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## The relationship between cortical structure and fibre diameter in primary and secondary wool fibres of yearling Perendale sheep

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### ABSTRACT

Midside skin and wool samples were taken from 32 Perendale ewes at 12 months of age to investigate the relationship between fibre cortical structure and fibre diameter in fibres derived from primary and secondary follicles in sheep with varying loose wool bulk values. Bulk was correlated to mean crimp frequency, staple length, mean fleece fibre diameter, follicle curvature and proportion of para-mesocortex. Medullation was present in only 2.8% of fibres and was not significantly related to bulk. Primary fibres were coarser than secondary fibres (37.4 $\mu$ m vs 31.9 $\mu$ m). The proportion of fibres with a bilateral cortical cell type arrangement decreased with increasing fibre diameter. The mean proportion of para-mesocortex was less in primary than secondary fibres (19.2% vs 24.1%). There was a significant within sheep relationship between proportion of para-mesocortex and fibre diameter. While primary and secondary fibres were different in mean fibre diameter and cortical structure, the relationship between cortical structure and fibre diameter did not differ between the two follicle populations. Consequently it appears unnecessary to differentiate between primary and secondary fibres in future studies of fibre cortical structure and wool bulk.

**Keywords:** Perendale; loose wool bulk; wool follicle type; cortical structure.

### INTRODUCTION

The development of Romcross type wool with superior manufacturing and end product performance of carpet and knitting yarns relies on the development of an understanding of the biological basis of the highly desirable bulk characteristic. Variations in wool bulk have been associated with differences in the dimensions and structure of wool follicles and fibres, in particular fibre crimp (Sumner *et al.*, 1993). The biological basis of crimp is not well understood. It is thought to arise from the bilateral configuration of orthocortical cells on the outside of the crimp curve with a cluster of paracortical cells on the inside of the crimp curve (Fraser and Rogers, 1955). Mesocortical cells lie between the ortho and paracortex. Deviations from the bilateral arrangement tend to occur with increasing fibre diameter. Cortical cell arrangement can also vary between sheep within a breed, between breeds, and may be influenced by seasonal factors (Orwin *et al.*, 1984).

Sheep skin contains two types of anatomically distinct fibre producing follicles referred to as primary and secondary follicles. In non-Merino breeds fibres produced by primary follicles tend to be larger than fibres produced by secondary follicles (Carter and Clarke, 1957). There is also a negative relationship between fibre diameter and the proportion of paracortex in individual fibres of both Merino (Nagorcka and Mooney, 1982) and Perendale (Orwin and Woods, 1980) wool. In Perendales the proportion of paracortex is positively related to crimp, and therefore bulk (Sumner *et al.*, 1993). Some aspects of fibre growth in primary and secondary follicle populations are under independent genetic control (eg fibre dimensions in the Drysdale breed (Stephenson, 1959)). As loose wool bulk is strongly inherited (Sumner *et al.*, 1989)

this study was initiated to establish whether interrelationships between cortical cell organisations and dimensions of fibres in low and high wool bulk sheep differ between primary and secondary follicle populations.

### MATERIALS AND METHODS

#### Sampling

Wool and skin samples were taken from 15 sheep born in 1990 and 17 sheep born in 1991 at hogget shearing in mid October. The sheep were from a flock of Perendale ewes varying in loose wool bulk grazed at the AgResearch Whatawhata Research Centre. Sampled hoggets were selected across the range of yearling loose wool bulk values by restricted randomization. The hoggets were live weighed, a midside wool sample and a snip skin biopsy sample taken prior to shearing. Skin samples were fixed in buffered 10% formalin. Individual sheep fleece weights were recorded at shearing.

#### Wool measurements

Staple length (SL) and total number of crimps along the staple were measured from the greasy midside sample, and crimp frequency (CFreq) calculated. The midside sample was scoured and measured for loose wool bulk (Bigham *et al.*, 1984), mean fibre diameter (FD(F)) and fibre diameter variation (FDSD(F)) (Lynch and Michie, 1976).

#### Skin measurements

Longitudinal sections (5mm) were cut by hand with a razor blade from half of each skin biopsy sample and stained with 0.25% Nile blue sulphate. Three sections from each sheep

were graded for extent of follicle curvature (FolC), from 1 = straight to 7 = bent and intertwined (Maddocks and Jackson, 1988) by three independent assessors and the average grade calculated. The remainder of each skin biopsy was processed through an ethanol gradient and embedded in wax. Transverse skin sections ( $7\mu\text{m}$ ) were oxidised with performic acid, stained with 0.1% janus green (S.G. Munro, CSIRO Division of Animal Production, unpublished) and counterstained with 0.04% light green. This staining procedure distinguished fibre cortical structure by rendering the fibre cuticle and para-mesocortical cells blue and the surrounding tissues and orthocortical cells light green. Medulla were unstained. The intensity of blue staining ranged from very dark in paracortex to light blue in mesocortical cells. In view of the difficulty of distinguishing paracortex from mesocortex the combined area (para-mesocortex) was measured. Images of these sections were viewed on a computer screen at 41x magnification. Fifty primary and 50 secondary follicles were scanned and measured for the area of the fibre, area of the para-mesocortex, area of medulla and length of the minor axis across the fibre. The proportion of para-mesocortex (%P-M) and medulla in each fibre was calculated as a percentage of fibre cross sectional area. Fibre diameter was calculated from the minor axis (FD(H)) taking account of the image magnification. The arrangement of cortical cell types in the cross sectional image was classified as bilateral, lobate cellular or bilobate (Fig. 1) (Orwin *et al.*, 1984).

**FIGURE 1:** A stylized representation of the classifications of cell type arrangement. The dark areas represent para-mesocortex and the white areas orthocortex.



The ratio of secondary to primary follicles (S/P) was calculated from classifying approximately 300 follicles per sheep. Individual sheep S/P were used to weight individual measurements of FD(H), %P-M, number of fibres with medulla and cortical cell type arrangements from each sheep to calculate mean within sheep values for these characteristics.

### Statistical Analysis

Analysis of variance, regression and REML analyses were used to evaluate relationships between measured follicle and fibre characteristics.

### Results

There was no between year effect in mean live weight, fleece weight or any of the measured follicle or fibre characteristics. The means, combined over years for each of the measured variables, are given in Table 1. The selected sheep covered a range of loose wool bulk values from 17.1 to 34.3 cm<sup>3</sup>/g. The mean between sheep S/P ratio of  $4.8 \pm 0.5$  lies between reported S/P values for the Perendale's parent breeds; Cheviot  $4.5 \pm 0.2$  and Romney  $5.5 \pm 0.3$  (Carter and Clarke,

1957). The pooled within sheep mean FD(H) calculated from the skin sections ( $32.8 \pm 3.3\mu\text{m}$ ) was not significantly different from that obtained by standard FFDA measurement, FD(F) ( $33.6 \pm 2.2\mu\text{m}$ ) (Table 1). Of the characteristics in Table 1, Bulk was significantly correlated at the 1% level with FolC ( $r = 0.83$ ), SL ( $r = -0.73$ ), CFreq ( $r = 0.64$ ) and FD(F) ( $r = -0.48$ ). %P-M was correlated with Bulk ( $r = 0.39$ ) at the 5% level. Correlations between Bulk and all other measured characteristics were not significant.

**TABLE 1:** Between sheep mean and standard deviation of live weight, greasy fleece weight, and follicle and fibre characteristics (n = 32).

Characteristic	Abbreviation	Mean $\pm$ SD
Live weight (kg)	LW	45.0 $\pm$ 6.7
Loose wool bulk (cm <sup>3</sup> /g)	Bulk	24.4 $\pm$ 4.7
Greasy fleece weight (kg)	GFW	3.4 $\pm$ 0.5
Staple length (mm)	SL	122 $\pm$ 24
Crimp frequency (crimps/cm)	CFreq	1.6 $\pm$ 0.5
Follicle curvature grade (1 - 7)	FolC	2.8 $\pm$ 0.9
Secondary to primary follicle ratio	S/P	4.8 $\pm$ 0.5
Fibre diameter (mean) (FFDA) ( $\mu\text{m}$ )	FD(F)	33.6 $\pm$ 2.2
Fibre diameter (SD) (FFDA) ( $\mu\text{m}$ )	FDSD(F)	7.3 $\pm$ 0.7
Fibre diameter (mean) (Histology) ( $\mu\text{m}$ )	FD(H)	32.8 $\pm$ 3.3
Fibre diameter (SD) (Histology) ( $\mu\text{m}$ )	FDSD(H)	6.0 $\pm$ 1.2
Proportion of para-mesocortex (%)	%P-M	23.3 $\pm$ 4.2
Proportion of para-mesocortex (SD) (%)	%P-MSD	8.0 $\pm$ 1.5
Proportion of fibres with medulla (%)	Med(%)	2.8 $\pm$ 5.0
Proportion of medulla in fibres with medulla (%)	%Med	2.3 $\pm$ 1.6
Proportion of fibres with bilateral cell arrangement (%)	%Bilat	80.8 $\pm$ 15.8
Proportion of fibres with lobate cellular cell arrangement (%)	%LC	18.7 $\pm$ 15.8

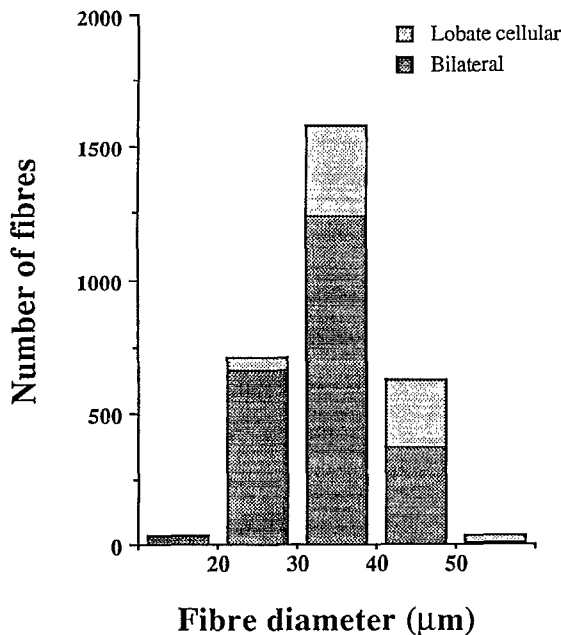
Pooled within sheep means of fibre cortical characteristics in primary and secondary fibres are listed in Table 2. There were more fibres with medulla in the primary than the secondary population (5.0% vs 2.1%,  $P < 0.01$ ) (Table 2). Within medullated fibres the mean proportion of the fibre cross section occupied by medulla was not significantly different between primary and secondary fibres. The presence of medulla was correlated with increasing fibre diameter ( $r = 0.91$ ,  $P < 0.001$ ) but not with any of the other characteristics in Table 1. Medullation was not considered further as a fibre characteristic likely to be associated with bulk in Perendales.

The proportion of fibres with the bilateral arrangement (%Bilat) decreased with increasing fibre diameter ( $r = -0.98$ ,

**TABLE 2:** Pooled within sheep means of fibre cortical structure characteristics for primary and secondary fibres. The meaning of abbreviations are given in the text and Table 1.

Characteristic	Follicle type		SED	Significance
	Primary	Secondary		
FD(H)	37.4	31.9	0.85	***
FDSD(H)	4.5	5.8	0.23	***
%P-M	19.2	24.1	1.19	***
%P-MSD	7.3	8.1	0.37	*
Med(%)	5.0	2.1	2.08	**
%Med	2.9	2.7	0.41	NS
%Bilat	70.5	83.0	4.70	*

**FIGURE 2:** Frequency of cortical cell arrangement within individual fibres by fibre diameter steps of 10µm.



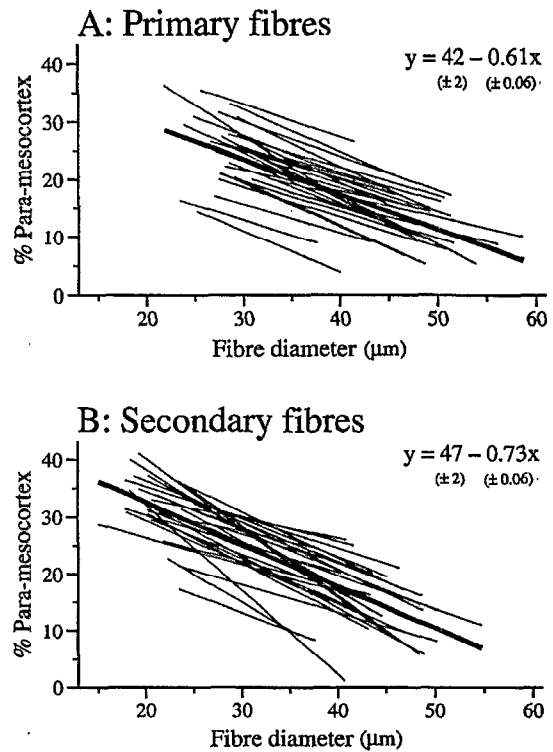
$P < 0.001$ ) (Fig. 2) but was unrelated to bulk. Mean %Bilat differed between primary and secondary fibres (70.5% vs 83.0%,  $P < 0.05$ ), (Table 2).

As reported for some non-Merino sheep breeds (Carter and Clarke, 1957), the mean FD(H) of primary fibres was significantly greater than the mean FD(H) of secondary fibres (37.4µm vs 31.9µm,  $P < 0.001$ ). This diameter difference explains the mean %Bilat difference between fibre types. Converse to FD(H), the mean %P-M was less in primary fibres than secondary fibres (19.2% vs 24.1%,  $P < 0.001$ ) (Table 2). Variation in FD(H) and %P-M in primary fibres was significantly less than in secondary fibres (4.5µm vs 5.8µm,  $P < 0.001$  and 7.3% vs 8.1%,  $P < 0.05$ , respectively) (Table 2). The maximum diameters of primary and secondary fibres were similar but secondary fibres had a 10µm greater range than primary fibres. Relationships between FD(H) and %P-M for primary and secondary fibres are shown in Fig. 3. The mean slopes of  $-0.61 (\pm 0.06)$  and  $-0.73 (\pm 0.06)$  for primary and secondary fibres respectively were not significantly different. The pooled within sheep relationship encompassing both primary and secondary fibre data was  $\%P-M = 46.1 (\pm 0.7) - 0.71 (\pm 0.06) \text{FD(H)}$ .

**Discussion**

The non significant differences between mean FD(F) and FD(H) measurements indicate that the wool fibres were not affected by histological processing. Bulk, SL, CFreq and FD(F) represent characteristics of wool fibres grown over 10 months prior to sampling. The other variables associated with bulk, namely FolC and %P-M, are characteristics of follicle and fibre cortical structure measured in the skin at time of shearing. Of the characteristics measured FolC, SL and CFreq were the most strongly correlated with bulk confirming the results of Sumner *et al.*, (1993). The results of this trial also indicate a small significant negative correlation between fibre diameter and bulk.

**FIGURE 3:** Fibre diameter versus proportion of para-mesocortex for each sheep and the pooled within sheep relationship (dark line), in (A) primary fibres and (B) secondary fibres. The length of each line covers the range of values for each sheep.



Fibre crimp is thought to arise from a bilateral arrangement of para and orthocortex which appears to be produced by a specific pattern of cell division and differentiation evident in curved follicles (Fraser, 1964). The present study found a between sheep increase in FolC and CFreq with increasing Bulk but not an associated increase in %Bilat. However, within sheep increasing %Bilat was strongly correlated to decreasing FD(H) while between sheep decreasing FD(F) was correlated to increasing Bulk. It is not only the presence of the observed bilateral cell arrangement but also the frequency and shape of the resultant fibre crimp which therefore influences measured bulk. Bilateral cell arrangement is only one of several factors that may affect fibre crimp, which may explain why %Bilat alone was not sufficient to detect between sheep interrelationships between cortical cell arrangement, and CFreq and Bulk. %P-M in the cortex was however correlated to FD(H). Therefore as the mean slopes shown in Fig. 3 are not significantly different, primary and secondary fibres of similar diameter appear to be indistinguishable on the grounds of cortical structure.

Finer fibres tended to have more %P-M and greater %Bilat that is believed to contribute to fibre crimp. In the larger fibres the %P-M area of cortex was less intensely stained, indicating the presence of more mesocortex compared to smaller fibres. The spectrum of para to meso cortical cell types may differ in keratin protein structure sufficient to influence the extent of fibre twisting and formation of the helical type crimp associated with bulk. The differential distribution of members of the keratin KAP5 gene family reported in the wool fibre cuticle is thought to be related to

follicle type (Jenkins and Powell, 1994). It has been suggested that the pattern of expression of other keratin genes may also differ between follicle types (Jenkins and Powell, 1994). The results of this study show no difference in the amount of para-mesocortex, medulla or cell type arrangement between primary and secondary fibres of the same diameter from sheep with either high or low bulk wool. The data therefore suggests that, while any differential expression of keratin genes resulting from selection for bulk may be related to fibre diameter, it would not differ between follicle populations. Consequently it is unnecessary to differentiate between follicle populations in future histological studies of the role of fibre cortical morphology in the expression of wool bulk.

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