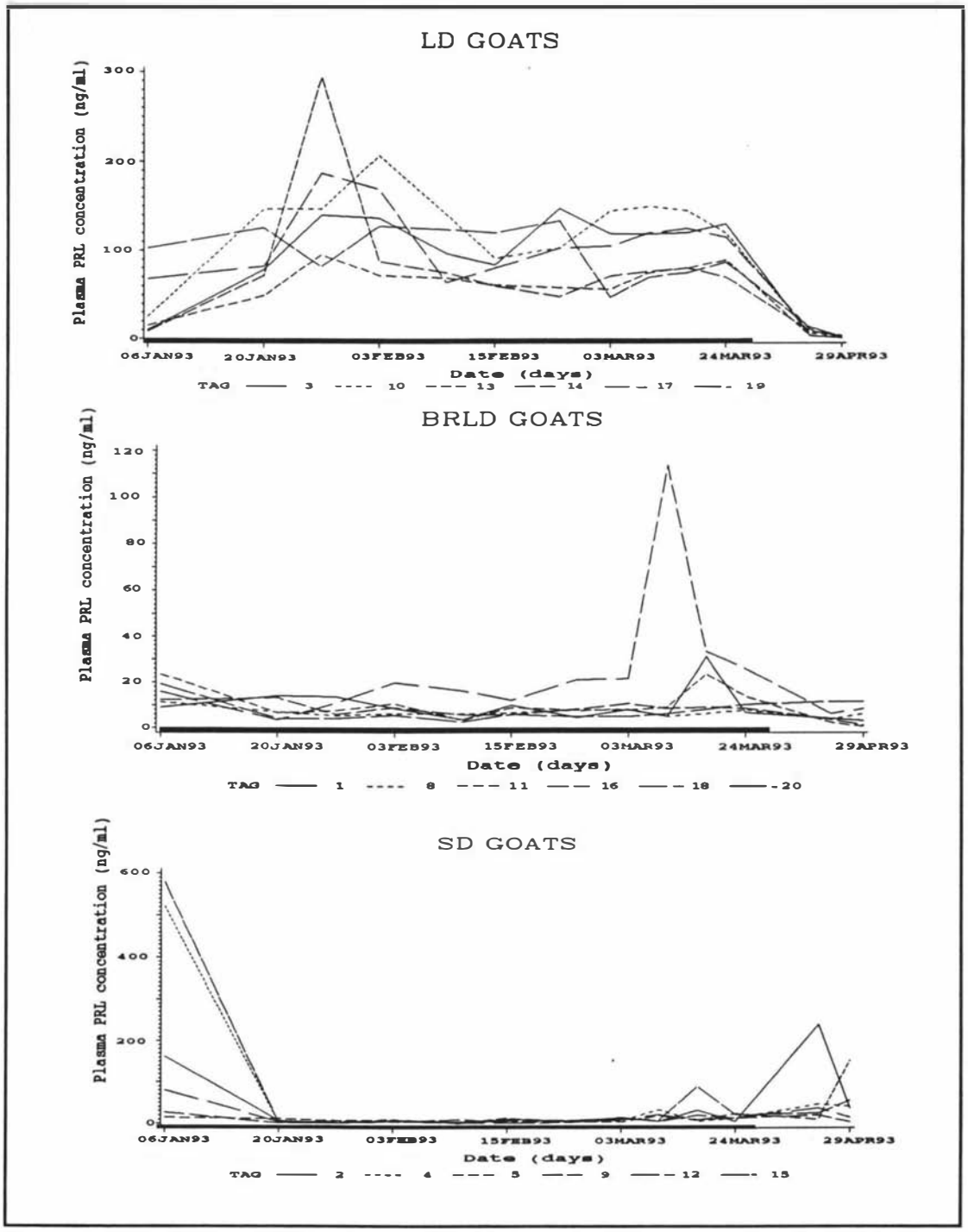

Figure A7.1: Plasma PRL concentrations in individual goats treated with short photoperiods (SD goats), long photoperiod (LD goats) and with both long photoperiod and long-actinng bromocryptine (BRLD goats). Horizontal bars represent peroid of treatment.

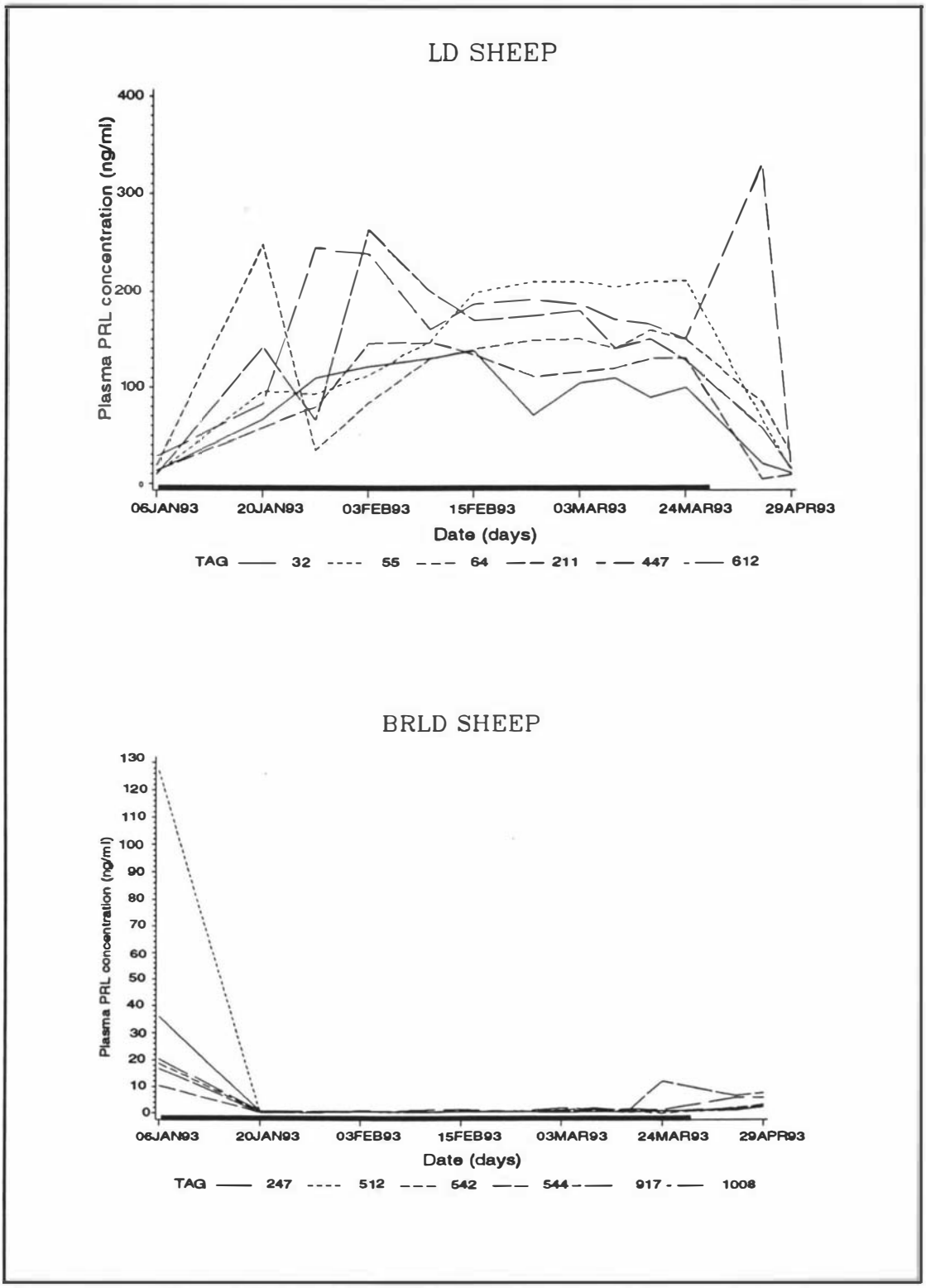

Figure A7.2: Plasma PRL concentrations in individual sheep treated with long photoperiod (LD sheep) and with both long photoperiod and long-acting bromocryptine (BRLD sheep). Horizontal bars represent period of treatment.

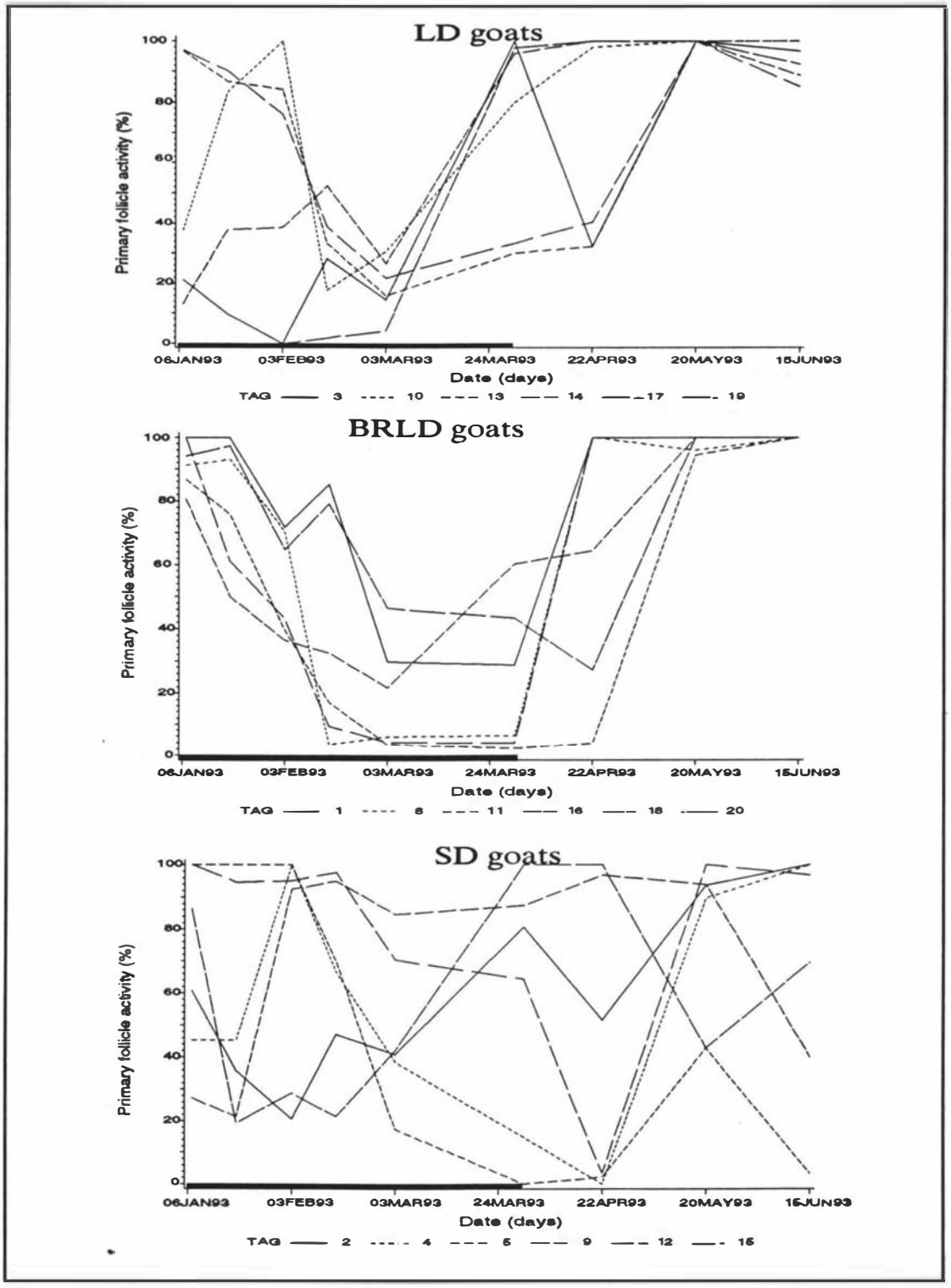

Figure A7.3: Mean primary follicle activity in individual goats treated with short photoperiods (SD goats), long photoperiod (LD goats) and with both long photoperiod and long-acting bromocryptine (BRLD goats). Horizontal bars represent period of treatment.

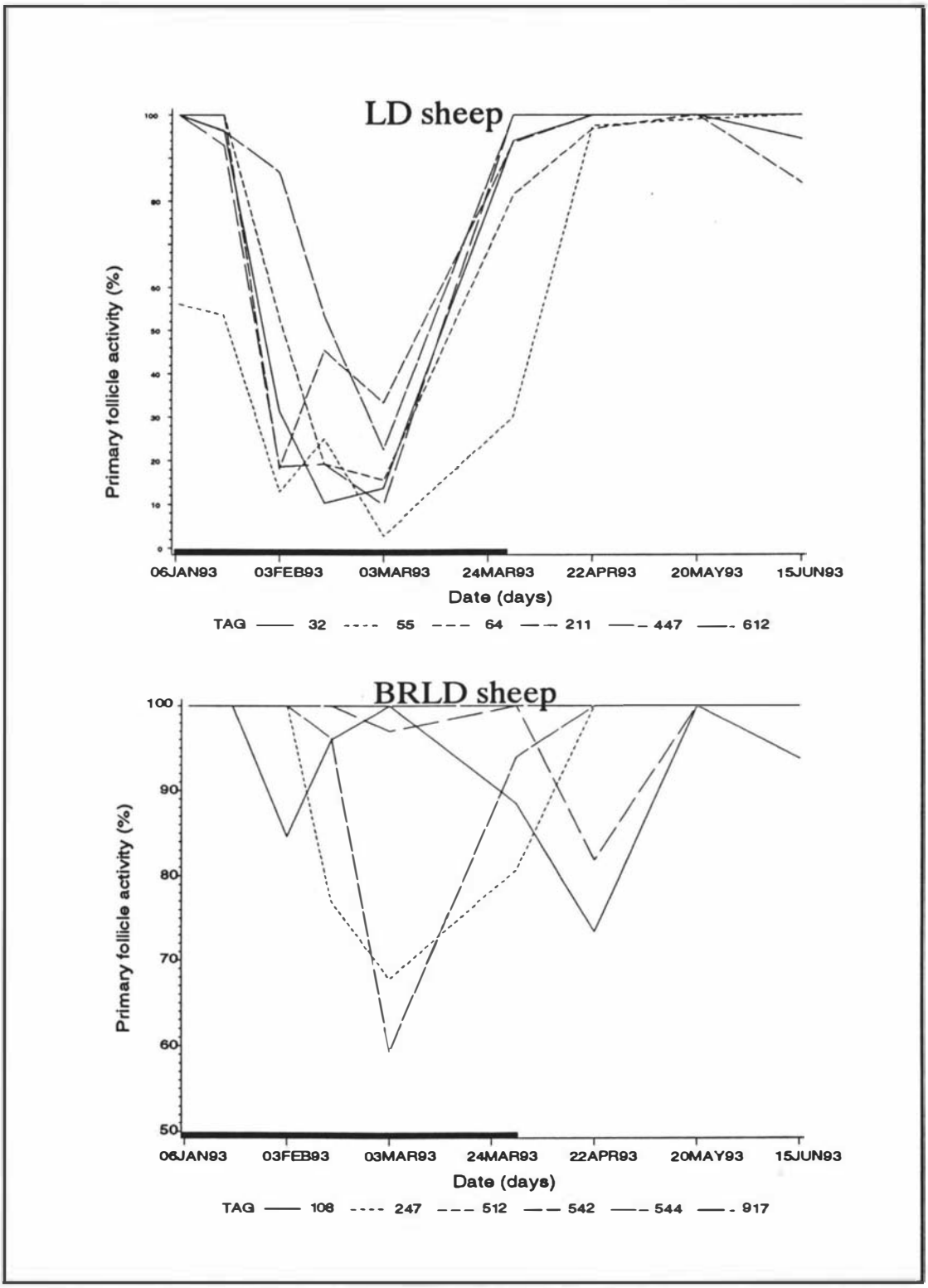

Figure A7.4: Mean primary follicle activiity in individual sheep treated with long photoperiod (LD sheep) and with both long photoperiod and long-acting bromocryptine (BRLD sheep). Horizontal bars represent period of treatment.

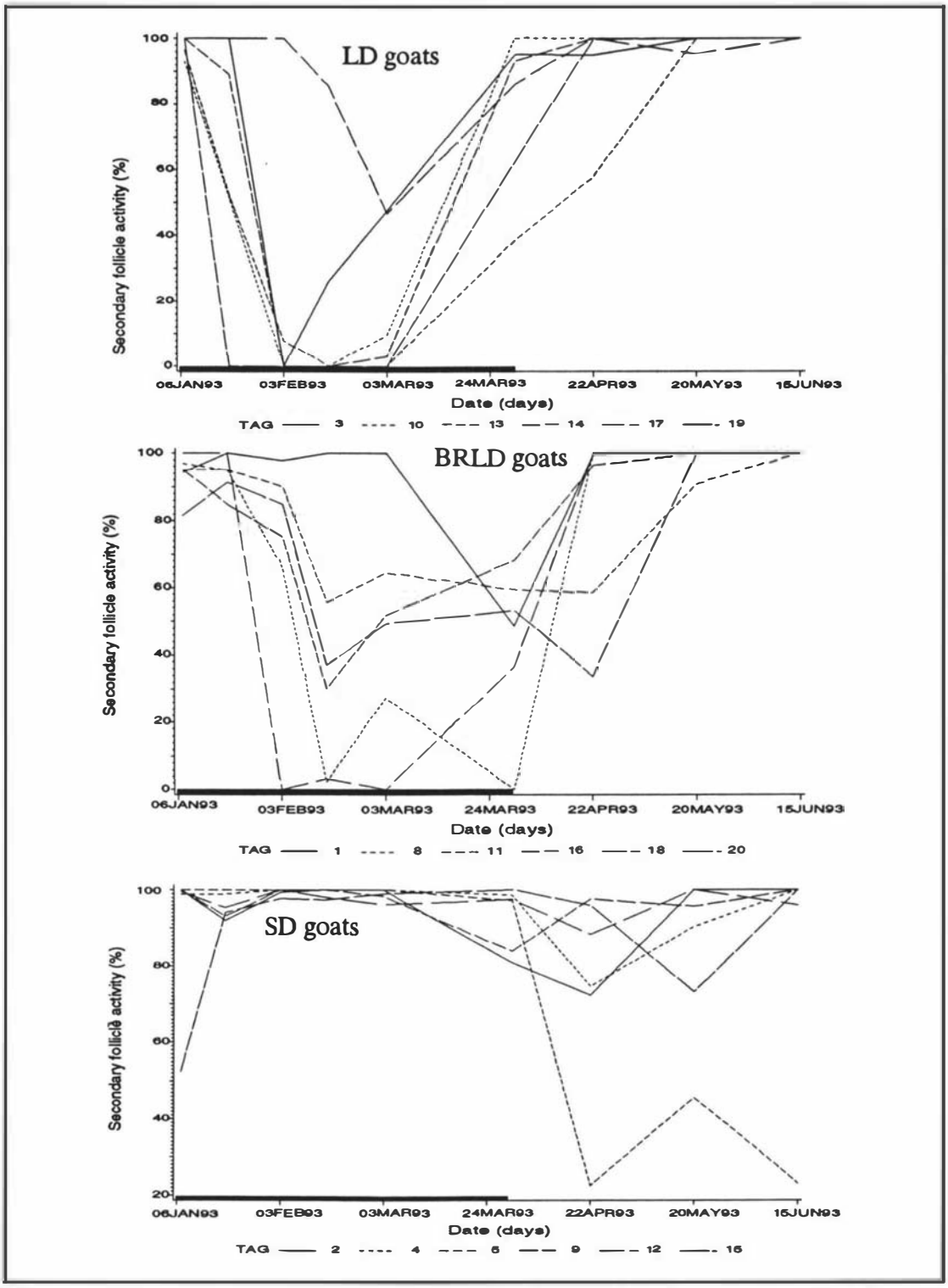

Figure A7.5: Mean secondary follicle activity in individual goats treated with short photoperiods (SD goats), long photoperiod (LD goats) and with both long photoperiod and long-acting bromocryptine (BRLD goats). Horizontal bars represent period of treatment.

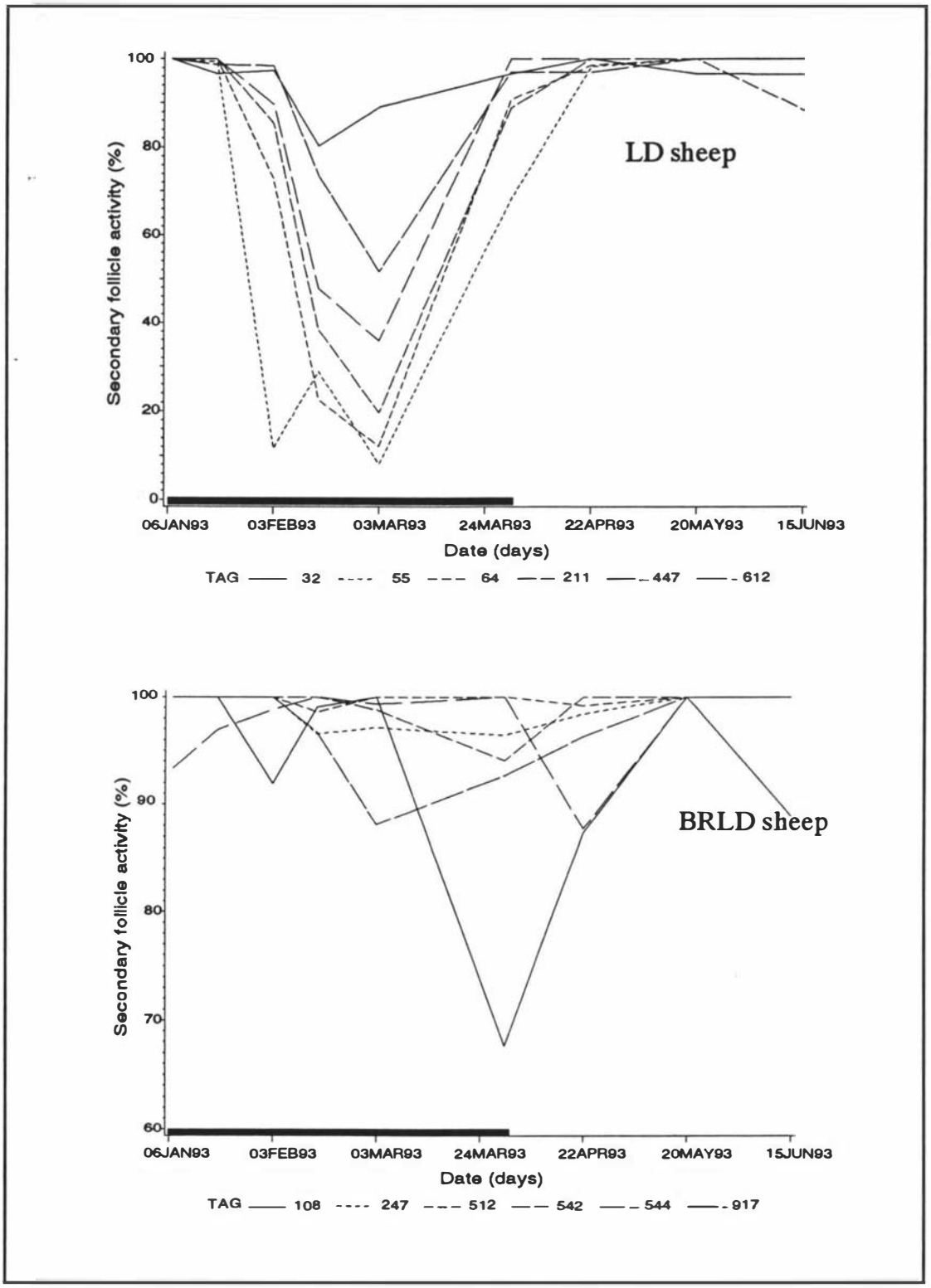

Figure A7.6: Mean secondary follicle activiity in individual sheep treated with long photoperiod (LD sheep) and with both long photoperiod and long-acting bromocryptine (BRLD sheep). Horizontal bars represent peroid of treatment.