REGIONAL HISTOLOGY OF STRATUM GERMINATIVUM OF THE SKIN IN LARGE WHITE YORKSHIRE PIGS

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Abstract

Histology of the stratum germinativum of the skin from different regions of the body in Large White Yorkshire pigs was studied using 12 animals of six to ten months of age. Stratum basalis was made up of a single layer of columnar cells which became cuboidal towards the apex of the dermal papilla. Cells of stratum basalis rested on a basement membrane made up of collagen and reticular fibres. Clear cells could be located in the stratum basalis and stratum spinosum. These cells possessed the characteristics of the Langerhan's cells. Stratum spinosum was the thickest layer of the epidermis. Cells of this layer were large, irregular and polyhedral with distinct cell boundaries. Thickness of this layer was maximum in the snout and was thinner at the dorsal and ventral neck regions and in the abdominal regions. Here the cell boundaries were indistinct and the nucleus showed peripheral condensation of chromatin. At the carpal region, the cells were comparatively smaller and closely packed. Cytoplasmic staining property of the cells of the stratum spinosum varied in different layers. Prekeratin granules were detected in the upper layers of stratum spinosum.

Key words: *Pig, Stratum germinativum, Regional histology*

The stratum basale and stratum spinosum of the skin comprise the stratum germinativum. The proliferative and regenerative abilities of the epidermal cells of the skin suggest a key role of the keratinocyte stemcells and progenitor cells in the tissue maintenance, repair and renewal. Banks (1993) noticed that the thickness of stratum germinativum varied in different regions of the mammalian integument. Because dermatologic, cutaneous, pharmacologic and toxicological studies utilise the skin from swine, it is important to realise the remarkable similarities and significant differences existing between the skin of pigs and human beings. The high prolificacy, short generation interval, fast growth rate and other biological advantages contribute to the selection of pig as a biological experimental model in the field of research. Hence, the present work was undertaken to study the regional histology of stratum germinativum in Large White Yorkshire pigs.

Materials and Methods

Histological studies were conducted on the skin of Large White Yorkshire pigs of six to ten months of age. Skin samples from eight body regions were collected from 12 animals (six each from either sex) from the Meat Technology unit of Kerala Agricultural University, Mannuthy. The age, sex and bodyweight of the animals were recorded. Skin samples of 2 cm2 area were collected immediately following exsanguination from eight areas of the body viz., the snout, dorsal nasal, dorsal neck, ventral

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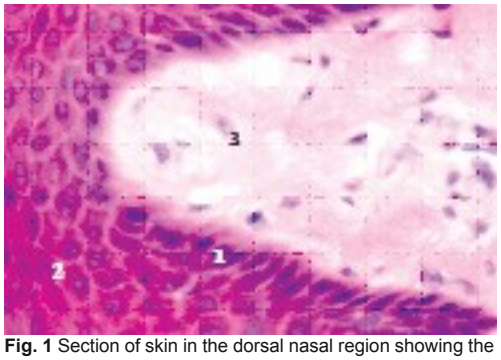

Fig. 1 Section of skin in the dorsal nasal region showing the layers of the stratum germinativum. H& E. x 400
1. Stratum basalis, 2. Stratum spinosum, 3. Dermal papilla

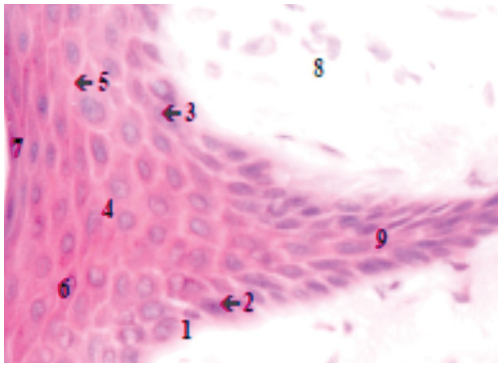

Fig. 2 Section of skin in the dorsal nasal region showing rete peg. H& E. x 400
1. Stratum basalis, 2. Nucleus, 3. Nucleolus, 4. Stratum spinosum, 5. Clear zone, 6. Flattened cells of Stratum spinosum, 7. Stratum granulosum, 8. Dermal papilla 9. Rete Peg

neck, dorsal abdomen, lateral abdomen, ventral abdomen and carpal regions and fixed in 10% neutral buffered formalin. The tissue pieces were processed in high melting paraffin (melting point, 58-60oC). Sections of 4 to 5 µm thickness were taken and stained using Haematoxyllin and Eosin; Ayoub-Shklar method for keratin and prekeratin (Luna, 1968) and PAS Alcian blue method for mucosubstance (Singh and Sulochana, 1996). Micrometrical parameters were measured using an ocular micrometer and the regression wise comparison of these parameters was done by one-way Analysis of variance Technique (Cochran and Cox, 1992).

Results and Discussion

The epidermis was formed of four layers, viz., stratum basalis, stratum spinosum, stratum granulosum and stratum corneum except in snout, dorsal nasal and ventral abdominal regions, where an additional layer, the stratum lucidum was noted. Stratum germinativum composed of both stratum basalis and stratum spinosum of the epidermis and these layers

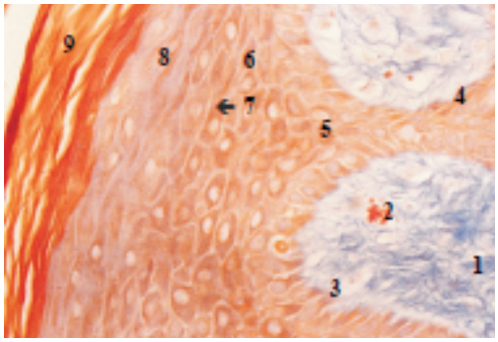

Fig. 3 Section of skin in the neck dorsal region. Ayoub-Shklar method for keratin and prekeratin x 400
1. Dermal papilla, 2.Capillaries, 3.Cytoplasmic processes of the basal cells, 4. Basement membrane, 5.Deeper layer of Stratum spinosum

contributed to the renewal of epidermal cells. The epidemis projected into the dermis as the rete pegs, which were abundant in the snout and the dorsal nasal.

Stratum basalis

Thickness of the stratum basalis in different regions of the body in both male and female pigs varied considerably. Stratum basalis was made up of a single layer of columnar cells with their axes perpendicular to the basal lamina (Fig. 1). The columnar cells measured about 18.50 µm in height and 9.25 µm in width. The nucleus was oval in shape with a prominent nucleolus of 11.10 µm in length and 5.55 µm in width (Fig. 2). Height of these cells gradually reduced towards the apex of the dermal papilla and they became cuboidal in shape (Fig. 1). These cuboidal cells measured 14.80 µm in height and 11.10 µm in width. Nucleus became almost spherical with a diameter of 4.44 µm. From the lower surface of the stratum basalis cells, cytoplasmic processes grew into the papillary layer of the dermis (Fig. 3). In the carpal region, all the basal cells were almost cuboidal in shape. Montagna (1962) opined that in early embryonic stage, the cells of stratum basalis were cuboidal with a clearly stainable large nucleus and smooth basal surface. This contributed to the easy peeling of the epidermis from the dermis. Later, the basal cells became columnar and the lower surface of the cells became serrated as the cytoplasmic processes invaded into the dermis. Copenhaver *et al.* (1971) suggested that the irregular boundaries of the epidermis and its underlying connective tissue as well as the half desmosomes accounted for the adherence between epidermis and dermis.

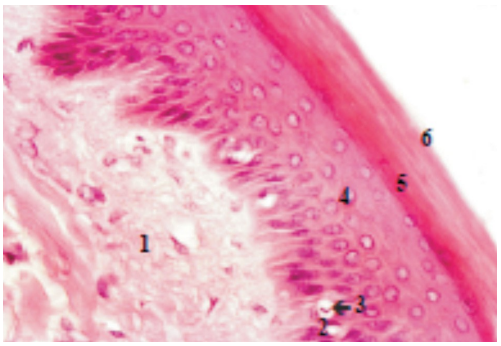

Fig. 4 Section of skin in the ventral abdominal region. H& E. x 400

1. Papillary dermis, 2. Clear cells, 3. Reniform nucleus 4. Stratum spinosum, 5.Stratum lucidum, 6.Stratum corneum

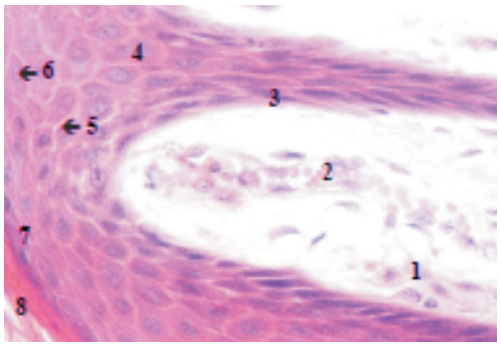

Fig. 5 Section of skin in the dorsal nasal region. H& E. x 400

1. Dermal papilla, 2. Capillaries, 3. Stratum basalis, 4. Stratum spinosum, 5. Inter cellular processes between adjacent cells, 6. Nucleolus, 7. Stratum granulosum, 8.Stratum corneum

Morris and Hopewell (1990) observed that the basal portion of the epidermis was considerably undulated in pigs.

Cells of stratum basalis rested on a basement membrane made up of collagen (Fig. 3) and reticular fibres. The basement membrane showed a positive reaction for PAS-alcian blue staining. The basement membrane separated the basal layer from the dermis. No blood vessels could be noticed in the epidermis. Sharma *et al.* (1996) demonstrated a thick collagenous basement membrane in the skin of the yak. Urmacher (1990) explained basement membrane as a thin PAS positive layer.

Nucleus of some of the cells of stratum basalis and stratum spinosum showed stages of mitosis. Monteiro-Riviere (1998) reported that some basal cells could act as stem cells with the ability to divide and produce new cells, whereas others primarily served to anchor the epidermis. Identification and functional characterisation of murine and human epidermal stem cells have

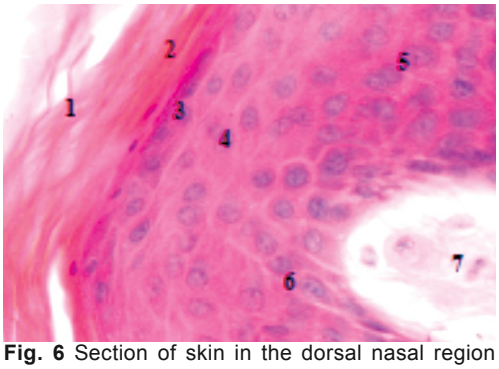

Fig. 6 Section of skin in the dorsal nasal region showing superficial layers of epidermis. H& E. x 400

1. Stratum corneum, 2. Stratum lucidum, 3. Stratum granulosum, 4. Peripheral cells of stratum spinosum, 5. Middle layer of stratum spinosum, 6. Deeper layer of stratum spinosum, 7. Dermal papilla

been made by Kaur (2006), which provided insights into the fundamental process of the tissue renewal and repair in the epidermis.

Clear cells could be located in the stratum basalis and stratum spinosum (Fig. 4). These cells were relatively larger and appeared lighter than the keratinocytes. Cytoplasm of these cells was clear and the nucleus was indented, sometimes had a reniform appearance (Fig. 4). These cells possessed characteristics of the Langerhan's cells. Copenhaver *et al.* (1971) reported in detail the Langrerhan's cells in the upper layer of stratum Malpighii. These cells possessed a dark stained nucleus surrounded by apparently clear cytoplasm. In sections stained by gold chloride method, these were blackened and were revealed as stellate or dendritic cells. Their slender processes penetrated the intercellular spaces among the prickle cells and were devoid of desmosomes attaching them to the neighboring cells. Langerhan's cells were interpreted variously such as worn-out melanocytes or as distinct series of cells derived from bone marrow. Electron micrographs showed that the characteristic Birberck granules of Langerhan's cells were different from melanin pigment.

Among the regions under study, the dorsal and ventral neck regions possessed a higher concentration of the clear cells compared to the other regions. Urmacher (1990) reported that the Langerhan's cells were the immunologic cells of the skin, which were needed to induce proliferative and cytotoxic T-cell responses by recognizing and presenting antigen to immunocompetent T-cell

lymphocytes. Langerhan's cells also possessed nonspecific esterase activity and expressed ATPase staining of the plasma membrane.

Stratum spinosum

Stratum spinosum was the thickest layer of the epidermis. Cells of this layer were large, irregular and polyhedral with distinct cell boundaries and had an average diameter of 19.20 µm (Fig. 2). A clear zone separated adjacent cells of this layer from one another. The fine lines across this clear zone formed the intercellular connections and gave a prickled appearance to this layer (Fig. 5). Copenhaver *et. al.* (1971) and Urmacher (1990) reported that the plasma membranes of adjacent cells were normally in close apposition throughout most of their extent, but during tissue processing; the cells shrunk and got pulled apart, leaving a clear intercellular space. The cells of stratum spinosum possessed a large, round to oval vesicular nucleus with a diameter of 11.10 µm. Nucleus possessed a distinct nucleolus (Fig. 5). Nuclei of the some of the cells of the deeper layers were in various stages of mitosis. These cells became flattened as they moved towards the surface with their long axes parallel to the surface (Figs. 2 and 3) and the cells measured 25.90 µm in length and 13.00 µm in width. The new cells formed by mitosis migrated outward and eventually sloughed from the superficial layer of the stratum corneum.

According to Bloom and Fawcett (1975), the prickling appearance of this layer was formed by the short processes or spines that were attached to similar projections from adjacent cells. Because the cell membranes at the sites of end to end junction of these processes could not be resolved with light microscopy, these structures were formerly called "intercellular bridges" in the belief that they represented open communications between the epidermal cells. But the electron microscopic studies revealed that there was no protoplasmic continuity between the cells. Instead, these short processes met end to end or side to side and were firmly attached by a well developed desmosome which appeared as a dense dot or granule in each 'bridge'. Monteiro- Riviere (1998) reported that desmosomes connected adjacent cells of stratum spinosum and these cells to the cells of stratum basalis below.

Thickness of the stratum spinosum varied in different body regions. At the snout region, in the area corresponding to the tip of the dermal papilla, this layer was 12 to 16 cell-layers thick and at the region of rete peg the thickness of this layer varied from 45 to 50 cell-layers. In the case of human beings, maximum thickness of stratum spinosum was observed at palms and soles (Urmacher, 1990). Morris and Hopewell (1990) found that the stratum spinosum at the interpapillary region was about four cells thick in pig. In dorsal nasal region, the thickness of the stratum spinosum at the region of the dermal papilla and rete peg was four to six cells and ten to twelve cells respectively (Fig. 2). In other body regions, thickness of this layer was comparatively less and ranged from two to four cells-thick and six to seven cells-thick, respectively (Fig. 4). The cell boundaries and the prickling appearance at the intercellular region were more distinct in the snout and carpal regions. Stratum spinosum was thinner at the dorsal and ventral neck regions and in the abdominal region. Here the cell boundaries were indistinct and the nucleus showed peripheral condensation of chromatin. Copenhaver *et al.* (1971) correlated the basophilia with the ribosomal content of cytoplasm, hence the cells were considered as active protein synthesising cells. Prekeratin granules were present in the upper layers. The uppermost layers of stratum spinosum contained small granules as reported by Urmacher (1990) in human beings. These granules were composed of lipids and neutral sugars conjugated to proteins and lipids. Their function is to provide epidermal lipids, which increase the barrier properties of the cornified layer and aid in desquamation process. It is also the site of synthesis and storage of cholesterol. At the carpal region, the cells were comparatively smaller (14.80 µm), and closely packed with clear cell boundaries and possessed an oval nucleus (7.40 µm).

Cytoplasmic staining property of the cells of the stratum spinosum varied in different layers. In the deeper layers, the cells were faintly basophilic and towards the middle, they became eosinophilic and closely packed. The peripheral cells were larger and the cytoplasm showed basophilic granules (Fig. 6). Prekeratin granules could be detected in the upper layers of stratum spinosum (Fig. 3).

Banks (1993) noticed that the thickness of stratum germinativum varied in different regions of the mammalian integument. It was usually thick in hairless areas of the body and rather thin in heavily haired region.

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