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Prenatal wool follicle development in Romney, Merino and Merino-Romney cross sheep

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ABSTRACT

The prenatal development of wool follicles has been described separately for the Merino and Romney but a comparative study of both breeds and their cross has not been undertaken. This study compared the rate and timing of follicle development in midside skin samples from Romney, Merino and Merino-Romney cross (MxR) fetuses slaughtered at weekly intervals from day 76 to 144 of gestation.

The developmental pattern of total number of follicles per unit area of skin differed between genotypes as indicated by a significant (P < 0.001) genotype x gestational age interaction. The total follicle density of each genotype increased from day 76 to day 105 at which time the follicle density of the Romney peaked at 229 follicles/mm². Total follicle density of the MxR continued to increase to between 420 and 510 follicles/mm² between day 104 and 122 of gestation and the Merino reached a peak of 1170 follicles/mm² at day 125 of gestation. The density of immature (i) and mature fibre-bearing (t) primary follicles (P) did not differ (P > 0.10) between genotypes over time, with each genotype reaching a maximum density of P(i) by around days 83 and 90 of gestation. Primary follicles (P) began to reach maturity by gestational day 90 in the MxR and by day 97 in the Merino and the Romney (P > 0.10). In all genotypes, secondary follicle (S) initiation began at around day 90 with a small proportion starting to form fibres by day 105 of gestation. There was a significant interaction between genotype and gestational age for both the density of S+ (P < 0.0001) and the density of S+ (P < 0.01). Specifically, S+ increased to 175 follicles/mm² in the Romney, 440 follicles/mm² in the MxR and 900 follicles/mm² in the Merino at 105, 105 and 125 days gestation respectively, after which S+ density stayed relatively constant up to birth.

These results indicate that follicle initiation and fibre formation in different genotypes occurs at similar ages during fetal growth. The high total follicle density in the Merino is due to a high S density which, in turn, is due to S initiation continuing for longer than in either the Romney or MxR. These data provide a model for the identification of certain physiological factors which affect secondary follicle initiation and development.

Keywords: follicle initiation; follicle density; fetal development; fetus; lamb; fetal genotype.

INTRODUCTION

Follicle density is an important factor controlling wool quality and quantity. An understanding of the determinants of follicle density might enable wool producers to manipulate the type of wool that sheep are able to produce whilst maintaining other genetically-based production characteristics, such as suitability for meat production, of the original breed.

The number of wool follicles in sheep is determined prior to birth. The prenatal development of wool follicles has been described separately for the Merino (Carte 1943, Carte and Hardy 1947; Hardy and Lyne 1956) and Romney (Galpin 1935; Stephenson 1957). These studies indicate that there are no marked differences in the rate of development of the follicle population or the age at which different stages of follicle development are completed. However, follicle density has not been quantified in fetal Merinos and the age at which genotypic differences in follicle density occur has not been identified.

This study was undertaken to examine follicle density and pattern of development in the fetal Romney, Merino and Merino-Romney cross sheep in order to determine the period during which genotypic differences in follicle density occur.

MATERIALS AND METHOD

Adult Romney ewes were mated to Romney rams and Merino and Romney ewes were mated to Merino rams, resulting in three fetal genotypes - Romney (n = 20), Merino-Romney cross (MxR; n = 20) and Merino (n = 21). Two ewes within each fetal genotype were assigned randomly to be slaughtered at weekly intervals from 76 to 146 days of gestation.

On the assigned day, the ewes were taken directly from pasture and slaughtered by captive bolt and exsanguination. Fetuses were removed from the ewe, euthanased, weighed, and a 10mm trephine biopsy of skin was taken from the midside and fixed in Bouins fluid. The skin was removed from the fixative 4 to 6h later and stored in 70%(v/v) ethanol.

Skin samples were embedded in paraffin, sectioned (8μm) transverse to the follicle and stained by the Saépiic method modified from Auber (1952). Sections were examined with a
light microscope and measurements were made at the level of the sebaceous gland using image analysis. The number of primary (P) and secondary (S) follicles per unit area was counted and mature fibre-bearing (f) and immature (i) follicles were identified within six fields of view for each skin sample. Total follicle density (P_{fi} + S_{fi}) total primary follicle density (P_{fi}), mature primary follicle density (P_f), total secondary follicle density (S_{fi}) and mature secondary follicle density (S_f) were then estimated. From these, the percentage of mature P (100 x P_{fi}/P_{fi}) and mature S (100 x S_{fi}/S_{fi}) and the ratio of secondary to primary follicles (both S_{fi}/P_{fi}) and mature S_{fi}/P_{fi} were calculated.

The surface area of each fetus was estimated from the following equation:

Surface area (cm²) = 3.5 x (fetus weight (g))^{0.77}
(Malan and Curson 1936).

Total follicle number of the fetus was calculated from the product of surface area (expressed in mm²) and follicle density.

Statistical analysis

Fetuses were assigned to 10 nominal gestational age groups and the effects of genotype and age group and the 2-way interaction on the follicle density estimates and ratios were examined using analysis of variance. In cases where the 2-way interaction was non-significant, a model containing only the main effects was fitted to the data. Least square means were estimated and genotype differences were examined. Duncans multivariate analysis was used to test age group differences.

RESULTS

Follicle density

Total follicle density (P_{fi} + S_{fi}), S_{fi}, density and S_{fi}/P_{fi} differed between genotypes with age, as indicated by a significant genotype x gestational age interaction (P < 0.0001 in each case). The P_{fi} + S_{fi} density increased from day 76 of gestation in all genotypes, until day 105 of gestation in the Romney, day 122 of gestation in the MxR and day 125 of gestation in the Merino. S_{fi} density increased from day 90 of gestation (Fig. 1) and followed a similar pattern to that described for total follicle density, reaching peak density on days 105, 122 and 125 of gestation for the Romney, MxR and Merino, respectively, after which there was a decline in S_{fi} density in all genotypes. In the final period of gestation (stage 10, between 139 and 146 days of gestation) there was a significantly greater density of S_{fi} in the Merino than in the other two genotypes (P < 0.01; Table 1). S_{fi}/P_{fi} also increased from 90 days of gestation and then appeared to plateau from day 110 of gestation in the Romney, day 122 in the MxR and day 125 in the Merino (Fig. 2).

There was no interaction between genotype and age when P_{fi} density was analysed (P > 0.1). Each genotype reached a maximum density of P_{fi} between days 83 and 90 of gestation (Fig. 3). There was a genotype effect on the density of P_{fi} (P < 0.05) with the Merinos having significantly more primary follicles per unit area of skin than the Romney (P < 0.05) or the MxR (P < 0.05) but there was no

FIGURE 1: Density of secondary follicles in midside skin of Romney (♦), Merino-Romney cross (O) and Merino (■) fetuses at different gestational ages.

FIGURE 2: Ratio of secondary to primary follicle densities (S/P) in midside skin of Romney (♦), Merino-Romney cross (O) and Merino (■) fetuses at different gestational ages.

TABLE 1: Density (mm⁻²) of secondary S(f-i) and mature secondary S(f) follicles in midside skin of Romney, Merino-Romney cross (MxR) and Merino fetuses between day 139 and 146 of gestation and the number of animals in each group (n) (least square means ± the standard error of the least square mean).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>S(f-i)</th>
<th>S(f)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romney</td>
<td>100.4 ± 71.63a</td>
<td>85.1 ± 13.56a</td>
<td>2</td>
</tr>
<tr>
<td>MxR</td>
<td>342.69</td>
<td>100.99</td>
<td>1</td>
</tr>
<tr>
<td>Merino</td>
<td>667.4 ± 50.65b</td>
<td>72.9 ± 9.59b</td>
<td>4</td>
</tr>
</tbody>
</table>

difference between the Romney and the MxR (P > 0.10). There was no significant interaction between age and genotype (P > 0.10) when the total number of P was analysed, and there was no significant genotype effect when a model was fitted without the 2-way interaction (P > 0.10).

FIGURE 3: Density of primary follicles in midside skin of Romney (♦), Merino-Romney cross (O) and Merino (■) fetuses at different gestational ages.
Follicle development

The density of $P_{P0}$ + $S_{P0}$ did not differ between genotypes with age ($P > 0.10$) but there was a significant genotype effect ($P < 0.05$). The MxR had significantly more mature P follicles per unit area of skin than the Merino ($P < 0.005$), but the density of mature P follicles in the Romney did not differ from the Merino or MxR.

There was no significant difference between genotypes in the gestational age at which the P began to reach maturity ($P > 0.10$). There was also no effect of genotype on the $P_{P0}$ density ($P > 0.10$) with P beginning to produce fibres by day 90 in the MxR and by day 97 in the Merino and the Romney. There was no interaction between genotype and age in the percentage of P containing fibres ($P > 0.10$) nor was there any genotype effect. At least 90% of P were producing fibres by day 104 of gestation in the three genotypes.

In all genotypes, S initiation began at around day 90 with some S starting to form fibres by day 105 of gestation (Fig. 4). There was a significant interaction between genotype and gestational age for both $S_{P0}$ ($P < 0.01$) and the percentage of mature S ($P < 0.05$). In the final stage of gestation, between day 139 and 146, there was no significant difference in $S_{P0}$ between genotypes (Table 1). Seventy five percent of S were producing fibres by day 146 of gestation in the Romney, 30% of S had fibres in the MxR and only 15% of S had fibres in the Merino.

**FIGURE 4:** Density of mature secondary follicles in midside skin of Romney (●), Merino-Romney cross (O) and Merino (■) fetuses at different gestational ages.

![Density vs Age](image)

**DISCUSSION**

This study is the first to quantify and compare prenatal follicle development in the Romney, Merino and their cross (MxR) in samples taken concurrently in the same environment. As expected from earlier studies of genotypic differences in follicle populations (e.g. Carter and Clarke 1957a, b) and follicle development (e.g. Stephenson 1957, 1958), total follicle density varied between genotypes over time. This was due to differences in $S_{P0}$ density, rather than $P_{P0}$ density, since $S_{P0}$ density followed similar patterns of change to total follicle density in all of the measured characteristics.

The density of $P_{P0}$ increased to a peak and then declined in a similar manner in all genotypes over time (Fig. 3) but the Merinos had more $P_{P0}$ per unit area of skin than the other two genotypes. However, when the total number of P was calculated from estimates of skin surface area (Malan and Curson 1936) there was no genotype effect. This suggests that the $P_{P0}$ density was less affected by growth and the associated surface area changes in the Merino than in the other two genotypes. Likewise, the total number of $P_{P0}$ remained constant after day 90 of gestation and the majority of P were producing fibres by day 104 of gestation in the three genotypes studied. Correction for surface area expansion over time showed that the decline of follicle density in all genotypes resulted from surface area change. Thus changes in $P_{P0}$ density after 90 days of gestation reflected skin expansion accompanying growth.

Constancy in the number of $P_{P0}$ with time after 90 days of gestation validates the use of $S_{P0}$/$P_{P0}$ as a measure of the development of the $S_{P0}$ population (Schinske 1955). Fig. 2 indicates the change in $S_{P0}$/$P_{P0}$ with gestational age and a clear developmental pattern is obvious. $S_{P0}$/$P_{P0}$ of the Romney increased from day 90 to day 110 of gestation. The stability of $S_{P0}$/$P_{P0}$ after this suggests no more S were initiated, whereas in the Merinos, $S_{P0}$/$P_{P0}$ increased until day 125 of gestation. The MxR initiated S for almost as long as the Merinos. Thus, it appears that Merinos have a higher follicle density than the other genotypes because of a longer period of S initiation.

The results show that there are no marked differences between genotypes in the timing of the onset of development phases as suggested by previous studies (Stephenson 1957). Initiation of S began at similar ages (Fig. 1) and both P and S began to produce fibres at similar times in the three genotypes (Fig. 4). Stephenson (1957) concluded that there was no difference between the Merino and Romney in the age at which different stages of follicle development were complete. The present study showed that in contrast to Stephenson's results, the S population continued to develop and mature for a longer period in the Merino than the Romney, with the MxR intermediate.

This study has identified the critical period for the development of different follicle populations in the Merino, Romney and their cross as between days 90 to 125 of gestation. It is this period which should be the focus for future studies to determine physiological factors controlling secondary follicle development.

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