Characteristics of pelt follicles and their relationship with pelt quality in fat-tailed Gray Shirazy sheep

Mazaher SAFDARIAN1, Majid HASHEMI1,2, Mohammad Reza HASHEMI1
1Department of Animal Science Research, Fars Agricultural and Natural Resources Research and Education Centre, Agricultural Research Education and Extension Organization, Shiraz, Iran
2Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization, Shiraz, Iran

Abstract: The purpose of the present investigation was to measure pelt follicle characters after birth and to determine the relationship among pelt and follicle characters in fat-tailed Gray Shirazy sheep. Skin samples were taken from 150 lambs at 1 and at 120 days of age and from their parents (18 rams and 150 ewes) using a biopsy punch (8 mm in diameter) from the right mid-side of the body. Several sections of 8 µm were then prepared, mounted, and stained on glass slides to determine follicle characteristics. The least square means of primary (P) follicle density/mm² in skin of 1- and 120-day-old lambs were 14.6 and 7.1 for males and 15.4 and 7.0 for females, respectively. The least square means of secondary (S) follicle density/mm² in skin of male and female lambs were 33.3 and 34.7 for 1-day-old and 21.7 and 20 for 120-day-old lambs, respectively. S/P ratios in 1- and 120-day-old lambs were 2.9 and 3.6 in males and 2.7 and 3.4 in females, respectively. There was no significant difference between sex of lambs for follicle density/mm² of skin and S/P ratio in lambs of 1 and 120 days old. The correlation coefficient between 1-day-old follicle characters and final score of pelt was not significant, but for 120-day-old follicles it was moderate and significant. Correlation coefficients between pelt traits in newborn lambs were significant. Results indicate that although the density of follicles and the S/P ratio were moderate in fat-tailed pelt lambs, there was no correlation between them and the final score of pelt. Further studies are needed to determine whether other follicle characters like follicle curvature, follicle depth, and follicle group size would have a high correlation with final score of pelt to assess skin characters objectively.

Key words: Follicle characters, pelt traits, correlation, Gray Shirazy sheep

1. Introduction
Pelt quality is very important in animal production because together with the size of skin it can determine the price of the skin. Pelt quality is a composite trait, including color and purity, structure and length of guard hair, and density of the wool. The characteristics that cause a pelt to be classified as high quality are difficult to quantify. One skin might be classified as high quality due to a silky appearance of the pelt and another due to the high density and structure of the wool. It is therefore of interest to understand which single traits influence the classification of skin as high quality.

Karakul sheep has been raised to produce valuable meat, wool, and milk and high-quality pelts in Fars, Markazi, and Khorasan provinces of Iran and it is called Gray Shirazy, Zandy, and Karakul in these regions, respectively. Although selection is always ongoing for better pelt grades, this sheep has rarely been raised only for pelt production since 1968. Newborn lambs usually have tightly curled black, gray (mixture of black and white wool), brown, and sur-color (mixture of brown and white wool) fur and may be slaughtered for pelt production soon after birth. The finest pelts are often obtained from unborn lambs. The value of a pelt is determined by its particular pattern, which will appeal to the selective buyer. The difference between high- and low-quality pelts could be made with minute deviations in a particular pattern at birth. A few days of postnatal development can result in major changes in the coat that make the pelt totally unfit for utilization in trade (1).

The lamb growth rate of Karakul sheep is moderate to high and they produce good-quality meat. These sheep are sometimes categorized as ‘broadtail’ or ‘fat-tailed’ sheep, because they store fat in their tails for adaptation and survival in harsh and dry conditions. The wool product of Karakul sheep has been classified as carpet wool and is a mixture of coarse and fine fibers, with color varying from black to various shades of gray and brown. Traditionally,
pelt characteristics have been assessed by pelt assessors and are considered as indirect criteria in sheep selection programs by breeders. Inaccuracy and inconsistency of subjective scoring methods can result in some problems for the assessment of pelt characteristics (2,3). Therefore, correlations among pelt characteristics, follicle characters after birth, and some quantity traits that can be measured directly on the lamb need to be studied. Sheep and goats have two distinct types of fiber-producing follicles within their skins. Primary follicles (P) are characterized by an associated sudoriferous or sweat gland, an often bilobed sebaceous gland, and an erector pili muscle, whereas secondary follicles (S) are only associated with a monolobed sebaceous gland. The follicles in adult animals are arranged within groups consisting typically of three P follicles and a variable number of S follicles. The long coarse outer and the fine inner coats of double-coated breeds are produced by P and S follicles, respectively. Fibers produced from P follicles tend to be large in diameter (4). The proportion of S to P follicles, expressed as the S/P ratio, is 2 for double-coated breeds and 30 for the developed single-coated Merino sheep. In general, a greater ratio will be equal to finer fleece. A considerable proportion of S and possibly a number of P follicles appear during the first month after birth. Almost all P follicles are mature at birth, while initiated S follicles continue to mature until 2 to 3 months after birth. The follicle population will stabilize at 4 months of age (5). However, Hocking-Edward et al. (6) believed that all secondary follicles are present at birth and no new follicles are initiated after birth in sheep.

No better method of assessing Karakul pelts exists presently, other than subjective scoring. It is likely that it can still be used for a long time. However, modeling should ideally be done by judiciously studying and applying biological principles and then selecting the one yielding results to the best of one's liking. For this reason, the objective of the present investigation was to determine the relationship among assessed pelt characteristics in Gray Shirazy sheep, primary and secondary follicle density in 1- and 120-day-old lambs, and the S/P ratio to understand the sequence of follicle density after birth and to find a quantitative trait with high correlation between it and pelt characters for evaluating pelts accurately.

2. Materials and methods
Skin samples used in this study were collected from the flock of Gray Shirazy (Karakul) sheep at the Aliabad-Kamin Research Station, Shiraz, Iran. This station is located at latitude 29°59'N, 53°7'E and an altitude of 1790 m from mean sea level. The average temperature in summer and winter is 22.8 °C and 6.9 °C, respectively, with mean annual rainfall of 350 mm. Data used for measuring follicle characters were obtained from pelt traits of 150 lambs at the age of 1 and 120 days old, which were born at the research station and were fully evaluated by pelt assessors, and also from their parents (18 rams and 150 ewes). The lambs were born during the spring and were evaluated subjectively for pelt traits (curl type, curl breadth, pattern, luster, color, and final pelt score) by a pelt assessor according to the method described in the guidelines of the Karakul Pelt Assessment Committee of Iran (7). Based on these guidelines, the score of curl breadth in different colors of pelt ranges between 5 and 10 (a score of 5 denotes inferior while a score of 10 denotes excellent), along with scores for luster (5–10), pattern (5–20), pelt size (0–5), skin thickness (0–5), and curl type (4–40), and then the final score of the pelt based on the scores given to the above characteristics is given to the lamb.

Two skin samples were taken with a biopsy punch (8 mm in diameter) from the right mid-side of lambs within 24 h after birth and when they were 120 days old. Samples were also taken from their parents (150 ewes and 18 rams) after the fibers had been sheared off. Specimens were fixed in 10% buffered neutral formalin. Tissue processing was performed automatically using Histokinette (Cambridge Instruments Company). Processed skin samples were embedded in paraffin using Leukhardt blocks. Embedded skin samples were sectioned in the transverse plane to the follicle line at 8 µm using a base sledge microtome (model: Leica rm 213s, Nussloch, Germany). Before staining, all sections were deparaffinized, immersed in Histo-Clear for 2 min, and rehydrated in a graded series of ethanol to water. A special tetrachrome stain, sacpic (8), was used to demonstrate follicular tissue compounds (Figures 1 and 2).

Figure 1. Transverse section through the skin sample indicating follicle groups (sacpic, 10×).
The best section from each slide was selected for determining the S/P ratio. The numbers of secondary follicles were counted in 20–25 follicular groups and the mean of S follicles was estimated in each follicular group. For numbering secondary and primary follicles per mm² of section area of skin, a glass slide and light microscopy adjusted to a magnification of 10× were used. Data were analyzed using Statistical Analysis Systems (9). A linear model (GLM procedure) was initially fitted to evaluate the influence of sex of lamb and age of dam on follicle characteristics. Correlation coefficients between quantitative traits and correlation coefficients between quantitative and qualitative traits were determined by the Pearson and the Spearman correlation coefficients method, respectively.

3. Results
Least square means (LSM ± standard deviation) of primary and secondary follicle density/mm² and S/P ratio of skin samples from 1- and 120-day-old lambs and their parents are presented in Table 1. There was no significant difference between sex of lambs for follicle density/mm² of skin and S/P ratios in 1- and 120-day-old lambs (P > 0.05). Age of dam had no significant effect on follicle characters in this study (P > 0.05). The differences between follicle characters in 1- and 120-day-old lambs and their parents were significant (P < 0.05).

The LSM (±SD) of pelt characteristics in Gray Shirazy lambs that were assessed immediately after birth by pelt assessors are presented in Table 2. The LSM scores of curl breadth, luster, pattern, pelt size, skin thickness, curl type, and final score in lambs were 7.6, 7.5, 12.2, 4.2, 4.2, 27.7, and 71, respectively. Sex of lambs had no significant effect on score of pelt characteristics in this study (P > 0.05). Correlation coefficients between pelt traits and follicle characteristics are presented in Tables 3 and 4. The correlation coefficients between 1-day-old follicle characters and final scores of pelts were not significant (P > 0.05).

Figure 2. Transverse section through the skin sample indicating a follicle group in 1-day-old lambs, 120-day-old lambs, and parents (P = primary follicles, S = secondary follicles) (sacpic, 10×).
> 0.05), but in 120-day-old pelts they were moderate and significant (P < 0.05). The correlations between follicle characters were significant (P < 0.01), except those between the S/P ratio in 1-day-old lambs and follicle density in 120-day-old lambs, secondary follicle density in 1-day-old lambs and primary follicle density in 120-day-old lambs, and primary follicle density in 1-day-old and S/P ratio in 120-day-old (P > 0.05).

As shown in Table 4, correlation coefficients between pelt traits in newborn lambs were significant at P < 0.01 and P < 0.05, except for skin thickness with curl type, curl breadth, and luster, which were not significant (P > 0.05).

4. Discussion
The results of the present study indicated that the mean of primary follicle density/mm² was 15, 7 (54% decrease),

Table 1. Least square means (±SD) of primary and secondary follicle density/mm² skin and S/P ratios in 1- and 120-day-old lambs and their parents. Different superscript letters mark significant differences.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Lambs</th>
<th>Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day old</td>
<td>120 days old</td>
</tr>
<tr>
<td></td>
<td>Male (n = 73)</td>
<td>Female (n = 77)</td>
</tr>
<tr>
<td>Primary follicle density</td>
<td>14.6 ± 3.6</td>
<td>15.4 ± 3.0</td>
</tr>
<tr>
<td>Secondary follicle density</td>
<td>33.3 ± 6.0</td>
<td>34.7 ± 7.2</td>
</tr>
<tr>
<td>S/P ratio</td>
<td>2.9 ± 0.5</td>
<td>2.7 ± 0.7</td>
</tr>
</tbody>
</table>

Table 2. Least square means of scores (±SD) of pelt traits of Gray Shirazy lambs.

<table>
<thead>
<tr>
<th>Pelt traits</th>
<th>Range score</th>
<th>Male lambs</th>
<th>Female lambs</th>
<th>LSM</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Curl type</td>
<td>4–40</td>
<td>27.63 ± 8.0</td>
<td>27.66 ± 7.5</td>
<td>27.65 ± 7.7</td>
<td>0.10</td>
</tr>
<tr>
<td>Curl breadth</td>
<td>5–10</td>
<td>7.67 ± 1.7</td>
<td>7.52 ± 1.9</td>
<td>7.60 ± 1.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Luster</td>
<td>5–10</td>
<td>7.60 ± 1.6</td>
<td>7.34 ± 1.8</td>
<td>7.48 ± 1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Pattern</td>
<td>5–20</td>
<td>11.90 ± 3.8</td>
<td>12.40 ± 4.2</td>
<td>12.20 ± 3.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Pelt size</td>
<td>0–5</td>
<td>4.31 ± 0.9</td>
<td>4.10 ± 1.1</td>
<td>4.15 ± 1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Skin thickness</td>
<td>0–5</td>
<td>4.13 ± 1.0</td>
<td>4.22 ± 1.0</td>
<td>4.18 ± 1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Final score</td>
<td>0–100</td>
<td>71.15 ± 14.0</td>
<td>70.70 ± 14.0</td>
<td>70.83 ± 14.0</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 3. Phenotypic correlation coefficient among final scores of pelts and follicle characters of Gray Shirazy lambs.

<table>
<thead>
<tr>
<th>Characters</th>
<th>$S/P$ ¹</th>
<th>SFD ¹</th>
<th>PFD ¹</th>
<th>$S/P$ ²</th>
<th>SFD ²</th>
<th>PFD ²</th>
<th>FSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S/P$ ¹</td>
<td>1</td>
<td>0.65**</td>
<td>-0.50**</td>
<td>0.52**</td>
<td>0.10**</td>
<td>0.09**</td>
<td>0.02**</td>
</tr>
<tr>
<td>SFD ¹</td>
<td>-1</td>
<td>0.91**</td>
<td>0.45**</td>
<td>0.35**</td>
<td>0.03**</td>
<td>0.01**</td>
<td></td>
</tr>
<tr>
<td>PFD ¹</td>
<td>-</td>
<td>-1</td>
<td>0.11**</td>
<td>0.05**</td>
<td>0.45**</td>
<td>0.01**</td>
<td></td>
</tr>
<tr>
<td>$S/P$ ²</td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>0.70**</td>
<td>-0.40**</td>
<td>0.28**</td>
<td></td>
</tr>
<tr>
<td>SFD ²</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.85**</td>
<td>0.22**</td>
<td></td>
</tr>
<tr>
<td>PFD ²</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>1</td>
<td>0.26**</td>
<td></td>
</tr>
<tr>
<td>FSP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

ns: Not significant. *Significant at P < 0.05. **Significant at P < 0.01.
¹: Follicle characters at 1 day old. ²: Follicle characters at 120 days old. FSP = Final score of pelt. S/P = Secondary/primary ratio. SFD = Secondary follicle density. PFD = Primary follicle density.
and 3.3 (48% decrease) in skin samples of newborn lambs, 120-day-old lambs, and adults, respectively. The mean of secondary follicle density/mm² of skin samples in newborn lambs, 120-day-old lambs, and adults was 34.15, 20.85 (39% decrease), and 9.9 (48% decrease), respectively. Percentage of reduction in the number of P follicles for newborn to 120-day-old lambs was higher than that for S follicles (54% vs. 39%), but the percent of reduction for 120-day-old lambs to adults was the same for both types of follicles (48%). The decrease in number of P follicles was greater than the decrease in S follicle density for newborn to 120-day-old lambs. This might be due to the fact that almost all of the initiated P follicles are mature at birth, and they were counted on slides prepared from the pelts of newborn lambs. Thus, while the body surface increases with the growth of the lamb, the P follicular population remains constant, and a decrease in the number of follicles per unit area would be expected. As some initiated S follicles continue to mature until the lamb is 2 to 3 months old (5,10), and as skin surface area increases, those S follicles that mature after birth gradually appear. They were counted in pelt slides from 120-day-old lambs. Although a greater proportion of decrease in S follicles is related to the increase in skin surface area, the low reduction in density/mm² of skin compared to P follicles could be due to maturation of S follicles after birth. Mobini et al. (11) found that the mean S/P ratio was 0.17 to 1.89 in adult Bakhtiari sheep while it was 3.5–3.7 in other Iranian sheep breeds (14). In some other sheep breeds such as native Omani (16), Barki, Sannen, Togenburg, Lori (12), and Merino hybrids (17), the mean S/P ratios were 0.6, 2.4, 3.9, 2.4, 2.26, and 4.8, respectively. The highest S/P ratio was found in Merino sheep, which was 16.5 (18). There were no differences among S/P ratios or secondary and primary follicle numbers in Gray Shirazy sheep (Karakul) as a pelt sheep breed and some other sheep breeds other than Merino sheep. Therefore, it can be concluded that these follicle characters appear to have no effect on pelt characters in pelt sheep and there is a need to study the effect of other follicle characters such as follicle curvature, follicle depth, and follicle group size on pelt characters.

The number of S follicles in the fetus reaches a peak at about 135 days of gestation and then there is a significant decline until birth (6,19), but the secondary follicle population can continue to develop during the postnatal period. In contrast, the primary follicle population becomes stabilized at approximately 100 days of gestation and this could be the reason for the increased S/P ratio. Prenatally, however, the trend is not absolutely consistent (1).

In the terms of production, despite lamb pelting, increases in ewe body weight after subsequent lambing shortens lambing intervals and leads to higher annual lambing rates and heavier subsequent lambs; however,
in Iran, pelt production by Gray Shirazi sheep is not an important product (mutton is the primary product), and over the years pelt assessors have assessed pelt characteristics and breeders have emphasized the importance of considering pelt characteristics as indirect criteria in sheep selection programs. Consequently, based on our results (Table 2), LSM values of pelt characteristics in Gray Shirazi lambs were moderate to high. For example, LSMs of curl type score and final score were 27.65 ± 7.7 (range: 4–40) and 70.83 ± 14 (range: 0–100), respectively. This shows that more than 50% of lambs had a score of higher than 20 for curl type.

Despite high correlations between some pelt and follicle characters, selection based on indirect characters has not always resulted in expected responses. For instance, Jackson et al. (20) and Rendel and Nay (21) found no increase in clean wool weight when they selected for increased S/P ratio, follicle depth, follicle density, or total follicle number, despite follicle characters changing in the expected direction with selection.

In conclusion, although subjective evaluation of important pelt traits is fairly inaccurate and inconsistent (3), at present there is no other method for assessing pelt traits and it will most probably still be used in the future. Therefore, it can be concluded that in order to reduce the possibility of errors in pelt evaluation by pelt assessors, it is better to assess the characters that have high correlations with final score, e.g., curl type (0.91). This has several advantages. First, the pelt assessor focuses entirely on one trait, which can raise the accuracy of the assessment. Second, the time needed for evaluation of pelt characteristics and subsequent costs will be reduced. Third, training farmers for pelt assessment will be much simpler and more understandable.

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