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Research

Keywords: Histology, Stereology, Scanning electron microscopy, immunohistochemistry

Posted Date: May 12th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-26867/v1>

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**The architecture and structure of alpaca skin (*Vicugna pacos*): impacts on
industry and wool trade**

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Abstract

Background: Alpacas are reared mainly for fiber extraction, which is a highly valued product in the textile industry. For this reason, this work aims to evaluate the morphological and quantitative aspects of the light and dark alpaca skin of Huacaya and Suri alpacas, comparing the structure and architecture of the scapular, costal and lateral femoral skin. Biopsies were collected from the skin of 12 alpacas from the Pacamarca Experimental Fund, located at Puno - Peru. The samples were weighed and fixed in 10% aqueous formalin solution for histological procedures. The histological sections were stained with Hematoxylin eosin, Picrossirius red and Masson Trichromic and immunostained for types I, III and IV collagen and S100.

Results: The derma presented sebaceous and sweat glands, as well as follicular groups with primary and secondary hair follicles. Each follicle had a hair called fiber, some with medulla and some without, but both surrounded by cortex and cuticle. The skin presented similar immunostaining for type I, II and IV and S100. Collagen III was detected only in the derma. The total volume of the skin, derma, follicular groups and sebaceous and sweat glands was estimated by stereology for the three body regions for both Huacaya and Suri alpacas. The total volume of skin (V_{ref}) and total volume of follicular groups (V_{GF}) were different for body regions. Femoral region showed higher values for V_{GF} . Colour and breed were also different for V_{ref} and total volume of derma (V_D).

Conclusion: Comparing the two breeds the femoral region presented higher fiber production. Dark animals had more derma and it was reported close relationship between total skin volume and their fractions volumes: derma, follicular groups and sweat glands.

Keywords: Histology, Stereology, Scanning electron microscopy, immunohistochemistry.

Background

Alpacas (*Vicugna pacos*) are a group of South American camelids, found in the upper Andean regions of South America. There are two varieties: the Huacaya and Suri. Huacaya represents 85% of the total alpacas in Peru and is characterized by its abundant, long and curly fiber, which has a spongy appearance. Suri is characterized by its smooth, slightly wavy, silky fiber. Alpaca fleece is one of the most valuable animal products in the textile industry and has a wide range of colors, almost 23 shades of white, beige, brown, gray and black [1, 2]. Alpaca fiber diameter is important because it determines the commercialization price, which is complemented with the weight of the fleece [3-10].

The dermis is composed of collagen and elastic fibers, being responsible for the resistance and elasticity and this is where the blood vessels, lymphatics, nerves, hair

follicles, sebaceous and sweat glands are located [11]. There is a negative correlation between follicular density and fiber thickness. The main histological characteristic of thin fibers is the larger number of secondary follicles, as it has been widely demonstrated that animals with smaller fiber thickness have a higher number of secondary follicles [12-14].

The fleece varies according to the body region, with the thinnest, longest and more dense in the dorsal, costal, sacral, brachial and femoral subregions. 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 [15-17].

For these reasons, the integument of three body regions of the two varieties of alpacas (Huacaya and Suri) was evaluated to establish comparisons between them and to define which of these regions would produce the best fiber for commercial purposes.

Methods

Alpaca skin biopsies were collected from the Pacamarca Experimental Fund in Llalli, Melgar, Puno - Peru, from Inca Tops SA. Twelve alpacas of both sexes, aged 5 to 19 months, were used (Six Huacaya variety and six Suri variety alpacas, divided into two groups for light and dark tones respectively). Skin biopsies were performed with an 8 mm punch in three body regions: scapular, costal and lateral femoral and fixed in 10% formaldehyde. They were later cut into 2mm thick fragments. Of the total fragments generated, $\frac{1}{4}$ was destined for scanning electron microscopy, $\frac{1}{4}$ for microscopic analysis, $\frac{1}{4}$ for immunohistochemical analysis and $\frac{1}{4}$ for stereological analysis. The distribution of fragments in these four sets was made following the pattern of systematic uniform random sampling (SURS).

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 then 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 [18]. 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 °C 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).

Stereology

Total volume of the skin (reference volume)

The 2mm-fragments, sampled previously, were embedded in paraffin and 5µm sections were collected on slides and stained with Masson trichrome. The distance between sections was 100 µm. The total volume of the skin (V_{ref}) was estimated using the Cavalieri Method [19, 20]. The image sections were captured using a digital camera connected to the light microscope (Nikon Eclipse 80i, Japan). The following formula was applied to estimate the total volume of skin (V_{ref}):

$$V_{ref} = \sum pS \times a(p) \times t \times k$$

$\sum pS$: points hitting the whole skin fragment; $a(p)$: area of points (square distance between points), t : section thickness; k : the distance between the sections.

Tissue shrinkage

The 8mm-biopsy-fragments were weighed using a digital precision balance (model JK3202B) and their wet weights were converted into volumes considering a tissue density of 1.06g/cm³. After the estimation of the total volume of the skin by the Cavalieri method above, the tissue shrinkage was calculated as 15%.

Total volume of the derma, follicular groups, sebaceous and sweat glands

The total volume of the derma (VD), follicular groups (VGF), sebaceous (VGSe) and sweat glands (VGSu) and the fraction volume were calculated applying the following formulae:

$$\text{➤ } VvD = \sum pD / \sum pP$$

$$\text{➤ } VvGF = \sum pGF / \sum pP$$

$$\text{➤ } VvGSe = \sum pGSe / \sum pP$$

$$\text{➤ } VvGSu = \sum pGSu / \sum pP$$

($\sum pD$): total points hitting the derma

($\sum pGF$): total points hitting the follicular groups

($\sum pGSe$): total points hitting the sebaceous glands

($\sum pGSu$): total points hitting the sweat glands

($\sum pP$): total points hitting the skin

The total volume was estimated multiplying each fraction volume by the reference volume. A three-way analysis of variance was carried out including factors of Breed, colour, region of the body and their interactions. Significant differences were tested using the tukey test at 5%. Correlations and principal component analyses between characteristics were calculated. Analysis were carried out in SAS v9.4 (Cary, North Carolina).

Results

Scanning electron microscopy

The two constituent layers of the skin were visualized: the epidermis and the dermis. In addition, there was the presence of adipose tissue-associated hypodermis (Fig. 1a). In the dermis, it was possible to observe the hair bulbs and the follicular groups,

which determine the grouping of hair follicles, surrounded by the connective tissue of the dermis (Fig. 1b and Fig. 1c).

The fiber was observed emerging from the epidermis, with the presence of the outermost layer, the cuticle, arranged in scales (Fig. 1d). At the junction between the epidermis and the dermis, it was possible to observe a cluster of cells irregularly arranged in the epidermis, called the basal layer. Still in the epidermis, the *stratum corneum* was seen, which is the last layer (Fig. 1d and Fig. 1e). In the papillary dermis, there was a group of six fibers emerging from the same hair follicle (Fig. 1f). Adjacent to the hair follicles were the sebaceous glands (Fig. 1g).

Histology

In transverse sections, it was seen that alpaca skin is composed of the epidermis and dermis. It was also possible to visualize the hypodermis or subcutaneous tissue as shown in Figures 2a and 2b. The epidermis and dermis were separated by the basal or germ layer of the epidermis (Fig. 2c and Fig. 2d).

The dermis was located between the epidermis and the hypodermis and consisted of the sweat glands as shown in Fig. 2e. The sebaceous gland consisted of rounded cells and acidophilic nuclei, which were arranged near a hair follicle. The dermis contained follicular groups, structured by a set of hair follicles grouped into a species of nests (Figs. 2e-2g). In the dermis, the sebaceous gland was visible, that is a tubular gland located inside a follicular group (Fig. 2g). The hair follicles usually contained more than one hair shaft or fiber inside. They were called composite follicles, which are seen in Fig. 6h, where it was possible to verify the presence of four fibers without marrow in a single hair follicle. Primary follicles consisted of three structures: the medulla internally, the cortex and the cuticle surrounding the hair cortex. Three layers of the hair follicle were observed: the inner sheath, consisting of a cuticle, a granular epithelial layer (consisting of flattened

cells), and a pale epithelial layer (outer layer of cuboid cells), followed by the outer sheath. The last layer was the vitreous membrane (Fig. 2i).

In the reticular dermis, hair bulbs surrounded by dense connective tissue were observed, which did not emerge evenly (Fig. 2j and Fig. 2k). The hair bulb contained the hair papilla or dermal papilla. The cells of the central portion of the matrix formed the hair medulla and, surrounding the dermal papilla, melanocytes within the hair cortex were identified (Fig. 2l). In the longitudinal sections, the dermis contained collagen fibers, compared to the epidermis which did not present this constitution. No collagen was found highlighted in the blue color of the dermis (Figs. 3a-3c).

The hair follicles were arranged into follicular groups located in the dermis and surrounded by collagen fibers as shown in Figs. 3d-3f. The hair follicles, found in the deep dermis, had a higher quantity of blue-stained collagen. Longitudinal sections of the skin were made, and it was observed that, in the dermis, the follicular groups were surrounded by a larger quantity of collagen (Figs. 3i-3l).

From the Picrosirius Red staining performed on the transverse histological sections of the skin, the dermis and hypodermis showed collagen fibers (Figs. 4a-4c). These were confirmed by the polarization technique, again showing the collagen fibers dispersed by the dermal tissue (Fig. 4d and Fig. 4e). This technique allowed the differentiation of collagen fiber types, with red and orange fibers compatible with type I collagen fibers interspersed with green fibers, indicating reticular fibers or type III collagen fibers (Fig. 4f).

In both Picrosirius Red staining and polarized light analysis, follicular groups delimited by collagen fibers were observed. However, in the hair follicles only collagen fibers are observed in the outer layer. Most collagen fibers were red to orange and therefore considered collagen type I fibers (Figs. 4g-4l).

Immunohistochemistry

In the four groups of alpacas: light-fiber Huacaya (Fig. 5 a, e, i, n, r), dark-fiber Huacaya (Fig. 5 b, f, j, o, s), light-fiber Suri (Fig. 5 c, g, l, p, t) and dark-fiber Suri (Fig. 5 d, h, m, q, u), when antibodies for Collagen Type I, Collagen Type IV and S100 were used, the structures of the epidermis, some collagen fibers of the dermis, hair follicles, sebaceous glands and sweat glands were marked, which was observed for the scapular, costal and lateral femoral regions.

Quantification

Vref and VGF were significantly different for body region (Table 1), with femoral region showing higher values than scapula, but costal was not different from femoral or scapula for VGF (Table 2). There were also significant interactions between colour and breed for Vref and VD. Dark coats had significantly lower values in Huacaya than Suri breeds for both traits while VD was lower in light than dark coats within the Suri breed (Table 3).

Correlations between Vref and VD were high and medium with VGSu, while others were low (Table 4).

Table 1. Level of Significance and summary of analysis of variance for coat traits in alpacas

| | Vref | VGF | VD | VGSe | VGSu |
|---------------------|--------|--------|--------|--------|--------|
| Source | Pr > F |
| Breed | 0.00 | 0.09 | <.0001 | 0.32 | 0.15 |
| Colour | 0.52 | 0.60 | 0.12 | 0.24 | 0.91 |
| Breed*Colour | 0.05 | 0.62 | 0.00 | 0.29 | 0.75 |
| Region | 0.02 | 0.00 | 0.30 | 0.48 | 0.38 |
| Breed*Region | 0.40 | 0.24 | 0.70 | 0.31 | 0.93 |
| Colour*Region | 0.53 | 0.38 | 0.89 | 0.52 | 0.75 |
| Breed*Colour*Region | 0.23 | 0.37 | 0.47 | 0.32 | 0.52 |
| | | | | | |

| | | | | | |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| R ₂ | 0.58 | 0.52 | 0.66 | 0.32 | 0.21 |
| cv | 19.34 | 21.09 | 30.06 | 177.83 | 47.11 |
| Mean mm ³ | 4.6 *10 ⁹ | 2.3 *10 ⁹ | 1.6 *10 ⁹ | 5.5 *10 ⁷ | 1.7 *10 ⁸ |

R₂ – determination factor, CV – coefficient of variation; the total volume of skin (Vref), total volume of the derma (VD), follicular groups (VGF), sebaceous (VGSe) and sweat glands (VGSu)

Table 2. Means by Region for total volume of skin (Vref) and follicular groups (VGF) in alpacas in mm³

| Region | Vref | VGF |
|---------|------------------------|-----------------------|
| Femoral | 5.2*10 ⁹ a | 2.8*10 ⁹ a |
| Costal | 4.4*10 ⁹ ab | 2.0*10 ⁹ b |
| Scapula | 4.2*10 ⁹ b | 2.2*10 ⁹ b |

Means in the same column followed by different letters are significantly different using the Tukey test (P<0.05)

Table 3. Interaction between coat colour and breed in alpacas for the total volume of skin (Vref), total volume of the derma (VD) in mm³

| | | Light | Dark |
|------|---------|-----------------------|------------------------|
| Vref | Huacaya | 4.2 *10 ⁹ | 3.8*10 ⁹ a |
| | Suri | 4.7 *10 ⁹ | 5.6*10 ⁹ b |
| VD | Huacaya | 1.3*10 ⁹ | 1.1*10 ⁹ a |
| | Suri | 1.7*10 ⁹ A | 2.5*10 ⁹ Bb |

Means in the same column followed by different lower case letters and in the same row followed by upper case letters are significantly different using the Tukey test (P<0.05)

Table 4. Correlations between coat traits in Alpacas

| | VRef | VGF | VD | VGSe |
|-------|------|------|------|------|
| VvGF | 0.72 | | | |
| VvD | 0.85 | 0.31 | | |
| VvGSe | 0.11 | 0.14 | 0.12 | |
| VvGSu | 0.56 | 0.37 | 0.51 | 0.10 |

Total volume of skin (Vref), total volume of the derma (VD), follicular groups (VGF), sebaceous (VGSe) and sweat glands (VGSu)

The principal component analysis showed a strong relationship between all traits except VGSe (Fig. 6).

Discussion

In the macroscopic analysis of alpaca skin and fiber fragments, two layers of the skin were observed: the epidermis and the dermis. Inside the follicular groups containing the hair, the structure was similar to that found in other mammals. The skin is an important organ as it is a protective barrier which acts as a defense of the body to the external environment [19, 20, 15, 23, 24, 11]. The set of hairs that emerge from the epidermis form the fleece [16, 17], protecting animals in extremely cold climates.

Unlike other authors, it was not possible to view all epidermis layers. The basal stratum contained cubic cells. In turn, the *stratum corneum* of dead keratinized cells was observed, which appeared as a detached layer from the rest of the other strata of the epidermis. However, our data are in agreement with the previous cited authors [11, 14, 18, 21, 22, 25, 26] and the alpaca epidermis constitutes a very thin layer in the scapular, costal and lateral femoral regions, more precisely parts of the lateral regions of the thoracic limb, thorax and pelvic limb.

The dermis was also analyzed, which had two parts, the papillary dermis, closer to the epidermis and the reticular but deep dermis, near the hypodermis. The difference between both portions was not evident. In the dermis, we found glandular tissue composed of sebaceous glands and sweat glands. The sweat glands were visualized as tubular glands responsible for the thermal regulation and elimination of toxic substances. They produce sweat, mostly composed of water, mineral salts, some proteins and fatty substances [11, 12, 15, 22, 23, 25, 27, 28].

In the present study, we evaluated the costal, scapular and lateral femoral regions, which were associated with the hair follicles. These were composed of rounded

cells with spherical nuclei. In other studies, where other regions were analyzed, the authors did not find these glands in the interdigital space [27].

For alpacas, this gland is extremely important because it confers softness, texture and resistance to its fibers [10-14, 21-25], which makes a difference to the analysis of fiber quality, determining the factors required and required by the textile industries [29-30].

In the reticular dermis, the hair bulbs were surrounded by dense connective tissue with an oblique disposition. These did not to emerge uniformly. Surrounding the dermal papilla were melanocytes within the hair cortex. The hair cuticle and two root sheaths were also observed.

It was possible to separate the follicular groups of hair follicles, which could be primary or secondary, being responsible for the formation of primary and secondary hair, respectively. Hair follicles were found both inside and outside the follicular groups. Authors found one to three primary follicles within a follicular group, and between 20 and 33 secondary follicles [3, 31-33].

Collagen is a matrix protein, that gives rigidity and consistency to the skin, making dermis structures more stable inside the dermal tissue, serving as a basis for sustaining and nourishing hair [15, 21, 23, 26]. Masson's trichrome stains showed blue-stained collagen fibers compatible with type I collagen fibers. These bypassed the follicular groups, offering support to them as well as the other structures in the dermis. In the case of the slides stained with Picrosirius Red and observed under polarized light, the dermis was visualized, which presented fibers of red color, indicating collagen type I fibers. In the deepest dermis, the presence of red, orange and green polarized fibers was observed, the latter being type III collagen fibers, or reticular fibers, which are small and provide a supporting the whole structure.

Through scanning electron microscopy analysis, it was possible to evaluate the components present in the integument, as well as the microstructures, but higher resolutions would be needed to identify them. It was possible to observe the two layers of the skin separated by the basal layer. In the epidermal layer, we could visualize the *stratum corneum*. This layer is the last superficial layer of the epidermis responsible for the protective barrier [11, 15, 19-23].

Through scanning electron microscopy, it was possible to see that there were follicles with only one hair or a set of fibers emerging from the same follicular group, as also described by [11, 19, 20, 25]. As hairs emerge they come together to share the same follicle. This is because secondary follicles are responsible for the cover hair, which help maintain thermal regulation [16, 17]. Therefore, they do not need to be very thick, just be numerous to make up the total body coat of the animal.

Scanning electron microscopy was used to determine the hair diameter of the costal region. This is the region considered as representative of the alpaca integument [3,10, 23, 32]. In the light and dark alpacas of the Huacaya and Suri varieties, the diameter ranged from 12.65 μm to 55.00 μm . [34] measured fibers of various species, finding diameters varying from 18 μm to 30 μm for alpacas; <15 μm for vicuñas; from 15 μm to 18 μm for guanacos; > 20 μm for llamas; from 12 μm to 18 μm for cashmere (double fleece goats); from 22 μm to 30 μm for mohair (single fleece goats); 17 μm for Merino sheep; > 30 μm for Scottish Blackface sheep; from 10 μm to 13 μm for Angora rabbits. According to Peruvian Technical Standards, for alpacas a fiber of at most 21.50 μm is the most valued fine fiber for the textile industry. This is because it provides a softer and more comfortable fiber for the production of fabrics, whereas the average fiber is 26.60 μm to 29 μm , and the thickest fiber of 31.60 μm is not used in the textile industry because they are not comfortable fibers to produce clothing.

The presence of different matrix proteins from the three body regions of the tegument of the light and dark alpacas of the Huacaya and Suri varieties was analyzed. First, descriptions were made for the various types of collagen (I, III and IV), where the marking for type I and type IV collagen was observed throughout the epidermal and dermal tissue, together with their glandular structures, and hair follicles. In the case of collagen III, only the marking for dermis collagen fibers was observed.

The S100 protein was also observed, important for the integument, because it is present in melanocytes, cells derived from the neural crest and is also present in Langerhans cells and keratinocytes. In this study, all skin structures such as epidermis, dermis, sebaceous and sweat glands showed positive labeling for this protein. Hair follicles were more marked than in the other structures of the integument. For the three body regions of the light and dark alpacas of the Huacaya and Suri varieties, the presence of melanocytes is similar and this marker may express any of its pigment forms such as eumelanins (dark colors like brown or black) or pheomelanins (reddish or yellow colors) [34].

More follicular groups and skin volume were seen in the femoral region indicating higher fiber production in this region. In dark skinned animals, more skin volume was seen in the Suri compared to the light colored animals and Dark Suri produced more derma than light-skinned animals or Huacaya. The principal component analysis shows the close relationship between total volume of the derma (VD), follicular groups (VGF), skin volume (Vref) and sweat glands (VGSu). This indicates that for fiber production, selection for one of these traits should increase the others. This may be useful for breeders when selecting animals for reproduction in breeding schemes.

Conclusions

Our study concluded that the femoral region presented higher fiber production. Dark animals had more derma and it was reported close relationship between total skin volume and their fractions volumes: derma, follicular groups and sweat glands.

Acknowledgments

Thanks are due to CAPES for funding and scholarships.

Authors' contributions

LMDN e MAM conceived and designed the experiment
LMDN, JB, MK, ADC, ACOV performed and analysed the immunohistochemistry
LMDN, THCS, SPG, CMM performed the stereological design and analysis
LMDN, ACOC, THCS, CMM, MAM analysed the data and wrote the paper

Funding

Coordination for the Improvement of Higher Education Personnel (CAPES) - Brazil

Availability of data and materials

All analyzed data from this study are included in this manuscript

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.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interest

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Legend of figures

Fig 1. Scanning electron micrograph. Sections to the skin of the Huacaya and Suri alpacas in A show the hair (p), the dermis (d) and the hypodermis (h). Follicular groups (gf) (B). In C, in greater increase, the follicular groups (gf). In D, the hair with the cuticle (arrow) is observed, also in E, the epidermis (e) and the dermis (d), separated by the basal layer of the epidermis (arrow). In F, the fibers of a follicular group (gf) emerging from the hair follicle in the papillary dermis. In G, follicular group fibers (gf) and sebaceous glands (gse) (arrow). Skin longitudinal sections, the follicular groups in the dermis (gf) (H), in I, greater increase of the follicular groups (gf). In J, sweat gland (gsu) indicated by the arrow between two follicular groups (gf). In K, follicular group (gf) composed of four hair follicles, accompanied by the sebaceous gland (gse). In L, the follicular group (gf) on the side is observed the primary follicle (fp). In M, primary follicle (fp) containing medullary primary hair (p) with visible cuticle in the form of layers (arrow). In N, the primary follicle (fp) with the medullary primary hair (p). In O, the hair follicle layers and the hair (p).

Fig 2. Photomicrograph of the skin. In A and B the dermis (d) and hypodermis (h). In C and D, the epidermis (e) and the dermis (d), the junction between the epidermis and the dermis (arrow). In E, follicular groups (gf), and sebaceous gland (gse). In F, longitudinal section to the skin with follicular groups (gf). In G, follicular group (gf) with sweat gland (gsu) inside. In H, hair follicle (fp) containing fibers (f) inside. In I, hair follicle containing a fiber (f) with marrow (*). In J, K and L, hair bulb (b). Staining: Hematoxylin eosin.

Fig 3. Photomicrograph of the skin. The structures stained in blue represent the collagen fibers. In A and B, skin cross sections; epidermis (e), dermis (d) and junction of epidermis with dermis (arrow). In C hair follicle (fp). In D, dermis (d) and follicular groups (gf). In E, follicular groups (gf) with sebaceous glands (arrows) and within the circle the sweat gland, just as in F. In G and H, hair bulb (b). Skin longitudinal sections; follicular groups (gf), hair follicle with marrow (*), in I-L. Staining: Masson thricrome.

Fig 4. Photomicrograph of the skin cross sections (A-F). In A, dermis (d), follicular groups (gf) and hypodermis (h). In B, the arrow indicating the separation between the epidermis (e) and the dermis (d). In C, follicular groups (gf) and hypodermis (h). In D, follicular groups (gf), collagen fibers are observed in red for dermis (d) and hypodermis (h). E, hair follicles (fp), the dermis (d) with red collagen fibers and the arrow indicating division with the epidermis (e). In F, follicular groups, surrounded by red and orange collagen fibers, hypodermis (h). Longitudinal cuts to the skin (G-L). In G, follicular group (gf), containing sebaceous glands indicated by arrows. In H, follicular groups (gf) with sweat glands (gsu). In I, sweat glands within the circle. In J, follicular group (gf) surrounded by collagen fibers, in the arrows sebaceous glands, which were not observed in polarized light. In K, dermal collagen fibers in red and orange surrounding the follicular groups (gf) and sweat glands (gsu), also in L, follicular groups (gf) and sebaceous glands within the circle. Staining: Picrossirius red.

Fig 5. Lateral femoral skin longitudinal sections immunostained 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).

Fig 6. First two principal components for coat traits in alpacas. Total volume of skin (Vref), total volume of the derma (VD), follicular groups (VGF), sebaceous (VGSe) and sweat glands (VGSu).

Figures

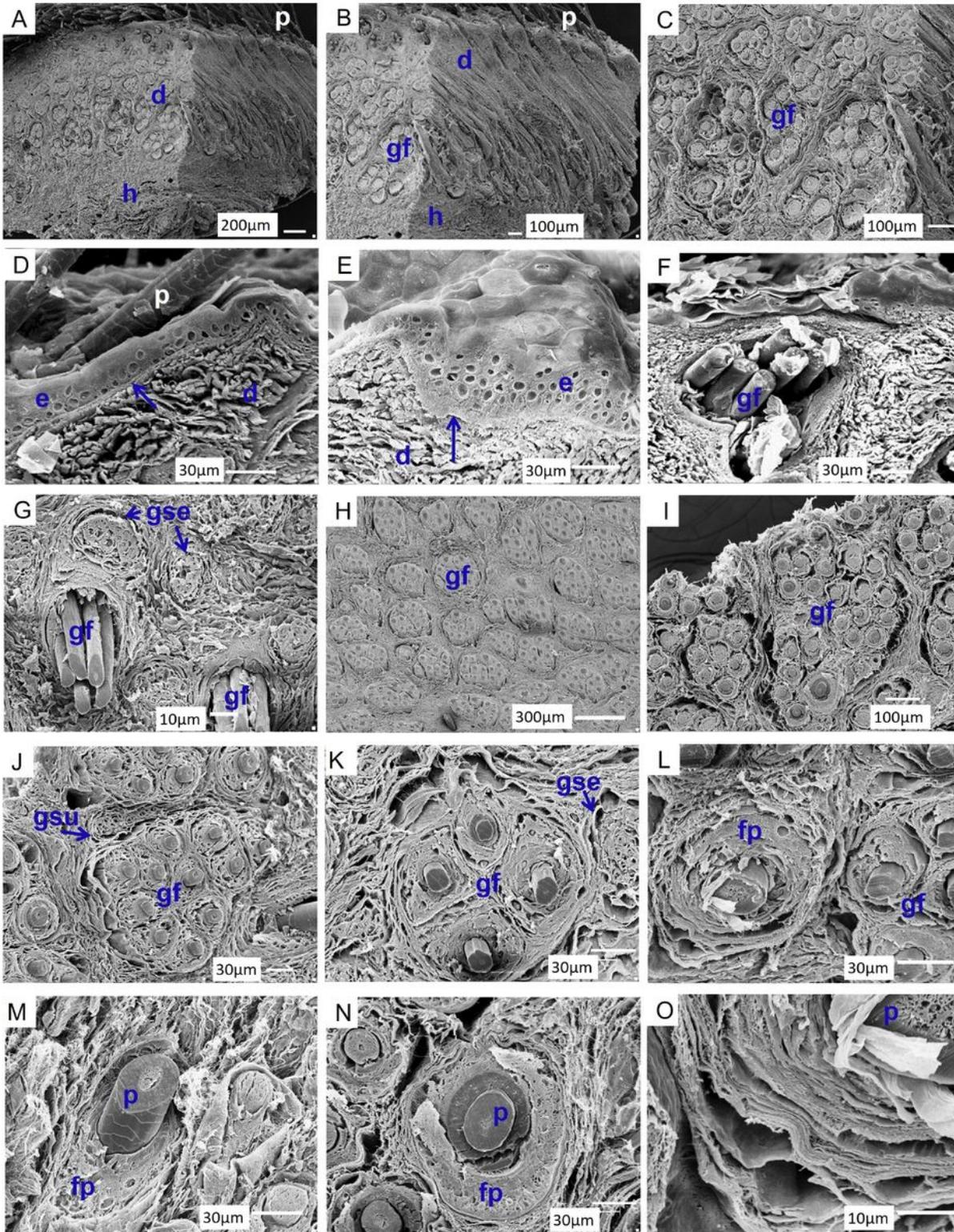


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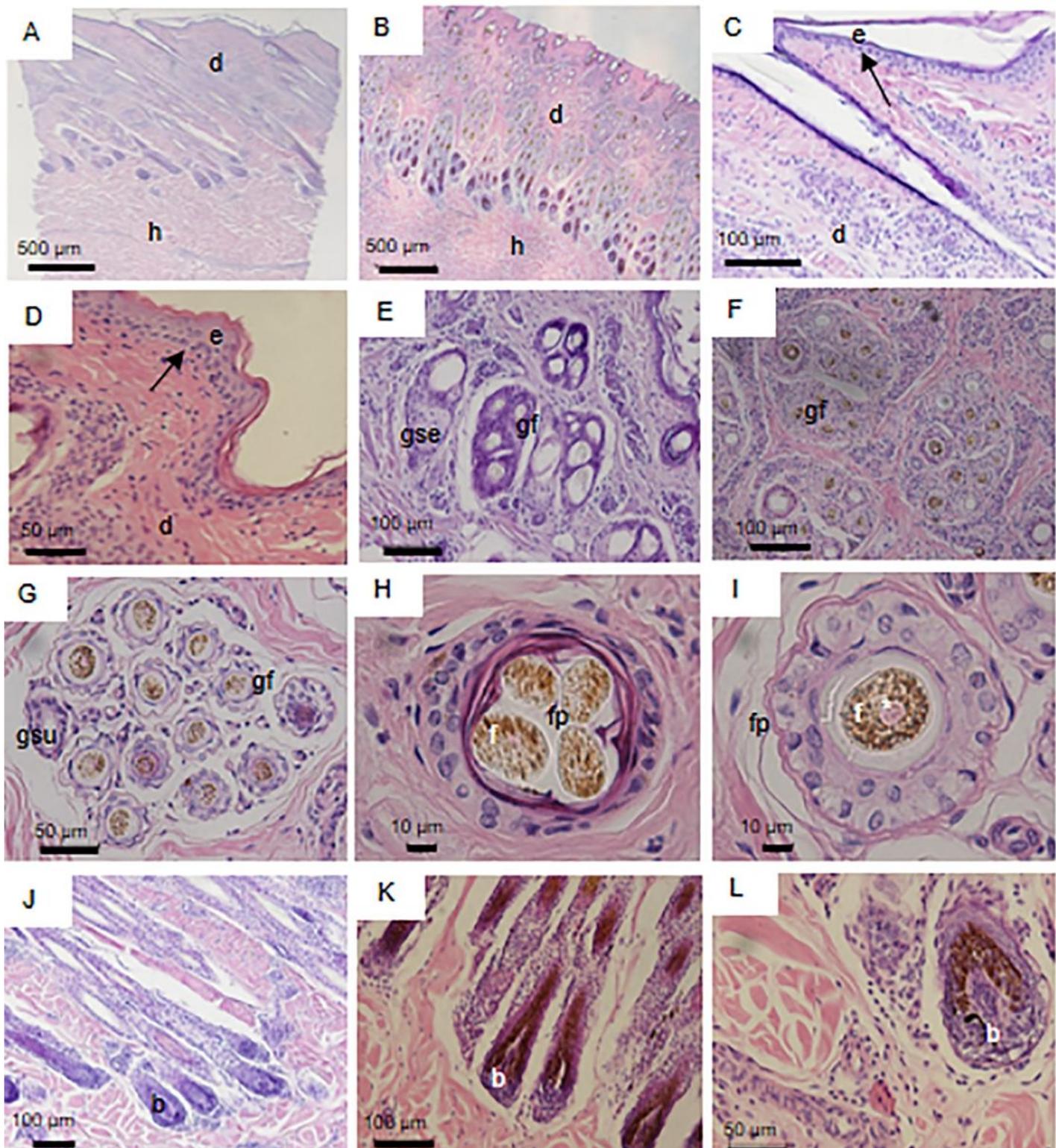


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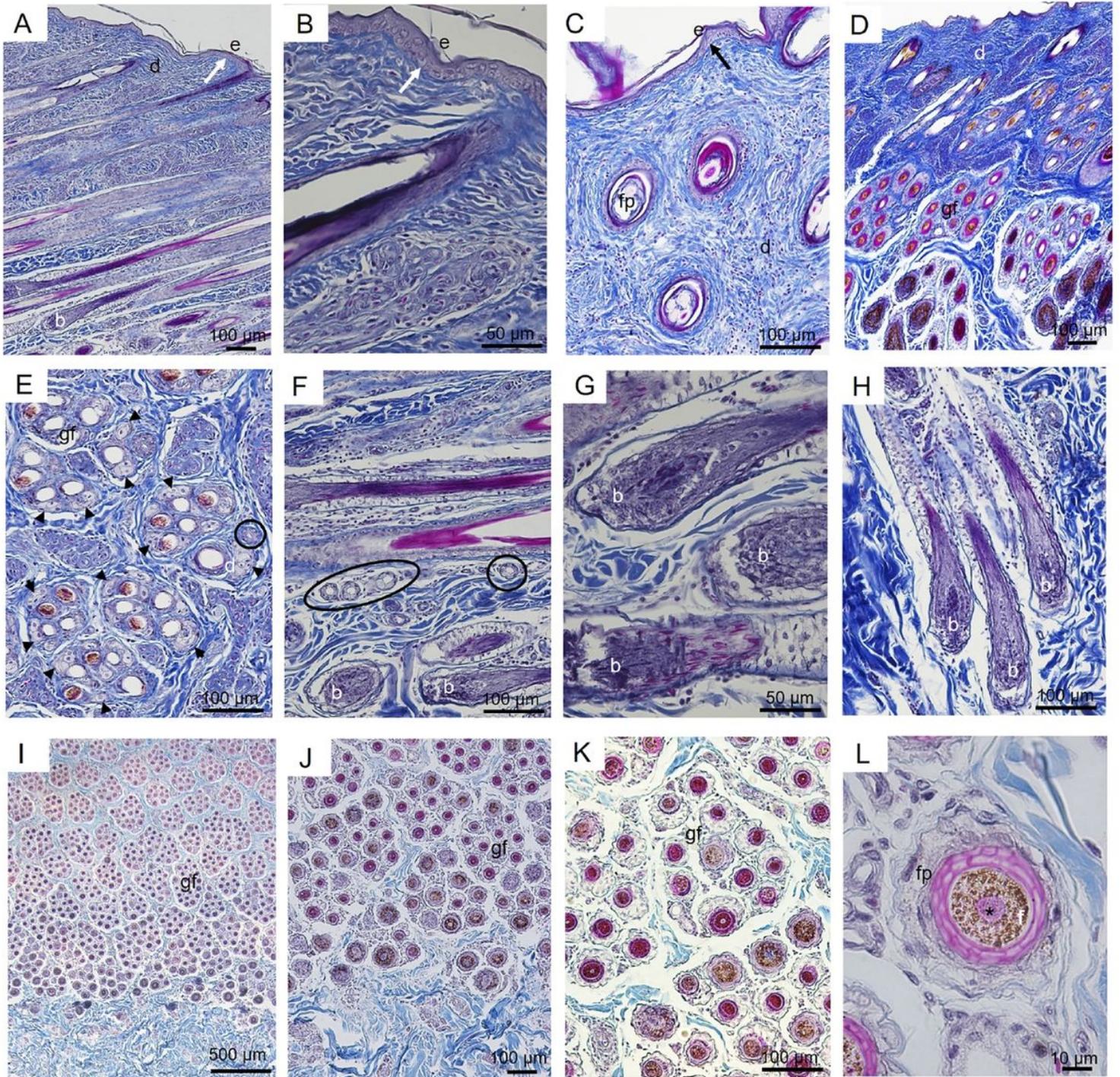


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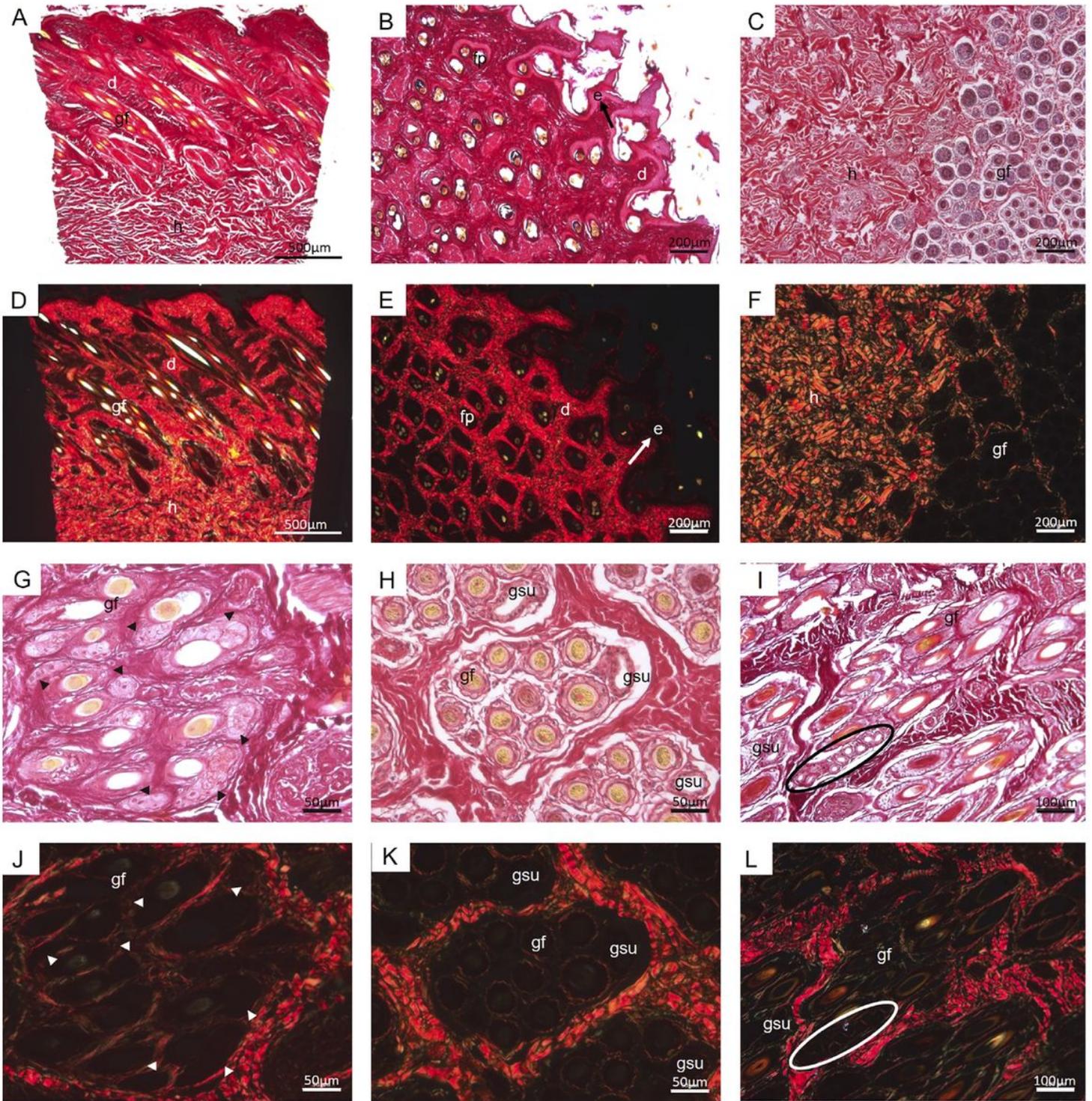


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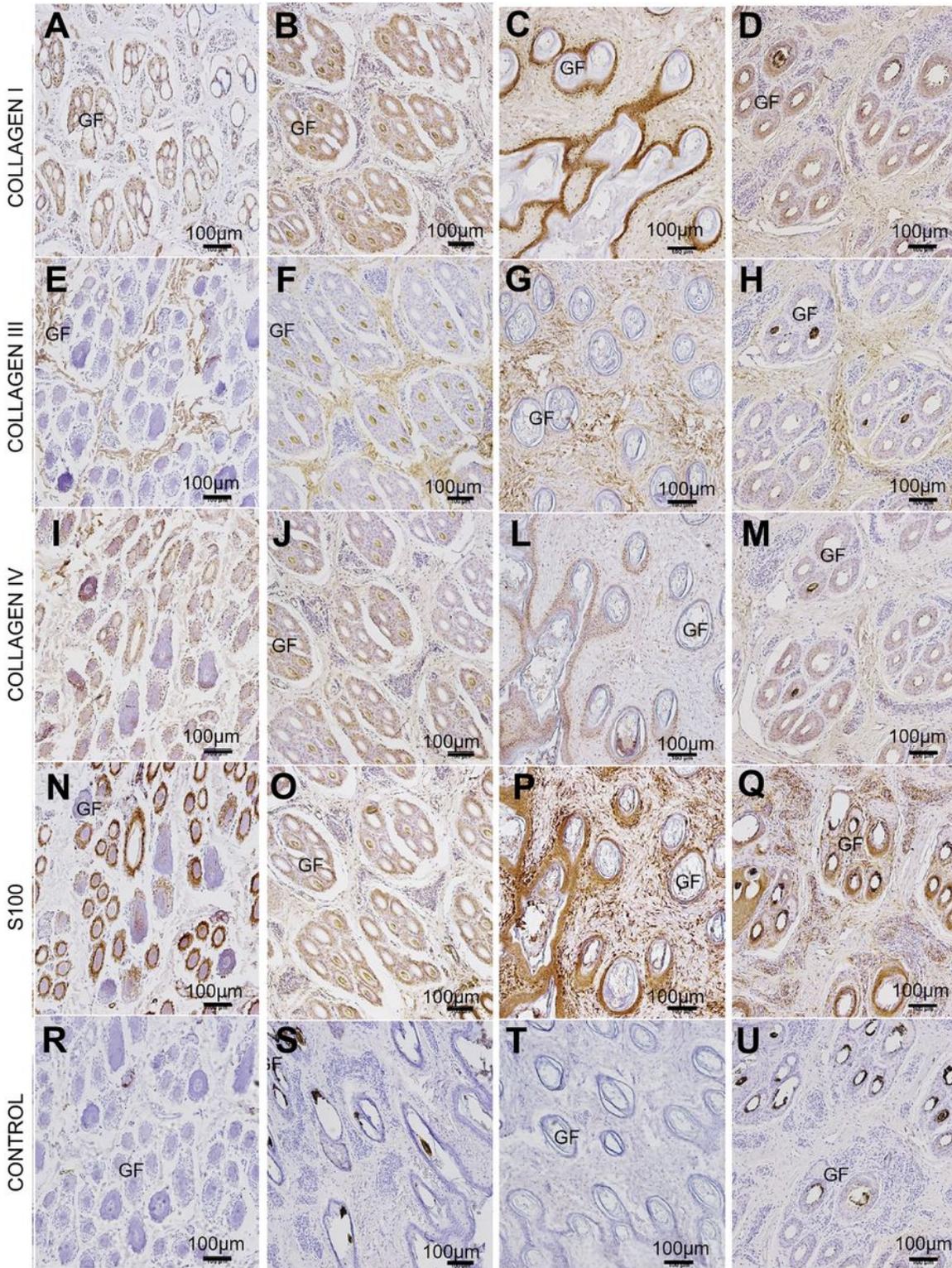


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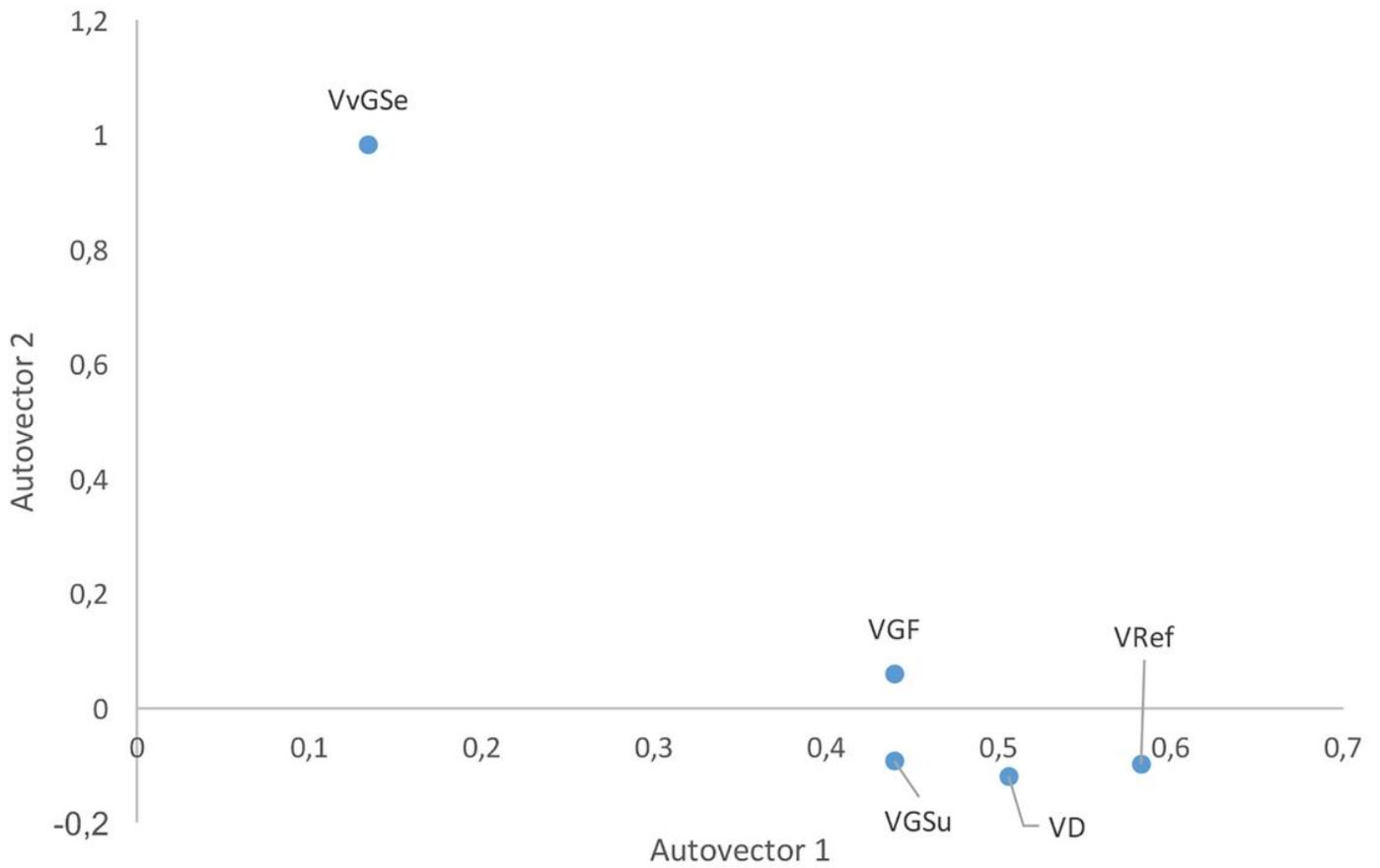


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