A comparison of protocols for the photoperiodic induction of synchronised wool follicle growth cycles

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ABSTRACT

The decline in wool growth in long-woolled sheep breeds during winter is associated with a reduction in follicle mitotic rate that is comparable to early catagen, the regressive stage of the follicle growth cycle. The identification of morphological or biochemical markers which indicate the onset of catagen would be facilitated by an experimental protocol that induces a synchronous entry into catagen. Four different photoperiod manipulations were compared, all involving short-to-long day transitions with New Zealand Wiltshire sheep. The percentage of primary (P) and secondary (S) follicles in the growth and resting phases of the cycle were assessed histologically from skin biopsies, and blood samples were collected to determine circulating prolactin (PRL) by radioimmunoassay.

In control sheep, plasma PRL levels increased over the spring to a maximum in December (254 ± 45 ng/ml). In short day (8L:16D) treated sheep, plasma PRL levels were suppressed (< 23 ng/ml) but rose rapidly after photoperiod treatment was terminated. In sheep maintained in short-days from 25 July to 22 October and released into either natural photoperiod, or into artificial long days (16L:8D), mean follicle activity declined by 58 ± 14% (P) and 49 ± 10% (S) during November and December. This reduction was coincident with, and did not differ significantly from, a natural spring decline in follicle activity observed in control sheep (55 ± 2% P; 56 ± 18 % S). Short day treatment beginning on either 25 July or 5 October and ending on 6 January caused a rapid synchronised drop in follicle activity of 65 ± 7% (P) and 58 ± 7% (S) 6 weeks after exposure to natural long-day photoperiod. Contemporary P and S follicle activities in all untreated sheep remained at 100%.

Conditioning with short days for 3 months in late spring and early summer followed by sudden exposure to long days increased PRL and induces synchronous entry of wool follicles into catagen.

Keywords: Wiltshire sheep; animal model; wool follicle; prolactin; histology; seasonality.

INTRODUCTION

Seasonal variation in wool growth of long-woolled sheep breeds leads to reduced winter fibre diameter, length growth rate and staple strength (Orwin, 1989). These changes in fibre morphology are associated with a reduced rate of cell proliferation in the wool follicle bulb (Holle, 1992). A comparable reduction in mitotic rate also occurs in early catagen, the regressive stage of the follicle growth cycle (Parry *et al.*, 1995). In shedding breeds, such as the NZ Wiltshire, bulb mitotic activity ceases completely during late catagen with the formation of a brush end that anchors the fibre within the resting (telogen) follicle. During spring, reactivation of follicle growth and production of new fibres results in the shedding of the fleece (Parry *et al.*, 1991). Such breeds are convenient models for the study of wool growth as the large seasonal changes in follicle morphology can be quantified using histological techniques.

The identification of morphological and biochemical markers associated with follicle growth would be facilitated by an experimental technique which induces synchronised catagen so that follicles can be collected at predictable stages of the cycle. Exposure of NZ Wiltshire sheep to a short-day photoperiod from mid-winter induces a follicle growth cycle after release into natural midsummer long day photoperiod (Pearson *et al.*, 1993a). The present study compares this protocol with other photoperiod manipulations to develop an optimal model for the investigation of mechanisms controlling changes in seasonal wool production.

MATERIALS AND METHODS

Trial 1

Sixteen, non-pregnant, 3 year old NZ Wiltshire ewes were randomly allocated to 4 groups (n=4). Each group was housed indoors at Flock House Agricultural Centre, Bulls, from 25 July 1991 as follows. Two groups were maintained for 3 months under short-day photoperiod (8L:16D) until 22 October. Of these, one was released into natural spring daylength (SDND), and the other into an artificially extended long-day photoperiod (16L:8D) until 6 January using incandescent electric lighting (SDLD). The third group was kept in short days for 24 weeks (SD5) before release into natural summer daylength on 6 January 1992, while the fourth (control) group was maintained under the natural photoperiod (C1).

Trial 2

In 1992 at Ruakura Research Centre, Hamilton, 12 non-pregnant, 1 year old NZ Wiltshire ewes were randomly allocated to 2 groups. All sheep were housed indoors from 5 October. One group (n=6) was subjected to short day photoperiod (8L:16D) for 3 months, and released to natural summer daylength on 6 January 1993 (SD3). A control group (n=6) was maintained under natural photoperiod (C2).

Plasma Prolactin

Blood samples were collected fortnightly (Trial 1) or 3 weekly (Trial 2) during short day treatment then weekly for 10

(SDND), 11 (SD5) and 17 (SDLD) weeks post-treatment in Trial 1, and for 10 weeks post-treatment in Trial 2. Blood (10 ml) was collected into EDTA vacutainers at 11 am by jugular venipuncture. Plasma was separated by centrifugation and stored at -20° C until assayed in duplicate for prolactin (PRL) by radioimmunoassay as described previously (Nixon *et al.*, 1993).

Skin Histology

Skin snip biopsies were collected from the right mid-side of all sheep at 4 weekly (Trial 1) and 3 weekly (Trial 2) intervals during short day treatment and then weekly for 13 weeks (Trial 1) or fortnightly for 12 weeks (Trial 2) post-treatment. The skin samples were fixed in phosphate buffered 10% formalin, wax embedded, sectioned (8 μm) and stained by the Sacpic method. Each skin sample was assessed for the proportion of active primary (P) and active secondary (S) follicles in transverse section (Nixon, 1993).

Statistical Analysis

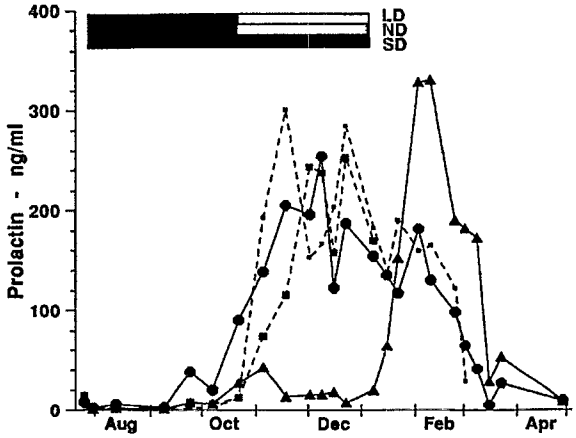
Differences in follicle activity and PRL concentration (log 10 transformed) between treatment groups were examined using general linear models with repeated measures over time (SAS Institute, USA. 1987). Treatment group differences in parameters related to the induced cycles were examined by analysis of variance (Table 1). The duration of the cycle was calculated as the time taken for follicle activity to fall and then recover to greater than 90%. Data are expressed as means ± SEM.

RESULTS

Prolactin

In Trial 1, C1 PRL levels increased from October. Plasma PRL concentrations in the 3 short day treatment groups remained low (<15 ng/ml) until after exposure to natural or artificial long days (Fig 1) when levels increased rapidly ($p<0.01$). Groups C1, SDND and SDLD had similar peak PRL levels of 254 ± 45, 243 ± 51, 300 ± 78 ng/ml, occurring on 9 December, 2 December and 18 November respectively. PRL levels in the SD5 group were suppressed until 6 January but then rose ($p < 0.001$) to 328 ± 41 ng/ml after exposure to natural long days for 4 weeks.

FIGURE 1: Mean plasma prolactin levels (ng/ml) in C1 (—●—), SDND (- - -□- - -), SDLD (- - -■- - -), and SD5 (—▲—) sheep in Trial 1. Short day (8L:16D) treatment duration for SDND, SDLD and SD5 groups is shown by horizontal bars.



Mean PRL levels in C2 sheep increased ($p < 0.02$) from 37 ± 12 ng/ml in October to a peak of 164 ± 39 ng/ml on December 23 (Fig 2). In SD3 sheep, PRL concentrations were low during treatment (<23 ± 8 ng/ml) ($p<0.01$), increasing within one week of exposure to long days to a maximum of 225 ± 75 ng/ml in early February ($p < 0.01$).

Follicle Activity

There were no significant differences between the follicle activity patterns (Figs 3a and b) of C1, SDND and SDLD groups in Trial 1. Following short day treatment, exposure to artificial (SDLD) or natural long days (SDND, SD5) follicle activity decreased in all groups (Figs 3a and b, Table 1). Follicle activity also fell in the C1 group in November but remained high in the SD5 group throughout short day treatment. There were no group differences between follicle activities at the start of the natural or induced cycles or in the extent of the follicle activity decline (Table 1). The mean durations of the P and S follicle growth cycles were shorter in

TABLE 1: Mean primary and secondary percentage follicle activities of (± SEM) immediately prior to the subsidiary growth cycle (C1 and C2) or at release from short day treatment (SDND, SDLD, SD5 and SD3); the decline in percent follicle activity during the subsidiary or induced cycle (averaged from the maximum declines of individual sheep which varied in timing); and the duration of the subsidiary (C1 and C2) or induced cycle (SDND, SDLD, SD5 and SD3). One sheep in the SDND group with 0% follicle activity on release from short days was excluded from the tabulated results. Significance of differences between treatments within trials is indicated by: ns nonsignificant; * $p < 0.05$; ** $p<0.01$; *** $p<0.001$.

	% Follicle Activity Prior to Growth Cycle		Decline in % Follicle Activity During Cycle		Duration of Cycle (Weeks)	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
Trial 1						
SDLD	77 ± 20	83 ± 17	59 ± 21	52 ± 12	15.8 ± 1.3	10.3 ± 1.5
SDND	74 ± 17	77 ± 18	57 ± 23	44 ± 22	15.3 ± 1.8	15.0 ±2.3
SD5	96 ± 4	99 ± 1	77 ± 11	66 ± 13	7.0 ± 1.0	5.2 ± 0..5
C1	69 ± 11	78 ± 13	55 ± 2	56 ± 18	17.5 ± 0.9	12 ± 1.5
Significance	ns	ns	ns	ns	***	**
Trial 2						
SD3	99 ± 1	100 ± 0	69 ± 8	66 ± 9	9.2 ± 1.0	7.6 ± 0.4
C2	88 ±4	98 ± 1	26 ± 8	6 ± 4	10.0 ± 1.1	–
Significance	*	ns	**	***	ns	–

FIGURE 2: Mean plasma prolactin levels (ng/ml) in C2 (—●—) and SD3 (- - -■- - -) sheep in Trial 2. Short day (8L:16D) treatment duration for the SD3 group is shown by the horizontal bar.

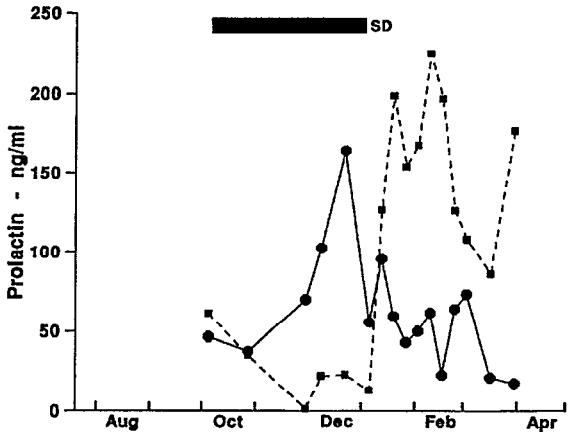
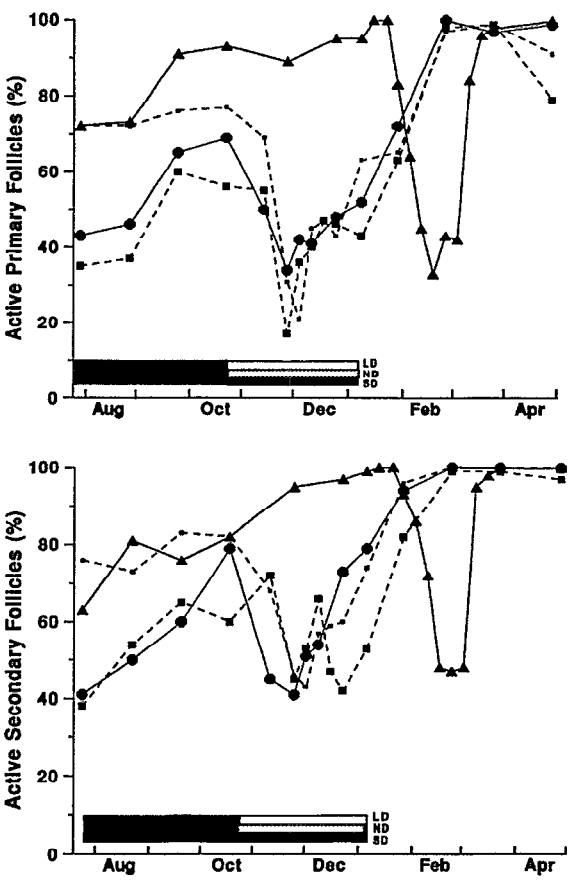


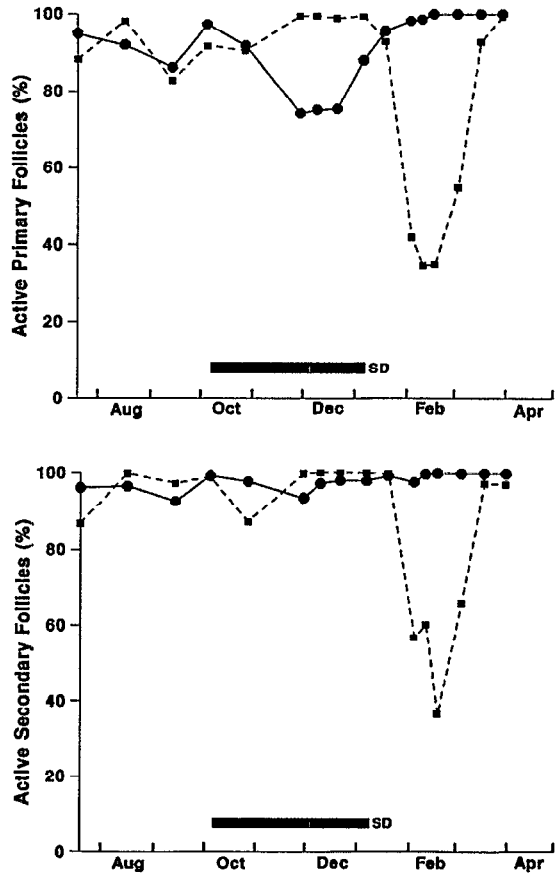
FIGURE 3: Mean primary follicle activity (3a) and secondary follicle activity (3b) levels (%) in C1 (—●—), SDND (- - -■- - -), SDLD (- - -●- - -), and SD5 (—▲—) sheep in Trial 1. Short day (8L:16D) treatment duration for SDND, SDLD and SD5 groups is shown by horizontal bars.



the SD5 group compared with the other groups (P $p < 0.001$; S $p < 0.01$) (Table 1).

In group C2, P follicle activity declined ($p < 0.05$) (range 42 - 96%) in November and December, before resumption of growth ($p < 0.05$) in January (Fig 4a). Mean C2 S follicle activity was high (range 93 - 99%) for the duration of Trial 2 (Fig 4b) although one animal showed evidence of decreased

FIGURE 4: Mean primary follicle activity (4a) and secondary follicle activity (4b) levels (%) in C2 (—●—) and SD3 (- - -■- - -) sheep in Trial 2. Short day (8L:16D) treatment duration the SD3 group is shown by the horizontal bar.



activity during November and December. In SD3 sheep, few P and S follicles were in telogen (follicle activity range 87 - 100%) during short day treatment but the percentage of anagen follicles declined rapidly between 2 and 4 weeks after exposure to long days (P $p < 0.01$; S $p < 0.05$). Minimum follicle activity was achieved at 6 weeks (P 35 ± 9% ; S 36 ± 7%). By 10 weeks, growth had resumed in almost all follicles ($p < 0.001$) (Figs 4a and b, Table 1). The decline in follicle activity was greater in the SD3 group compared with the C2 group (P $p < 0.01$; S $p < 0.001$).

DISCUSSION

In the present study short day treatment suppressed the increase in PRL normally observed in the spring, as previously reported (Pearson *et al.*, 1993a). After sudden exposure to either natural or artificial long day photoperiod, PRL levels increased and wool follicles regressed. Brush ends were first observed 3-4 weeks after exposure to long days and by 6 weeks minimum follicle activity was reached. These and other data (Craven *et al.*, 1994; Pearson *et al.*, 1993b), together with the recent localisation of PRL receptors to wool follicles (Choy *et al.*, 1995), imply that PRL has a role in regulating wool growth in NZ Wiltshires. Increasing daylength and rising plasma PRL during the spring may mediate the entrainment of follicle growth cycles in sheep as in other species (Ling, 1970; Allain, et al. 1994).

Natural follicle growth cycles were observed in control animals during November and December. These were more evident in Trial 1 (C1) than in Trial 2 (C2) (Figs 3 and 4). Neither SD3 or SD5 groups, which were maintained in short days, showed evidence of follicle cycling over this period. Similar cycles have been previously noted in Wiltshire Horn (Ryder, 1969) and Soay sheep (Ryder, 1971). These were termed "subsidiary cycles" to distinguish them from the decline in winter follicle activity. Variability in subsidiary follicle activity patterns between the C1 and C2 groups and between individual sheep within these groups (data not shown) may reflect variation due to age, year and genetic susceptibility to photoperiodic cues (Ryder, 1971; Parry *et al.*, 1991; Craven *et al.*, 1994).

Artificial short-to-long day photoperiod transitions resulted in similar follicle responses in all 4 treatment groups (Figs 2 and 3, Table 1). However, imposition of short days during late winter and spring occurred when follicle activities were rising from winter minima. Release into long days in early November (SDND and SDLD groups) occurred while a significant proportion of follicles were still inactive and coincided with the subsidiary cycle of untreated controls. Hence skin samples collected from SDND, SDLD and untreated sheep during November and December contained follicles in widely varying stages of the growth cycle. On the other hand, more than 95% of wool follicles in the SD3 and SD5 groups were in anagen when released from short days in January. The duration of the induced cycle was less in the SD5 group compared with other Trial 1 treatment groups (Table 1) indicating that the degree of synchronisation was improved.

The present study shows that it is possible to induce follicle regression and reactivation in a predictable manner after an initial preconditioning period of short days. There appeared to be little difference in follicle responses following 24 weeks of short day treatment (SD5) compared with 12 weeks (SD3). Hence, short day treatment commencing in early October, with release into long days in January, is an appropriate protocol for synchronising follicle growth cycles. However it should be noted that not all follicles in either control group had resumed growth in January following the subsidiary cycle. When an experimental design requires the collection of contemporary anagen follicles, it would be necessary to extend the period of short day treatment until complete resumption of growth had occurred in untreated controls.

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