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Pashmina follicle dynamics during winter months in Ladakh

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Abstract
The experiment was conducted to study the changes in follicle diameter of pashmina fibre during winter months of Ladakh. Skin biopsies were taken from six Changthangi goats at monthly interval from November 2017 to February 2018. Routine histology followed by H&E staining revealed significant (p<0.05) variation in the pashmina follicle (PF) diameter during winter months (range = 26.580 to 103.278 µ). Average PF diameter during winters was found to be 60.474 ± 14.119 µ lower than other cashmere goats and thus explains the fine quality of pashmina produced by Changthangi goat. The PF diameter was significantly larger in November (64.957 ± 15.838 µ, p<0.05) and showed a decreasing trend in succeeding months. The study indicates that PF starts its growth phase before winters. PF actually regressed after November arguably to conserve energy otherwise needed for pashmina growth during the food scarcity period. We recommend studying the annual cycle of PF covering whole year to identify time points corresponding to the different stages of growth (Anagen), regression (catagen) and quisence (telogen) to better qualify the animal for hair cycle research.

Keywords: Pashmina, hair cycle, Changthangi goat, follicle diameter

Introduction
Cashmere (locally called Pashmina) is the worlds' most sought after natural fibre. It is produced by Cashmere goat (*Capra hircus*) as undercoat and shed annually. The outer main coat of guard hair develops from primary follicles and pashmina from secondary follicles [1]. The goat has a specific geographical distribution being limited mainly to China, Mongolia, Afghanistan and Iran [2]. Even though India contributes less than 1% of the world's Pashmina, it is of the highest quality, with a fineness of 11-14µ and occupies a unique position in world trade. Indian cashmere goat- Changthangi goat is raised by the nomads called Changpas in Ladakh.

Hair follicle is one of the nature's most fascinating structures [3] and has the distinction of being the only organ that cycles throughout the life time of mammals. Every mammalian hair cycle through growth (Anagen), regression (Catagen) and rest (Telogen). Hair follicle is also an ideal systems biology research model [4]. Much of our understanding in hair cycle has come from murine models [5]. However because of the great differences in the rodent and human hair follicles [6], domestic animals have gained increased biomedical relevance in hair research. Cashmere goat presents an excellent model for studying hair cycle because its secondary follicles bear an annual cycle that exhibits seasonal rhythms with a well-defined duration of fibre growth [7]. In fact Cashmere goat is contributing significantly to elucidate the molecular mechanisms involved in hair cycling using deep sequencing [8-10]. All the works however refer to Chinese cashmere goat. Despite the repute of Changthangi goat for producing the world's finest cashmere, the potentially best model has been ignored. Even the annual cycle of pashmina has not been delineated in Changtangi goat. Identification of exact time points corresponding to different stages of hair cycle under study becomes the first and fundamental step in this direction. Follicular characteristics are important guides to accurately classify the hair cycle into distinct phases [11]. Follicle diameter is one of the most important parameters to study hair follicle dynamics [12]. Secondary follicle characteristics at different stages of Chinese cashmere growth are well documented [13], even to ultrastructural level [14]. However the rhythm of the cashmere cycle depends on photoperiod [15], indicating that changthangi pashmina cycle must vary from Chinese cashmere cycles documented. The present study was conducted to study the changes in follicle diameter of pashmina fibre during winter months of Ladakh.

Materials and Methods

Sampling and Staining

Six Changthangi goats of two-year old (three females, three males) were studied. All animals were kept under the same conditions of natural photoperiod and natural temperatures. They were maintained at High Mountain Arid Agricultural Research Institute (HMAARI) Stakna Leh (Ladakh). Biopsy samples of skin of Changthangi goats were taken from right flank region under local anesthesia with 1% lignocaine (2 mg/kg) at monthly interval from November 2017 to February 2018. All aspects of animal research were conducted in accordance with the guidelines set by the Institution Animal Ethics Committee.

Whole samples fixed in a solution of 10% neutral buffered formalin. They were serially dehydrated using acetone as dehydrating agent and benzene as clearing agent. The tissues were embedded in paraffin wax and cut at 5 µ thickness. The sections were stained with hematoxylin and eosin (H&E).

Measurements and Statistical Analysis

The images of sections of skin were taken with the Radical Microscope RLX-4 equipped with Genoptik Progress Camera and PF diameter was measured with VideoTest Size5.0. A total of 150 PF was counted from randomly selected follicle groups for each month. Data were subjected to one-way analysis of variance (ANOVA) between different months.

Results and Discussion

We present first report on variation in diameter of PF in Changthangi goat during most critical part of the annual cycle. We studied the skin histology from cross sections and transverse sections using H&E staining. The technique could demonstrate groups of pashmina follicles around main primary follicles. Sebaceous glands could be easily resolved. Sample H&E stained images from four months of winter are presented in figures 1-4. The results are summarized in Table 1.

Table 1: Diameter of pashmina follicle in Changthangi goat in winter months

Month of the Year	Pashmina follicular Diameter (µ) (Mean±SD)	Replications	Maximum (µ)	Minimum (µ)
November	64.957±15.838	150	103.278	35.681
December	59.766±14.220	150	96.793	30.134
January	58.349±13.837	150	97.314	29.721
February	58.827±11.353	150	84.972	26.580
Overall	60.474±14.119	600	103.278	26.580

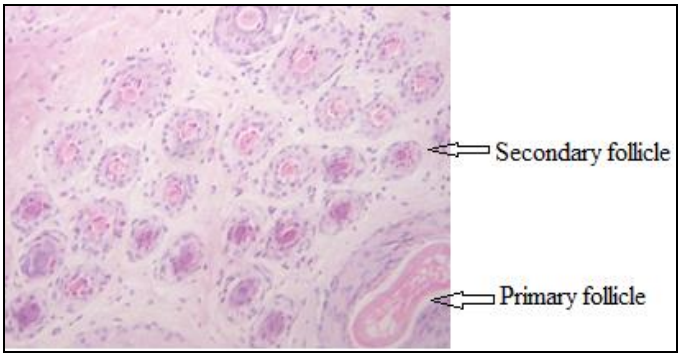

Fig 1: Cross section of skin showing a large group of secondary follicles around a primary follicle for November (H&E, magnification10x) November

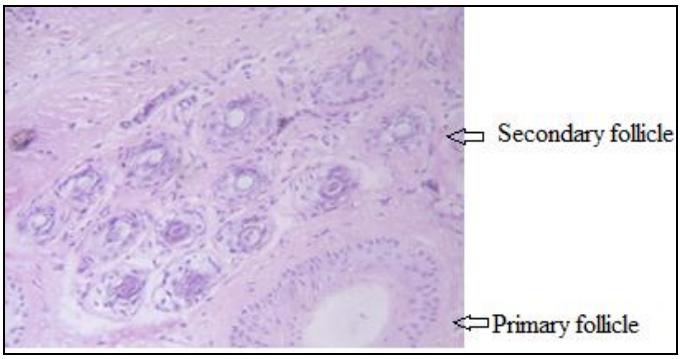

Fig 2: A moderately sized group of secondary follicles in cross section of skin for December (H&E, magnification10x)

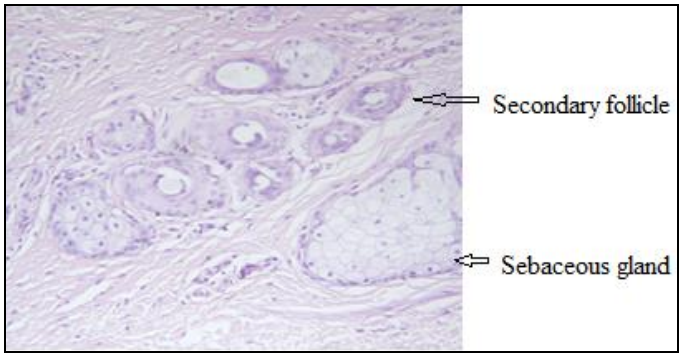

Fig 3: A small group of secondary follicle in cross section of skin for January (H&E, magnification10x)

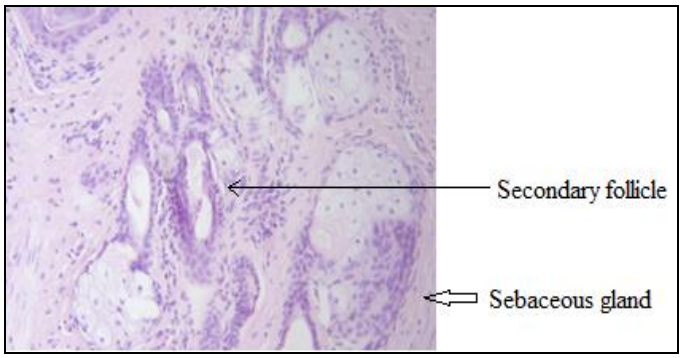

Fig 4: Transverse section of skin for February showing small secondary follicles (H&E, magnification10x)

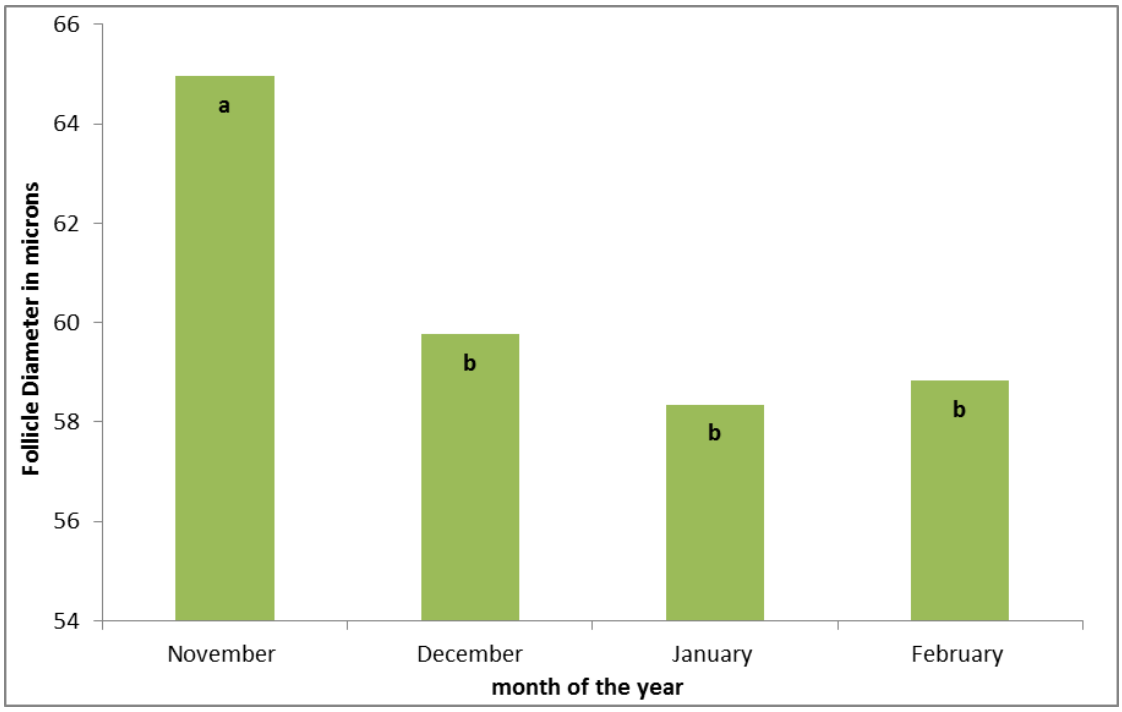

Fig 5: Variation in diameter of pashmina follicle in Changthangi goat in winter months

The PF diameter varied during winter months from as low as 26.580µ to 103.278µ. Such a wide range of follicular diameter signifies the complex follicular dynamics. Overall Average diameter of PF during winters was found to be 60.474±14.119µ. Follicle diameter is one of the most important parameters to study hair follicle dynamics [13]. The diameter of hair follicles in cashmere goats has a reported range 80-130 µ [16]. Average follicle diameter of secondary follicles in Hexi cashmere goats is 65.606 ± 17.620µ [13] We observed a smaller follicle diameter 60.474±14.119 µ than reported in Hexi chinese goat (75.739±23.971 µ) [13] for winter months of November to February. Small follicle diameter explains the finer quality of pashmina produced by Changthangi goat. The follicle diameter reported in the present study is less than the average follicle diameter reported in changthangi goat (78.89±0.40) [17]. This may be due to the fact that PF diameter varies between different regions of the body/skin [17]. A more important reason is that PF were in regression phase in most part of the period of study. As evident from figure 5, diameter of PF was significantly larger in November and decreased in succeeding months. PF diameter didn't change significantly from December to February. The study indicates that PF starts its growth phase before winters. The time point of PF growth initiation needs to be defined by extending the study to summer and autumn months. Thus PF actually regressed from November to December and the regression phase continued till February. Whether it continued the regression phase afterwards and for how long is a significant question and demands extending the study to succeeding months. The PF regression during harsh winter should not be surprising. The winters in Ladakh are extremely harsh and virtually no quality food is available to the animals. Thus it may be argued that PF regression is a part of adaptation strategy of Changthangi goats to conserve energy otherwise needed for pashmina growth during the food scarcity period.

Conclusion and Recommendations

We conclude that follicle diameter is one of the most important parameters to study hair follicle dynamics. Present study indicates pashmina growth starts well before winter. Pashmina follicles were in regression phase in December to February. It is recommended to study the whole annual cycle of PF covering whole year to identify time points/periods corresponding to the different stages of hair cycle. This will allow for better standardization of studies, for example investigating gene and protein expression patters during specific stages.

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