DISTINCTIVE FEATURES OF THE PIGLET SKIN REVEALED BY LIGHT AND TRANSMISSION ELECTRON MICROSCOPY

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Abstract. The present work was aimed to study the piglet skin by light and transmission electron microscopy. This investigation has considered six samples of the abdominal skin of six male two-month-old piglets, which were collected from the El-Amria abattoir, Alexandria governorate, Egypt. The skin constitutes three layers: the epidermis, dermis, and hypodermis. Some hair follicles appeared among epidermal cells. Melanin granules appeared scattered among the basal cells; in addition the granular layer was discrete and comprised of one layer of flattened keratinocytes. This paper presents a novel record by transmission electron microscopy (TEM) for the presence of the Merkel cells that were observed with neuroendocrine granules and different densities in the two-month-old male piglet. In addition, Langerhans-like cells appeared in between the keratinocytes of the stratum spinosum cells. The Langerhans-like cells had lucent cytoplasm with Birbeck granules (tennis-racket shape) around the nucleus, melanin granules. Also, there were numerous tonofilaments and Merkel cells in the keratinocytes of the stratum spinosum. Measurements for the epidermis and dermis thickness were 49.6±6 and 1005 ±19 µm. Our study concluded that pigskin is applicable, reliable animal model for human skin.

Keywords: Piglet; Skin; Light microscopy; transmission electron microscopy.

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Introduction

Skin tissue is the biggest organ of the body, consists of a compound layered structure, which provides a barrier between the body and the outside environment. However, this barrier remains slightly open and permeable to the environment to allow an exchange of heat, air, fluids containing matter of low molecular weight [30]. The pigskin has a role in the Egyptian tanning industry, where it is used in the manufacturing of light leather [10]. Porcine skin has been applied in varied study fields involving pharmacology, toxicology, and immunology [7]. Pigskin is a familiar model for human skin in health, plastic surgery, and cosmetics researches [16,23].

The literature of the skin of the domestic pig (one week to 14-weeks old) displays a variety of the ultrastructure of the epidermis and dermis. Small anchoring fibrils extend from the stratum basalis into the dermis. Mitochondria have condensed beneath the cellular nuclei of the stratum basalis. Upper stratum spinosum and stratum granulosum cells contain membrane-coating granules. The stratum corneum contains cells of varying densities. The epidermal non-keratinocytes of human skin were the melanocytes and Langerhans-suchlike cells [19].

Analogous to humans, the porcine epidermis has a stratified, multi-layered, keratinizing epithelium. Keratinocytes have organized in four layers separated from the dermis by an undulating basement membrane [13]. Porcine skin has been applied in numerous cases as a model for human skin [27]. That includes studies on injury healing [2,12,26], burns [1,24], and transdermal penetration, delivery, and toxicology [5,9,18,25,32].

This study aims to describe the ultrastructure of the skin of two-month-old piglets by light and transmission electron microscopy.

Materials and Methods

Study Design. Six skin samples of six male two-months-old piglets, which were collected from the El-Amria abattoir, Alexandria governorate, Egypt have been considered in the study.

Light microscopy. Small pieces (0.3-0.5 mm) were taken from the skin of the abdominal region of the male piglet. The samples were fixed in 10 formalin. After fixation, the samples were considerably washed in 70 alcohol (3 X 24 hrs) to get relief of the fixative before the other step of tissue processing. The tissue samples were also dehydrated in graded series of ethanol (80, 95, and absolute), cleared in xylene and impregnated, and embedded in paraffin wax. The paraffin blocks were sliced to 6μm thick and stained by Harris hematoxylin and eosin stain (H&E) [4].

Transmission electron microscopy. Small pieces (1 mm3) have taken from the skin of the abdominal region of the male piglet were directly fixed in a 6% of phosphate-buffered glutaraldehyde (pH 7.4, and 4°C) for 6 hrs (19). After the first fixation, tissues were washed in 0.1 M phosphate buffer every 15 min for 2 hrs. The samples were fast dehydrated by

adding increasing concentrations of ethanol, transferred to propylene oxide, and put overnight in an 11 mixture of propylene oxide and epoxy Araldite. Semi-thin sections (1mm) were sliced and stained with toluidine blue and examined with light microscopy to define areas fit for transmission electron microscopy. Ultrathin sections (60-100 nm) were also cut by a glass cutter with an L.K.B. Microtome and stained with uranyl acetate followed by lead citrate [11]. The ultrathin sections have been investigated with a JEOL transmission electron microscope operating at 100 Kv.

Measurements. The obtained images were analyzed by ImageJ (version1.534 December 2020) application to measure the different structures.

Results and Discussion

Light microscopy (LM). The skin of the two-month-old male piglet is composed of three layers: the epidermis, dermis, and hypodermis (Figure 1). Some hair follicles were among the epidermal cells (Figure 2). The epidermis consisted of stratified squamous keratinized epithelium. The basal cells have arranged at one row were consisted of low columnar to cuboidal basophilic cells with large nuclei, which were situated perpendicular to the basement membrane. Melanin granules appeared scattered among them (Figure 3). The spinosum cell layers (Malpighian layer) were polygonal in shape and arranged in several layers with round nuclei. The granular layer had one layer of flattened keratinocytes. However, the stratum granulosum was not apparent in all skin regions, particularly in the thin skin region. The corneum layer consisted of dead unnucleated keratinocytes (Figures 4 and 5).

Figure 1. Light photomicrograph of the piglet skin showing the following; epidermis (Ep) consists of the dermis (D) and hypodermis (H), the dermis contains the papillary dermis (dp), reticular dermis(rd) and sweat gland (Sw), hair shaft can be seen above epidermis (arrow). Stain H&E.

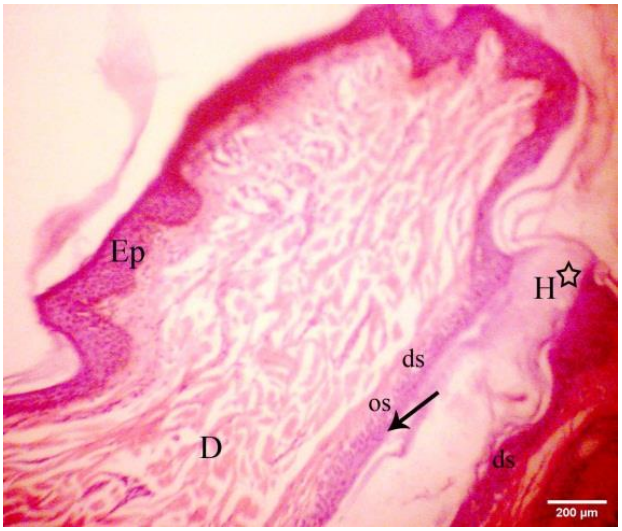

Figure 2. Light photomicrograph of the piglet skin composed of the following; epidermis (Ep), dermis (D). Inside the hair follicles (H), infundibulum (a strike), dermal sheath (ds), and the outer (os) and inner root sheath (arrow) were seen. Stain H&E

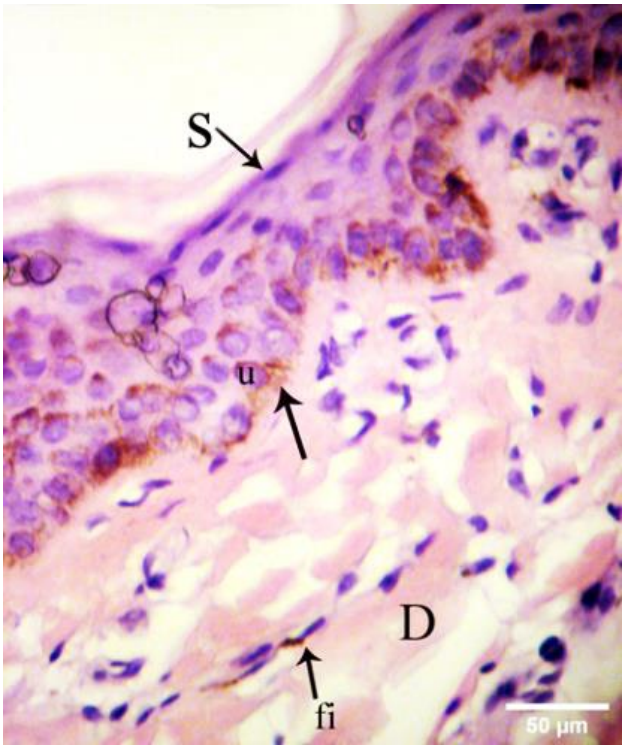

Figure 3. Light photomicrograph depicting the melanin granules (arrow) around, squamous keratinocyte (s), undifferentiated keratinocyte found among basal cell (U), the dermis (D) containing fibroblast (fi). Stain H&E. Micro

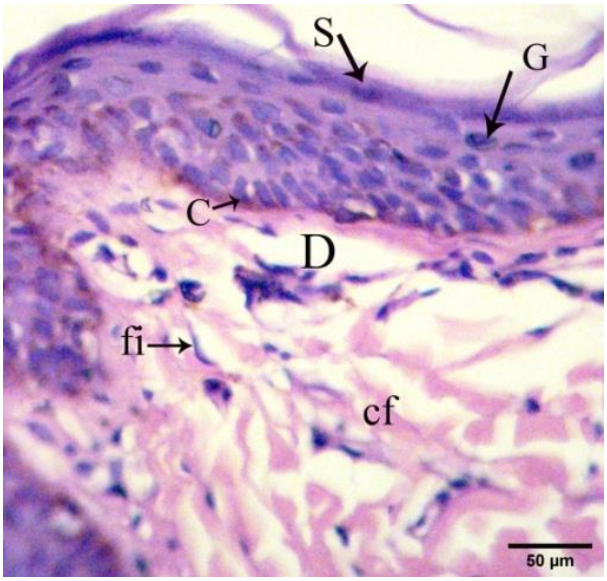

Figure 4. Light photomicrograph depicting that epidermis composed of the following; basal columnar cells (C), spinosum cells (p), granular cell (G), and squamous keratinocytes(S). The dermis (D) was enclosed numerous collagen fibers (cf) and fibroblasts (fi). Stain H&E. Micro

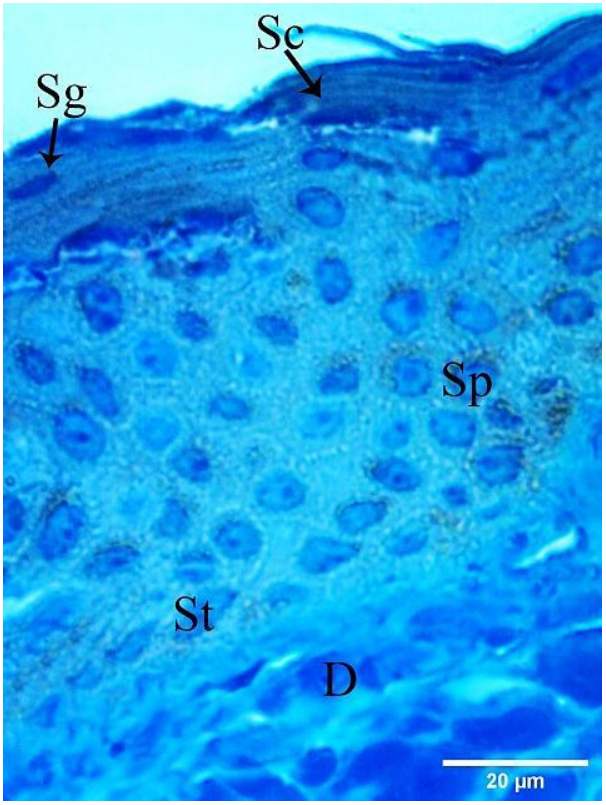

Figure 5. Light photomicrograph depicting the layers of the epidermis as the following: stratum basalis (St), stratum spinosum cell (Sp), stratum granulosum (Sg), and stratum corneum (Sc), Dermis (D). Stain Toluidine blue.

The dermis had two layers, the papillary dermis, and reticular dermis, both consisting of collagen bundles, fibroblast, and numerous blood vessels (Figure 6). Mast cells appeared around the blood vessels (Figure 7).

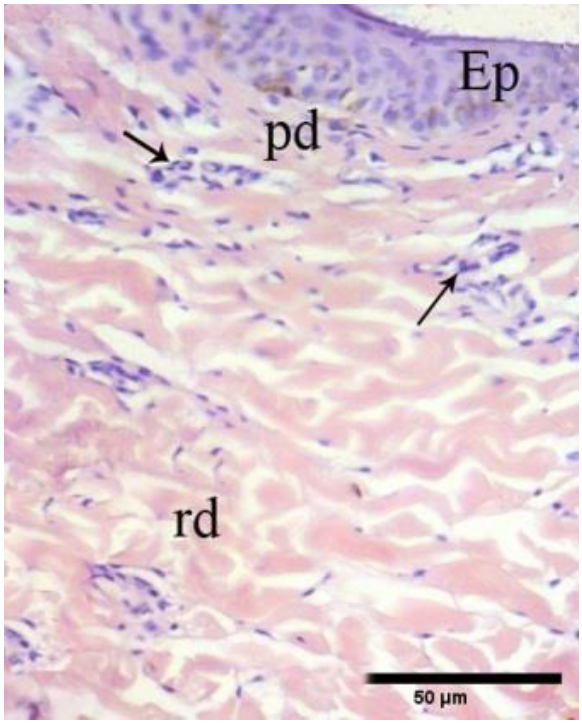

Figure 6. Light photomicrograph showing the epidermis (Ep) and dermis that composed of papillary dermis (pd) and reticular dermis (rd), numerous fibroblasts could be seen (arrow). Stain H&E

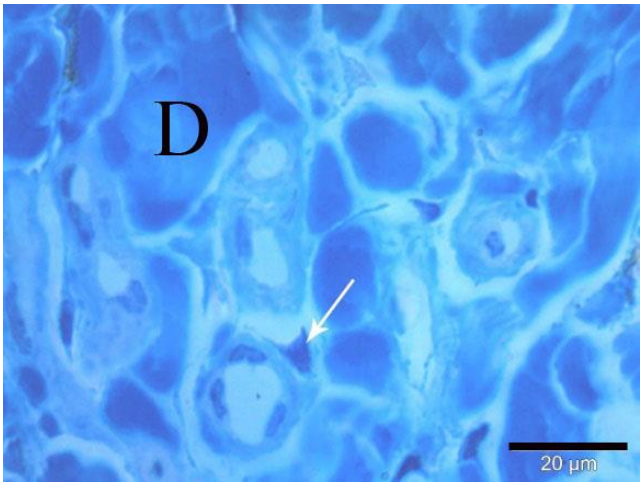

Figure 7. Light photomicrograph denoting the special structure dermis (D) that was the mast cell (arrow). Stain Toluidine blue

Transmission electron microscopy (TEM). In the two-month-old male piglet, the epidermis consisted of stratum basalis, stratum spinosum, stratum granulosum, and stratum corneum (Figure 8). The lower portion of the stratum basalis cells was deeply corrugated and invaginated into the underlying basal lamina, Also, spaces were found in between the basal

cells (Figures 9; 10). Interdigitation of adjacent cell processes at their lateral surfaces was seen. Nuclei of basal cells were oval occupying a large space of the cytoplasm.

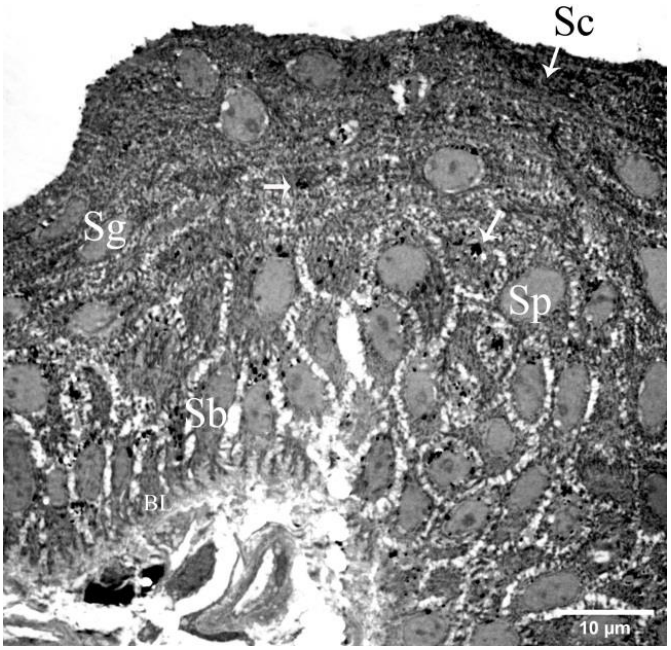

Figure 8. Transmission electron micrograph of the piglet skin showing the epidermal layers like the following: the stratum basalis (Sb), the stratum spinosum cell (Sp), the stratum granulosum (Sg), and the stratum corneum (Sc), the epidermis was attached to the basal lamina (BL) and scattered melanin granules (white arrow) at all the layers

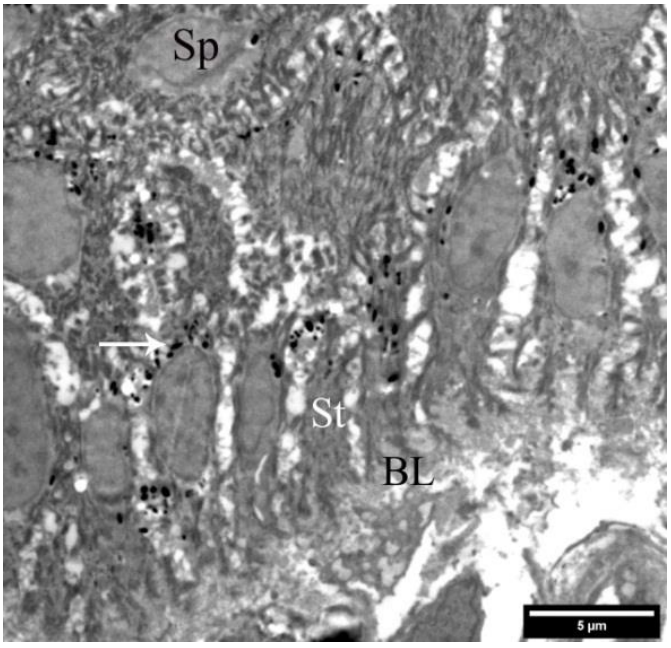

Figure 9. Transmission electron micrograph of the piglet skin showing the stratum basalis (St) attached to the corrugated basal lamina (BL), and related stratum spinosum cell (Sp) dorsally, and melanin granules (white arrow) were scattered around the stratum basalis cells

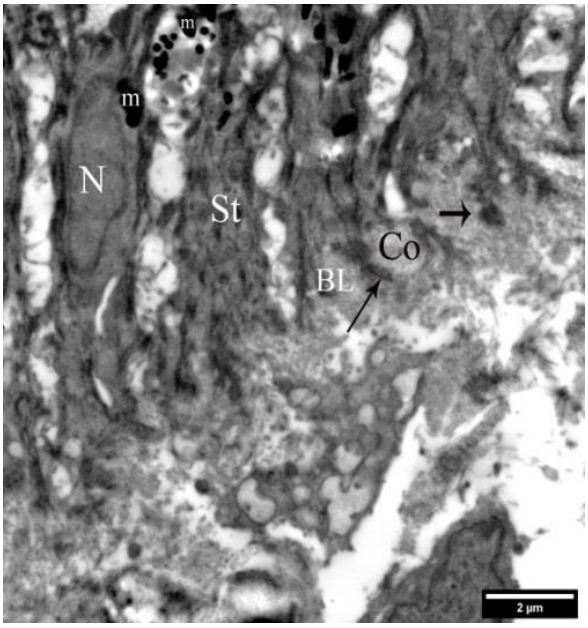

Figure 10. Transmission electron micrograph depicting the corrugated basal lamina (BL, hemidesmosomes (arrow), stratum basalis cells (St), melanin granules (m), nucleus (N) of the basal cell was perpendicular to the basal lamina and collagen fibrils (Co) were seen under the basal lamina

The keratinocytes of the stratum spinosum have linked by desmosomes. Their cytoplasm had numerous tonofilaments, and sometimes melanin granules appeared. The Langerhans-like cells appeared in between keratinocytes of the stratum spinosum cells. The Langerhans-like cell has lucent cytoplasm with few racket shape granules (Birbeck granules) around the nucleus. It has no junctional complex with the adjacent cells (Figures 11; 12).

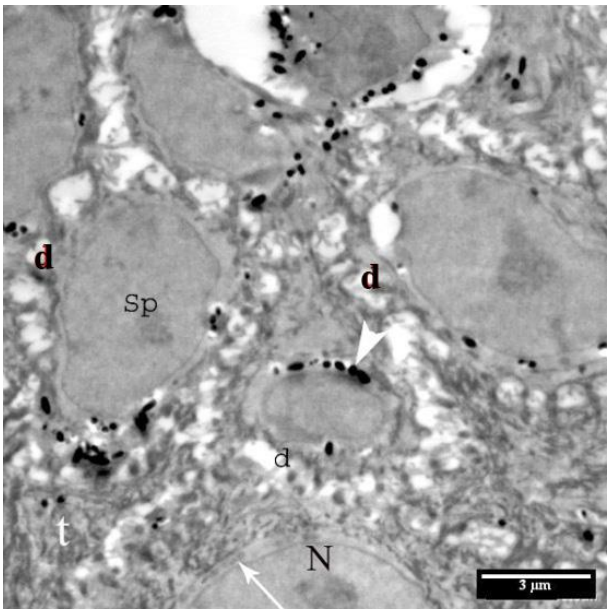

Figure 11. Transmission electron micrograph depicting the stratum spinosum (Sp) that explained the desmosomes (d), tonofilaments (t), melanin granules (arrowhead), Langerhans-like cells (white arrow), and nucleus (N)

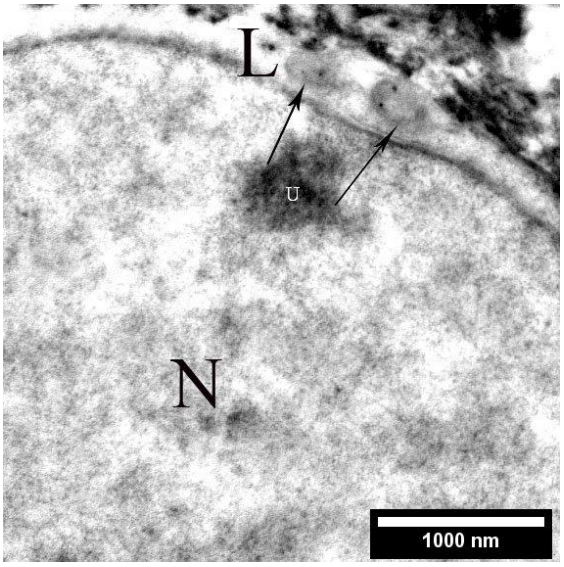

Figure 12. Transmission electron micrograph showing Langerhans-like cells (L) with clear cytoplasm, nucleus (N), Birbeck granules (arrow), and nucleolus (U)

Merkel cells were apparent with neuroendocrine granules with different densities, some of them were electron-dense, and the others were electron-lucent. Few filaments have located near the nucleus (Fig.13).

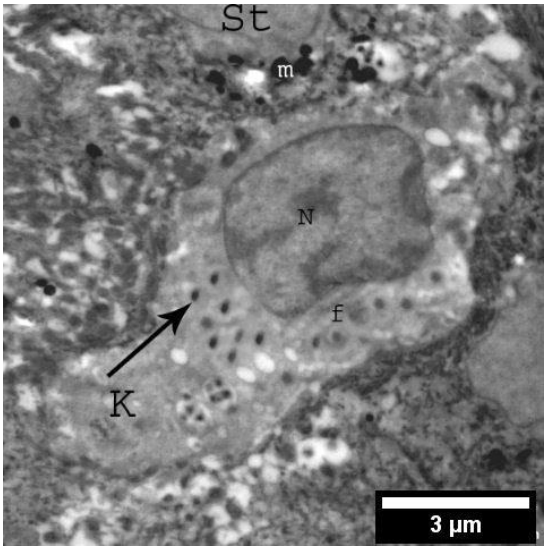

Figure 13. Transmission electron micrograph showing the Merkel cells (K) containing neuroendocrine granules (arrow), few filaments (f), nucleus (N), and the melanin granules (m) were seen adjacent to the Merkel cells

Measurements. The thickness of the epidermis varied from 25.8 ± 7 µm dorsal to the dermal papillae and 49.6 ±6 µm. The thickness of the dermis was 1005 ±19 µm, and their dermal papillae thickness was 27±12 µm. The number of the layers of the stratum basalis, stratum spinosum, stratum granulosum, and stratum corneum were 1, 4, 3, and 9 layers, respectively; and the thickness of the stratum basalis, stratum spinosum, stratum granulosum, and stratum corneum were 8.6 ± 0. 5, 39.8 ±0.8, 8 ± 0 .9 and 8 ± 0 .9µm, respectively (Fig.14 and Table 1).

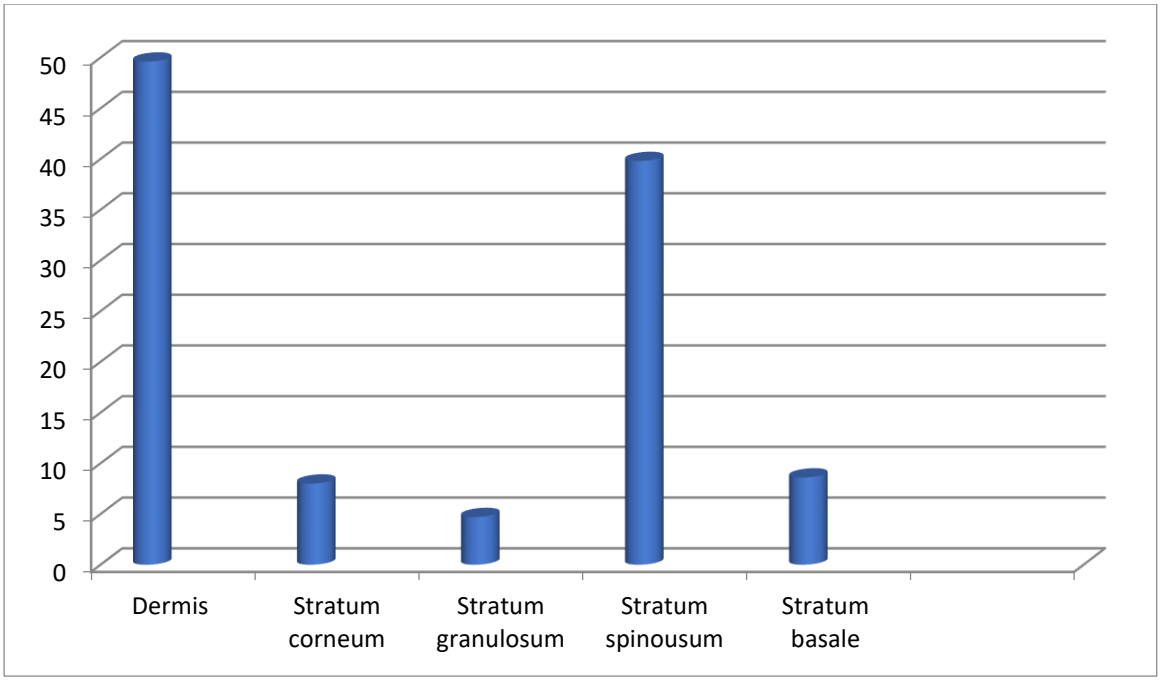

Figure 14. Measurements of the different parameters of the epidermis by µm.

Table 1. Measurements of the different parameters (mean ± *SD*) of the dermis and epidermis count cell layers.

The thickness of the epidermis (µm)	49.6 ±6
The thickness of the epidermis dorsal to the dermal papillae (µm)	25.8 ± 7
The thickness of the dermal papillae	27 ± 12
The thickness of the dermis (µm)	1005 ±19
The thickness of the stratum basal (µm)	8.6 ± 0. 5
The thickness of the stratum spinosum (µm)	39.8 ± 0.8
The thickness of the stratum granulosum (µm)	4.7 ±1.4
The thickness of the stratum corneum (µm)	8± 0 .9
Number of layers of the stratum basalis	1 ± 0.0
Number of layers of the stratum spinosum	4 ± 0.6
Number of layers of the stratum granulosum	3 ± 0.9
Number of layers of the stratum corneum	9 ± 0.5

The two-month age of the piglet was the age of marketing in Egypt. Egyptian authorities culled 400,000 pigs as a preventive measure against swine flu, despite many local and international health organizations arguing that pigs were not the source of swine flu [3]. Consequently, the swineherd sells piglets at two months old, while the weaning age ranges from 4 to 7 weeks [28]. In this study, we found that the epidermis of the male piglet consisted of stratified squamous keratinized epithelium. The basal cells were low columnar to cuboidal basophilic cells with large nuclei. The spinosum cell layers were polygonal in shape and arranged in several layers with round nuclei. The granular layer was discrete and comprised of one layer of flattened keratinocytes. The corneum layer consisted of dead unnucleated keratinocyte, similar results were found in human skin [7; 13]. In our paper, the focus of attention was on TEM. The lower portion of the stratum basalis cells was deeply corrugated and invaginated into the underlying basal lamina in the two-month-old piglets and that was

similar to that reported previously in the 14-week-old domestic pig. The epidermal-dermal junction was frequently irregularly convoluted. The amplitude of these convolutions tended to grow somewhat with age, peaking at 7 weeks. Since the ultrastructure of the epidermal-dermal junction in the pig appears like that depicted in humans, the pig may act as a model for studying wound healing or disease states of this region [19]. Porcine keratinocytes have been isolated and used for wound healing studies [8; 15; 26; 29; 31], including studies involving gene therapy [21].

This study showed the Langerhans-like cells among keratinocytes of the stratum spinosum layers. They had lucent cytoplasm with few racket shape granules (Birbeck granules) around the nucleus. As in humans, the porcine Langerhans-like cells contain Birbeck granules and express CD1 [17]. The Langerhans-like cells cytoplasm contained fine filaments and no typical Langerhans cell granules were identified [19]. In 6-months-old white crossbred pigs, the presence of Birbeck granules in porcine Langerhans cells and the absence of tonofilaments and melanocytes have been reported [22]. The Langerhans-like cells are specialized antigen-presenting cells residing within mammalian multilayered epithelia at the steady-state and play a central role in regulating cutaneous immune responses [7; 20].

Merkel cells were observed with their neuroendocrine granules which had different densities. The Merkel cells with neuroendocrine granules have been found in pig snout epidermis [6], while some authors have reported that the Merkel cells were not found in pig skin [19]. Also, there was no reactivity of antibodies on the porcine skin, either in the epidermis or in the hair follicles [7]. This result suggests that Merkel cells in flank porcine skin are rarer than in human skin. The majority of the porcine skin cells express antigens that detected immunohistochemically with antibodies recognizing their human counterparts; however, the subset of antibodies that recognize specific cells/structures in humans did not react in the porcine skin specimens. This absence of reactivity is due to the absence of the corresponding cells such as melanocytes, Merkel cells, eccrine sweat glands from the specific porcine site such as the flank. It seems that some porcine antigens are phylogenetically different from the antihuman antibodies [7; 20].

Our work reveals that the thickness of the dermis of the two-month-old piglet was 1005 ± 19 μm that was less than that was recorded at the caudal abdomen of the adult pig that was 1167 ± 78.8 μm while in the human abdomen, it was 1831.5 ± 29.6 μm [14]. The thickness of the epidermis of the two months piglet was 49.6 ±6 μm less than that was recorded at the caudal abdomen of the adult pig was 53.4 ± 2.2 μm while in the human abdomen was 59.9 ± 8.7μm [14]. Due to limited access to human skin explants, animal skin is frequently used for cosmetics and plastic surgery. The most relevant, dependable animal model for human skin is the pig skin, as recommended by the Scientific Committee on Consumer Safety opinion [23].

Conclusion

Our paper presents the two-month-old piglet integument with Langerhans-like cells seen in between keratinocytes of stratum spinosum cells. Langerhans-like cell has lucent cytoplasm that had few Birbeck granules around the nucleus less than expected where they are many in the different animals, melanin granules and many tonofilaments at the keratinocytes of the stratum spinosum and Merkel cells. These results may be of value in the identification of pig skin.

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Author Contributions

Conception and design: Mahmoud Elghoul, Mohamed Alsafy, Samir El-Gendy, and Amira Derbalah. Acquisition of data: Mohamed Alsafy, Samir El-Gendy, and Amira Derbalah. Analysis and interpretation of data: Mahmoud Elghoul, Mohamed Alsafy, Samir El-Gendy, and Amira Derbalah. Drafting the article: Mohamed Alsafy, Samir El-Gendy, and Amira Derbalah. Revising article for intellectual content: Mohamed Alsafy, Amira Derbalah, and Samir El-Gendy. Final approval of the completed article: Amira Derbalah, Mohamed Alsafy, Samir El-Gendy, and Mahmoud Elghoul.

Disclosure of Conflict of interest

The authors have no disclosures to declare.

Compliance with Ethics Standards

This study followed the guidelines for the care and use of laboratory animals and animal welfare and ethics. Institutional ethical permission was granted by Alexandria University (nonASPA, ethical review number 1911 161206).

References

[1] Abdullahi, A., Amini-Nik, S. & Jeschke, M. (2014). Animal models in burn research. *Cellular and molecular life sciences*, 71(17) 3241-3255.

[2] Ansell, D. M., Holden, K. A. & Hardman, M. J. (2012). Animal models of wound repair: Are they cutting it? *Experimental dermatology*, 21(8) 581-585.

[3] Audi, N. (2009). Culling pigs in flu fight, Egypt angers herders and dismays UN. *New York Times.* 30.

[4] Bancroft, J. D. & Gamble, M. (2008). *Theory and practice of histological techniques.* 6th edition (Elsevier Health Sciences) pp. 121-134.

[5] Barbero, A. M. & Frasch, H. F. (2009). Pig and guinea pig skin as surrogates for human in vitro penetration studies: a quantitative review. *Toxicology in vitro*, 23(1) 1-13.

[6] Beiras, A., García-Caballero, T., Espinosa, J. & Gallego, R. (1986). Staining of Merkel cells of pig snout epidermis using the uranaffin reaction: Morphometric analysis of neuroendocrine granules. *Differentiation*, 32(1) 89-92.

[7] Debeer, S., Le Luduec, J.-B., Kaiserlian, D., Laurent, P., Nicolas, J.-F., Dubois, B. & Kanitakis, J. (2013). Comparative histology and immunohistochemistry of porcine versus human skin. *European journal of dermatology,* 23(4) 456-466.

[8] Eldardiri, M., Martin, Y., Roxburgh, J., Lawrence-Watt, D. J. & Sharpe, J. R. (2012). Wound contraction is significantly reduced by the use of microcarriers to deliver keratinocytes and fibroblasts in an in vivo pig model of wound repair and regeneration. *Tissue Engineering Part A.*, 18(6) 587-597.

[9] Godin, B. & Touitou, E. (2007). Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. *Advanced drug delivery reviews,* 59(11) 1152-1161.

[10] Haddad, S. S. G. (2018). The status of leather supply chain in Egypt: An exploratory study for the innolea projects. In: *International Conference on Advanced Materials and Systems* (The National Research & Development Institute for Textiles and Leather-INCDTP) pp. 105-110.

[11] Hayat, M. (2000). *Basic Techniques For Transmission Electron Microscopy*. 3rd edition (Academic Press).

[12] Jung, Y., Son, D., Kwon, S., Kim, J. & Han, K. (2013). Experimental pig model of clinically relevant wound healing delay by intrinsic factors. *International wound journal,* 10(3) 295-305.

[13] Kanitakis, J. (2002). Anatomy, histology and immunohistochemistry of normal human skin. *European journal of dermatology,* 12(4) 390-401.

[14] Khiao In, M., Richardson, K. C., Loewa, A., Hedtrich, S., Kaessmeyer, S. & Plendl, J. (2019). Histological and functional comparisons of four anatomical regions of porcine skin with human abdominal skin. *Anatomia Histologia Embryologia,* 48(3) 207-217.

[15] Kiwanuka, E., Hackl, F., Philip, J., Caterson, E. J., Junker, J. P. & Eriksson, E. (2011). Comparison of healing parameters in porcine full-thickness wounds transplanted with skin micrografts, split-thickness skin grafts, and cultured keratinocytes. *Journal of the American College of Surgeons,* 213(6) 728-735.

[16] Lawson, L., Freytag, L. & Clements, J. (2007). Use of nanocarriers for transdermal vaccine delivery. *Clinical Pharmacology & Therapeutics,* 82(6) 641-643.

[17] Liu, Y., Chen, J.-y., Shang, H.-t., Liu, C.-e., Wang, Y., Niu, R. & Wei, H. (2010). Light microscopic, electron microscopic, and immunohistochemical comparison of Bama minipig (Sus scrofa domestica) and human skin. *Comparative medicine,* 60(2) 142-148.

[18] Mahl, J. A., Vogel, B. E., Court, M., Kolopp, M., Roman, D. & Nogués, V. (2006). The minipig in dermatotoxicology: methods and challenges. *Experimental and Toxicologic Pathology,* 57(6) 341-345.

[19] Monteiro-Riviere, N. & Stromberg, M. (1985). Ultrastructure of the Integument of the Domestic Pig (Sus scroh) from One through Fourteen Weeks of Age. *Anatomia Histologia Embryologia,* 14(2) 97-115.

[20] Nfon, C. K., Dawson, H., Toka, F. N. & Golde, W. T. (2008). Langerhans cells in porcine skin. *Veterinary immunology and immunopathology*, 126(3) 236-247.

[21] Pfützner, W., Joari, M. R., Foster, R.-A. & Vogel, J. C. (2006). A large preclinical animal model to assess ex vivo skin gene therapy applications. *Archives of dermatological research*, 298(1) 16-22.

[22] Romano, J. & Balaguer, L. (1991). Ultrastructural identification of Langerhans cells in normal swine epidermis. *Journal of Anatomy*, 179 43.

[23] SCCS. (2010). Basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients. In *the Scientific Committee on Consumer Safety adopted this opinion at its 7th plenary meeting.*

[24] Sheu, S.-Y., Wang, W.-L., Fu, Y.-T., Lin, S.-C., Lei, Y.-C., Liao, J.-H. & Yao, C.-H. (2014). The pig as an experimental model for mid-dermal burns research. *Burns*, 40(8) 1679-1688.

[25] Simon, G. A. & Maibach, H. I. (2000). The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations–an overview. *Skin Pharmacology and Physiology*, 13(5) 229-234.

[26] Sullivan, T. P., Eaglstein, W. H., Davis, S. C. & Mertz, P. (2001). The pig as a model for human wound healing. *Wound repair and regeneration*, 9(2) 66-76.

[27] Tang, W. & Bhushan, B. (2010). Adhesion, friction and wear characterization of skin and skin cream using atomic force microscope. *Colloids and Surfaces B: Biointerfaces*, 76(1) 1-15.

[28] Van der Meulen, J., Koopmans, S., Dekker, R. & Hoogendoorn, A. (2010). Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal functionality. *Animal*, 4(10) 1653-1661.

[29] Vardaxis, N., Brans, T., Boon, M., Kreis, R. & Marres, L. (1997). Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. *Journal of Anatomy*, 190(4) 601-611.

[30] Verma, D. D., Verma, S., Blume, G. & Fahr, A. (2003). Particle size of liposomes influences dermal delivery of substances into skin. *International journal of pharmaceutics*, 258(2) 141-151.

[31] Yan, G., Sun, H., Wang, F., Wang, J., Wang, F., Zou, Z. & Su, Y. (2011). Topical application of hPDGF-A-modified porcine BMSC and keratinocytes loaded on acellular HAM promotes the healing of combined radiation-wound skin injury in minipigs. *International journal of radiation biology*, 87(6) 591-600.

[32] Yu, M., Guo, F., Ling, Y., Li, N. & Tan, F. (2015). Topical skin targeting effect of penetration modifiers on hairless mouse skin, pig abdominal skin and pig ear skin. *Drug delivery*, 22(8) 1053-1058.