

Normal progressive developmental stages of the pilosebaceous unit in the albino rat's skin - A light microscopic study

Bijo Elsy¹, Aijaz Ahmed Khan²

¹Department of Anatomy, College of Medicine, King Khalid University, Abha, Saudi Arabia, ²Department of Anatomy, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

Corresponding author: Dr. Bijo Elsy, E-mail: bijobaby22@yahoo.com

ABSTRACT

Background: Basic histological knowledge of the different components of the pilosebaceous unit is important in the pathologic interpretation of hair disorders. **Aim and Objectives:** Due to paucity of detailed microscopic features and photomicrographs of the pilosebaceous unit (PSU) at one place, this study aimed to review the normal developmental stages and provide microscopic features, along with the photomicrographs of different components of the pilosebaceous unit that could prove useful in certain hair conditions and therapeutic procedures. **Materials and Methods:** Tissue samples were obtained from the site of experimentally induced incisional and excisional skin wounds in such a way that parts of normal skin around these healing skin wounds were included. These samples were processed for routine paraffin serial sectioning. These sections were stained with routine and special stains. Histological features were recorded under different magnification of the Trinocular microscope. **Study Limitation:** The study presented is a part of another research study related to skin wound healing in adult albino rats. **Results:** The general structure of the pilosebaceous unit was shown by routine Haematoxylin and Eosin staining. While the presence and distribution of collagen fibres were demonstrated by Masson's Trichrome and PicroSirius Red with Fast Green staining, type III collagen (reticular) fibres were observed in PicroSirius Red with Silver Nitrate staining and that of elastin fibres were demarcated by Aldehyde Fuchsin with Fast Green staining method. **Conclusion:** In certain conditions and procedures, such as hair disorders, hair transplantation, and follicular drug delivery, where detailed histology of the pilosebaceous unit is required, the present study wherein many special stains were used could prove very useful for quick and easy identification of different types of cells and fibres associated with the pilosebaceous unit.

Key words: Arrector pili muscle; Hair; Hair follicle; Pilosebaceous unit; Sebaceous gland

INTRODUCTION

The pilosebaceous unit (PSU) is a complex structure within the skin that is responsible for hair growth and lubrication of the epithelium [1]. The hair follicle (HF) generates hair and is a reservoir of multipotent stem cells, having the self-renewing capacity during hair cycles, also plays an important role in thermoregulation, physical and immunological protection [2]. Hair follicles also play a vital role in the repair of skin wounds by providing new epithelial cells [3-5]. A complex interaction and communication are known to occur between the epidermis and dermis during embryogenesis [6]. Pilosebaceous units are also known

to contain some drugs targeting sites as well. Therefore, this study aims at focusing on normal cyclic changes of follicle formation and microscopic features of different components of the PSU through routine and special stains, to help better understand and plan certain conditions and procedures such as hair transplantation, detection of hair disorders and follicular drug delivery.

MATERIALS AND METHODS

For the study of progressive stages of development in the pilosebaceous unit, skin tissue samples were secured from the sites of experimentally induced incisional and excisional skin wounds in such a way that parts of

How to cite this article: Elsy B, Khan AA. Normal progressive developmental stages of the pilosebaceous unit in the albino rat's skin - A light microscopic study. Our Dermatol Online. 2020;11(3):231-237.

Submission: 03.05.2020; **Acceptance:** 18.06.2020

DOI: 10.7241/ourd.20203.2

normal skin surrounding these healing wounds were included. These samples were a part of a research study that included a total of forty eight adult albino rats of either sex, each weighing 230-320g, and were performed in the Department of Anatomy, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Surgical Procedures for the Collection of Skin Samples

Horizontal full-thickness incision was made at 2.95 ± 0.17 cm in length on the shaved right mid-thigh region. This skin incision was re-approximated with 3-0 Vicryl (2metric-NW2401) absorbable sterilized surgical needled suture USP (synthetic; braided coated polyglactin 910 violet; from Ethicon, manufactured in India by Johnson and Johnson Ltd, Aurangabad). This re-approximated full-thickness incisional skin wound was used for the study of incisional (primary intention) skin wound healing [7]. Dorsal surface of the thoracic region for full-thickness excisional skin wound of 8.5 ± 0.48 mm diameter (an area equivalent to 46.74 ± 0.32 mm²) on pinched skin fold for the study of excisional (secondary intention) skin wound healing [8].

The skin samples were processed for paraffin sectioning. 5µm thick sections were stained with Haematoxylin and Eosin (H & E), Masson's Trichrome (MT), PicroSirius Red with Fast Green (PSRFG), PicroSirius Red with Silver Nitrate (PSRSN), Periodic Acid Schiff with Haematoxylin (PASH), Verhoeff Van Gieson (VVG) and Aldehyde Fuchsin with Fast Green (AFFG). Histological features under x10, x20, x40, and x100 objective lenses of Trinocular microscope (Olympus, BX40; Japan) were recorded by a digital camera (Sony 18.2 MP, Japan).

Study Limitation

The present study is a part of another research study related to skin wound healing in adult albino rats.

Ethics Statement

The principal study was conducted in the Department of Anatomy, approved by the Institutional Animal Ethical Committee (No. 8937/2014), Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. The animal groups, surgical procedures, and tissue samples for the present study remained the same as those of the principal study.

RESULTS

The general structure of the pilosebaceous unit was shown by routine Haematoxylin and Eosin staining. While the presence and distribution of collagen fibres were demonstrated by Masson's Trichrome and PicroSirius Red with Fast Green staining, type III collagen (reticular) fibres were observed in PicroSirius Red with Silver Nitrate staining and that of elastin fibres were demarcated by Aldehyde Fuchsin with Fast Green staining method. All features of the pilosebaceous unit and its developing stages were depicted by the following figures (Fig. 1 – 11).

Hair placode, Hair germ or follicular bud, Hair peg and Bulbous peg (Fig. 1); Dermal papilla, different components of a hair shaft, bulb and bulge (Fig. 2); Pilosebaceous unit or the follicular unit and Arrector pili muscle (Fig. 3); Segments of the hair follicle (Fig. 4); Perifollicular dermal sheath consists of type III collagen (reticular), elastin fibres (Fig. 5); Components of the hair follicle and hair shaft (Fig. 6); Collagen fibres in the connective tissue sheath (Fig. 7); Elastin fibres in the connective tissue sheath (Fig. 8); Arrector pili muscle (Fig. 9); Progression of growth of the pilosebaceous unit (Fig. 10); Features of the Sebaceous gland (Fig. 11).

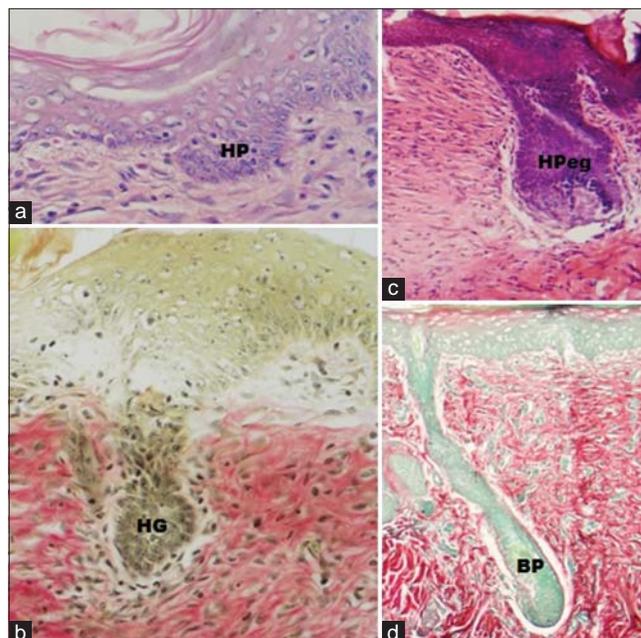


Figure 1: Different developmental stages of hair follicles. a- HP: Hair Placode, Stain: H & E, Magnification x40; b- HG: Hair Germ, Stain: VVG, Magnification x40; c- HPeg: Hair Peg, Stain: H & E, Magnification x20; d- BP: Bulbous Peg, Stain: PSRFG, Magnification x20.

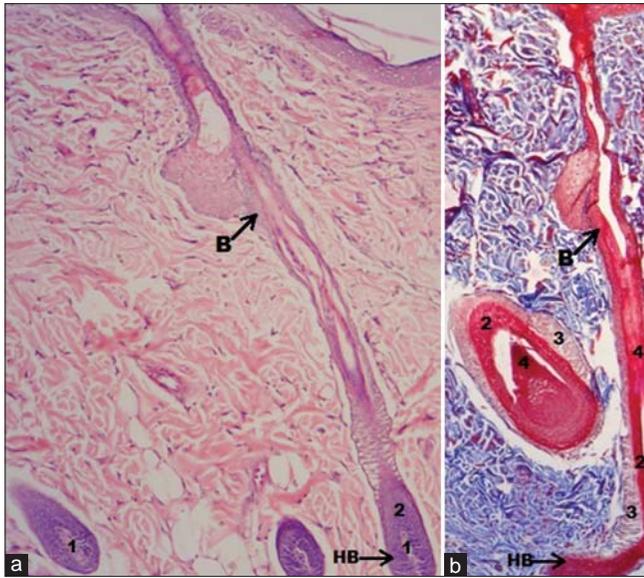


Figure 2: Hair follicle. B→: Bulge, HB: Hair Bulb, 1: Dermal papilla, 2: Inner root Sheath, 3: Outer root sheath, 4: Hair shaft. a- Stain: H & E, Magnification: x20; b- Stain: MT, Magnification x20.

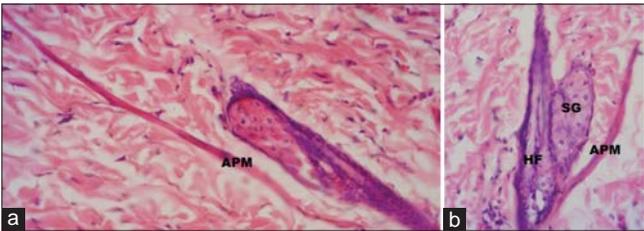


Figure 3: PiloSebaseous Unit (PSU). APM: Arrector Pili Muscle, SG: Sebaceous Gland, HF: Hair Follicle. (a and b)- Stain: H & E, Magnification: x40.

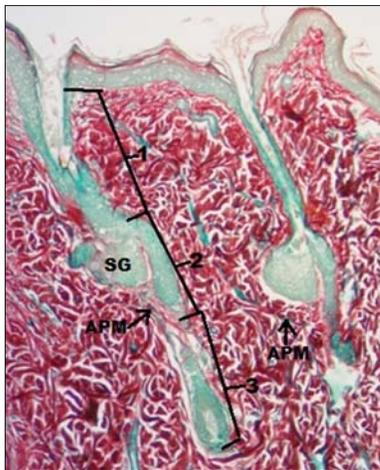


Figure 4: Parts of Hair follicle. 1: Infundibulum, 2: Isthmus, 3: Inferior segments, SG: Sebaceous Gland, APM: Arrector Pili Muscle. Stain: PSRFG, Magnification: x20.

DISCUSSION

Hair follicle (HF) morphogenesis and cycling occur as a result of interactions between epithelial and

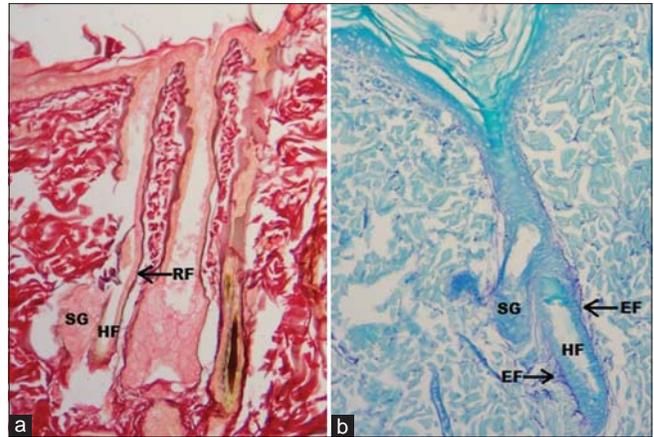


Figure 5: Fibres around the hair follicle. RF: Reticular fibres (Black Colour), SG- Sebaceous Gland, HF- Hair Follicle, EF: Elastin Fibres (Violet Colour). a- Stain: PSRSN, Magnification: x20; b- Stain: AFGG, Magnification x20.

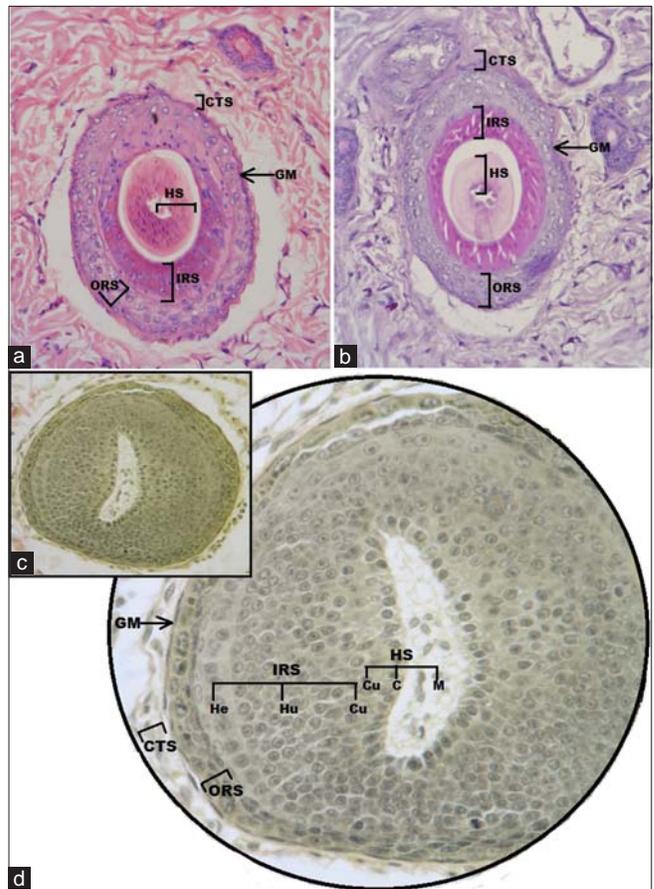


Figure 6: Cross-sections of the hair follicle. CTS: Connective Tissue Sheath, ORS: Outer Root Sheath, IRS: Inner Root Sheath, HS: Hair Shaft, GM: Glassy Membrane. d: IRS- He: Henle's layer, Hu: Huxley's layer, Cu: Cuticle; HS-M: Medulla, C: Cortex, Cu: Cuticle. a- Stain: H & E, Magnification: x40; b- Stain: PASH, Magnification x40; c- Stain: VVG, Magnification: x40; d- Stain: VVG, Magnification: x100.

mesenchymal cells [9]. Hair follicle (HF) morphogenesis and cycling occur as a result of interactions between epithelial and mesenchymal cells [9]. In the embryonic

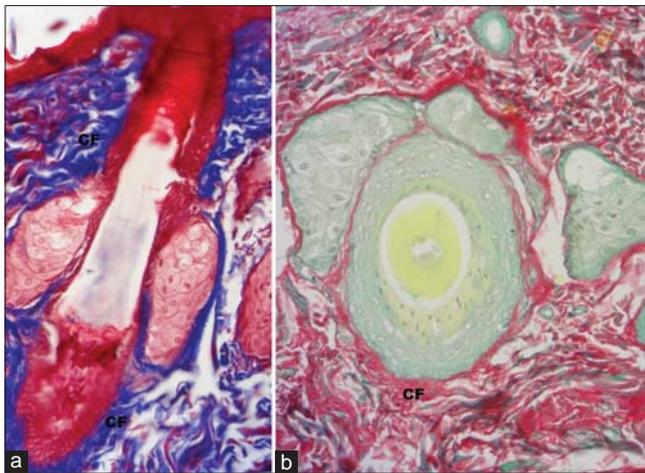


Figure 7: a- Longitudinal section of the hair follicle; CF: Collagen Fibres (Blue Colour); Stain: MT, Magnification: x40. b: Cross-section of hair follicle; CF: Collagen Fibres (Red Colour); Stain: PSRFG, Magnification: x40.

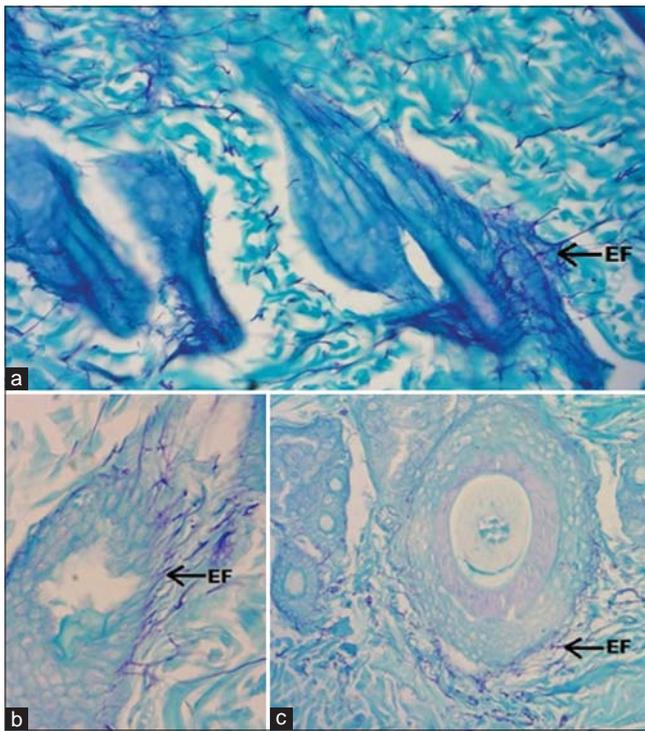


Figure 8: EF: Elastin Fibres (Violet Colour). (a and b): Longitudinal section of hair follicle, a-Magnification: x40; b- Magnification: x100. c- Cross-section of the hair follicle, Magnification: x40. Stain: AFFG

murine model study [10], the hair follicle development is divided into three stages; they are hair placode formation or induction, hair follicle organogenesis and cytodifferentiation. In this present study, all three stages of hair follicle morphogenesis were observed in adult rat skin in different weeks of the skin wound healing process. In the induction stage, local epidermal thickening forms the hair placode (Fig. 1a), and signals from placode cells lead to the development of the dermal condensate [6].

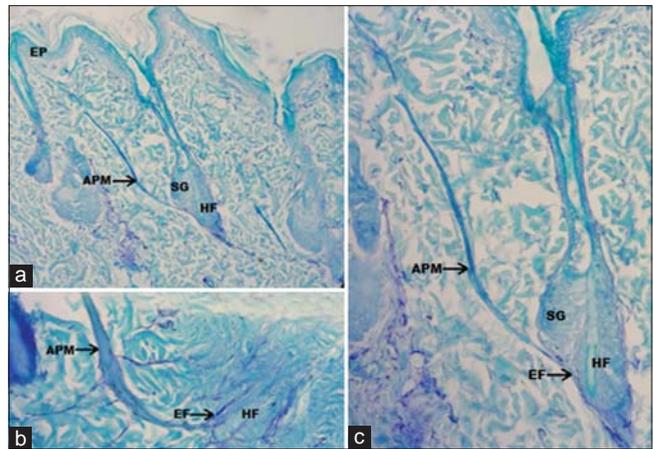


Figure 9: EP: Epidermis, APM: Arrector Pili Muscle, SG: Sebaceous Gland, HF: Hair Follicle, EF: Elastin Fibres (Violet Colour). a- Magnification: x10; b- Magnification: x40; c- Magnification: x20. Stain: AFFG.

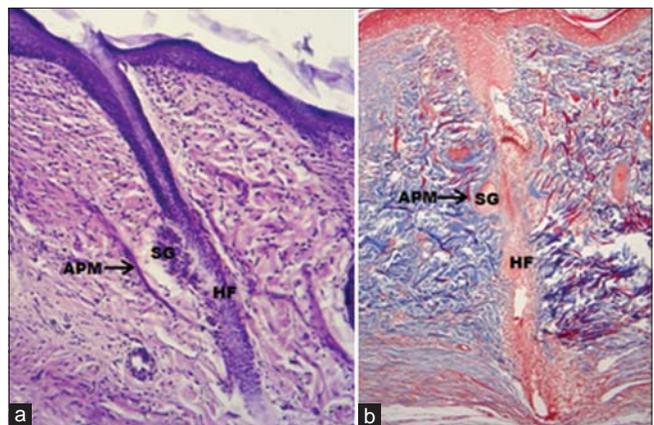


Figure 10: Progression of growth of Pilosebaceous unit. HF: Hair Follicle, SG: Sebaceous Gland, APM: Arrector Pili Muscle. a- Stain: PASH, Magnification: x20; b- Stain: MT, Magnification: x20

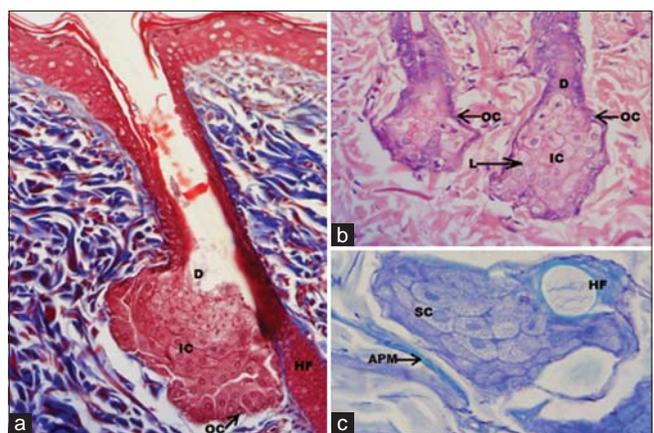


Figure 11: Sebaceous Gland-OC: Outer Cells, IC: Inner Cells, L: Lobule, D: Duct, SC: Sebocyte. HF: Hair Follicle, APM: Arrector Pili Muscle. a- Stain: MT, Magnification: x40; b-Stain: H & E, Magnification: x40; c- Stain: AFFG, Magnification: x40.

In organogenesis, condensation of mesenchymal cells is known as hair germ or follicular bud (Fig. 1b). Dermal

proliferation and invagination of the hair germ lead to the formation of hair peg and bulbous peg (Figs. 1c and 1d). During cytodifferentiation, the bulbous peg surrounds the condensed dermis to form the dermal papilla (DP) [11,12] (Fig. 2). El-Sayed et al [13] described the prenatal development of all stages of hair follicle morphogenesis in rat's semi-thin skin section by using the routine staining method. However, in our study, we found all these events in adult albino rat's full-thickness skin during the wound healing process in different weeks by using both routine and special staining methods.

In embryogenesis and postnatal life, this dermal papilla regulates the growth and development of the hair follicle [14]. Signals from the DP induce its surrounding epithelial cells to differentiate into the inner root sheath (IRS) and different components of a hair shaft (HS) [2] (Fig. 2).

The pilosebaceous unit or follicular unit consists of hair and its follicle, arrector pili muscle, and sebaceous gland [3,15]. During embryogenesis, hair follicle cells and sebaceous gland originate from the ectoderm but the dermal papillae, the inner and outer root sheaths develop from the mesoderm [9]. Dermal papillae contain numerous small blood vessels, myelinated and non-myelinated nerve twigs. These blood vessels provide nutrition to the growing hair follicle [16]. All these features had been observed in the rodent's hair follicle study [17]. In the present study, we observed only components of the pilosebaceous unit (Fig. 3), but the blood vessels and nerve fibres in the dermal papillae could not be visualized.

The hair follicle has three segments: Infundibulum or dermal pilary canal that is part of the invaginated epidermis extending up to the opening of the sebaceous duct; Isthmus, from the opening of the sebaceous duct to the site of attachment of the arrector pili muscle, and Inferior segment, which extends from the insertion of the arrector pili muscle to the hair bulb [18,19]. Infundibulum and isthmus are permanent regions of the follicle but the lower inferior segment is a variable region. The present study has also found all these segments of the hair follicle (Fig. 4), and this similar finding has recently been reported in a comparative study describing the rodent's hair follicle [17]. Below the infundibulum, the perifollicular dermal sheath consists of type III collagen (reticular) and elastin fibres [19]. While in the rodent's skin study [17] type I and type III collagen and elastin fibres were demonstrated in the dermis, but in the present study collagen (reticular) (Fig. 5a) and elastin fibres (Fig.

5b) have been shown very much in the perifollicular dermal sheath by using special staining methods.

During the skin wound healing process in the adult albino rat, we found all components of the hair follicle. They are the outer root sheath (ORS), inner root sheath (IRS), hair shaft, extracellular matrix, dermal papilla, and connective tissue sheath (CTS) (Fig. 6). A specialized basal lamina called the glassy membrane or dermal sheath is commonly present between connective tissue sheath and follicular epithelium [20] (Fig. 6). Another study [13] reported all these components of hair follicles in the 19-day prenatal development stage of the rat. In this present study, we observed that the CTS is connected to the dermis and consists of collagen fibres running in various directions along with some elastin fibres, which possibly helps in the movement of hair follicles [19,21]. The presence of collagen and elastin fibres in the connective tissue sheaths of the rat's hair follicles had been reported by Maynard and Downes [22]. Available photomicrographs of the mice skin study [23] focused on only a general arrangement of collagen fibres by Masson's trichrome and elastin fibres by Verhoeff method fibres in the skin. The present study mainly focused on the distribution of fibres around the hair follicles themselves. The collagen fibres were visualized by Masson's Trichrome and PicroSirius Red with Fast green stainings (Fig. 7) and elastin fibres were observed with Aldehyde Fuchsin with Fast green staining method (Fig. 8).

Dermal papillae, hair melanocytes and matrix are localized at the proximal end of the hair follicle to form the bulb (Fig. 2). The bulge is an extension of ORS and contains localized epithelial and melanocytic hair follicle stem cells [9,15] (Fig. 2). Experimental group in the nude mice skin study [24], the newly formed hair follicle contains the IRS, ORS, hair bulb and matrix.

The basic ultrastructural features of rodents and human skin are remarkably similar at physiological and anatomical levels [17]. Interestingly, all hair follicle's epithelial components and layers of the hair shaft in the adult albino rat's skin were also noticed in the present study (Fig. 6). The epithelial components contain inner (internal) and outer (external) root sheaths. Outer root sheath (ORS) is made up of rounded and nucleated double-layered cells, but in the upper part of the follicle, it contains multilayered cells and the arrector pili muscle attached to this sheath [16]. The inner root sheath (IRS) supports the deeper part of the hair follicle and extends up to the level of isthmus. This

sheath contains three layers from within outwards are Cuticle, Huxley's layer, and Henle's layer [5,16]. The cuticle layer consists of keratinized squamous cells which interlock with hair cuticle to stabilize the hair and direct the upward growth of the IRS and hair shaft [5]. Cells in the Huxley's layer containing one to three layers of flattened nucleated cells, but Henle's layer consists of a single layer of cubical cells with flattened nuclei [20]. Hair shaft consists of medulla, cortex and cuticle from within outwards. The medulla is present only in thick hairs and consists of irregular shaped cornified cells containing vacuoles and air cavities present within and between the cells [19]. In the cortex, the cuboidal cells differentiate into keratin-producing cells. The cuticle is formed by cornified squamous cells, acts as a protective barrier and provides strength and protection to the hair shaft [16,25]. All microscopic features of the epithelial components and hair shaft of the present study were found to be in agreement with another rat's skin histological study [22].

The arrector pili muscle (APM) (Fig. 3) is a bundle of smooth muscle cells, attached from the bulge region of the hair follicle to the basement membrane of the epidermis marked by the presence of elastin fibres at both ends [19] (Fig. 9). Numerous nerve fibres are present at the site of muscular attachment of the muscle to the follicle [26]. Contraction of the arrector pili muscle helps in the elevation of hair and squeezing the sebum into the hair follicle, which was found to be in agreement with the findings reported earlier in the animal study [17].

Sebaceous gland develops from the ectodermal cells in the wall of the hair follicle [27] (Fig.10). The gland consists of lobules with sebocytes and ducts. One or more lobules are connected to the base of the follicular infundibulum by a single duct [28]. Each lobule contains outer or basal and inner cells. The small outer, cuboidal cells are a single layer of proliferative cells that rests on the basement membrane and large rounded inner cells filled with lipids [19]. The gland secretes sebum, which can be characterized by antimicrobial activity [25], thermoregulatory role and maintains the softness of the skin and hair. The features of the rat's sebaceous gland had been reported by Maynard and Downes [22], which are very similar to those observed in the present study (Fig.11).

In the present study, we observed the events of hair follicle morphogenesis and microscopic features of the pilosebaceous unit in the adult albino rat during different weeks of skin wound healing. Due to the paucity of photomicrographs of events of hair follicle

morphogenesis and the pilosebaceous unit in one place, we used the routine and special staining methods to explain most of the microscopic features supported with photomicrographs. The special staining methods that were used proved to be very useful for quick identification, determining the location, orientation and density of different types of cells, and fibres associated with the pilosebaceous unit.

CONCLUSION

The review and observations of the histological features of all stages of the follicle formation in the adult albino rat's skin are expected to be useful in the interpretation of certain hair disorders, application of follicular drug delivery, and the hair transplant procedure.

ACKNOWLEDGEMENTS

All kinds of support availed from the Department of Anatomy, JN Medical College, Aligarh Muslim University are gratefully acknowledged.

Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

REFERENCES

1. Schepeler T, Page ME, Jensen KB. Heterogeneity and plasticity of epidermal stem cells. *Development*. 2014;141:2559-67.
2. Welle MM, Dominique J. The Hair Follicle. A comparative review of canine hair follicle anatomy and physiology. *Toxico Patho*. 2016;44:564-74.
3. Murphrey MB, Agarwal S, Zito PM. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL); Jul 14, 2019. Anatomy, Hair.
4. Taub AF, Pham K. Stem cells in dermatology and anti-aging care of the skin. *Facial Plast Surg Clin North Am*. 2018;26:425-37.
5. Erdoğan B. Anatomy and physiology of hair. *Intech Open Scie*. 2017:1-17.
6. Gnedeva K, Vorotelyak E, Cimadamore F, Cattarossi G, Guisto E, Terskikh VV, et al. Derivation of hair-inducing cell from human pluripotent stem cells. *PLoS One*. 2015;10:e0116892:1-25.
7. Elsy B, Khan AA, Maheshwari V. Effect of vitamin E isoforms on the primary intention skin wound healing of diabetic rats. *Our Dermatol Online*. 2017;8:369-75.
8. Elsy B, Khan AA, Maheshwari V. Therapeutic potential of d- δ -tocotrienol rich fraction on excisional skin wounds in diabetic rats. *Our Dermatol Online*. 2017;8:376-84.

9. Coulson-Thomas VJ, Gesteira TF, Esko J, Kao W. Heparin sulfate regulates hair follicle and sebaceous gland morphogenesis and homeostasis. *J Biol Chem*. 2014; 289:25211-26.
10. Hu MS, Borrelli MR, Hong WX, Malhotra S, Cheung ATM, Ransom RC, et al. Embryonic skin development and repair. *Organogenesis*. 2018;14:46-63.
11. Rishikaysh P, Dev K, Diaz D, Qureshi WM, Filip S, Mokry J. Signaling involved in hair follicle morphogenesis and development. *Int J MolSci*. 2014;15:1647-70.
12. Grubbs H, Nassereddin A, Morrison M. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL). Jan 6, 2020. Embryology, Hair.
13. El-Sayyad HIH, Abdraboh ME, Aljebali AMA. Positive impact of fish oil on diabetic and hypercholesterolemic skin disorders. *J Nutri Health*. 2015;1:1-8.
14. Zhou L, Wang H, Jing J, Yu L, Wu X, Lu Z. Regulation of hair follicle development by exosomes derived from dermal papilla cells. *Biochem Biophys Res Commun*. 2018;500:325-32.
15. Paniagua Gonzalez LM, Tschen JA, Cohen PR. Ectopic Sebaceous glands in the hair follicle matrix: case reports and literature review of this embryogenic anomaly. *Cureus*. 2018;10:e3605.
16. Brown TM, Krishnamurthy K. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Oct 27, 2018. Histology, Hair and Follicle.
17. Sundberg JP, Booth CJ, Nanney LB, Feleckman P, King JR LE. Skin and Adnexa, Chapter 24. *Comparative Anatomy and Histology a Mouse, Rat and Human Atlas*, 2nd Edn. 2017; 511-531.
18. Martel JL, Miao JH, Badri T. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Apr 28, 2020. Anatomy, Hair Follicle.
19. Standring S. *Gray's Anatomy. The anatomical basis of clinical practice*. Elsevier Churchill Living stone; 2016, 41st Edn:147-55.
20. Gartner LP. *Text book of histology E book*. Elsevier; 2020, 5th Edn:345-349.
21. Morisaki N, Ohuchi A, Moriwaki S. The role of neprilysin in regulating the hair cycle. *PLOS ONE*. 2013;8:e55947, 1-10.
22. Maynard RL, Downes N. *Anatomy and histology of the laboratory rat in toxicology and biomedical research. the skin or the integument*, Chapter 24. 2019; 303-14.
23. Souza APB, Oliveira MMR, Andrade RR, Amorim RFB, Bocca AL, Borin MF. The in vivo effect of L-arginine on skin elasticity in mice. *Braz J Pharm Sci*. 2017;53:e 00045,1-11.
24. Zhang M, Ye Y, Zhao P, Bai L, Li X. Preliminary studies of hair follicle regeneration by injections of epidermal stem cells and dermal papilla cells into nude mice. *Cell Tissue Bank*. 2020;21:321-7.
25. Hoover E, Alhadj M, Flores JL. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Aug 10; 2019. Physiology Hair.
26. Torkamani N, Rufaut NW, Jones L, Sinclair RD. Beyond goose bumps: does the arrector pili muscle have a role in hair loss? *Int J Trichology*. 2014;6:88-94.
27. Devi VS. *Inderbir Singh's Human Embryology*. 2018, 11th Edn:121-122.
28. Requena L, Sangüeza O. Embryology, Anatomy, Histology, and Physiology of the Sebaceous Glands. In: *Cutaneous Adnexal Neoplasms*. Springer, Cham; 2017:755-64.

Copyright by Bijo Elsy, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Source of Support: Nil, Conflict of Interest: None declared.