Histological studies on wool follicles
by Mushtaq Ahmad Khan

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree
MASTER OF SCIENCE in Animal Science (Wool Technology)
Montana State University


Abstract:
In order to determine if supplemental feeding of sheep on winter range would favorably affect the wool follicles, histological observations were made in the skin of twenty-four mature animals on two feed levels both from Columbia and Rambouillet breeds. Six animals were put into each feed level from both the breeds. The high feed level group received two pounds of thirty percent protein pellet every second day, while the low fed group was maintained on range grazing only. Animals in the high feed level group gained weight while those in the low fed lost weight, especially those carrying twin lambs. A highly significant ($P<.01$) effect of feed treatment was observed on the live body weight of animals. There was no significant feed treatment effect on the number or size of wool follicles nor on the sebaceous glands. However, there was a highly significant ($P<.01$) difference between number of follicles among the two breeds.

Breed effect was also noted on the size of follicle ($P<.06$). Individual differences were also noted regardless of breed or treatment.

There was a highly significant correlation ($P<.01$) between the size of follicle and grease fleece weight, grease fleece weight and grade of wool and grade of wool and size of follicle. A highly significant negative correlation ($P<.01$) existed between the number of follicles and the grade of wool.

Also a negative correlation was obtained between the number of follicles and the grease fleece weight which was approaching significance at ' the 5 percent level. Body weight gain or loss did not show any significant effect on size or number of follicles.

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## ABSTRACT

In order to determine if supplemental feeding of sheep on winter range would favorably affect the wool follicles, histological observations were made in the skin of twenty-four mature animals on two feed levels both from Columbia and Rambouillet breeds. Six animals were put into each feed level from both the breeds. The high feed level group received two pounds of thirty percent protein pellet every second day, while the low fed group was maintained on range grazing only. Animals in the high feed level group gained weight while those in the low fed lost weight, especially those carrying twin lambs. A highly significant ($P < .01$) effect of feed treatment was observed on the live body weight of animals. There was no significant feed treatment effect on the number or size of wool follicles nor on the sebaceous glands. However, there was a highly significant ($P < .01$) difference between number of follicles among the two breeds. Breed effect was also noted on the size of follicle ($P < .06$). Individual differences were also noted regardless of breed or treatment.

There was a highly significant correlation ($P < .01$) between the size of follicle and grease fleece weight, grease fleece weight and grade of wool and grade of wool and size of follicle. A highly significant negative correlation ($P < .01$) existed between the number of follicles and the grade of wool.

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## INTRODUCTION

Sheep were among the first animals domesticated by man. This animal supplies wool for clothing, meat for food and in certain parts of the world milk for drinking. Continuous efforts have been made to improve both wool and mutton to give the greatest financial return to the sheep grower. The chief value of wool is its ability to be spun into yarn. Other animals i.e., camel, goat and alpaca, produce valuable textile fibers. For general purposes other fibers are not as useful as the wool because the character of the fiber is not adapted to as many uses as wool, and the production of these fibers is inadequate to meet the world's needs. Sheep are found in nearly every inhabitable part of the world (Darlow *et al*. 1934).

Because wool is the most valuable animal fiber, efforts by research workers should be made to improve the quality as well as the quantity. It is encouraging to note that in recent years the attention of research workers in this field has been diverted from the fiber itself to the follicle which produces it. Various workers have studied the different types of follicles, their pre-natal and post-natal development, density per unit area and the ratio of different types of follicles over different regions of the body. Most of the research work is directed toward morphological studies of the follicle and its accessories. Some workers have studied the effect of environments on wool growth but very little information is available on the effect of these factors on follicle growth and development.

Most of the work of histological observations of follicular growth and development has been conducted in Australia, New Zealand and England. No preliminary information could be found on histological studies of American breeds of sheep.

This study was initiated as a preliminary investigation on the histology of wool follicles and their associated structures in Rambouillet and Columbia breeds of sheep on two different feed levels.

## REVIEW OF LITERATURE

In addition to the feed required for growth and maintenance, sheep require energy for wool production. Other factors such as temperature, age and season also affect wool growth. An understanding of the structure and population ratios of the skin follicles are essential for a complete understanding of the type and number of fibers produced in developing and mature animals. Little attention has been given to follicle development beyond the limit of morphology. This is partly due to the technical difficulties and expense of using histological preparations. Making good preparations with sufficient ease and consistency has been the greatest obstacle in studying the follicle population and development. Within the last twenty years the work on skin preparations has advanced considerably and new techniques have been developed for biological research on fleece growth (Carter, 1955).

### Morphology

The skin of sheep is composed of minute cells and consists of two main parts; the first, an under-skin or dermis, and overlying this is an outer skin or the epidermis. These two parts of the skin retain their identity throughout the growth of the animal. The epidermis is formed by constant growing and dividing of a very active layer of cells called the basal layer. There is a constant wear on the outermost layer of the epidermis and this is replaced by cells derived from the basal layer. The initial stages of formation of the wool follicle take place in the basal layer. At certain positions in the skin, the basal layer thickens and by a process of very active cell division, invaginates into the dermis. This invagination forms the wool follicle with its accessories. As this tubular invagination grows downward, two outgrowths appear from it, usually on the same side. These are the sebaceous gland and the sweat gland or the sudoriferous gland. The bulbous base of the follicle becomes turned in and grows upward to form a dome like s-shaped asymmetrical body of actively dividing cells called the papilla. This part is well supplied with blood vessels. The basal layer is continuous over the dome of the papilla and the basal cells in this region are actively dividing. The cells thus divided gradually elongate and give rise to the wool fiber. The tip of the growing fiber together with its inner root-sheath is pushed upward through the follicle plug by pressure of new cells being formed continuously from the dome of the pappila. The arrector pilli muscle, which is attached at its lower end to the follicle sheath and at the upper end to the basal layer, is usually on the same side of the follicle as the sebaceous gland. Contraction of this muscle causes the hair to stand at its end in human and certain other animals. In sheep it is inserted too high up on the follicle and consequently is not functional. The sweat gland opens into the neck of the follicle just above the sebaceous gland. The structure of the human hair follicle differs from the wool follicle of sheep because the sweat gland in human opens directly on the skin surface having no association with the follicle. In sheep the sweat gland lies between the two main lobes of the sebaceous gland. The sebaceous gland of sheep secretes a complex mixture of esters called the sebum or wool grease which is an oily, semiliquid material which gives wool a greasy touch (Anonymous, 1950).

<u>Terminology and classification</u>

Carter (1955) states that until the middle of the 19th century, it was customary to regard the arrangement of hair follicles in the mammalian skin in a disorderly fashion. A German worker, Nathusius (1866), demonstrated the existence of some form of group pattern which was later established by other German workers (Spottel and Tanzer, 1923). The clear terminology in the development of wool follicles was still lacking when in 1923 Spottel and Tanzer described the follicles as "Leithaar and Cruppenhaar" meaning primary and secondary. These two German authors traced the development of the follicles in the foetal stages and demonstrated the earlier origin of Leithaar or the primary follicles. Wildman (1932) has used these terms to distinguish between the early and the late developing follicles in the foetuses of various British breeds of sheep. Duerden (1936) in his report noted two major types, namely primary and secondary follicles.

A compromise in terminology was then put forward by Wildman and Carter (1939) which was accepted by workers in this field. The same classification is still in use; except when classifying follicles into various types the following symbols and terms like X, C, P, Y, x, L, y, U, O, S, B and D have also been used. This classification was suggested by Hardy and Lyne (1956). The various symbols like X (first formed), C (central), P (primary), Y (later formed), x (associated with PCX), L (lateral), y (associated with PCY), U (unbranching), O (original), B (branching), S (secondary), and D (derived), are easy to use to designate various types of follicles. Carter (1955), using the same terminology expressed total follicle population as P + S and any sample of skin population as $^{n}P + S$.

The follicles lie at an acute angle to the skin surface and usually have a common slope. In many breeds of sheep, especially the Merino, the earliest growing follicles are arranged in groups of three and such a grouping is called the trio arrangement. The follicles forming the "trio" are known as primary follicles and characteristically have all the accessory structures present with each follicle, i.e. sebaceous gland, sweat gland and arrector muscle. The secondary follicles differ from the primaries in having an incomplete set of accessory structures, the sweat gland and usually the arrector muscle being absent; the sebaceous gland may be present but it is smaller in size than that of primary follicles (Anonymous, 1950).

The grouping of these follicles has been shown to consist of one or two follicles per group by Narayan (1961). But the proportion of these incomplete trio groups was considered to be too small to make a difference in the primary lateral/primary central ratio. He found that in the hairy group (Malpura and Sonadi breeds) the PL/PC ratio, the mean S/P ratios and the percentage of trio groups are lower but the percentage of couplets and solitary groups is higher than in the carpet wool group (Chokla, Magra, Nali, Jaisalmere, and Marware breeds). It appears that in the hairy breeds the trio arrangement is not a predominant feature of the grouping of the primary follicles.

The origin of later secondary follicles by branching from earlier ones was first reported by Tanzer (1926) in Karakul sheep. Frolich, et al. (1929) without citing a reference indicated that additional observations had been made by Tanzer (1926) on follicle branching in other breeds including the Merino. Bundles of secondary follicles with common openings at the skin surface were found in the adults of many breeds of sheep examined by Spottel and Tanzer (1923). The number of bundles and the number of follicles per bundle increased progressively from the primitive Moufflon to the fine-wooled Merino. Duerden (1939) postulated that secondary follicles were formed from a wedge of unidentified epidermal tissue which later became divided laterally into separate follicles. In his illustration from a 16 week old Romney Marsh foetus he states, "The follicles of a group appear as if arising by branching or budding; this is not so, but each group contains several closely adjacent follicles." Carter (1943) followed Duerden (1939) in his description of the secondary follicle development. Hardy and Lyne (1956) reported the majority of these follicles arise by simple budding from other discrete secondary follicles which are already undergoing differentiation.

Lyne (1957) studied the skin samples from the mid-lateral region of the body of four medium-wooled Merino rams (age 20-21 months), two inbred Merino ewes (age seven years) and one Corriedale ewe (age more than seven years). He found the presence of a few bundles of primary wool follicles in all the sheep examined except one ram. The bundles consisted of two to six primary follicles with a common neck and opening at the skin surface. A normal sudoriferous gland and arrector pilli muscle were associated with each of the bundles. The follicles were usually separate at the level of the junction of sebaceous glands, although occasionally they were contained within a common outer root-sheath. The method of formation of bundles of primary follicles is unknown. Lyne (1957) hypothesized that they develop by branching in a manner similar to that described for secondary follicles by Hardy and Lyne (1956).

Wildman (1957) suggests that care should be exercised in identifying follicles as either primary or secondary during counts made to determine the S/P ratio. In follicle population studies, particularly in adult skin and in Merino skin, the classification of some follicles was difficult and he classed such follicles separately as "doubtful identity". He points out that further evidence is required in classifying follicles before Lyne's (1957) S/P determination can be established. The follicles may not have developed by branching as Lyne (1957) assumes; because they are smaller than the large obvious primaries, have no sweat glands and may be identified later than the original primary follicles. If this is so, the fact that these and the original primary follicles lead into a common neck loses its significance in assigning the smaller follicles to one category because they may have all the characteristics of secondary follicles. In his opinion, further observations in the differentiation of these follicles should be made to assess their significance in population studies.

Orwin (1961) has reported the occurrence of branching follicles in the skin from two-year-old New Zealand Romney ewes. Wildman (1961) points out that there appears to be some confusion in Orwin's (1961) communication concerning the evidence required to demonstrate conclusively whether follicles are branched, or are separate follicles which have coalesced. Orwin's (1961) photographs show more than one fiber in a common sheath at sebaceous gland level and at higher levels. Wildman (1961) indicates the evidence presented by Orwin (1961) is not adequate to conclude that one follicle has been derived from another by branching. These follicles may have been separately initiated and coalesced so the fibers come to lie in a common sheath and a common follicular mouth. He suggests that skin sections at lower levels than that of the sebaceous glands should be made. The follicles should then be traced up and observations confirmed by vertical sections before deciding whether a follicle has been derived from another one by branching or has coalesced with it. Wildman (1961) does not suggest that branching does not occur in breeds of sheep other than the Merino. In thousands of serial skin sections from English Romney sheep and lambs examined at all levels, he has found coalesced follicles but not true branched follicles.

Follicles with multiple fibers were ascribed by Flemming (cited by Pinkus, 1951) to "partial merging" of several embryonic papillae, rather than to "budding" of an originally normal papilla. This is considered to be an anomaly as suggested by Auber and Ryder (1955). Until the factors leading to the formation of these follicles have been determined, these considerations must remain a possibility (Orwin, 1961).

<u>Development and factors affecting it</u>

Different workers have studied the pre-natal and post-natal development of both types of follicles. Carter (1943) studied the embryological material from South African Merino foetuses and concluded that the primary follicles develop during the 35th to 85th day of pre-natal life (pre-trio and trio periods). The secondary follicles develop thereafter. In most breeds of sheep the follicle development in any but a few special regions of the skin is almost negligible during the first fifty days of pre-natal life. Table I gives details of different stages of follicle development and its accessory structures. He also reported that the primary follicles first appear at the poll and face of the foetus and then on the rest of the body.

TABLE I. GENERAL COURSE OF DEVELOPMENT OF A TYPICAL FOLLICLE GROUP. (Carter 1955).

| Phase | Period | Foetal age (days) | Pre-natal sequence (approximate) | | | Post-natal sequence (approximate) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Follicle initiation | Gland initiation | Fibre and smooth muscle initiation | Fibre types (juvenile) | Fibre types (adult) | |
| Primary or Protophase | Pre-trio period | 50 | Primary (central) follicles initiate | | | Halo hairs<br>↓<br>Super-sickles | PRIMARY (outer coat) (LEITHAAR)<br>Bristle | Stichelhaar |
| | | 60 | | | | ↓<br>Hairy-tip-curly-tip | Kemp<br>Outer hair<br>Over hair<br>Protective hair | Mittelhaar |
| | | 70 | | Primary (central) tubular gg. | | ↓<br>Sickles | Awn<br>Heterotype<br>Transitional hair | Grannenhaar<br>Stammhaar |
| | Trio period | 80 | Primary (lateral) follicles initiate | Primary (lateral) tubular gg.<br>Primary (central) acinous gg. | | ↓<br>Curly-tips | Intermediate hair | Seitenhaar |
| Secondary or Neophase | Post-trio period | 90 | Early<br>↑ | Primary (lateral) acinous gg. | | Curly-tips | | |
| | | 100 | | Early secondary acinous gg. | Primary (central) fibres and smooth muscle initiate<br>Primary (lateral) fibres and smooth muscle initiate | | SECONDARY (inner coat) (GRUPPENHAAR) | |
| | | 110 | Secondary follicles initiate | | Primary (central) fibres emerge<br>Primary (lateral) fibres emerge | | Under hair<br>Under fur<br>Down hair, etc. | Beihaar<br>Flaumhaar<br>Nebenhaar<br>Wollhaar |
| | | 120 | | | Early secondary fibres initiate | | | |
| | | 130 | | | Early secondary fibres emerge | | | |
| | | 140 | | | Later secondary fibres initiate | | | |
| | | 150 (birth) | ↓<br>Late | | | ↓<br>Histerotrichs | | |

The general pattern of differentiation results in an orderly array of follicles, differing in their size and the complexity of their structure according to the line of their initiation and the rate of growth in the following general order: central primary, lateral primaries, early secondaries, late secondaries. Structural distinctions within the group only persisted for periods of time with varying clarity according to the breed or genotype (Carter, 1955).

Carter and Hardy (1947) showed that at birth all the primary follicles have reached maturity as judged by keratinized fiber in the follicle. They were unable to find any evidence that would suggest the initiation of new primary follicles after the 85th day of pre-natal life. Barton (1951) discovered that it was not until 115 days after conception, or 33 days before birth, that the secondary follicles were visible. Secondary follicles continue to be produced for a short period of time after the lamb is born.

Pohle et al. (1945) examined fleeces of 69 lambs of Rambouillet, Corriedale, Targhee, and Columbia breeds that were classed as hairy at docking time. Samples from the fleeces of these lambs were examined by the cross-section method at monthly intervals until yearling age at the U. S. Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho. They concluded that the number of hair constituted only a fraction of the total population and these diminished rapidly as the lambs increased in age, indicating that the primaries ceased producing. Fewer hair were found on the side than on the thigh (britch) or back. Fiber diameter decreased between 6 and 11 months of age, showing a slight increase thereafter. This may have been due to weaning and subsequent poor feeding or to inherent properties of the follicle. The yearling fleeces of the hairy lambs were compared to those classed as non-hairy at docking. No important differences were found among the yearling fleeces of hairy and non-hairy lambs in hairiness, mean fiber diameter and variability or percentage of medullation.

Grandstaff and Blunn (1944) reported that the average amount of kemp in the fleeces of Navajo lambs decreased from 15 percent at 28 days of age to 4.5 percent at 84 days and then maintained a fairly constant level until the lambs reached yearling age. They also found highly significant differences between sheep and between the position, the sample was taken in the percentage of wool, hair and kemp fibers. In another experiment, Grandstaff and Wolf (1947) found significant differences between ages, years and lambs within years in both breeds. They claim that a reasonably accurate selection for wool characters can be made at an age of 112 days when the fiber content of the fleece has reached a fairly constant level; meaning that maturation of secondary follicles has fully occurred.

A study of the follicle types, ratios and the populations was made in four different breeds of Indian sheep by Narayan (1960). He made comparisons between follicle and fiber counts per unit of the skin area on the shoulder, side and the britch regions of the body. He established that there was a significant breed difference in wool follicle densities in different regions of the body. He suggests that adverse pre-natal conditions adversely affect the growth of secondary follicles. He mentioned that any adverse condition influencing the initiation or maturation of secondary follicles, even to a small extent, will tilt the balance in favor of primary follicles and the adult fleece will then be composed of fibers from a higher percentage of primary follicles than the secondary follicles. The britch region had the highest percentage of total and secondary follicles with medullated fibers.

Schinckel (1955) suggests that in considering differences between follicle population densities, body size must be taken into consideration to determine whether differences lie in total follicle population on the whole sheep, or merely in mechanical changes in density due to changes in body size. He found that the total follicle population was the highest on the shoulder while the britch value was below that for the side but not significantly so. The density of primary follicles became less per unit area as the animal grew in age. Because all the primary follicles are fully developed and producing fibers at birth, the decrease is a direct result of skin expansion accompanying growth. The greatest decrease in follicle density took place between birth and one month of age. The major increase in skin expansion also took place up to four months of age and then small decrease in follicle density was measured up to sixteen months of age.

He also found that little follicle development occurred during the first weeks after birth and that this period of inactivity was longer than the usual 1 to 2 days a lamb takes before it starts growing. During the second and third week of age the greatest activity in the development of the secondary follicles was observed. After the third week the secondary follicles continue to develop but at a much reduced rate.

Schinckel (1953) also studied the wool follicle development in the early post-natal life. He took skin samples from the mid-side at various ages ranging from 10 to 426 days of age. Large differences in the S/P ratio were found at 10 days of age. He feels that this difference appears to be too large to have developed since birth, and strongly suggests that the late pre-natal development of secondary follicles may be affected by foetal environment.

His results also indicated that lambs from twin births have a retarded maturation of secondary follicles compared with single lambs. The S/P fiber ratio of twins was also permanently below that of singles. It was not determined to what extent the difference between single and twins resulted from post-natal nutrition. There is experimental data (Schinckel and Short, unpublished data, 1956) to show that restricted post-natal nutrition delays the maturation of secondary follicles but they are not sure if this is a permanent depression of the secondary follicle development.

Ferguson _et al_. (1956) found that depression of wool growth rate accompanied the depression of follicle maturation caused by thyroid deficiency. This did not appear to be a consequence of the lowered follicle population but rather it appeared to be independent of it. It is suggested that the two effects may be associated with different metabolic defects which occur to different relative extents in different lambs. Stephens (1940) claims poor nutrition leads to depression of secretion of thyrotrophic hormone and hence of thyroxine in the guinea pig, and this effect, if present in the sheep, may account at least in part for the delayed follicle development of lambs fed on a restricted diet.

From Wallace's (1948) extensive studies on growth of lambs before and after birth in relation to nutrition, it appears that during the 56 to 84 day foetal stage, the specific growth rates of carcass, organs, and skin are very similar. During the 84 to 112 day interval, the specific growth rate of skin exceeds those of the carcass and organs, but from 112 to 140 days, the rates are again approximately the same. Between birth and 112 days, the specific growth rate of the skin is considerably lower than that of the carcass or organs but is considerably greater from 112 to 332 days after birth. During this period considerable growth of fleece takes place.

Short (1955) traced the maturation of secondary follicle population from birth to six months of age by comparing the mature fiber producing secondary to primary follicles. He found that in lambs the secondary follicles were present at birth. The rate at which the secondary follicles mature reaches a maximum at 7 - 21 days after birth, with approximately 65.0 percent of secondaries growing fiber by 28 days. In his opinion the adult secondary follicle population may be permanently affected in two ways. Firstly, the number of secondary follicles initiated to the time of birth may be restricted by adverse foetal environments. Secondly, the number of initiated secondary follicles which mature may be restricted by the early post-natal environments, particularly up to 21 days. In another experiment Short (1955) studied the effects of adverse maternal nutrition on the fleece structures. Although the post-partum feeding of ewes was ad libitum, restricted nutritional regime during gestation had a delayed impact on the post-natal growth of lambs, resulting in restricted final S/P ratio in the offspring of poorly fed ewes. However, at 200 days of age, the lambs with fewer mature follicles per unit area of skin grew fibers both longer and coarser than lambs with higher fiber densities. This evidence supports the observations that the wool production per unit area of skin is independent of the fiber density, and further substantiates the hypothesis of competition between follicles for the precursors of wool keratin. Stephenson (1959) studied the developmental effects influencing the rate of primary anlagen formed in the foetus. The major factor controlling the initiation of new primary follicle anlagen on different regions of the body was the rate of skin expansion. There is probably a competition effect among anlagen during this phase of development. It was also shown that during the period of primary anlagen initiation, foetuses with greater surface area have a greater total number of primary anlagen.

Davenport and Ritzman (1926) reported age and feed level had a modifying effect on fleece weight. They found that advancing age, state of health, level of subsistence and exposure to changes in weather conditions affect the growth of wool to such an extent that an unfavorable combination of these factors may vary the fleece weight by as much as 50 percent. Darlow et al. (1934) stated that a fattening ration acts as a stimulus to the sebaceous glands in the production of wax. They also suggested that the organs which are concerned with the secretion of wool fiber may be affected by such influences as drastic changes in the level of feeding for only a short period of time. The function of these organs is easily disturbed when sheep become abnormal in health. The growth of wool is reduced when ration is insufficient for maintenance and the amount of wool fiber produced seems to be affected to a greater extent than the body weight.

In another experiment they found appreciable differences in the changes of crimps per inch among the different lots but they did not know whether these differences could be attributed to the difference in rations. Results