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Wool follicle characteristics in sheep with differing ventral wool length phenotypes

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ABSTRACT

Recent interest in sheep requiring less on-farm fleece management has lead to the development of sheep with long wool covering the trunk, but only short wool on the belly and breech. We have characterised developmental and structural features of the skin, including follicle density, primary to secondary follicle ratio and follicle activity state at various body sites of bare- and woolly-bellied ewes, and their foetuses. At 70 days of gestation, foetuses of bare-bellied ewes had developed fewer follicles than woolly-bellied sheep (1.7 ± 0.27 versus 2.8 ± 0.21 follicles per mm^2; $P < 0.01$). In adults, similar densities and ratios of primary to secondary follicles occurred in bare- and woolly-bellied ewes in each body site studied including dorsal, midside, anterior and posterior fleece positions, as well as the belly, breech and face. However, the proportion of inactive follicles in late autumn was greater in bare-belly adult ewes, not only on the belly ($15.5 \pm 1.9\%$ versus $4.9 \pm 2.9\%$; $P < 0.05$), but also across the fleece which exhibited anterior-posterior and dorsal-ventral gradients in follicle activity. The short wool phenotype was thus associated with altered follicle cycles, but no differences in adult follicle densities. However, wool production might be compromised, as follicle activity varied across the whole fleece of bare-belly sheep.

Keywords: bare-bellied sheep; hair cycle; follicle density; follicle development; topobiology.

INTRODUCTION

Fleece cover across the sheep's body should be optimised to maximise the production of high quality wool, while minimising economic and husbandry costs associated with harvesting the fleece. Wool grown on the belly, is usually poor in quality, often being short, variable and heavily stained and therefore classified as lower value oddments (Scobie *et al.*, 1999; Scobie *et al.*, 2007). If left unshorn, long belly wool can increase the incidence of microbial contamination of sheep carcasses during meat processing. It requires 30% of shearer's time to remove the fleece for very little economic gain (Scobie *et al.*, 2006). As a proof of concept, a flock containing sheep with easy-to-harvest fleeces due to short-length belly wool (bare-bellies) has been bred. This composite-breed flock is derived from breeds as diverse as Border Leicester, Cheviot, Finnish Landrace, East Friesian and Wiltshire.

Initial studies have been carried out with the aim of identifying genes responsible for the differential wool growth patterns in these sheep. Homeobox genes including Msx genes and some canonical Hox genes are differentially expressed between belly and fleece sites and these developmental regulators could contribute to the bare-belly phenotype (Craven *et al.*, 2007). However, to fully understand the significance of such gene expression patterns we must firstly characterise the skin. In addition, as selection of these "easy-care" sheep is based on visual observation of wool growth on the belly and other traits, it is useful to understand the micro-anatomical characteristics associated with this gross phenotypic selection.

In this paper, we compare the development, density and cycling of wool follicle populations in skin from both foetuses predicted to exhibit the bare-belly and woolly-belly phenotype, as well as skin obtained from multiple body sites of adult ewes. We report that these phenotypic differences in belly wool are associated with temporal differences in follicle development, but by adulthood the follicles are similar in density but differ in cyclic behaviour.

MATERIALS AND METHODS

Animals and sampling

All animal experimental procedures were approved by the Ruakura Animal Ethics Committee. Skin samples were collected from sheep with either large or small dorso-ventral differences in fibre length such that they could be characterised as either "bare-bellied" or "woolly-bellied" phenotypes respectively. These sheep were developed in a selective breeding programme (Scobie *et al.*, 1997; Scobie *et al.*, 2007). The ewes were chosen from one flock on the basis of the wool covering on the belly prior to mating and records of belly bareness score (Scobie *et al.*, 2006) from previous years in the flock. Ewes which were described as woolly-bellied had been repeatedly scored with a bareness score of 2 or less, while those described as bare-bellied had repeatedly shown bare belly scores of at least 4 as an adult (lower scores may be observed in younger animals). A total of 35 ewes were selected, these were randomly separated into two groups with woolly bellies and two groups with bare bellies and each group was mated to a single sire of matching belly bareness phenotype. The four sires were selected from the same flock as the ewes, but were all two-tooths with a limited number of records for belly bareness score. The ewes were mated in March and pregnancy scanned in May. Four ewes from each phenotype that were carrying twin foetuses were selected for the histological examinations described below and gene expression studies described elsewhere (Craven *et al*., 2007).

The oestrus cycles of these ewes were synchronised using controlled internal drug release (CIDR) devices, and mating conducted as part of normal flock management. Four ewes of each phenotype (bare- and woolly-bellied), each carrying twins, were sacrificed from 22 May to 1 June 2006 at day 70 of gestation. Skin was collected from the midside and belly of foetuses, and from seven body sites of the ewes comprising the face, mid-dorsum (back), midside, belly, shoulder, hip (in line with the mid-side) and the breech (5 cm laterally from the anus). These sampling sites are shown pictorially in Craven *et al*. (2007).

Histology

Sub-samples were preserved in 10% phosphate buffered formalin and processed to wax for histological analysis. The developmental stage and density of follicles in foetuses were determined from longitudinal sections of skin according to the criteria described by Paus *et al*. (1999). Topographical differences in the density and ratio of primary to secondary follicles of adult ewes were determined from transverse sections of skin from the seven body sites. Equal shrinkage of biopsies during histological processing between phenotypes and body sites was assumed. In addition, the percentage of actively growing (anagen) or dormant (telogen) follicles was assessed as described elsewhere (Nixon, 1993).

Statistical analyses

Differences between animals and sites were assessed by analysis of variance. Overall mean values and SEM are reported.

RESULTS

Follicle development

At 70 days of gestation, foetal skin contained developing follicles that could be classified into the morphogenic stages I to IV (briefly described as placode, prepapilla, papilla, and hair cone respectively) according to criteria described by Paus *et al*. (1999) (Figure 1). Skin from foetuses of bare-bellied ewes had fewer initiating follicles with each

FIGURE 1: Wool follicle development is retarded in bare-bellied sheep. Number and developmental stages of wool follicles in skin of foetuses (at 70 days of gestation) predicted to exhibit bare- or woolly-bellied phenotypes. Error bars indicate standard error of mean.

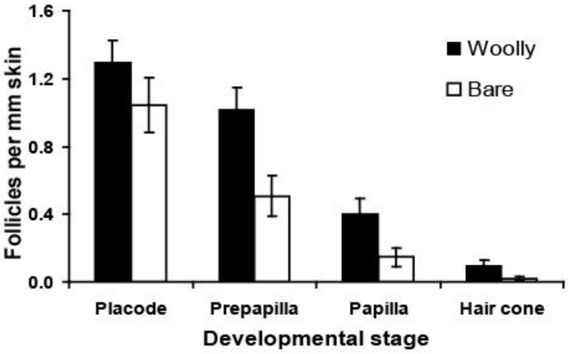
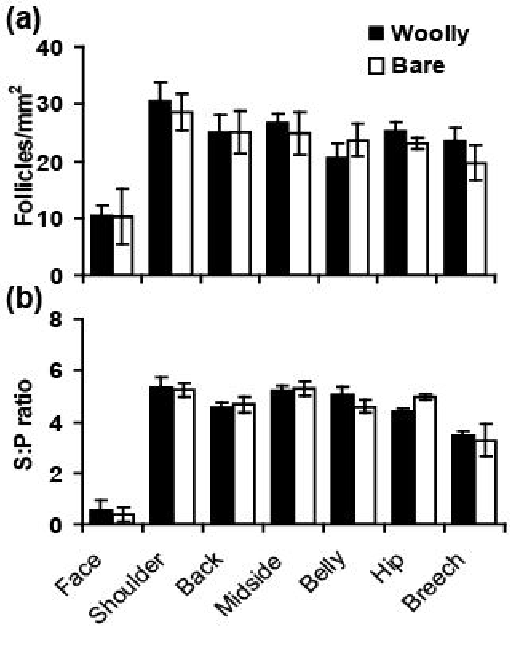


FIGURE 2: Bare- and woolly-bellied adult ewes have similar follicle densities. (a) Follicle density, and (b) ratio of secondary to primary follicles (S:P) in various body sites in sheep exhibiting a bare- or woolly-bellied phenotype. Error bars indicate standard error of mean.

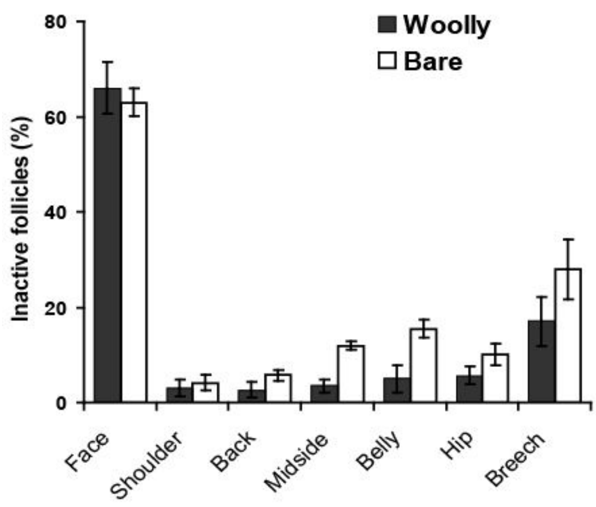


developmental category being under-represented. At this time, a total of 1.7 ± 0.27 and 2.8 ± 0.21 follicles per mm^2 ($P < 0.01$) for foetuses from bare-bellied and woolly-bellied ewes respectively were observed. No difference in follicle development was observed between male or female foetuses or between samples obtained from the midside or belly.

Adult follicle density and S:P ratio

No difference between the overall density of follicles or S:P ratio was noted between bare-bellied and woolly-bellied sheep (Figure 2). Differences

FIGURE 3: Wool follicle inactivity is greater in skin of bare-bellied adult ewes. Percentage of inactive (telogen) follicles in various body sites in sheep exhibiting a bare-or woolly-bellied phenotype. Error bars indicate standard error of mean.



between sites across the fleece were evident in both follicle density ($P < 0.002$) and S:P ratio ($P < 0.001$). The highest density of follicles was found on the anterior fleece region, and an anterior–posterior decrease in follicle density ($P < 0.001$) and S:P ratio ($P < 0.001$) were observed. Thus, the breech had fewer secondary follicles than elsewhere in the fleece. The face had the lowest density of follicles and contained few secondary follicles.

Adult follicle activity

The fleece of woolly-bellied ewes contained similar proportions of inactive follicles at each site (approximately 6%) (Figure 3). In contrast, ewes with bare-bellies had greater ($P < 0.05$) proportions of inactive (brush-end containing) follicles at most sites such that a clear dorso-ventral gradient in follicle activity was evident as follicles containing brush ends made up 6% of the dorsal population while 15% of belly follicles contained brush ends. Similarly, an anterior-posterior gradient of follicle activity within the fleece was also evident as fewer inactive follicles occurred on the shoulder and most on the breech. The face, however, contained high proportions of inactive follicles (Figure 3).

DISCUSSION

The current sheep farming trend is towards maximising quality meat production with minimal economic and husbandry inputs. Minimising the cost of shearing and crutching would reduce a major on-farm cost in maintaining the sheep flock. As belly wool provides small returns, a selective breeding programme produced composite-breed sheep with short wool on the belly to save 20 to 40% of shearing time and cost (Scobie *et al.*, 2006). Here, we have shown that this phenotypic selection results in sheep with a physiologically different integument, not only on the belly but across the wool-bearing regions as well.

The observations at 70 days gestation suggest a temporal shift in the developmental processes such that fewer primary follicles were formed in foetuses from bare-bellied ewes as compared to those from woolly-bellied ewes. Despite this early disparity, no difference in the final follicle density or S:P ratio was observed between adults exhibiting the short or long belly wool phenotypes. This might indicate a slightly delayed follicle initiation and a later “catch-up” of follicle formation in bare-bellied sheep.

These developmental alterations may reflect variation within the wide gene pool of these composite-breed sheep. On the other hand, similar temporal profiles of primary follicle development have been reported in Romney, Drysdale, Wiltshire and Merino sheep; although Merinos had a higher follicle density and Drysdales attained their full complement of follicles earlier (Hocking Edwards *et al.*, 1996). Secondary follicle development commences at similar ages in all these breeds, but not until after the 70 day sampling point in the present study, and continues for longer in Merinos, explaining the higher follicle density in that breed.

Surprisingly, no significant difference in the density or maturity of midside and belly follicles was observed. Although examination of greater numbers of animals might establish statistical differences in development between sites, as is commonly accepted (Lyne & Hollis, 1968), our results indicate that these structural variations are subtle and are unlikely to be solely responsible for the topographical differences in gene expression that occurs in the skin of these foetuses. Thus, when comparing levels of expression of growth and development regulator genes between sites (Craven *et al.*, 2007), it is important to consider follicle maturity and density. The present study suggests that the differential expression of genes in midside and belly skin at 70 days post-conception are likely to be site specific rather than arising directly from differing numbers of follicles.

Sheep exhibiting the woolly-bellied phenotype had approximately 6% of follicles in telogen ranging uniformly across the body. This percentage, which reflects one point of time during the natural decline in wool growth in autumn (Bigham *et al.*, 1978; Ryder & Stephenson, 1968), is somewhat greater than expected in most commercial strong wool breeds but reflects the parent breeds of these composite-breed sheep; namely Cheviot, Finnish Landrace, Border Leicester and especially Wiltshire, which all exhibit some degree of follicle cycling

(Bigham *et al.*, 1978; Ryder & Stephenson, 1968; Slee, 1965).

Not surprisingly, bare-bellied sheep exhibit higher levels of follicle cycling than woolly-bellied sheep. This difference between phenotypes is unlikely to be due to the minor difference in sampling times. Furthermore, longer durations of follicle quiescence and short growth periods are consistent with short staple length. This study demonstrates a gradation of follicle cycling across the fleece-bearing regions of the torso that is associated with the increased telogen on the belly. Increased follicle cycling means that more wool fibres cease growing, and is probably also associated with variable and weak fibres that will decrease the overall production and quality of the wool clip. It remains to be seen whether selection of appropriate breeding stock can produce bare-bellied animals that have uniform non-shedding fleeces on the remainder of the body.

These results describe follicle characteristics at only two time points in the life of the sheep. It remains possible that the differences between phenotypes in adults reflect shifts in the timing of seasonal wool cycles, rather than amplitude. Such questions could be resolved by a comprehensive histological study of follicle behaviour, sampling multiple sites over time, but this would involve an elaborate and invasive animal experiment. In the meantime, we conclude that the short-bellied wool phenotype was associated with differences in the timing of wool follicle development, which did not result in changes in adult follicle densities. However, uniformity between wool fibres with respect to fibre diameter and length in these sheep may be compromised as increased variations in follicle activity also occurred across the whole fleece bare-bellied sheep.

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REFERENCES

Bigham, M.L.; Sumner, R.M.W.; Elliott, K.H. 1978: Seasonal wool production of Romney, Coopworth, Perendale, Cheviot, and Corriedale wethers. *New Zealand journal of agricultural research* **21**: 377-382.

Craven, A.J.; Rufaut, N.W.; Scobie, D.R.; Nixon, A.J. 2007: Expression of the developmental regulators Msx1 and Msx2 in sheep skin varies with body region and wool growth status. *Proceedings of the New Zealand Society of Animal Production* **67**: 345-350.

Hocking Edwards, J.; Birtles, M.; Harris, P.; Parry, A.; Paterson, E.; Wickham, G.; McCutcheon, S. 1996: Pre- and post-natal wool follicle development and density in sheep of five genotypes. *Journal of agricultural science, Cambridge* **126** 363-370.

Lyne, A.; Hollis, D. 1968: The skin of sheep: a comparison of body regions. *Australian journal of biological science* **21** 499-527.

Nixon, A.J. 1993: A method for determining the activity state of hair follicles. *Biotechnic and histochemistry* **68** 316-325.

Paus, R.; Muller-Rover, S.; Van Der Veen, C.; Maurer, M.; Eichmuller, S.; Ling, G.; Hofmann, U.; Foitzik, K.; Mecklenburg, L.; Handjiski, B. 1999: Review article: A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis. *Journal of investigative dermatology* **113**: 523-532.

Ryder, M.L.; Stephenson, S.K. 1968: Wool growth. Academic Press, London, UK. p. 599-600.

Scobie, D.R.; Bray, A.R.; O'Connell, D. 1997: The ethically improved sheep concept. *Proceedings of the New Zealand Society of Animal Production* **5**: 84-87.

Scobie, D.R.; Bray, A.R.; O'Connell, D. 1999: A breeding goal to improve the welfare of sheep. *Animal welfare* : 391-406.

Scobie, D.R.; Nixon, A.J.; Rufaut, N.W. 2006: The biological basis of variation in sheep's fleece cover. *Proceedings of the New Zealand Society of Animal Production* **6**: 124-129.

Scobie, D.R.; O'Connell, D.; Morris, C.A.; Hickey, S.M. 2007: A preliminary genetic analysis of breech and tail traits with the aim of improving the welfare of sheep. *Australian journal of agricultural research* **58**: 161-167.

Slee, J. 1965: Seasonal patterns of moulting in Wiltshire Horn sheep. In: Lyne, A.G.; Short, B.F. eds. Biology of the skin and hair growth, Angus Robertson, Sydney, Australia. p. 545-563.