

# Histopathological and Ultrastructural Aspects of Normal Buffalo Skin (*Bubalus bubalis*, LINNAEUS, 1758)

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**Abstract**— Macroscopic and microscopic observations were performed on fragments of normal skin from the periauricular region of buffaloes. The analysis demonstrated that the skin of this species has a structural organization similar to that observed in other mammals, including humans, and consists of two main layers: the epidermis and dermis. Evaluations were conducted sequentially by light microscopy and electron microscopy, allowing characterization of the epidermis, basement membrane, and dermis, as well as their respective cellular and tissue subdivisions. The findings revealed morphological similarities between buffalo skin and that of other mammalian species.

**Keywords**— buffalo, skin, dermis, epidermis.

## I. INTRODUCTION

The buffalo *Bubalus bubalis* (LINNAEUS, 1758) was introduced into Brazil in 1890 from animals imported from the island of Trinidad to Marajó Island. Originating from the Asian continent, buffaloes were subsequently introduced into Europe, Oceania, and the Americas and are currently adapted to a wide variety of ecosystems worldwide (MARQUES & CARDOSO, 1997).

Since then, several breeding centers have been established throughout Brazil, contributing to the expansion of buffalo farming into different states and to increased imports of breeding females over the decades. According to IBGE data (2020), the Brazilian buffalo herd comprised approximately 1.5 million animals, with a strong concentration in the Northern region. The states of Pará and Amapá accounted for a substantial proportion of the national herd, confirming the importance of this region for buffalo production in the country. The survey recorded 1,103,630 animals in the Northern region; 131,493 in the Northeastern region; 201,304 in the Southeastern region; 94,337 in the Southern region; and 61,778 in the Central-Western region.

Worldwide, the main buffalo-producing countries are located on the Asian continent, particularly India, Pakistan, Indonesia, the Philippines, and China. In Europe, buffalo production is important in countries such as Italy, Bulgaria, and Russia, as well as in regions formerly comprising Yugoslavia.

These animals are raised mainly in natural grasslands and native pastures, although buffaloes also perform exceptionally well on firm ground and cultivated pastures. This adaptive capacity is of considerable economic importance because buffaloes are less selective regarding forage than other species of similar size. They can therefore utilize coarser feedstuffs and efficiently convert them into products of high nutritional value (MARQUES & CARDOSO, 1997).

This characteristic makes buffalo production particularly important in regions regarded as marginal or less favorable for conventional livestock farming, such as the seasonally flooded lowlands of the Amazon, coastal lowlands, wetlands of the

Southern region, and the Pantanal. In this context, buffalo farming represents an important alternative for meat and milk production, enabling productive use of previously underutilized areas and expanding opportunities to obtain animal protein at relatively low cost (MARQUES & CARDOSO, 1997).

According to the Brazilian Association of Buffalo Breeders, in 2024 the Brazilian buffalo herd was estimated at approximately 3 million animals, corresponding to approximately 1.4% of the national cattle herd. Despite this relatively small proportion, Brazil has the largest buffalo herd in the Western world, demonstrating the potential for expansion of this activity. Approximately 30% of buffalo farms are devoted to milk production, and this sector has shown substantial growth in recent years (BRAZILIAN ASSOCIATION OF BUFFALO BREEDERS, 2024).

Buffaloes have productive aptitudes similar to those observed in cattle of European and Indian origin and can be used for milk and meat production and as draft animals. Although they are naturally more rustic, they have a high capacity for domestication, a process dating back to prehistoric times, particularly in regions of Indochina.

Among the organs directly involved in the interaction between the organism and the environment, the skin stands out as one of the largest organs of the body and is responsible for covering the body surface. In addition to acting as a physical protective barrier against external agents, it performs several essential functions for maintaining organismal homeostasis.

The skin consists of different, closely related structures and cell populations. The epidermis is of ectodermal origin and, because it lacks its own blood supply, depends on the diffusion of nutrients from the underlying tissues. During differentiation, epidermal cells undergo progressive changes that culminate in keratinization and programmed cell death. Because of its extensive contact surface with the external environment, the skin is continuously exposed to different physical, chemical, and biological agents, making its integrity essential for protecting the organism. It also includes a connective tissue portion of mesodermal origin, the dermis. Below and continuous with the dermis lies the hypodermis, which, although it has the same origin as the dermis, is not part of the skin and merely supports it and connects it to the underlying organs (ABRAHAMSOHN, 2026).

## **II. OBJECTIVE**

To characterize the morphological, histological, and ultrastructural aspects of normal buffalo skin, identifying its main structures and comparing them with those observed in other mammals, particularly cattle.

## **III. MATERIALS AND METHODS**

### **3.1 Material**

Skin samples from the periauricular region of Murrah buffaloes were collected by excision for laboratory examination. Some were kept refrigerated, whereas others were fixed in 10% formalin buffered with calcium carbonate or in 2.5% glutaraldehyde and also refrigerated. Formalin-fixed samples were processed for light microscopy and embedded in paraffin, whereas glutaraldehyde-fixed samples were embedded in Spurr resin for transmission electron microscopy.

### **3.2 Methods**

#### **3.2.1 Routine hematoxylin and eosin (H&E) histological technique**

Organ fragments fixed in 10% buffered formalin were dehydrated, cleared, embedded in paraffin, and sectioned at a thickness of 5  $\mu$ m using a microtome. After deparaffinization in xylene, the sections were stained with hematoxylin and eosin, mounted under coverslips with Entellan, and examined using a conventional direct-light optical microscope.

#### **3.2.2 Electron microscopy technique: resin embedding of fragments followed by positive staining of ultrathin sections**

Skin fragments were processed according to standard resin-embedding procedures based on the methods of Gonzalez-Santander (1969). Freshly collected samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.0, post-fixed in 2% osmium tetroxide in 0.2 M phosphate buffer at pH 7.0, stained with 0.5% uranyl acetate, dehydrated through a graded acetone series (50–100%), and embedded in Spurr resin. After ultrasectioning of the blocks, the resulting ultrathin sections

were positively stained by sequential treatment with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) before examination with a Philips EM 208 transmission electron microscope.

## IV. RESULTS

Overall, the structures observed in buffalo skin were similar to those of other mammalian species, including humans (Figures 1–9). Two basic portions were therefore identified: the epidermis and dermis.

### 4.1 Epidermis

The epidermis is the outermost portion of the body and consists of several layers of closely apposed cells. These cells originate in the deepest layer and, as they move toward the surface, undergo necrobiosis associated with nutritional deficiency, forming a superficial keratinized layer (Figures 1, 2, 3, and 4).

In the region examined, the epithelium is thinner than in other areas of the animal. Consequently, fewer cells are present, although the layers can still be identified. From deepest to most superficial, these layers are the basal, spinous, granular, lucid, and keratinized layers.

#### 4.1.1 Basal layer

This epidermal layer receives the greatest nutrient supply from the dermal vessels (Figure 2). Under light microscopy, it appears as a single layer of prismatic to cuboidal cells. The cells have oval nuclei, finely dispersed chromatin, and a visible nucleolus. The short axis of the nucleus is perpendicular to the base of the cell, and the cells are numerous (Figures 1, 2, 3, 4, 5, and 6). Under electron microscopy, each cell has scant cytoplasm surrounding the nucleus (Figure 7).

#### 4.1.2 Spinous layer

It consists of three to five layers of polygonal or cuboidal cells in the deeper region, which become flatter as they approach the surface (Figures 1, 4, and 8).

Its characteristic appearance results from cell shrinkage during dehydration procedures. This shrinkage creates intercellular spaces, except in areas where cell processes are joined by desmosomes. Together with the tonofibril-rich cytoplasm, these areas produce a spiny appearance (Figures 4, 5, and 6). Nuclear shape follows cell shape: in cuboidal cells, the nuclei tend to be spherical, whereas in flatter cells the nuclei become elliptical. The nuclei remain centrally located. Electron microscopy reveals tonofibrils surrounding the nucleus and inserting into desmosomes, thereby forming intercellular bridges (Figure 8).

#### 4.1.3 Granular layer

Under light microscopy, it consists of one to two layers of very thin, polygonal squamous cells with a homogeneous, translucent appearance and slight eosin staining.

Its main characteristic is the presence of coarse basophilic granules in the cytoplasm (Figure 2). The nucleus is central and flattened, with its long axis parallel to the cell surface, and frequently exhibits chromatolysis. Electron microscopy reveals degenerating cells. The cell membranes are clearly visible, with finely serrated outlines, whereas the tonofibrils are barely detectable (Figure 9).

#### 4.1.4 Lucid layer

This layer is very thin and virtually absent from the region examined. Where present, it consists of clear, eosinophilic, flattened cells with indistinct or absent nuclei (Figure 4).

#### 4.1.5 Keratinized layer

Also known as the stratum corneum, this is the most superficial layer of the epidermis. Its thickness varies, and it consists of highly compact, flattened, anucleate cells (Figure 4). Under electron microscopy, the cytoplasm appears filled with fibrils, the cytoplasmic membrane is well defined, and the desmosomes are degenerating. These cells are cornified and undergo desquamation.

## 4.2 Basement membrane

Under light microscopy, the basement membrane can be observed as a thin sheet at the interface between the dermis and epidermis, appearing as a condensation of the dermis at its contact surface with the epidermis and forming a basal membrane (Figures 5 and 6).

It is more clearly demonstrated in toluidine blue-stained sections than in hematoxylin-eosin-stained sections. Under electron microscopy, it consists of a basal lamina into which very fine filaments are inserted (Figure 7).

## 4.3 Dermis

Also known as the corium, it lies immediately beneath the basement membrane. It has projections and indentations toward the epidermis, allowing the two layers to interlock.

The dermis can be divided into two zones with poorly defined boundaries: the papillary and reticular zones. It contains fibroblasts, collagen fibers, blood vessels, lymphatic vessels, and epidermal derivatives such as hairs and glands (Figures 1 and 2).

### 4.3.1 Papillary zone

This is the most superficial portion of the dermis and follows the contour of the basal layer, forming invaginations.

It consists of loose connective tissue, collagen fibers, and fibroblasts (Figure 1). The fibroblasts have large, pale, ovoid nuclei, finely dispersed chromatin, and a prominent nucleolus. This region contains numerous capillaries (Figure 2).

### 4.3.2 Reticular zone

This is the deepest portion of the dermis and borders the hypodermis.

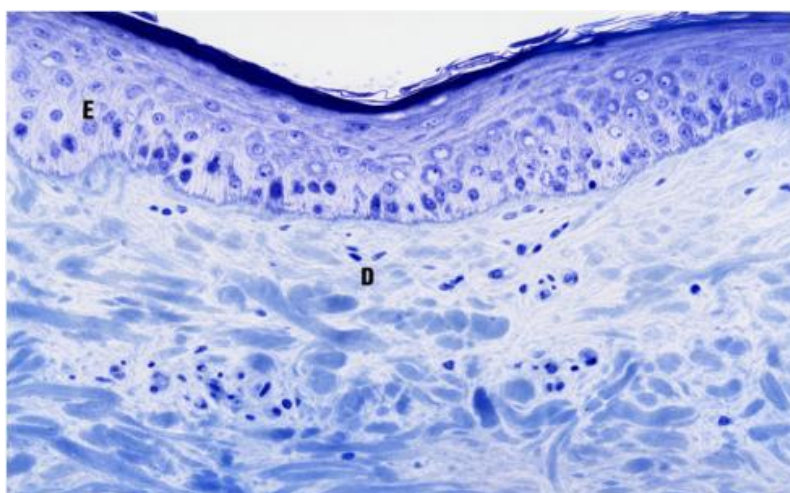
It consists of dense connective tissue with fewer cells and a greater amount of collagen and elastic fibers (Figures 1 and 2). This region contains sebaceous glands, hair follicles, and medium-caliber vessels (Figure 1).



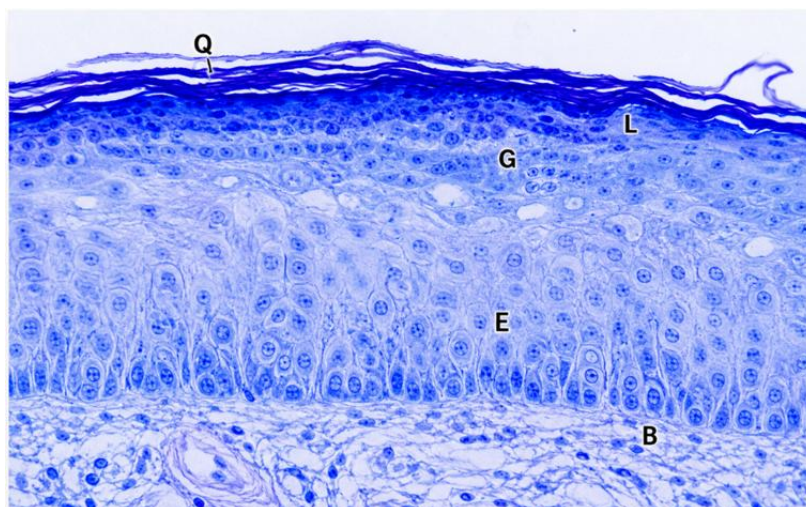
**FIGURE 1: Photomicrograph of a histological section (paraffin). General appearance of normal skin. The two portions, the dermis (D) and epidermis (E), are visible. In the reticular zone (r) of the dermis, numerous sebaceous glands (s), hair follicles (→), vessels (v), and collagen fibers (c) are observed; fibroblasts (f) predominate in the papillary zone (p). The epidermis consists of stratified squamous epithelium. Stain: hematoxylin and eosin. Magnification: 80×.**



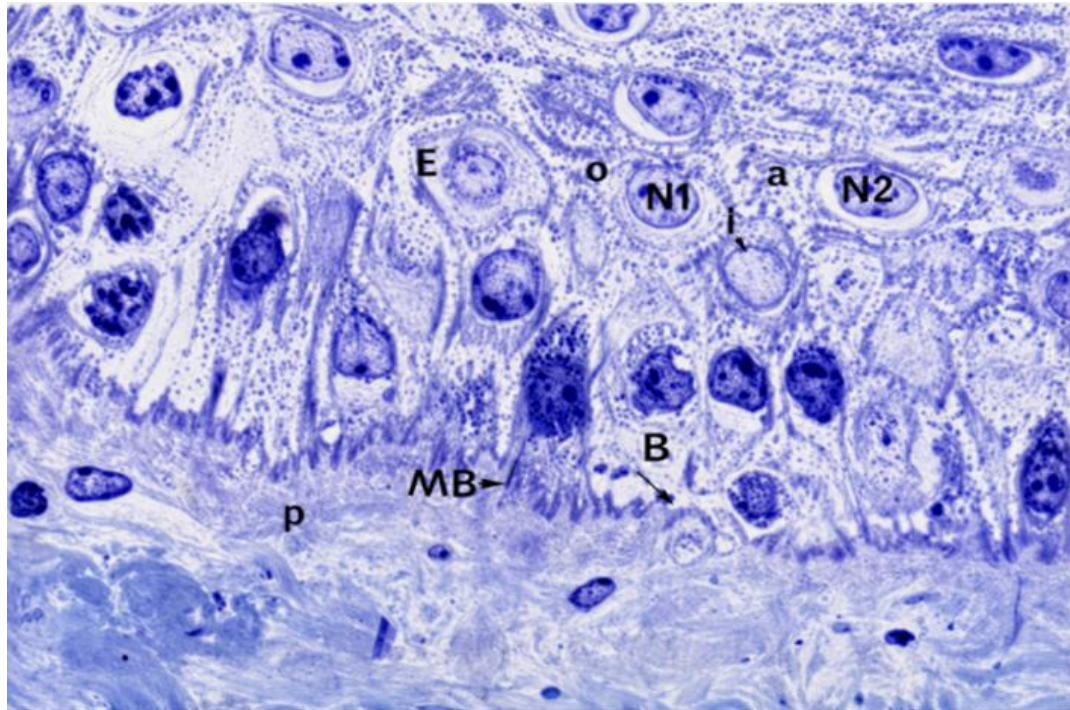
**FIGURE 2: Photomicrograph of a paraffin-embedded histological section of normal skin. The papillary zone (p) of the dermis, containing numerous capillaries (v), fibroblasts (f), and collagen fibers (c), and the epithelium, with the basal (B), spinous (E), granular (G), and keratinized (Q) layers, are visible. Stain: hematoxylin and eosin (H&E). Magnification: 160×.**



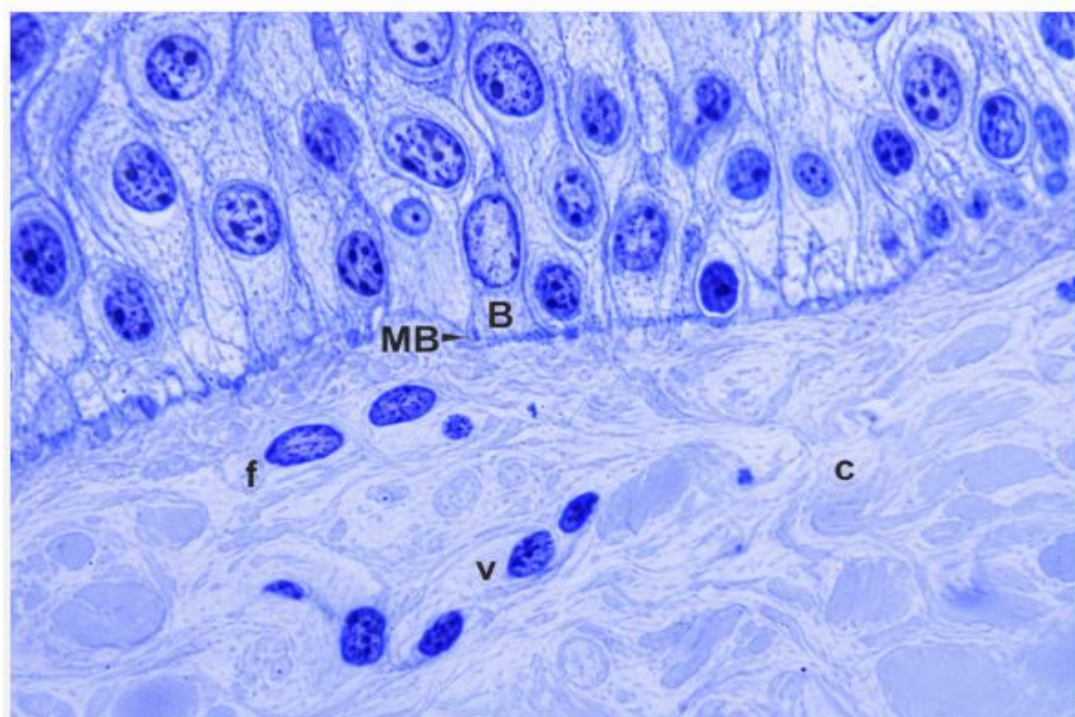
**FIGURE 3: Photomicrograph of a histological section (resin) showing the general appearance of normal skin. Portions of the dermis (D) and epidermis (E), with the characteristic stratified squamous epithelium, are visible. Stain: Toluidine blue. Magnification: 500×.**



**FIGURE 4: Photomicrograph of a resin-embedded histological section of the epidermis of normal skin, showing its different layers: basal (B), spinous (E), granular (G), lucid (L), and keratinized (Q). Stain: Toluidine blue. Magnification: 2,000×.**



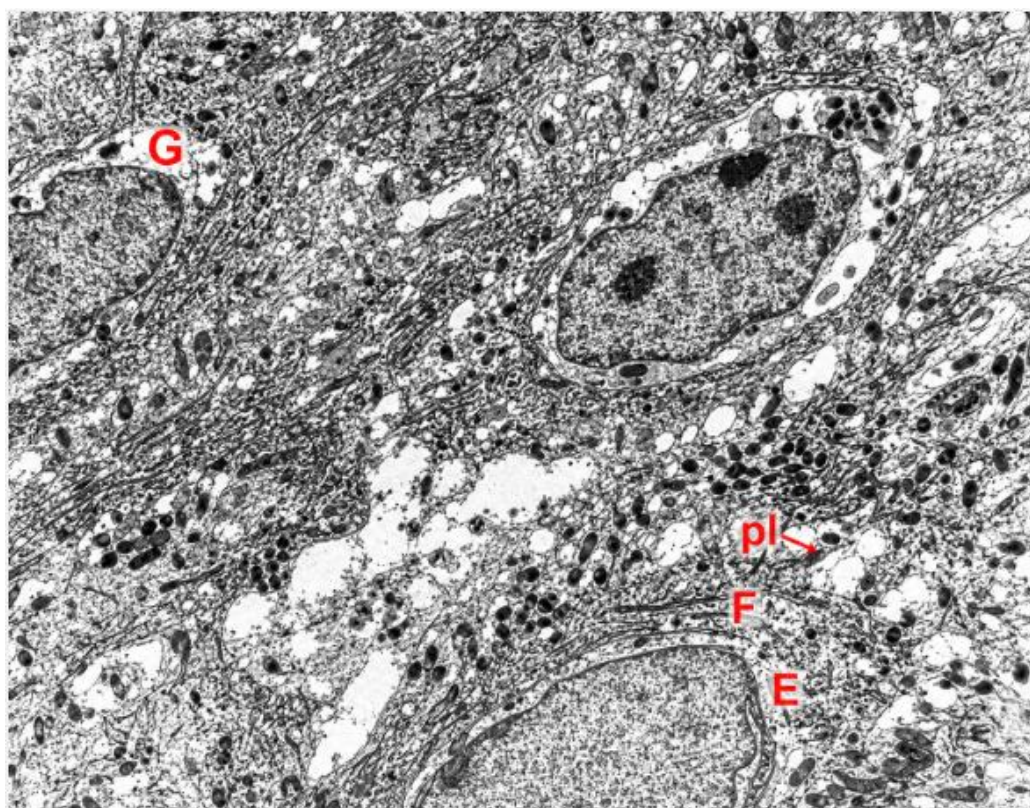
**FIGURE 5: Photomicrograph of a resin-embedded histological section of a portion of normal skin, showing the papillary dermis (p), basement membrane (MB), and epidermis with the basal (B) and spinous (E) layers. Intercellular bridges (i) and flattening of the cells and nuclei of this layer are visible; polygonal cells (o) with round nuclei (N1), and flattened cells (a) with oval nuclei (N2). Stain: Toluidine blue. Magnification: 2,000 $\times$ .**



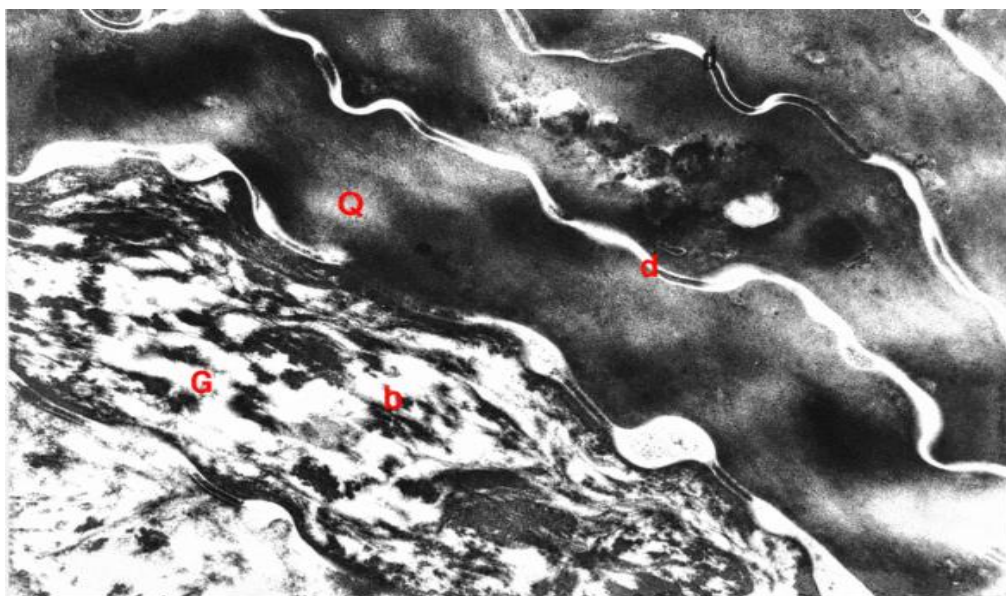
**FIGURE 6: Photomicrograph of a resin-embedded histological section of normal skin at the transition between the dermis and epidermis, showing collagen fibers (c), fibroblasts (f), and capillaries (v) in the dermis; the basement membrane (MB); and part of the epithelium, including the basal (B) and spinous (E) layers. Stain: Toluidine blue. Magnification: 2,000 $\times$ .**



**FIGURE 7: Electron micrograph of the transition area between the dermis (D) and epidermis (E), showing collagen fibers (c) in the dermis, the basement membrane (MB), and prismatic cells (m) of the basal layer and melanin granules (g) in the epidermis. Magnification: 5,800×.**



**FIGURE 8: Electron micrograph of the transition area between the granular (G) and spinous (E) layers, showing tonofibrils (F) surrounding the nucleus and intercellular bridges (pi) in the latter. Magnification: 5,850×.**



**FIGURE 9: Electron micrograph of the transition area between the granular (G) and keratinized (Q) layers, showing clear evidence of degeneration (D); cell boundaries and desmosomes (d) can be observed in both layers. Magnification: 39,000×.**

## V. DISCUSSION

In addition to protecting against physical and chemical injury and desiccation, the skin participates in the immune response through hypersensitivity reactions and antigen presentation by immunocompetent cells. Much of its structure consists of vascularized connective tissue that, when looser, facilitates the circulation of interstitial fluid. In buffaloes, the connective tissue of the dermis consists of bundles of collagen fibers interspersed with elastic and reticular fibers (Majeed et al., 1975; Cormack, 1987; Gayen et al., 1989).

Overall, the organization of buffalo skin was similar to that observed in cattle. Jenkinson & Nay (1975) also described this similarity among different bovids, noting that the main interspecific differences are related to skin thickness, the size of its components, and hair follicle density.

The epidermis of cattle and buffaloes consists of keratinized stratified squamous epithelium composed of the basal, spinous, granular, and cornified strata (Abdul Raheem et al., 2006; Abbasi et al., 2008). The stratum lucidum was not observed in either species, a finding like that reported by Hafez et al. (1955).

The stratum basale consisted of a single layer of basophilic cells ranging from cuboidal to columnar and resting on the basement membrane. The stratum spinosum comprised several layers of polyhedral cells joined by cytoplasmic processes and desmosomes. These structures contribute to the resistance of the epidermis to stretching, pressure, and abrasion, as described by Junqueira & Carneiro (2013).

Differences between cattle and buffaloes were observed mainly in the number of cell layers and epidermal thickness. In buffaloes, the stratum spinosum comprised six to eight cell layers in the dorsal region, 16 to 19 in the muzzle, and 10 to 12 in the other regions. In cattle, three to five layers were observed in the dorsal region, 14 to 17 in the muzzle, and seven to nine in the other regions. These results are consistent with the observations of Mulling (2000) in cattle.

The stratum granulosum also differed between the two species, consisting of three to five cell layers in buffaloes and one to three in cattle. The cells were flattened and contained basophilic keratohyalin granules, with progressive nuclear changes toward the stratum corneum, as described in buffaloes by Bagi & Vyas (1983).

The stratum corneum was thicker in buffaloes than in cattle in the different regions analyzed. In both species, it was thickest on the muzzle and thinnest in the dorsal region. The greater thickness found on the muzzle is probably related to greater abrasion in this region. The development of epidermal ridges also paralleled skin thickness, being more pronounced in regions with thicker skin and more evident in buffaloes. A similar result was reported by Abdul Raheem & Al-Hety (1999).

The epidermis and dermis are joined by the basement membrane, which follows the interdigitations between the two layers. In regions protected by a greater amount of hair, this interface has a more regular appearance, whereas in areas subjected to greater mechanical stress it is more undulating, as described by Dellmann & Brown (1976) and Al-Bideri (2003).

The dermis consists of connective tissue containing collagen, elastic, and reticular fibers, as well as hair follicles, sebaceous and sweat glands, arrector pili muscles, and blood vessels. It can be divided into papillary and reticular layers, although there is no clearly defined separation between them, as also observed by Panchal et al. (1999) in buffaloes.

Dermal thickness also differed between the two species and was greater in buffaloes. In both species, the cervical and dorsal regions were thicker, whereas the abdominal and thoracic regions had lower values. In buffaloes, numerous broad bundles of collagen fibers arranged in different directions were observed, whereas these fibers were less abundant in cattle.

Regarding the skin appendages, buffaloes had fewer hair follicles than cattle, a characteristic also described by Jenkinson & Nay (1975). Buffalo sebaceous glands had a multilobulated appearance, whereas those of cattle were predominantly bilobulated. This difference was observed in both the ano-caudal fold and cervical regions.

The lower density of hair follicles in buffaloes has been associated with the specific characteristics of the skin of this species and its thermoregulatory mechanisms. The dermis contains bundles of collagen fibers interspersed with elastic fibers, forming looser connective tissue with a considerable amount of interstitial fluid (Majeed et al., 1975; Gayen et al., 1989).

Reticular fibers showed a similar distribution in both species, forming fine networks adjacent to the basal layer, around hair follicles and glands, and in the region of the blood vessels. In the cervical region, no significant differences were observed between cattle and buffaloes in the distribution of collagen, elastic, and reticular fibers.

In the ano-caudal fold region, however, more evident differences were observed. In buffaloes, the collagen fiber bundles were more delicate and accompanied by a greater number of elastic fibers, whereas collagen fibers predominated in cattle. This finding is consistent with Gayen et al. (1989), who described a greater number of elastic fibers in regions where buffalo skin is thinner, such as the tail fold.

This difference between collagen and elastic fibers observed in the tail fold was not found in the cervical region, where fiber organization was similar between cattle and buffaloes. This result shows that the interspecific differences do not occur uniformly in all skin regions.

Mohan (1968) reported that buffaloes may exhibit a more intense reaction to tuberculin when it is administered in either the tail fold or cervical region. Considering the differences observed in the organization of the connective tissue in the ano-caudal fold of buffaloes, the greater looseness of the tissue in this region may favor a more evident local inflammatory response. However, this relationship should be interpreted with caution because the response to the tuberculin test does not depend solely on skin structure.

Transmission electron microscopy enabled a more detailed examination of the cellular organization of the epidermis and the components of the dermoepidermal junction and dermis in buffaloes and cattle. Although several histological studies have examined the skin of these species, relatively few reports specifically describe its ultrastructure, particularly in buffaloes. Some of the available information comes from studies of inherited skin diseases in which preserved skin regions or control animals were used to establish the normal ultrastructural pattern.

In the basal layer, keratinocytes rested on the dermoepidermal junction and were joined to one another by adhesion structures. In buffaloes, Riet-Correa et al. (1994) used transmission electron microscopy to demonstrate basal cells attached to the dermis, as well as a basement membrane zone containing basal lamina, hemidesmosomes, anchoring filaments, and anchoring fibrils. Although the study was conducted in Murrah buffaloes with hereditary mechanobullous dermatosis, these structures remained preserved, and an unaffected animal was also used as a control for ultrastructural evaluation. These findings provide an important reference for the organization of the dermoepidermal interface in *Bubalus bubalis*.

In cattle, the organization of the dermoepidermal junction was detailed by Medeiros et al. (2012) in a calf with junctional epidermolysis bullosa. Electron microscopy distinguished the lamina lucida and lamina densa and demonstrated the association of hemidesmosomes with the basal surface of keratinocytes. In the affected animal, separation occurred specifically within the lamina lucida, and the hemidesmosomes were reduced and poorly defined, reinforcing the importance of these structures in epidermal adhesion to the dermis. Thus, preservation of these structures in the samples examined may be considered a feature of dermoepidermal junction integrity.

Among keratinocytes, desmosomes were particularly evident structures associated with cytoplasmic intermediate filaments. In buffaloes, Riet-Correa et al. (1994) directly observed desmosomes and tonofilaments and demonstrated the attachment of tonofilaments to desmosomal adhesion plaques. In the affected animals described by the authors, loss of desmosomal adhesion caused widening of the intercellular spaces and suprabasal acantholysis, whereas tonofilaments frequently remained associated with the desmosomal plaques. This observation demonstrates the importance of the desmosome-tonofilament complex for the mechanical cohesion of buffalo epidermis.

A similar organization had previously been demonstrated in cattle by Jolly, Alley, and O'Hara (1973) in Angus calves with familial acantholysis. In that study, the changes were related to abnormal development of the desmosome-tonofilament complexes of basal and spinous cells. The authors observed abnormal aggregation of tonofilaments and impaired intercellular adhesion, whereas the basement membrane and dermis remained preserved. Thus, in both cattle and buffaloes, the available ultrastructural studies indicate a similar basic organization of the mechanisms responsible for adhesion between keratinocytes.

The structure of bovine desmosomes was subsequently examined at higher resolution by Owen et al. (2008) using desmosomes isolated from the stratum spinosum of the bovine muzzle and electron cryotomography. The authors demonstrated the closely apposed cell membranes, the intercellular region occupied by adhesion molecules, and cytoplasmic plaques associated with intermediate filaments. Although the muzzle is a specialized skin region, these observations confirm the structural organization of bovine desmosomes and their role in the mechanical resistance of the epithelium.

The tonofilaments observed in the cytoplasm of keratinocytes become progressively more evident during epidermal differentiation. Matoltsy (1975) demonstrated, by electron microscopy and biochemical methods, the close relationship among desmosomes, intermediate filaments, and keratinization. Keratin filaments form cytoplasmic bundles associated with desmosomal plaques, contributing to the formation of an intracellular network capable of distributing mechanical forces among epidermal cells. This relationship is consistent with the numerous tonofilaments observed by electron microscopy in the cells of the intermediate epidermal layers.

In the most superficial epidermal cells, the presence of keratohyalin granules represents a stage of keratinocyte differentiation preceding formation of the stratum corneum. The association between keratohyalin granules and keratin filaments was described ultrastructurally by Matoltsy (1975) and at high resolution by Lavker & Matoltsy (1971). The latter study was conducted on human and rat skin and should therefore be used as a comparative reference for granule morphology rather than as a specific description of bovine or buffalo skin. The scarcity of studies demonstrating these granules by electron microscopy in normal cattle skin, and particularly buffalo skin, limits more specific interspecific comparisons.

In the dermis, electron microscopy enabled the identification of fibroblasts associated with the fibrillar components of the extracellular matrix. Little specific information is available on the ultrastructure of dermal fibroblasts in normal cattle *in situ*. Yaeger et al. (1975) examined fibroblasts derived from calf skin by electron microscopy, although under culture conditions and in the context of dermatosparaxis. The authors demonstrated features related to the synthesis and processing of extracellular matrix components. This reference may therefore assist in identifying fibroblasts but should be used cautiously for direct comparisons with cells present in the normal dermis.

Collagen fibrils were the predominant fibrillar component of the dermis. In bovine skin, Naffa et al. (2019) directly examined collagen organization using different techniques, including transmission electron microscopy. The authors demonstrated large-diameter collagen fibrils in bovine skin and showed that their organization, diameter, and degree of association are related to the mechanical properties of the skin. These findings reinforce that dermal strength depends not only on the amount of collagen but also on the organization of collagen fibrils and bundles.

Another particularly useful demonstration of bovine collagen fibril morphology was presented by Carty et al. (2016). Although the study investigated dermatosparaxis in Limousin calves, the authors included skin from an age-matched control calf. Under electron microscopy, the control fibrils exhibited parallel organization and the characteristic cross-banding periodicity of collagen, whereas in the affected animals they were irregular and disorganized and showed loss of the periodic pattern. Thus, images from the control animal provide an appropriate reference for the ultrastructural appearance of normal collagen fibrils in bovine dermis.

Elastic fibers are less abundant than collagen structures but also participate in the three-dimensional organization of the dermis. Using scanning electron microscopy in different domestic mammals, including cattle, Meyer and Neurand (1987) demonstrated the elastic fiber network associated with dermal connective tissue bundles and interspecific differences in matrix organization.

At a more detailed ultrastructural level, Toyoshima et al. (1999) used immunoelectron microscopy in bovine tissues to demonstrate that mature elastic fibers have an amorphous central elastin component peripherally associated with microfibrils; they also identified the MAGP-36 protein at the elastin-microfibril interface.

The role of microfibrils in the organization of the elastic system of bovine skin was also demonstrated by Kielty et al. (1993), who examined fetal bovine skin and identified fibrillin-containing microfibrils. Although fetal tissue was used, the study confirms that characteristic microfibrillar components of the elastic system are present in the bovine dermal matrix and participate in its structural organization.

Overall, the ultrastructural findings indicate that buffalo and bovine skin share the same fundamental organization of the epidermis and dermoepidermal junction, including basal keratinocytes, desmosome-tonofilament complexes, hemidesmosomes, and anchoring structures, as well as a dermal matrix composed predominantly of collagen fibrils associated with fibroblasts and components of the elastic system. However, the available literature remains insufficient to establish specific ultrastructural differences between the normal skin of *Bubalus bubalis* and *Bos taurus*. This limitation is particularly evident for structures such as keratohyalin granules, dermal fibroblasts, and elastic fibers, for which virtually no comparative transmission electron microscopy studies exist between the two species. Comparative evaluation of these structures may therefore provide information that remains poorly documented regarding the ultrastructural organization of the skin of large ruminants.

## VI. CONCLUSION

Thus, despite the marked similarity in the histological organization of cattle and buffalo skin, differences were observed in epidermal and dermal thickness, the number of hair follicles, sebaceous gland morphology, and the distribution of collagen and elastic fibers. Some of these differences were more evident in the ano-caudal fold region, whereas the skin structure of the two species was more similar in the cervical region. These characteristics should be considered when comparing the cutaneous responses of cattle and buffaloes, particularly in intradermal tests.

Ultrastructural analysis demonstrated similar organization in the two species, revealing keratinocytes, desmosomes associated with tonofilaments, the basement membrane, fibroblasts, collagen fibrils, and components of the elastic system. No marked ultrastructural differences were observed between cattle and buffaloes, although the scarcity of studies, particularly in *Bubalus bubalis*, still limits more detailed comparisons. Thus, the findings of this study contribute to expanding knowledge of the histological and ultrastructural organization of the skin of these large ruminants.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

- [1] Abbasi, M., Gharzi, A., Karimi, H., & Khosravinia, H. (2008). Effects of sex on histological characteristics of various areas of skin in an Iranian native breed of sheep. *Journal of Animal and Veterinary Advances*, (7), 1503-1505.
- [2] Abdul Raheem, M. H., & Al-Hety, M. S. (1997). Histological and morphometrical study of the skin of black goat. *Iraqi Journal of Veterinary Sciences*, \*10\*, 59-71.
- [3] Abdul Raheem, M. H., Elias, A. M., & Ahmed, N. S. (2006). The correction factor of hair density in the skin of buffalo. *Iraqi Journal of Veterinary Sciences*, \*20\*(2), 101-106.
- [4] Abrahamsohn, P. (n.d.). 15-3 Skin. MOL - Online Microscopy, Institute of Biomedical Sciences, University of São Paulo. <https://mol.icb.usp.br/index.php/15-3-pele/>
- [5] Al-Bideri, A. W. M. (2003). *Ultrastructure and cellular organization in different anatomical sites of some adult Iraqi mammal's epidermis* [Master's dissertation, Al-Qadissiya University].
- [6] Brazilian Association of Buffalo Breeders. (n.d.). *Brazilian Association of Buffalo Breeders - ABCB*. <https://bufalo.com.br/>
- [7] Bagi, A. S., & Vtas, K. M. (1983). Morphological skin surti buffaloes compared to that of other breeds of buffaloes and ox and its role in thermoregulation. *Gujarat Agricultural University Research Journal*, \*12\*, 564-582.
- [8] Carty, C. I., Lee, A. M., Wienandt, N. A. E., Stevens, E. L., Alves, D. A., Browne, J. A., Bryan, J., Ryan, E. G., & Cassidy, J. P. (2016). Dermatosparaxis in two Limousin calves. *Irish Veterinary Journal*, \*69\*(15), 1-5.
- [9] Cormack, D. H. (1987). *Ham's histology* (9th ed.). J. B. Lippincott.
- [10] Dellmann, H. D., & Brown, E. M. (1976). *Textbook of veterinary histology*. Lea & Febiger.
- [11] Gayen, S., Prasad, G., & Sinha, R. D. (1989). Comparative histological studies on the skin of Indian buffalo and Black Bengal goats. *Indian Journal of Animal Sciences*, \*59\*, 920-924.
- [12] Gonzalez-Santander, R. (1969). *Electron microscopy techniques in biology*. Aguilar.

- [13] Hafez, E. S. E., Badreldin, A. L., & Shafei, M. M. (1955). Skin structure of Egyptian buffaloes and cattle with particular reference to sweat glands. *Journal of Agricultural Science*, \*46\*(1), 19-30.
- [14] IBGE - Brazilian Institute of Geography and Statistics. (2020). *Buffalo herd in Brazil*. <https://www.ibge.gov.br/explica/producao-agropecuaria/bubalinos/br>
- [15] Jenkinson, D. M., & Nay, T. (1975). The sweat glands and hair follicles of different species of Bovidae. *Australian Journal of Biological Sciences*, \*28\*(1), 55-68.
- [16] Jolly, R. D., Alley, M. R., & O'Hara, P. J. (1973). Familial acantholysis of Angus calves. *Veterinary Pathology*, \*10\*, 473-483.
- [17] Junqueira, L. C., & Carneiro, J. (2013). *Basic histology: Text and atlas* (12th ed.). Guanabara Koogan.
- [18] Kielty, C. M., Berry, L., Whittaker, S. P., Grant, M. E., & Shuttleworth, C. A. (1993). Microfibrillar assemblies of foetal bovine skin: Developmental expression and relative abundance of type VI collagen and fibrillin. *Matrix*, \*13\*(2), 103-112.
- [19] Lavker, R. M., & Matoltsy, A. G. (1971). Substructure of keratohyalin granules of the epidermis as revealed by high resolution electron microscopy. *Journal of Ultrastructure Research*, \*35\*(5), 575-581.
- [20] Majeed, M. A., Naseem, M., Toor, M. A., & Khan, L. R. (1975). Alcune particolarità strutturali della pelle di bufala. *Rivista di Zootechnia e Veterinaria*, \*6\*, 519-528.
- [21] Marques, J. R. F., & Cardoso, L. S. (1997). Buffalo production in Brazil and worldwide. In *The buffalo in Brazil: Proceedings of the Brazilian Symposium on Buffalo Production* (pp. 7-42). UFBA, School of Agronomy.
- [22] Matoltsy, A. G. (1975). Desmosomes, filaments and keratohyaline granules: Their role in the stabilization and keratinization of the epidermis. *Journal of Investigative Dermatology*, \*65\*, 127-142.
- [23] Medeiros, G. X., Riet-Correa, F., Armien, A. G., Dantas, A. F. M., Galiza, G. J. N., & Simões, S. V. D. (2012). Junctional epidermolysis bullosa in a calf. *Journal of Veterinary Diagnostic Investigation*, \*24\*, 231-234.
- [24] Meyer, W., & Neurand, K. (1987). A comparative scanning electron microscopic view of the integument of domestic mammals. *Scanning Microscopy*, \*1\*(1), 169-180.
- [25] Mohan, R. N. (1968). Diseases and parasites of buffaloes. II. Bacterial and fungal diseases. *Veterinary Bulletin*, (38), 647-659.
- [26] Mülling, C. K. W. (2000). Three-dimensional appearance of bovine epidermal keratinocytes in different stages of differentiation revealed by cell maceration and scanning electron microscopic investigation. *Folia Morphologica*, \*59\*(4), 239-246.
- [27] Naffa, R., Maidment, C., Ahn, M., Ingham, B., Hinkley, S. F. R., & Norris, G. E. (2019). Molecular and structural insights into skin collagen reveals several factors that influence its architecture. *International Journal of Biological Macromolecules*, \*128\*, 509-520.
- [28] Owen, G. R., Acehan, D., Derr, K. D., Rice, W. J., & Stokes, D. L. (2008). Cryoelectron tomography of isolated desmosomes. *Biochemical Society Transactions*, \*36\*(Pt. 2), 173-179.
- [29] Panchal, K. M., Vyas, K. N., & Vyas, Y. L. (1999). Histogenesis of skin and its adnexa of Surti buffalo. *Indian Journal of Veterinary Anatomy*, \*11\*(2), 158-160.
- [30] Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, \*7\*(1), 208-212.
- [31] Riet-Correa, F., Barros, S. S., Damé, M. C., & Peixoto, P. V. (1994). Hereditary suprabasilar acantholytic mechanobullous dermatosis in buffaloes (*Bubalus bubalis*). *Veterinary Pathology*, \*31\*(4), 450-454.
- [32] Roxo, E., Vasconcellos, S. A., Pinheiro, S. R., Baruselli, P. S., & Macruz, R. (1997). Comparative study of the skin structure of buffaloes (*Bubalus bubalis*) and cattle (*Bos taurus*). *Arquivos do Instituto Biológico*, \*64\*(2), 117-120.
- [33] Toyoshima, T., Yamashita, K., Furuichi, H., Shishibori, T., Itano, T., & Kobayashi, R. (1999). Ultrastructural distribution of 36-kD microfibril-associated glycoprotein (MAGP-36) in human and bovine tissues. *Journal of Histochemistry and Cytochemistry*, \*47\*, 1049-1056.
- [34] Yaeger, J. A., Church, R. L., & Tanzer, M. L. (1975). Structure of cultured fibroblasts from dermatosparax calves. *Veterinary Pathology*, \*12\*(1), 16-31.
- [35] Watson, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *Journal of Biophysical and Biochemical Cytology*, \*4\*, 475-478.