**Fleece structure and its relation to histological skin characteristics of Merino stud rams in Australia**

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**Abstract**

*812 Merino stud rams in 45 flocks throughout Australia between 1986 and 1995 were classified into 4 types based on skin and fleece structure.*

*Substantial differences in follicle and fibre characteristics between sheep types were observed. As a group, plain bodied sheep with very loose, thin skins and producing fleeces consisting of very long fibre bundles and long thin staples(SRS), had the highest density and S/P ratio measurements and the lowest measurements for diameter and standard deviation of primary fibres and secondary fibres. These rams represented only 9 % of the sheep surveyed.*

*The next ranked group on measurement was the semi SRS sheep which were plain bodied with loose skins and fleeces consisting of long, thin staples but no fibre bundles. These rams represented 28 % of the sheep surveyed.*

*As a group, sheep with wrinkly skins in which the inherent skin looseness had been over-ridden by skin thickening and wrinkle formation had low follicle density and S/P ratio values and high diameter and diameter variability for the primary fibres and secondary fibres; as did plain bodied sheep lacking skin looseness (flat skins). Both groups mostly produce fleeces consisting of large staples. The wrinkly rams and flat skin rams represented 32% and 30 % respectively of the rams surveyed .*

*Results are interpreted in terms of the pre-papilla cell hypothesis of follicle formation proposed by Moore and co-workers.*

**Introduction**

Pilot studies by one of us (JW) in 1987 showed that sheep with measurably high fibre density and length have straight and evenly seated wool follicles in the skin (reference). We have shown more recently (staple formation reference here) that for this sheep type (SRS) the follicle groups are arranged in orderly, well-spaced rows containing high numbers of follicles. The sheep are plain bodied with a very loose skin and produce fleeces consisting of long and closely packed fibre bundles and thin staples. The wool is very soft, lustrous and has high crimp amplitude (“deep” crimp) and low crimp frequency (“bold” crimp).

However, for Merino sheep generally there is large variation in follicle, fibre, fleece and body traits. Wool follicles vary widely in depth, curvature and shape. This wide variation for follicle patterning was first identified by Nay (1973) and has been illustrated and described by Maddocks and Jackson (1988). The follicle density, the ratio of secondary follicles to primary follicles, the relative sizes of primary and secondary fibres as well as the number and size of the sebaceous and suderiferous glands also vary widely between Merino sheep. Carter and Clarke (1957) and Carter (1965) have reported values for some of these characteristics.

This biological disorder appears to be a consequence of the long held practice of Merino studbreeders and sheepclassers selecting for increased skin fold (wrinkle) as a means of increasing the body surface area for growing wool. Skin wrinkle has strong genetic correlations with follicle curvature (0.68) and follicle unevenness (0.64) (Jackson, AB32 reference). Merino sheep with wrinkly skins have entangled and unevenly seated follicles in the skin, and because these are genetic correlations, so will subsequent generations if rams and ewes like this are used for breeding. Sheep with wrinkly skins produce fleeces consisting of thick, stiff and short staples of entangled fibres and “closed” backs. These features create the allusion of high density. It was the main ram type (32%) found in this survey of Merino stud breeding practices.

The survey was carried out between 1986 and 1995. The stud rams had already been selected by studbreeders and sheep classers. Our reclassification of the rams did not alter the breeding decisions already made.

**Materials and Methods**

*Sheep*

Merino and Poll Merino rams previously classed as stud sires by studbreeders and sheepclassers using alternative visual criteria to the SRS system, were tested. The rams were mainly 14 to 16 months of age. There were 544, 226 and 38 rams tested from 23 strong, 13 medium and 9 fine wool stud flocks respectively. The testing was carried out between 1987 and 1995.

*Classification of skin and fleece structure*

Each ram was classified according to the following types:

* plain bodied sheep with fleeces consisting of very long, and closely packed fibre bundles and thin staples. The wool is very soft, lustrous and has high crimp amplitude (“deep” crimp) and low crimp frequency (“bold” crimp). The skin is very loose. The sheep are referred to as “SRS” and expected to have high density and length.
* plain bodied sheep with fleeces consisting of long, thin staples. The wool is soft and semi-lustrous. The crimp amplitude is not as pronounced and the skin is not as loose as for SRS sheep. The sheep are referred to as “semi SRS” and expected to rank next best for density and length.
* relatively plain bodied sheep with fleeces consisting of wide and lightweight staples. The wool is non-lustrous and has low crimp amplitude. The skin is thick and taut. The sheep are referred to as “flat skin” and expected to have low density.
* wrinkly sheep with fleeces consisting of thick and stiff staples. The fleece is short in length and often excessively greasy. The skin is very thick and forms wrinkles all over the body. The sheep are referred to as “wrinkly skin” and are expected to have average to low density and high variation in fibre size, shape and alignment.

The classing types are described in more detail, along with photographic standards elsewhere (reference).

*Histology*

Paired midside skin samples were collected from the rams using circular skin trephines of one centimetre diameter. The samples were fixed in 10 % formol saline.

Prior to sectioning, the wool stubble and subdermal tissue was closely trimmed from the skin samples using fine curved scissors. A Mitutoyo ballpoint gauge (model …) was used to measure the compressed skin depth at four sites on one sample from each ram.

Histological preparation and measurements on horizontal skin sections from all of the tested rams and vertical skin sections from some of these rams were made as described by Maddocks and Jackson (1988) using the frozen section technique of Nay (1976).

Follicle density, secondary follicle to primary follicle ratio, primary fibre diameter and secondary fibre diameter, were measured or counted on horizontal skin sections cut at the mid-sebaceous gland level. For each ram, follicle density was counted for 10 fields of 1 square millimetre at … magnification. Secondary follicle to primary follicle ratio was counted on 10 follicle groups at …magnificaton. Primary fibre diameter was measured for 50 fibres and secondary fibre diameter was measured for 100 fibres at … magnification.

Follicle curvature and follicle evenness/unevenness were scored and follicle depth was measured on vertical skin sections using the methods of Nay (1973) as described by Maddocks and Jackson (1988).

**Results**

*Follicle, fibre and skin characteristics*

The means and standard errors for follicle density, follicles per group, density of follicle groups, primary and secondary fibre diameter and standard deviation of secondary fibre diameter and compressed skin thickness are listed in Table 1.

**Table 1. Mean values ( standard errors) for follicle, fibre and skin characteristics of Merino stud rams surveyed.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Characteristic | SRS | Semi SRS | Flat skin | Wrinkly skin |
| *Strong wool:* | | | | |
| No. of sheep | 63 | 138 | 152 | 191 |
| Follicle density | 83.6 (2.69) | 67.5 (1.36) | 51.8 (1.11) | 55.1 (1.13) |
| S/P ratio | 44.3 (0.73) | 33.5 (0.37) | 24.9 (0.32) | 28.4 (0.34) |
| Dp | 19.2 (0.24) | 20.3 (0.29) | 23.3 (0.37) | 23.6 (0.26) |
| Dp SD | 2.59 (0.07) | 3.28 (0.20) | 3.96 (0.20) | 3.91 (0.76) |
| Ds | 20.7 (0.20) | 22.4 (0.18) | 25.0 (0.32) | 25.5 (0.22) |
| DsSD | 2.79 (0.10) | 3.15 (0.06) | 3.77 (0.22) | 4.06 (0.07) |
| CST | **0.90 (0.05)**  **n = 20** | **1.01 (0.03)**  n= 68 | **1.04 (0.025)**  **n = 74** | **1.15 (0.032)**  **n = 82** |
| *Medium wool:* | | | | |
| No. of sheep | 8 | 82 | 71 | 65 |
| Follicle density | 87.4 (8.35) | 66.3 (1.60) | 61.2 (1.90) | 57.3 (1.76) |
| S/P ratio | 43.4 (1.58) | 35.4 (0.53) | 24.3 (0.45) | 29.3 (0.57) |
| Dp | 17.0 (0.78) | 19.0 (0.38) | 20.2 (0.44) | 21.2 (0.50) |
| Dp SD | 2.43 (0.22) | 2.98 (0.12) | 3.03 (0.10) | 3.89 (0.22) |
| Ds | 18.5 (0.65) | 20.7 (0.28) | 21.8 (0.22) | 22.2 (0.50) |
| DsSD | 2.2 (0.18) | 2.89 (0.07) | 2.95 (0.08) | 3.86 (0.29) |
| CST | **n =3** | **0.98 (0.03)**  **n = 35** | **1.10 (0.03)**  **n = 49** | **1.20 (0.06)**  **n = 34** |
| *Fine and superfine wool:* | | | | |
| No. of sheep | 4 | 11 | 21 | 6 |
| Follicle density |  | 67.9 | 58.7 | 60.5 |
| S/P ratio |  | 37.1 | 22.5 | 32.7 |
| Dp |  | 16.7 | 16.2 | 16.3 |
| Dp SD |  | 2.3 | 2.57 | 2.1 |
| Ds |  | 17.5 | 18.1 | 17.3 |
| DsSD |  | 2 | 2.25 | 2.3 |
| CST |  |  |  |  |

SRS rams had significantly higher values for follicle density and S/P ratio and significantly lower values for primary fibre diameter, secondary fibre diameter and skin thickness than the other three classing types. The primary fibres and secondary fibres were significantly more uniform in diameter for SRS and semi SRS rams than for flat skin and wrinkly skin rams. Semi SRS rams also had significantly higher values for follicle density and S/P ratio, and significantly lower values for primary fibre diameter, secondary fibre diameter and skin thickness than flat skin and wrinkly skin rams.

Neville, I could not think of a simple way of including P values in Table 1. Most of the results are significantly different at the P < 0.0001 level (\*\*\*). The non-significant differences were for Dp flat skin vs wrinkly skin, DpSD flat skin vs wrinkly skin (strong wool only), Ds flat skin vs wrinkly skin, DsSD flat skin vs wrinkly skin, CST flat skin vs wrinkly skin, Density flat skin vs wrinkly skin (medium wools only), and Dp SRS vs semi SRS (medium only)

**Discussion**

Large differences between the four classing types of Merino rams for follicle density, S/P ratio, Dp, DpSd, Ds and DsSD were found. The results were in close agreement with the predicted outcomes. Rams visually selected as likely to have high density and length of fibres (SRS) had the highest follicle density, highest S/P ratio and finest and most uniform primary fibres and secondary fibres. The semi-SRS group was next best, as forecasted. Rams visually classed as likely to have the lowest density of fibres (flat skin) had the lowest density and lowest S/P ratio. Rams visually classed as likely to have average to low density of fibres and high variation in fibre diameter (wrinkly skin) were also forecast accurately; the high fibre variability being similar to that of the flat skin group.

Fibre length was not routinely measured on the survey rams, even though it was included as a component of the classing method. However, there were ….. of the …. rams measured and fibre length was highest in the SRS group, then the semi SRS group, and lowest in the flat skin and wrinkly skin groups. (Neville, results to follow)

Follicle curvature and follicle depth were measured on a small proportion of the rams (Table…). The results show that the SRS and semi SRS groups had straight and evenly seated follicles in the skin, and the flat skin and wrinkly skin groups had highly curved and unevenly seated and often, the deepest seated follicles. (Neville, results to follow)

Skin looseness was an important component of the classing method. This component was estimated as being very loose (SRS), loose (semi SRS), absent (flat skin) or loose but tightened by skin folds (wrinkly skin). In sheep with no sign of skin wrinkle (plain bodied sheep), the very loose skinned group had the highest density and S/P ratio, followed by the loose skinned group, and lastly by the group where skin looseness was absent. In the wrinkly skin group, the skin was loose but “tightened” by skin thickening and skin fold development. The density and S/P ratio values of this group were lower than the density and S/P ratio values of the plain bodied sheep with very loose (SRS) and loose (semi SRS) skins (see Table …).

Skin thickness was measured on trimmed skin samples using a Mitutoyo ballpoint compression gauge. The trimmed samples included layers 1 to 3 of the five layers described for Merino sheep skin by Mitchell et at ( ). The classing types, as groups, ranked as predicted, for compressed skin thickness. SRS and semi SRS sheep were assessed as having thin skins, because it was considered to be a prerequisite for plain bodies (no skin wrinkle), and were found to measure accordingly. Flat skin sheep were visually assessed as likely to have thick skins but not as pronounced as wrinkly skin rams since the animals were relatively plain bodied. Wrinkly sheep were visually appraised as likely to have the thickest skins (even though there was an underlying looseness to the skin) and were found to measure as such. The extra thickness detected in these compression gauge measurements of the flat skin sheep and in particular, the wrinkly sheep, may be due to more collagen being laid down in the papillary layer (layer 2) and the reticular layer (layer 3) of the dermis, or an intermeshing (“hardening”) of the collagen in these two layers, or both factors. The overt appearance of skin wrinkling on the sheep’s body is due to the intermeshing of collagen microfibrils in layer 4 of the skin (Mitchell et al). Neither layers 4 or 5 were measured in this study.

The desired outcome for maximizing fleece weight and fleece quality from visual classing is likely to be realized where Merino sheep that are plain bodied very loose skin that are thin, not thick, are chosen. Skin folds (skin wrinkle), undermine these fleece improvements. Where skin wrinkle occurs, it is associated with decreases in follicle density and S/P ratio when compared with plain bodied Merinos with loose and thin skins (Table …). Skin folds develop in the foetal skin of the wool bearing surface at day 100 of gestation (Bogolyubsky, 1940) and have the potential to interfere with follicle development.

We hold a view that zero tolerance to skin wrinkle in the breeding of Merino sheep is essential. Skin looseness needs to be present on plain bodied sheep to avoid reductions in follicle density and S/P ratio. Skin looseness and its associated fleece structure provide visual classing tools that are easily and accurately used to provide improvements in follicle density, S/P ratio and fibre quality. We report elsewhere (reference) that these classing tools are also indicative of rapid improvements to fleece weight and wool quality.

Merino sheep always have been bred mainly for wrinkly skins. Photographic evidence and descriptions confirming this unsatisfactory situation can be found in many writings and more recently and comprehensively in the historical accounts of the breeding history of the Australian Merino given by Massy ( …., …).

Belschner et al (….), like us, may have come to the conclusion that zero tolerance to skin wrinkle in Merino sheep is necessary following their extensive field studies of the 1930s. They showed that non-mulesed, plain bodied sheep have low susceptibility to breech strike whereas wrinkly, non-mulesed sheep are highly susceptible. Belschner, in his advisory role to geneticists in the planning and design of the Merino selection experiments on clean fleece weight selection in the 1950s seems likely to have taken that position. But it did not happen. Skin wrinkle was included as a valid trait in these genetic selection experiments, albeit with a ceiling on the degree of skin wrinkle permitted. Skin folds (wrinkle) was included in the geneticists’ SWALK formula for calculating the wool producing potential of a Merino sheep.

Skin looseness, not skin folds, is the key component that should have been recognized and included in these long term experiments which took place in Australia continuously from 1952 to 2006. These genetic selection experiments report that wrinkle score is positively correlated genetically with S/P ratio (references). But it is the skin looseness, not the skin wrinkle, that is responsible. In fact, skin wrinkle is having a separate and negative effect in lowering S/P ratio and follicle density. Thick skins are associated high follicle depth, and high follicle depth is strongly correlated with high primary fibre diameter, low density, low S/P ratio and primitive regression toward a two-coated fleece type with its attendant problems of increased incidence of “hairy birthcoats (high Dp/Ds ratio), reduced wool softness, increased fleece colour and increased susceptibility to fleece rot and body strike (Jackson reference on Merino evolution here).

There were only 8 % of SRS rams identified in the large population of stud sires studied. Individual data for unclassed ewes, … to … months old, in 16 studs (Carter and Clarke 1957 and Carter, personal communication) revealed very few sheep with the high values for S/P ratios of the SRS type. One must conclude that traditional classing has not allowed the incidence of the SRS type to increase in the Australian Merino population.

The marked differences between visual classing types in follicle density, S/P ratio, primary fibre diameter and secondary fibre diameter raise the question of the developmental mechanism involved in creating the differences. Moore (1984), Moore and Jackson (1984), Moore et al (1989, 1996 and 1998) proposed that a population of pre-papilla cells committed to form follicle dermal papillae developed in the skin prior to follicle initiation. They postulated that the population of these cells is depleted by the formation of primary follicles, with the remaining cells forming the original and derived secondary follicles. They suggested that the number of cells available for the induction of secondary follicles is inversely related to the number and diameter of the primary fibres. It also follows that the number of secondary fibres should be inversely related to their fibre diameter. Such relationships would be with respect to the numbers and dimensions of the fibres during follicle development and may be accurately mirrored by the dimensions of the fibres measured in the adult sheep. …………. …….. ………

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