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Alpaca (*Vicugna pacos*) Skin Analyzed Under Architecture and Structure

Piel de Alpaca (*Vicugna pacos*) Analizada Bajo Arquitectura y Estructura

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SUMMARY: This study evaluated the morphology of alpacas skin. Biopsies were collected and samples were fixed in 10 % neutral buffered formalin for histological procedures. The sections were stained with hematoxylin and eosin, picrosirius red and Masson's trichrome. Types I, III and IV collagen were analyzed by immunohistochemistry. The derma presented sebaceous and sweat glands, as well as follicular groups with medullated fibers. Type I and type IV collagen were observed at epidermis and dermis as well as in glandular structures and hair follicles. The collagen III, was observed only in dermis.

KEY WORDS: Histology; Integument; Immunohistochemistry; Scanning electron microscopy.

INTRODUCTION

Alpacas (*Vicugna pacos*) are a group of camelids. Alpaca fiber diameter is important topic to be considered because it determines the commercialization price, which is complemented with the weight of the fleece (Antonini *et al*., 2004; Junqueira & Carneiro, 2013; Ministerio de Agricultura, 2019).

The hair follicles, as well as the blood and lymphatics vessels, nerves and sebaceous and sweat glands are found at the dermis, composed basically of collagen and elastic fibers (Torres de Jasaui *et al*., 2007). The main histological characteristic of the thin fibers is the larger number of secondary follicles, as it has been widely demonstrated that animals with smaller fiber thickness present a higher number of secondary follicles (Atlee *et al*., 1997; Moore *et al*., 2011; Montes *et al*., 2008).

The fleece varies according to the body region, with the thinnest, longest, and denser in the dorsal, costal, sacral, brachial, and femoral regions. It is known that 80 % of the fleece is made of delicate fibers, and 20 % of the thickest fleece is on the head, as well as in the carpal and tarsal regions (Tolosa *et al*., 2003; Grigg *et al*., 2004).

Taking into account the skin morphology, samples of alpaca integument was evaluated by different techniques; i. scanning electron microscopy, to visualize the arrangement of the hair follicle, ii. light microscopy for description of skin layers and components, iii. Immunohistochemistry to characterize the proteins of the skin. The description of the structure and architecture of alpaca skin is valuable to support research aimed at genetic improvement to produce high quality fibers.

MATERIAL AND METHOD

Alpaca skin biopsies were collected from the Pacomarca Experimental Fund in Llalli, Melgar, Puno - Peru (Inca Tops SA). The material was collected from twelve alpacas of both sexes, aged from 5 to 19 months. Skin biopsies were performed using an 8 mm punch. Fragments were collected and fixed in 10 % neutral buffered formalin.

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After fixation, the fragments were cut in 2mm thick and separated in three sets; (1) 1/3 of them was destined for scanning electron microscopy, (2) 1/3 for microscopic analysis and (3) 1/3 for immunohistochemical analysis.

Scanning electron microscopy. The alpaca skin fragments were washed with distilled water and then additional washes were made in ultrasonic distilled water. The fragments were dehydrated in increasing concentrations of alcohols (70 %, 80 % and 90 %). The samples were dehydrated in a critical point Leica EM CPD300 and placed on an aluminum disc (stub) to be gold plated in the Emitech K550 metallizer. Samples were analyzed using the LEO 435VP Scanning Electron Microscope (SEM) (Zeiss, Germany) from the Advanced Center for Diagnostic Imaging (CADI).

Light microscopy. Biopsies were dehydrated in an increasing series of ethanol concentrations (70 %, 80 %, 90 % and 100 %) followed by xylene. The fragments were then embedded in paraffin as described by Tolosa *et al*. (2003). Longitudinal and transverse skin sections were cut in an automatic microtome (Leica, RM2165, Germany). The sections were stained with hematoxylin and eosin (HE), Masson's trichrome and picrosirius red. The slides were analyzed under a Fluorescence Light Microscope (FLM) (Nikon Eclipse 80i, Japan). Those slides stained with Picrosirius Red were also observed under polarized light and images were acquired with AxioCam HRc camera (Zeiss, Germany) connected to an Olympus BX60 Microscope (Olympus, Japan).

Immunohistochemistry. The EnVision ™ Flex kit, High pH, (Link), a high-sensitivity visualization kit used together with Autostainer Link (code K8000, Agilent Dako, USA) was used. Tissue sections were deparaffinized and hydrated. The slides were submerged in Flex Target Retrieval Solution (50x) previously heated to 95 ºC in a water bath for 20 minutes, followed by cooling at room temperature for 20 minutes. The slides were washed twice for 5 minutes with Flex Wash Buffer (Agilent Dako, USA) (20x). Peroxidase-Blocking Reagent was added for 30 minutes at room temperature. Samples were washed twice with Flex Wash Buffer (Agilent Dako, USA), 5 minutes each. The sections were then incubated in a humid chamber overnight at 4°C, with the primary antibody (Collagen I clone (5D8-G9 / Col 1), GTX 60939, rabbit GeneTex brand polyclonal, at a 1:50 dilution; Collagen III (1E7-D7 / Col 3) GTX 60940, GeneTex mouse monoclonal mouse 1:50 dilution; LSBio (Life Span BioSciences Inc.). Collagen IV LS-B8763 rabbit polyclonal (IgG) at a 1:100 dilution; S100A4 LS-B11817 rabbit polyclonal LSBio (Life Span BioSciences, Inc) at a 1:100 dilution. For the negative control, the sections were incubated with phosphate buffered solution (PBS).

RESULTS

The epidermis and the dermis were visualized. The basal layer was observed at the junction between the epidermis and the dermis. The hair bulbs and the follicular groups were surrounded by the connective tissue. In the papillary dermis, fibers were seen in the same hair follicle. The fiber cuticle presented edges giving the fiber a serrated aspect. Most of fibers presented medulla (Figs. 1A, B, C).

The dermis and hypodermis showed collagen fibers stained in blue by Masson's trichrome and in red by picrosirius red. The follicular groups, the sweat glands and the sebaceous glands, visible as a tubular gland located inside a follicular group, were seen at the dermis and they were surrounded by red and orange collagen fibers (Figs. 1 D, E, F, G, H, I). Immunostaining for collagen type I and type IV was observed in the epidermis and dermis. In the dermis, the immunostaining for the three antibodies was observed in hair follicles, sebaceous and sweat glands. The collagen type III was only observed in dermal fibers (Fig. 2).

DISCUSSION

The fleece is composed by primary and secondary follicles. The differentiation occurs during foetal development (Moore *et al*., 1998). It is known that the fiber produced by a primary follicle is larger in diameter and lower in quality than the fiber produced by the secondaries (Moore *et al*., 1996). In alpacas follicles with only one fiber or a set of fibers emerging from the same follicular group were identified (Bustinza, 2001; Torres de Jasaui *et al*., 2007) and most of the fibers presented medulla.

Inside follicular groups sebaceous gland was identified, presented as tubular gland. In llamas they were smaller and less numerous when compared to sheep. According to Atlee *et al*. (1997), this difference could explain the less lanolin production in llama coats when compared to the sheep. For alpacas, this gland is extremely important because it confers softness, texture and resistance to its fibers (Banks, 1991; Atlee *et al*., 1997; Samuelson, 2007; Lacolla *et al*., 2010; Junqueira & Carneiro, 2013; Vélez *et al*., 2016), which makes a great difference to the analysis of fiber quality, determining the factors required by textile industries (Peña *et al*., 2013; Mucha & Janeczek, 2018).

The dermis is composed mainly of collagen type I and III. The dermis collagen arrangement provides strength, flexibility and protection of the deeper anatomical structure (Hashmi & Marinkovich, 2011; Green *et al*. 2014). In alpacas, type I and type IV collagens were observed in epider-

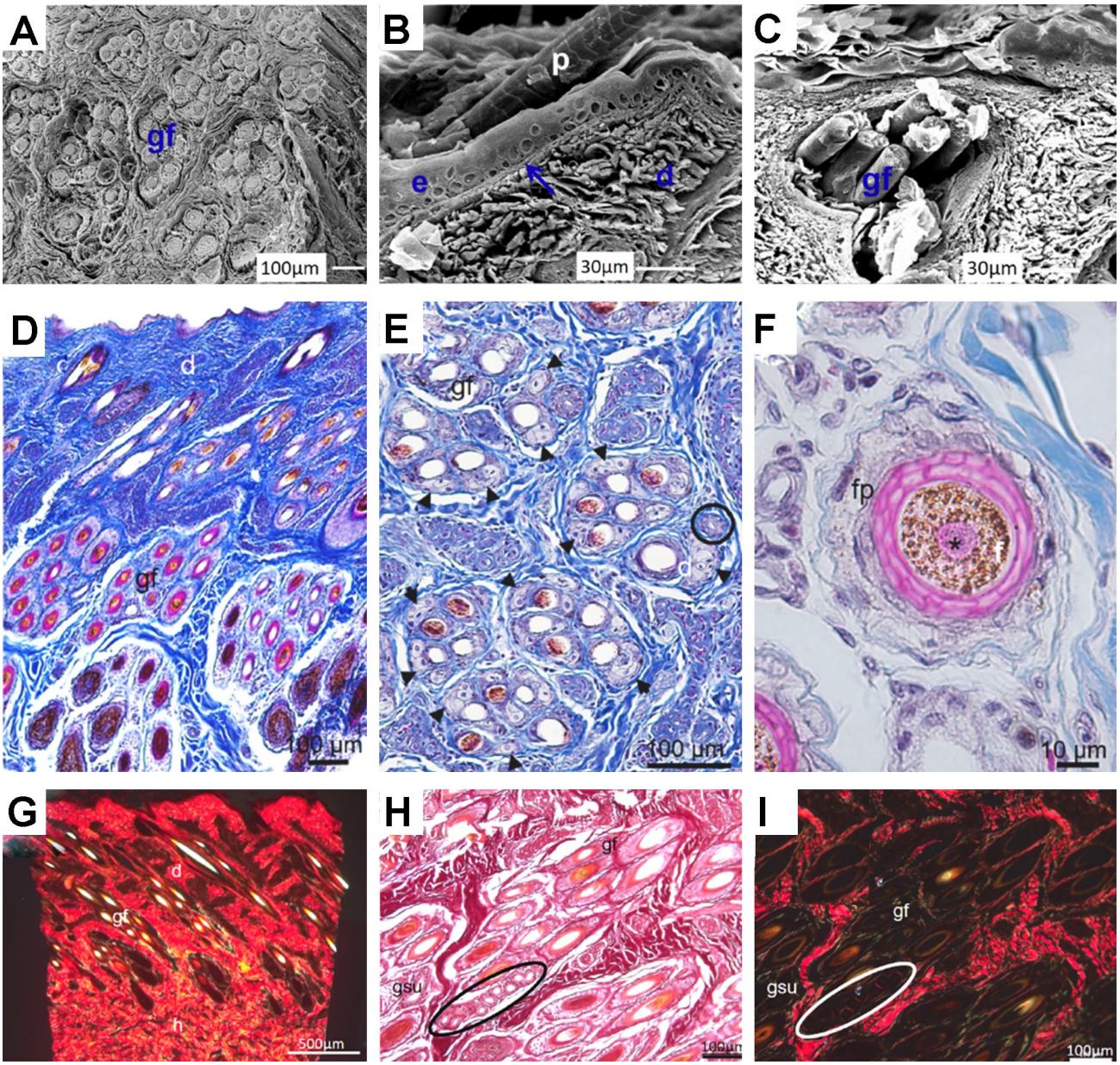

Fig. 1. Scanning electron micrograph of the skin. Follicular groups (gf) (A). Hair with the cuticle (p), epidermis (e), basal layer (arrow) and dermis (d) (B). The fibers of a follicular group (gf) emerging from the hair follicle (C). Masson's trichrome staining: dermis (d) and follicular group (gf) (D). Follicular group (gf) with sweat gland (arrow head) and sebaceous gland (circle) (E). Fiber with medulla (*) (F). Picrosirius red staining: derma (d), follicular group (gf), hypodermis (h), sweat glands (gsu) (circle) (G, H, I).

mis and dermis, as well as in glandular structures and hair follicles. Collagen III, was observed only in dermis.

Studies on skin morphology in camelids, as camels, llamas and guanacos has been reported by Atlee *et al*. (1997), de Lamo *et al*. (2001) and Dowling & Nay (1962). The description of the structure and the architecture of alpaca skin is valuable, because this animal is recognized to produce high quality fiber.

CONCLUSIONS

The Alpaca derma had collagen type I, III and IV with sebaceous and sweat glands close to the follicular groups. Fibers with medulla and some without were observed, but both were surrounded by cortex and cuticle. The hair follicles had only collagen type I.

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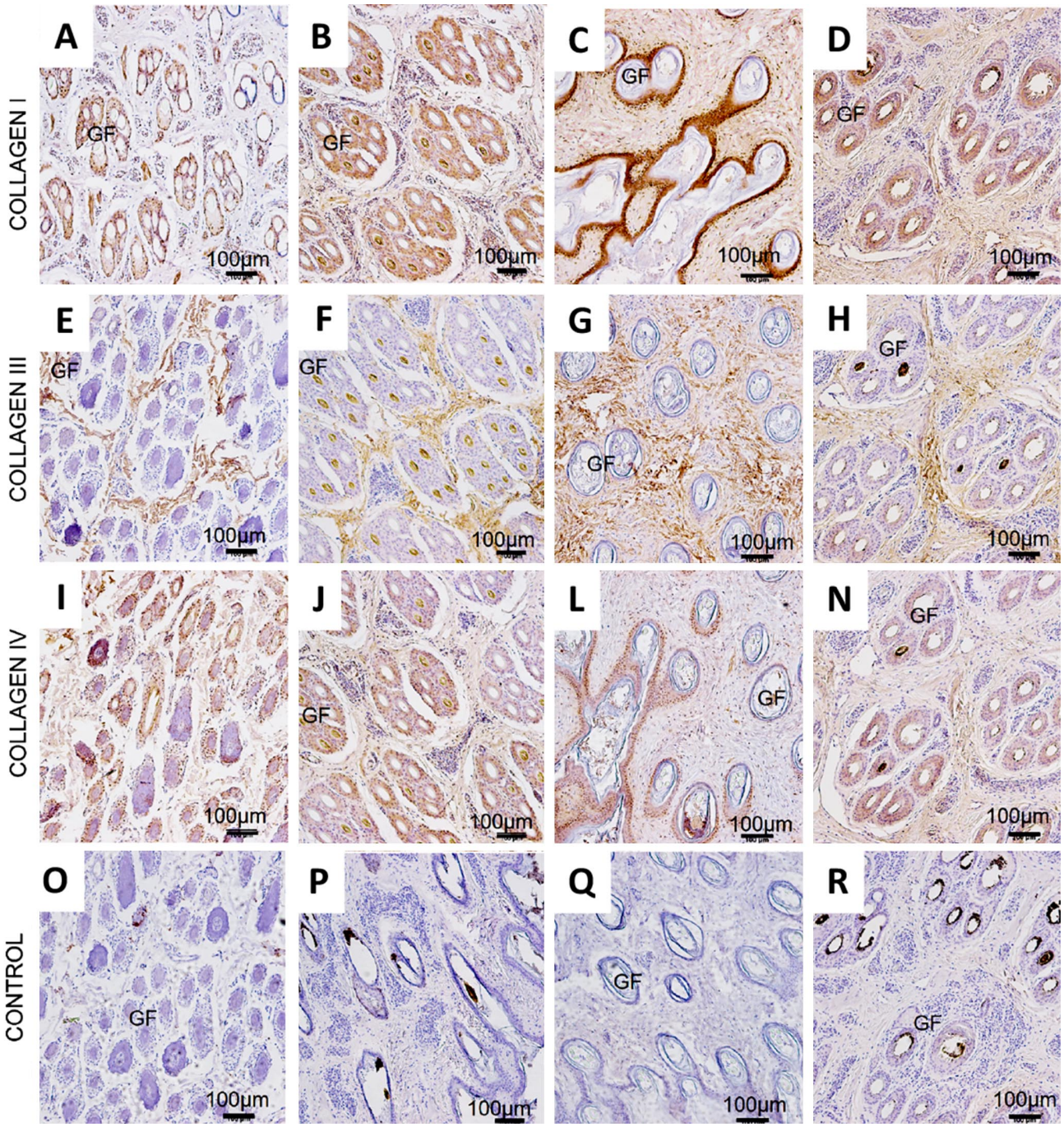

Fig. 2. Immunostaining collagen I (A-D) Collagen III (E-H), Collagen IV (I-M), S100 (N-Q), Control (R-U). In the images the follicular groups (gf) are observed in light-fiber Huacaya (A, E, I, N, R), dark-fiber Huacaya (B, F, J, O, S), light-fiber Suri (C, G, L, P, T), dark-fiber Suri (D, H, M, Q, U).

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Ethics approval. The experiment was approved by the Animal Use Ethics Committee of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo (CEUA/FMVZ) under No. 4781170317.

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RESUMEN: Este estudio evaluó la morfología de la piel de alpacas. Se recogieron biopsias y las muestras se fijaron en formalina tamponada neutra al 10 % para procedimientos

histológicos. Las secciones se tiñeron con hematoxilina y eosina, rojo picrosirius y tricrómico de Masson. El colágeno tipo I, III y IV se analizó mediante inmunohistoquímica. La dermis presentó glándulas sebáceas y sudoríparas, así como grupos foliculares con fibras medulares. Se observó colágeno tipo I y tipo IV en la epidermis y la dermis, así como en estructuras glandulares y folículos pilosos. El colágeno III, se observó únicamente en la dermis.

PALABRAS CLAVE: Histología; Integumento común; Inmunohistoquímica; Microscopía electrónica de barrido.

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