Investigations of wool follicle morphology and cell proliferation in sheep with different levels of wool production

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ABSTRACT

Preliminary results are given of a study of follicle differences between a progeny-tested fleeceweight (FWT) line of sheep and a control (CLT) flock which was conducted during winter. Measurements using immunocytochemistry and image analysis on skin biopsies were based upon the use of intracutaneously administered bromodeoxyuridine (BrdU) to assess the replicating cell population in wool follicles. The FWT animals showed a significant advantage over the CLT animals in number of replicating cells, papilla length, papilla area and area of replicating zone in the follicle bulb. There were no significant differences between the flocks in the ratio of primary to secondary follicles or in the number of active follicles.The mitotic density was similar in both flocks. The greater productivity of FWT sheep appears to be accounted for by a larger proliferative zone rather than a higher cell replication rate.

KEYWORDS Wool follicle, cell proliferation, BrdU, ICC, wool, selection, fleeceweight.

INTRODUCTION

Because the follicle bulb provides all the cells forming the fibre and the inner root sheath, wool production must be influenced by cellular events in the follicle bulb. The total output of the follicle is a direct function of the size (number of cells and shape) of the germinative region of the bulb and the proliferative activity of its cells.

Factors altering wool production, measured as volumetric output of fibre material, may be genetic as well as nutritional or seasonal. Response to these influences can be brought about either by changes in the follicle population, follicular dimensions or cell producing events in the bulb.

Relatively few studies have been made on medium or coarse-wool sheep. In the Romney, Fraser (1965) observed an increase in germinative tissue volume along with a reduction in mitotic density. The importance of the germinative tissue volume as an indicator for seasonal and nutritional changes was underlined in another study on Romneys, by Henderson (1965). Studies on Merino sheep have revealed an alternative selection response to increased wool production. Williams and Winston (1987) reported changes in the ratio of secondary to primary follicles. Small changes in the percentage of active follicles in the follicle population (Short *et al.*, 1965) and an increase in the germinative cell population have also been reported for the Merino (Short *et al.*, 1965, Wilson and Short, 1979, Hynd, 1989).

No study has yet investigated follicular changes in Romney sheep genetically selected for higher wool production and it is not clear if results obtained from studies on Merinos can be adapted to the Romney.

The present experiment was designed to examine the relative contribution of various follicle parameters to fibre output in sheep differing genetically in clean wool production per day during periods of low (winter) wool production. Sheep were taken from a closed Romney flock managed by the Department of Animal Science at Massey University, selected for high greasy fleeceweight (FWT) or at random (CLT) since 1956. Fleeceweight selected sheep show a 25 % advantage in wool production over their control flock throughout the year, this advantage, in relative terms, being greatest during winter (McClelland *et al.*, 1987). Follicle density and percentage of active follicles in the population as well as individual follicular influences on wool production were assessed.

MATERIALS AND METHODS

Skin biopsies were taken with a 10 mm diameter trephine from the clipped midside skin of 8 FWT and 8 CLT sheep (two-tooth rams) in August. Both flocks were kept outdoors, running together on the same pasture. Prior to sampling the sheep were locally injected with a solution of bromodeoxyuridine (BrdU) (Holle and Harris, 1992) into their clipped midside skin patches.

For the estimation of the secondary to primary follicle ratio (S:P ratio) and the percentage of active follicles, skin sections were cut horizontally at sebaceous gland level and stained with "SACPIC" trichrome stain (Auber, 1952; modified Holle, 1992). Follicle identification and terminology followed that of Hardy and Lyne (1956). Ten consecutive follicle groups on each skin sample were examined for assessment of primary and secondary follicles. Assessment of the percentage of active follicles was conducted by screening serial sections from the skin surface to the follicle bulb origin in 500 follicles per sample.

BrdU injected skin tissues were fixed and embedded in wax for subsequent sectioning and immunocytochemical detection of replicating bulb matrix cells (Holle and Birtles, 1990). About 20 representative bulbs in longitudinal skin sections were chosen from each animal for follicle bulb measurements and assessment of the number of replicating cells in the bulb. A light microscope with a video camera attached was used to produce a captured histological image on a computer screen which was measured using specially developed computer software (MARKLINE, Dr. A. Hall, Fruit and Trees, DSIR, Palmerston North).

Measurements undertaken on individual bulbs included:

- number of labelled, proliferating cells in the proliferative region of the bulb (NO)

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- bulb area (including dermal papilla) (A)
- dermal papilla area
- dermal papilla length

The germinative tissue zone (B) was calculated by subtracting dermal papilla area from follicle bulb area. The number of replicating cells per unit area of germinative bulb tissue (C) was calculated as a ratio of NO/B.

RESULTS

The fleeceweight selection line showed no advantage over their control line in the ratio of secondary to primary follicles or in the percentage of active follicles in the follicle population. They had, however, significantly more proliferating cells in their follicle bulbs, significantly larger bulb areas as well as larger proliferative zones in the follicle bulb, and significantly larger papillae. The density of replicating cells per unit replicating tissue area was similar in both flocks (Table 1.).

TABLE 1 Comparison of follicle population and follicle bulb variables of 8 FWT and 8 CLT rams (two-tooth) in August and significance levels for analysis of variance of group differences.

	FWT MEAN ± SEM	CLT MEAN ± SEM	p
S:P ratio	5.24 ± 0.24	5.37 ± 0.15	0.642
% active follicles	94.0 ± 4.08	95.2 ± 2.44	0.811
Number of replicating cells in the bulb [NO]	45.32 ± 0.77	36.24 ± 0.90	0.0001
Follicle bulb area (μm^2) [A]	9658.2 ± 231.6	8056.3 ± 275.9	0.0001
Germinative zone (μm^2) [B]	7783 ± 263	6438 ± 363	0.0008
Replicating cell density [C]	0.0063 ± 0.0001	0.0063 ± 0.0002	0.9524
Dermal papilla length (μm)	92.0 ± 1.38	81.56 ± 1.75	0.0001
Dermal papilla area (μm^2)	1874 ± 70.8	1603 ± 78.8	0.0104

[A] Follicle bulb area, includes dermal papilla
[B] Germinative zone = A - dermal papilla area
[C] Replicating cell density = NO B^{-1}

DISCUSSION

Because selection criteria demand a ceiling on fibre diameter (Turner *et al.*, 1968, Turner and Jackson, 1978), increased follicle density might well be a specific feature of the Merino. That is, in the Merino, increased growth could be achieved only by fibre length growth and follicle density changes. However, an increase in follicle density might not occur if there is no restriction on fibre diameter as for the New Zealand Romney sheep used in the present study.

Observed fluctuations in the percentage of active follicles within the population could not be attributed to treatment line effect. Thus, selective breeding for heavier fleece production appears to have no effect on the wool growth cycle in the Romney. The effects of selective breeding on follicle activity in the Merino does not appear to have been studied. But, in Merinos, as a response to increased nutrition, a small decrease in the percentage of inactive follicles with increased nutrition was observed by Short *et al.*, (1965).

In the present study FWT sheep showed advantages over CLT sheep through a greater number of proliferating cells as well as through greater bulb and dermal papilla size. FWT sheep have a bigger area of proliferating cells, an observation in accord with the findings of Williams and Winston (1987) in Merino sheep, for which "Fleece Plus" showed a larger area of mitotically active tissue. If follicles of FWT sheep possess more cells in their larger germinative tissue areas, it is also possible that they will show a greater number of proliferating cells. The present study shows that this does not necessarily mean that the proliferative density is greater. The mitotic density is the same in both flocks, an observation which contrasts with that of Fraser (1965), who reported a reduction in mitotic density. Since the increase in the number of replicating cells and the increase in area of germinative zone each accounted for the 25% between line differences in fleece production reported by McClelland *et al.*, (1987), it is unlikely that acceleration in cell proliferation rates take place in the follicles of FWT sheep.

A similar finding has been made recently by Hocking *et al.*, (1992) who showed that the genotype determines a difference in the volume of the mitotically-active tissue between finewool and strongwool Merinos. Although strongwool Merinos also showed a higher bulb cell production rate, wool production was best predicted by the volume of the germinative tissue and the follicle density.

If other control mechanisms influencing the maturing fibre are kept constant, size changes in the follicle bulb influence the net output of fibre. However, possible changes in proliferation rates or the proportion of matrix cells distributed to follicular structures other than the wool fibre must be taken into account, and will be measured in more detailed studies being undertaken now.

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