Growing wool in tissue culture - which fibre type is best?

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ABSTRACT

Individual wool follicles were isolated from adult sheep and maintained in tissue culture for three days. Follicles from Merino, Poll Dorset and Suffolk animals were classified as fine wool types and were compared with follicles from Romney, English Leicester and Drysdale animals which were termed a coarser wool type.

There were significant differences in both follicle survival and the rate of fibre growth between coarse and fine follicle types maintained *in vitro*. Only 48% of follicles from animals with fine fibre types survived in tissue culture. In contrast, 84% of follicles from coarse fibre types remained viable *in vitro* ($P<0.01$). The fibre growth rates of viable follicles was also significantly different between fibre types ($P<0.01$). Follicles isolated from animals classified as fine fibre types had a fibre growth rate of 108 ± 16µm per day compared with 170 ± 12µm per day fibre growth by animals with coarser fibre types.

In vitro fibre growth by animals with large, straight follicles such as those from the English Leicester and Drysdale animals was greatest and a high proportion remained viable. Furthermore, follicles from these animals were easy to isolate relative to finer fibre types. Dissection of finer follicles was considerably more difficult, due to the decreased follicle size, curvature and high follicle density; factors which may be responsible for their reduced viability *in vitro*.

Keywords: follicle culture; wool growth; fibre type; sheep breed; genotype.

INTRODUCTION

Sheep genotype is a major factor influencing wool growth *in vivo*. Clean wool production, fibre diameter and the seasonal variation of wool growth vary significantly with sheep breed (Bigham *et al*. 1978). Ross (1990) has reviewed breed differences, and Table 1 details typical ranges for fibre diameter and staple length for some common sheep breeds in New Zealand.

In addition to effects noted *in vivo*, sheep genotype may also influence wool growth and follicle survival *in vitro*. The ease with which follicles can be isolated is likely to depend on the toughness of the dermis, the size and curvature of follicles, and the degree to which the follicles are intertwined. These factors vary considerably between sheep breeds.

It seems reasonable that follicles which are easier to isolate are likely to be less damaged and more likely to remain viable, than follicles which are difficult to isolate. The work presented here examines the observation that follicles from coarse wool breeds appear easier to isolate than those from fine wool breeds.

MATERIALS AND METHODS

Skin strip biopsies (0.5mm x 30mm) were collected from the clipped mid-side of one adult animal from each of six breeds of sheep (Merino, Suffolk, Poll Dorset, Romney, English Leicester and Drysdale). Approximately 0.5ml of local anaesthetic (Lignovet™; 20% w/v lignocaine hydrochloride; C-Vet Ltd, UK) was injected intradermally into the area from which the sample was to be collected.

Individual follicles were isolated from the biopsy sample by microdissection (Messenger 1984) and maintained in 500µl of supplemented, serum-free nutrient medium modified from that described by Philpott *et al*. (1989). This consisted of William's Medium E supplemented with L-glutamine (1mM), insulin (10µg/ml), transferrin (10(g/ml), sodium selenite (10ng/ml) and hydrocortisone (10ng/ml) [Sigma Chemical Co]. Follicles were maintained at 37°C in a humidified atmosphere of 5% CO_2 / 95% air. The pH of the culture medium was adjusted to pH 7.0-7.2 prior to filter sterilisation.

Fibre growth *in vitro* was determined using a method termed fixed time analysis (Winder 1995). For this analysis, fibre plus follicle length was measured on two occasions - immediately after follicle isolation from the skin, and again following three days incubation of the follicle in nutrient medium. The fibre growth rate per day was then calculated as (final length-initial length)/3 days. Length measurements were undertaken by image analysis (Bioquant IV, R & M Biometrics, USA) at 144X magnification.

Follicles from the six animals used in this experiment were allocated to one of two groups - fine wool or coarse wool - on the basis of the fibre diameter and staple length data presented in Table 1. Follicles from the Merino, Poll Dorset and Suffolk animals were allocated to the fine wool group, with follicles from the Romney, English Leicester and Drysdale animals forming the coarse fibre group. An analysis of variance of the rate of growth of fiber from viable follicles, of the two groups was performed using the statistical package GENSTAT 5 (Rothamsted Experimental Station, UK). Previous studies (Winder 1995) have shown that the error of measurement is approximately

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TABLE 1: Fibre diameter and annual staple length data for a selection of sheep breeds, adapted from Ross (1990).

		Fibre diameter (µm)	Annual staple length (mm)
Finewool	- Merino	18-25	60-100
Downwool	- Poll Dorset	27-32	75-100
	- Suffolk	28-35	75-100
Longwool	- Romney	31-41	125-175
	- English Leicester	36-42	150-200
Carpetwool	- Drysdale	36-49	200-300

30µm per day. Wool follicles with a fibre growth rate of less than 30µm per day were therefore assumed to be non-viable and excluded from analysis of varience.

A χ^2 analysis was performed on all data (including wool growth rate values less than 30µm per day) to examine differences in *in vitro* follicle viability between fibre types.

RESULTS

Table 2 shows that there there were significant differences in both follicle survival and fibre growth, between coarse wool and fine wool follicles maintained *in vitro*. Only 48% of follicles from animals with fine fibre types survived in tissue culture. In contrast, 84% of follicles from coarse fibre sheep breeds remained viable *in vitro* (P<0.01).

TABLE 2: Fibre growth in vitro by follicles isolated from different fibre types. **P<0.01 by χ^2 analysis for differences in the proportion of viable follicles.

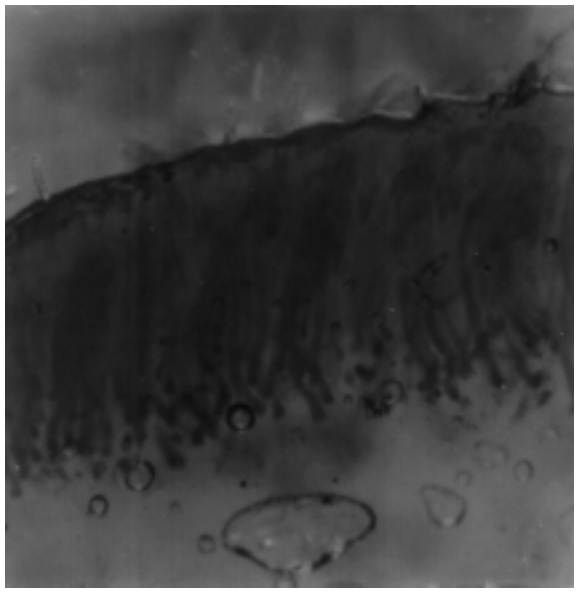
	number of follicles isolated	number of follicles viable	fibre growth rate mean ± s.e.m. (µm/day)	P value (growth rate)
Fine Wool Type	65	31**	108 ± 16	
Coarse Wool Type	128	107	170 ± 12	<0.001

The fibre growth rates of viable follicles was also significantly different between fibre types. Follicles isolated from animals classified as fine fibre types had fibre growth rate of 108 ± 16µm per day compared with 170 ± 12µm per day fibre growth by animals with coarse fibre types (P<0.01).

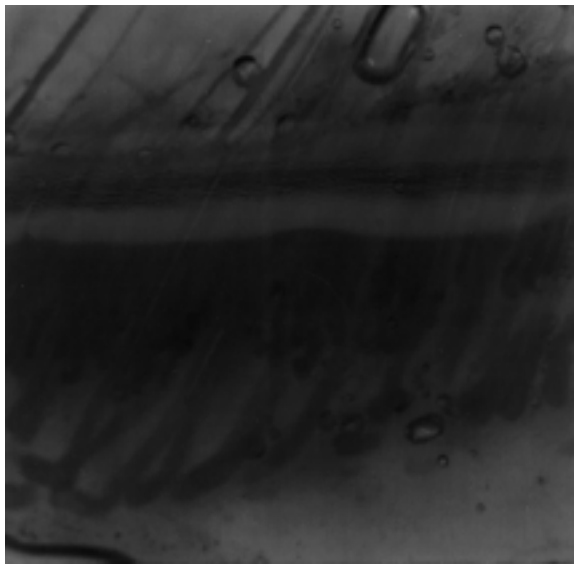
During the course of this study it was noted that the different animals varied in the ease of follicle isolation. Follicles from the coarser wool animals were easier to isolate because the follicles were large and lay parallel and comparatively straight, in the dermal tissue. Dissection of follicles from finer wool animals was considerably more difficult due to the decreased follicle size. In addition to follicle size, follicle curvature and density also influenced the ease of isolation. Follicle size, curvature and density differences can be seen in Figure 1 which shows typical skin sections from one animal of each of the fine and coarse wool types stained with Nile blue sulphate (Maddocks and Jackson 1988).

FIGURE 1: Histological slides showing follicles in the skin of animals representing the two fibre types examined in this study (20X magnification). (A) Poll Dorset (fine wool type); (B) Drysdale (coarse wool type). Wool follicles are darkly stained. Connective tissue and wool fibres remain unstained. In each slide, the skin surface is shown at the top, with follicle bulbs near the bottom of the photograph.

1(A)



1(B)



DISCUSSION

In this study, *in vitro* follicle viability was greater for follicles from coarser wool breeds of sheep (P<0.01). It was found that follicles from finer wool types were more difficult to isolate. This may have been responsible for their reduced viability *in vitro*. Animals from finer wool types generally produced follicles with a smaller diameter, greater density (Carter and Clarke 1957; Orwin 1988) and a higher degree of curvature. Isolation of large wool follicles was quicker, easier and more likely to result in a

viable follicle *in vitro*. Not only did coaser wool types have follicles with a greater diameter, many breeds with coarser wool (eg Drysdale) have clear size differences between large primary follicles and smaller secondary follicles (Figure 1(B)). Under these conditions, many large primary follicles can be isolated easily, over a short period of time and with minimal damage to follicles; factors which are likely to be an important in maintaining a high proportion of viable follicles during tissue culture.

During maintenance *in vitro* it was also shown that follicles from coarser wool breeds had a significantly greater rate of fibre growth ($P<0.01$). This concurs with the *in vivo* data of Ross (1990) presented in Table 1. Sheep breeds classified in this study as fine wool types (Merino, Poll Dorset and Suffolk) had a slower fibre growth rate *in vivo* compared with coarser wool types (Romney, English Leicester and Drysdale). This may reflect true phenotypic differences between breeds and deserves further attention as it suggests that genetic differences in wool growth are generated at the follicle itself, and are not merely a consequence of differences in nutrient supply or hormonal control.

The growth rate difference may be an effect of follicle size; in previous *in vitro* studies it has been shown that follicles with a larger bulb diameter produced fibre at a greater rate than smaller follicles (Hynd *et al.* 1992; Winder 1995); a trend which has frequently been observed *in vivo* (Black 1987; Orwin 1988).

Alternatively, differences in both follicle viability and fibre growth rates between fibre types may be due to differential effects of stress related growth factors released during the collection of skin strips, prior to follicle isolation. Plasma levels of cortisol are elevated in response to stress and Behrendt *et al.* (1993) demonstrated that an increased plasma cortisol concentration can affect wool growth *in vivo*. Cortisol seems unlikely to be responsible for the observed responses however as Hynd *et al.* (1994) found no effect of cortisol on the *in vitro* rate of wool production over three days. In addition, Scobie and Hynd (1995) reported that although sustained elevation of plasma cortisol reduces wool growth, cortisol elevation was required for approximately 29 hours before effects on mitotic rate within the follicle bulb were observed. These studies do not rule out the possibility of between breed differences. For instance, Behrendt *et al.* (1993) demonstrated that individual animals within one breed may have differences in their sensitivities to stress related hormones. Thus, it is possible that between breed differences occur.

In contrast to the delayed effects noted with cortisol, catecholamines have been shown to rapidly lower the rate of mitosis within the wool follicle (Scobie *et al.* 1994). It appears well worth investigating the possible roles of adrenaline or noradrenaline in the growth of wool *in vitro*, and potential between breed variation in susceptibility to these neuro hormones.

In summary, it is easier to isolate large, straight follicles such as those occurring in the skin of English Leicester and Drysdale sheep. Furthermore, a high proportion of these follicles remain viable in culture. Fibre growth from follicles maintained in tissue culture, appears to be correlated with bulb size, the rate of fibre growth by animals with large follicles being relatively high. Excessive curvature, small size and high density make follicle isolation difficult and lead to reduced viability *in vitro*. Effects of stress related hormones on fibre growth between animals and between different days of isolation, cannot be discounted. As follicles from coarser wool breeds are easier to isolate than those from finer wool breeds and are more likely to remain viable and produce wool fibre in culture; preferential isolation of large, primary follicles and the use of coarser wool breeds of sheep are recommended for future *in vitro* studies of wool growth.

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