ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(8): 303-308

www.veterinarypaper.com
Received: 18-07-2025
Accepted: 20-08-2025

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Histomorphometric and histochemical study of skin in cape hare *Lebus cabensis* and domestic rabbit *Oryctolagus cuniculus*, comparative study

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DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i8e.2498

Abstract
Objective: The purpose of this study was designed to describe and contrast the skin characteristics between hare and rabbit.
Materials and Methods: Twelve skin samples from each animal were taken for the study as soon as they were killed. Seven samples were obtained from each of the following skin regions: the back, face, thigh, abdomen, perineum, and upper lip. The samples were then preserved in 10% formalin for a full day. Standard histology procedures were used to prepare the sections.
Results: Sections of skin showed that the epidermis is a thin outer layer made up of four strata: *Corneum, granulosum, spinosum,* and basal. Each research animal's back and thigh skin had keratinized stratified squamous epithelium lining its epidermis, while other skin regions had non-keratinized stratified squamous epithelium. In every area of the skin, the hare's epidermis was thicker than the rabbit's. The hair follicles, sebaceous glands, and sweat glands are all found in the dermis, which is divided into two layers: papillary and reticular. Compared to the hare's skin, the rabbit's dermis showed a higher thickness of reticular and papillary layers. The basic alveolar sebaceous glands were found in each research animal's little skin. There were several tubule-coiled sweat glands dispersed across the dermis. While hare skin had sweet glands in every area of the skin in this investigation, rabbit skin had none, with the exception of the upper lips. Periodic Acid-Schiff produced a positive result for sweat and sebaceous glands, however alcian blue alone produced a positive result for sebaceous glands.
In conclusion: There were several differences in histological structure of skin, and variations in the thickness of skin between hare and rabbit.

Keywords: *Oryctolagus cuniculus*, Streptozotocin (STZ), urolithiasis, diabetic, rats, rabbit

Introduction
In the domains of experimental toxicology, experimental pharmacology, and human dermatology, research animals are a popular concept in experimental animal medicine [1, 2]. Throughout the world, the Cape hare, also called a desert hare, as nocturnal herbivore. Because they have been kept as pets, are prized for their flesh and fur, and are seen as economically valuable creatures. Every rabbit in the area has a specific purpose. High-quality rabbit skins are used to make fur clothing and trims for cosmetic and therapeutic purposes [3-5]. The epidermis and dermis of laboratory animals' skin are connected to underlying structures like bone and muscle through the subcutis [6-8]. Defense, immunological protection, external senses, thermal regulation, wound healing, wound perception, and excretion are all functions of the skin, a complex structure that is regarded as one of the largest and most significant systems of an animal's body. The layers of the skin vary depending on the species, habitat, and area of the body [9, 10, 12], Hair follicles, sebaceous glands, and sweat glands are examples of skin appendages that invade the dermis in all animals and give each person particular physiological roles [13-15]. To control the animal's body temperature, the sweat glands release a fluid that contains salts and water. Sebum keeps the animal's skin supple and hydrated [16-18].

In contrast of the skins and hides of various animal species, laboratory skins haven't received enough attention. Although they have unique qualities, such as brittleness, low thickness, and a distinctive look, they compete with other types of skins or hides in the areas of their industrial usages, but they are not utilized enough. Inadequate skinning, insufficient surface area, the challenge of combining skins into bigger parts are the primary causes of laboratory skin's restricted industrial applicability. The purpose of this study was to compare the skin characteristics of two laboratory animals that live in different environments because laboratory domestication, proper skinning techniques, handling of skins, and the use of contemporary tanning processes may increase marketing of the laboratory skins.

Materials and Methods

- **Ethics statement:** All relevant procedures were carried out in compliance with the Veterinary Medicine College's Animal Care and Use Commission at Al-Muthanna University on March 6, 2024.
- **Samples collection:** Seven skin samples, each measuring 1 cm^3, were taken from the back, face, neck, thigh, abdomen, perineum, and upper lip of domestic rabbits *(Oryctolagus cuniculus*) and cape hares *(Lebus cabensis*) aged 1 to 1.5 years. Animals were bought from Al-Muthanna province, quit by a ketamine and xylazine a drug overdose then the living beings were sacrificed. Depilatory sodium sulfate cream was applied for 10 minutes after the skin's hair was removed using an unhairing procedure [3]. Hematoxylin and Eosin (H&E), Masson's trichrome, Periodic Acid Schiff (PAS), Alcian blue pH 2.5, and Companied Alcian blue plus periodic acid schiff (AB-PAS) were the stains used to compare the neutral and acidic mucopolysaccharide after the skin specimens were fixed in 10% formalin for 48 hours and then treated using a standard histological procedure [19].

Statistical analyses

Two-way ANOVA was used for the statistical analysis. At $p<0.05$, a significance level was chosen. Histological layer measurements were made using an ocular micrometer, followed by compliance and a stage micrometer, also use of an object-oriented lenses to determine the overall thickness of the epidermis, dermis, corneal, granulosum, spinosum, and basal layers. For each slide of each distinct area of the hare and rabbit skin, mean and standard error are calculated for ten per microscopic field 10x [20].

Results and Discussion

According to the current study, the skin of hares and rabbits has a fundamental structure consisting of a thin superficial layer called the epidermis and a deep layer called the dermis. This structure is similar to that of laboratory animals [3-5] and cattle [21]. However, the skin of other animals was separated into three layers: the hypodermis, thick dermis, and thin epidermis [22]. The epidermis was covered in stratified squamous epithelium, either keratinized or non-keratinized, and was arranged in four rows from the inner to the outer layers, which were the corneal, spinosum, granulosum, and basal layers (Figure 1, 2, 3). This was in contrast to [3, 4, 23], but differed from [18], which mentions the sratum lucid in *Millivora capenesis* skin. A thick layer of keratinized squamous epithelial cells covers the epidermis externally in the neck and back regions of the skin (Figure 1, 4, 5), while the non-keratinization layer is present in other skin regions (Figure 6, 7). In addition, the thickness of the hare's epidermis is greater than that of the rabbits, with significant difference ($p\leq0.05$) (Table 1). Simple columnar cells and dark-colored, elongated nuclei make up the basal layer, which also contains melanocytes. Granules of the melanin pigment extend downward about hair follicles bulbs (Figure 1, 2). Table [1] mentioned that there is a significant difference in thickness of the basal layer between the skin regions and between hares and rabbits.

Numerous polyhedral cells make up the spinosum layer, whereas the granulosum layer is made up of fragmented patches of cells that are one cell thick and flat. The cells also have big nuclei and noticeable basophilic granules. There was no discernible difference between the stratum spinosum and granulosum layers of the epidermis because the cells of the stratum spinosum connected by desmosomes, which start the keratinization process and move into the stratum granulosum with little space between them. The granulosum layer, which is situated directly beneath the corneal layer, is a prominent and continuous layer that gives the skin its flexibility and strength [24]. According to Table [1], the thickness of spinosum and granulosum layers varies significantly across the research animals. The last layer, the corneal layer, was rather thick and appeared as several cornified layers, which are dead cell layers that have been squamous layers. These cells are rigid, flat, and have a network-like appearance, and they are filled with dense keratin (Figure 1, 2), Significant variations exist in the corneal layer's thickness across the various skin locations and between the study animals' skins (Table 1). The stratum corneal plays a crucial role in maintaining hydration; if there is insufficient water retention, dry skin may result. The thickness of the epidermal layer varies by body region and may be caused by water retention in the hair follicles and light absorption by the neck region [22, 24, 25]. According to age and gender, Branchet *et al*. [26] described the thickness of epidermis in various body parts [27]. Stated that older dogs had thicker epidermis than younger dogs, and that the quantity of sweat gland and sebaceous gland secretory units and skin thickness varied by skin part. These variations might result from the skin's protective function in preventing water loss.

In the rabbit, the dermis was thicker than in the hare because of the density of dense irregular connective tissue and fibers. The dermis was separated into two layers: superficial papillary and deep reticular layers. The dermis layer was made up of dense connective tissues that contained hair follicles, the blood vessels, the sweat glands, the sebaceous glands, and arrector pili muscles (Figure 8, 9, 10). Primary and secondary follicles for hair are the two types found in both mammals. Primary hair follicles and groups of secondary hair follicle make up the follicles. All body parts had hair follicles, while rabbits and hares had compound hair follicles. This was consistent with the results of [28]. While the current result was inconsistent with the results of [17], who maintained that the hair follicle in domestic animals were of the simple type, compound hair follicles were formed of important primary hair follicle and clusters of fewer secondary follicles surrounding them (Figure 2, 11, 12).

The reticular layer, which comprises thick connective tissue and striated muscle, is compact and papillary was composed of loose connective tissue that is superficially and extremely vascular (Figure 4, 6, 7). The sebaceous glands, which were simple or unilobular and small alveolar glands, were always connected to hair follicle and directly above a sweat gland, between a hair follicle and pili muscles. They

were lined by stratified cuboidal cells and they reacted positively to PAS and alcian blue in all skin regions. Sweat glands were tubular glands that aggregated in large numbers near the intersection of the papillary layer and reticular layer of the dermis. They were tubular glands that aggregated in large numbers near the papillary layer and reticular layer of the dermis.

According to Table 1, the dermis thickness varies significantly between skin regions as well as rabbit and hare skin. Striated muscles are responsible for the force, motion, and posture of the tail; thickness of skin layers varied depending on the area of the body. Rabbits are more susceptible to heat stress because they have fewer sweat glands, which makes heat disposal more difficult. In rabbits, heat stress has a negative impact on growth, reproduction, milk production, immunity and wellness status, feed consumption overall utilization, and welfare and adaption [4]. Rabbits are creatures that are homoeothermic. It should be possible for them to regulate their body temperature within a specific range. Rabbits' absence of sweat glands results in incredibly poor thermoregulation. The presence of thick insulating fur on the skin further prevents heat loss in rabbits, which are extremely sensitive to high ambient temperatures [8]. The study's follicles were compound (Figure 6, 8), which contrasts with [17], which noted that hair follicles in domestic animals were simple in type, as were sebaceous glands, sweat glands, and blood vessels via erector pilli muscles present in all animal skins. Rabbit skin had no sweat glands, with the exception of the upper lips, and hare skin had sweet glands in all the skin regions. Rabbit skins are unsuitable for the fur industry because of their thick dermis and loose connective tissue fibers [30, 31]. Due to the necessity of retaining water and preventing energy loss through heat, the thickness of the dermis layer in various animal parts, as well as the distribution of sweat and sebaceous glands, differ from those of other animals. The dermis is also crucial for sensation, as it contains receptors for pain, temperature, touch, pressure, protection, and thermoregulation [32]. The sebaceous glands, an exocrine gland close to the hair follicles, secrete an oily or waxy substance to lubricate the skin and hair, and there are numerous blood vessels around hair follicles. Because animals hibernate, the skin in the abdomen area has a thick layer of adipose tissue. Additionally, there are blood arteries close to the hair follicles that provide the skin with nutrients and oxygen (33-35). As the water in sweat evaporates, skin's surface cools. Sweat glands secrete fluid to control the animal's body temperature. Additionally, sweat aids in grasping by slightly moisturizing the skin [36].

Table 1: Measurement the thickness of skin wall layers of hare and rabbit (µm)

Skin Region	Animal	Epidermis Total (µm)	Corneum (µm)	Granulosum–Spinosum (µm)	Basal (µm)	Dermis (µm)
Back	Hare	37.9±0.2	12.2±0.03	23.1±0.04	3.1±0.09	151.1±0.1
	Rabbit	34.8±0.3	11.2±0.02	21.6±0.03	2.4±0.01	162.1±4.2
Face	Hare	26.7±0.4	-	25.4±0.05	1.8±0.05	180.6±3.2
	Rabbit	22.4±0.5	-	21.6±0.06	1.4±0.04	186.6±1.3
Thigh	Hare	32.2±0.6	11.1±0.03	20.1±0.07	1.9±0.03	144.1±2.3
	Rabbit	28.8±0.7	8.2±0.02	18.2±0.08	2.1±0.07	167.1±1.4
Perineum	Hare	24.6±0.8	-	23.1±0.04	1.9±0.06	152.1±2.3
	Rabbit	21.3±0.4	-	19.1±0.07	1.9±0.08	171.1±2.3
Abdomen	Hare	22.4±0.3	-	20.2±0.03	2.1±0.05	197.1±1.4
	Rabbit	20.5±0.2	-	18.1±0.08	2.9±0.04	201.1±2.3

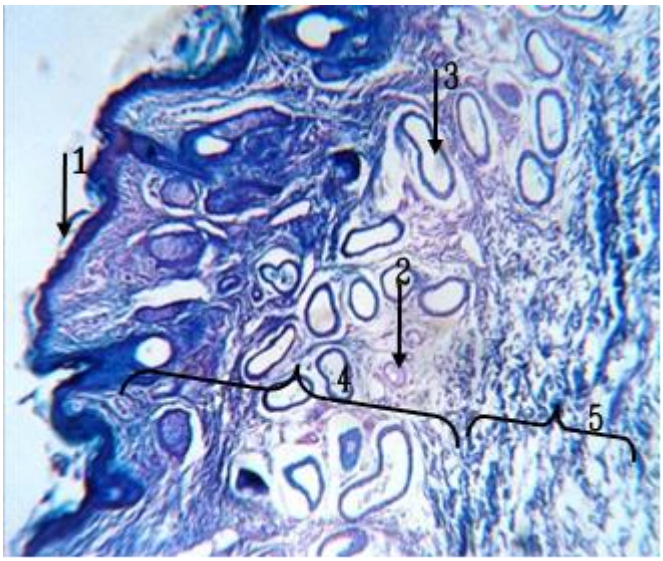

Fig 1: Microscopic section of back region skin of hare; epidermis [1], blood vessel [2], duct of sweet gland [3], superficial layer of dermis [4], reticular layer [5], masson trichrome, 100X

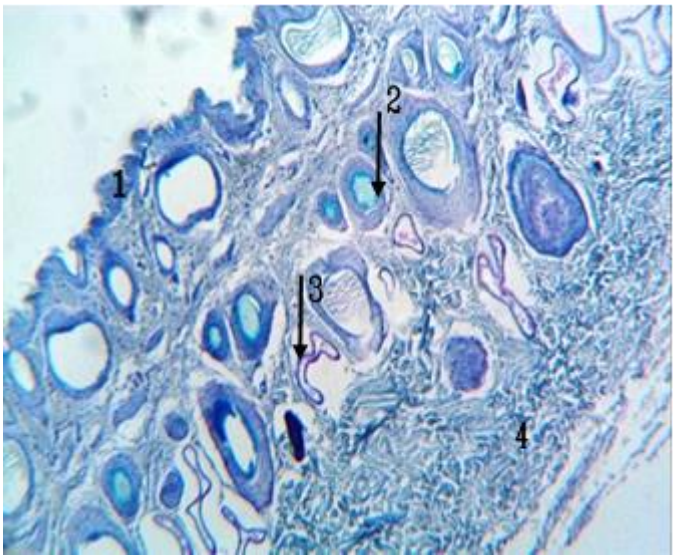

Fig 2: Microscopic section of back region skin of rabbit**;** epidermis [1], hair follicle [2], sweet gland [3], connective tissue [4], PAS-AB, 100X

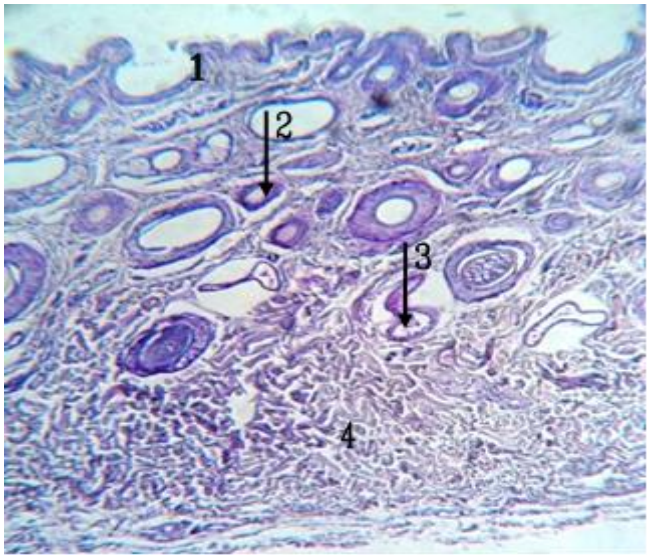

Fig 3: Microscopic section of face region skin of hare; epidermis [1], hair follicle [2], duct of sweet gland [3], connective tissue of dermis [4], H@E, 100X

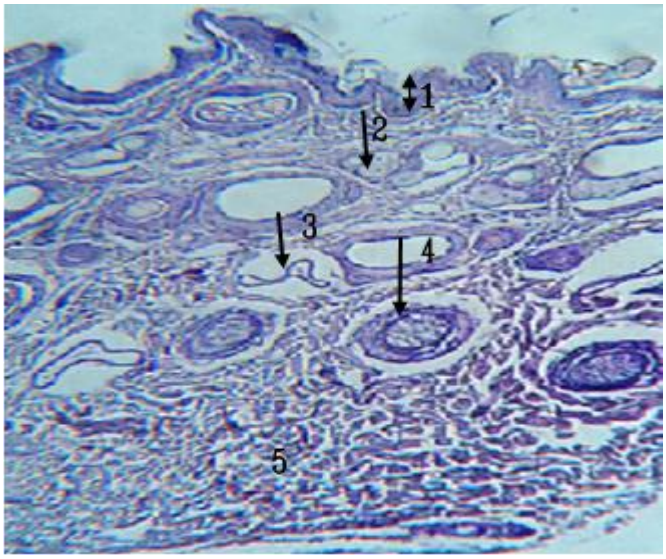

Fig 4: Microscopic section of face region skin of rabbit; epidermis [1], sebaceous gland [2], sweet gland [3], hair follicle [4], connective tissue of dermis [5], H@E, 100X

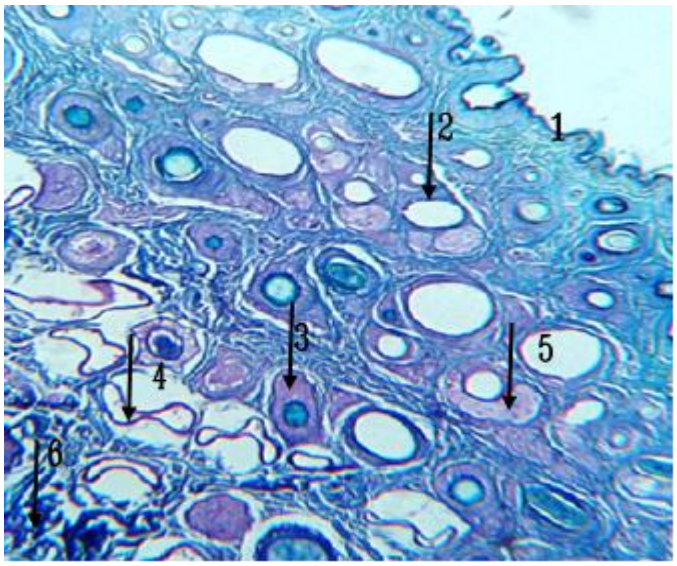

Fig 5: Microscopic section of perineal region skin of hare**;** epidermis [1], adipose tissue [2], hair follicle [3], sweet gland [4], sebaceous gland [5], connective tissue of dermis [4]**,** PAS, 100X

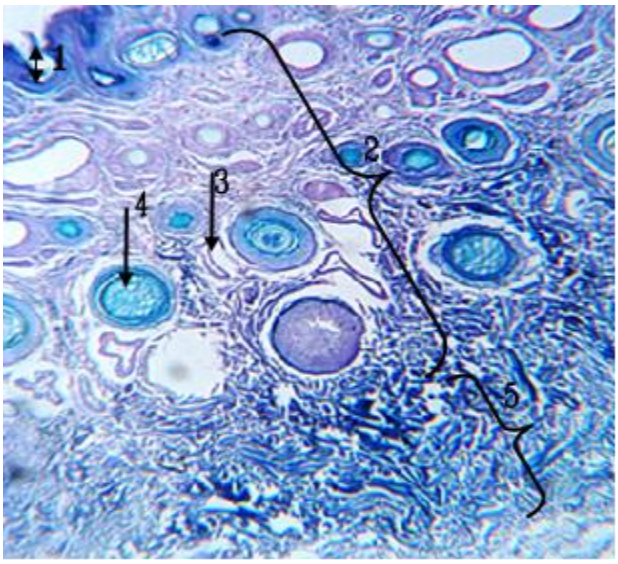

Fig 6: Microscopic section of perineal region skin of rabbit; epidermis [1], connective tissue of dermis [2], sweet gland [3], hair follicle [4], connective tissue of dermis [5], PAS, 100X

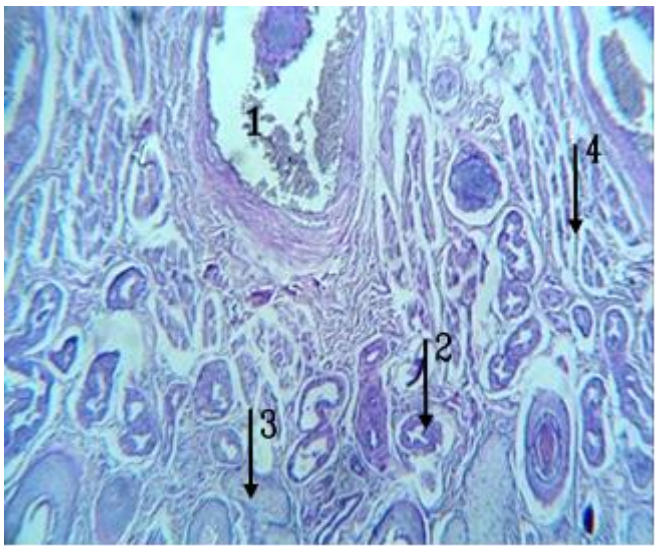

Fig 7: Microscopic section of thigh region skin of hare**;** blood vessels [1], connective tissue [2], sebaceous gland [3], smooth muscle [4], masson trichrome, 100X

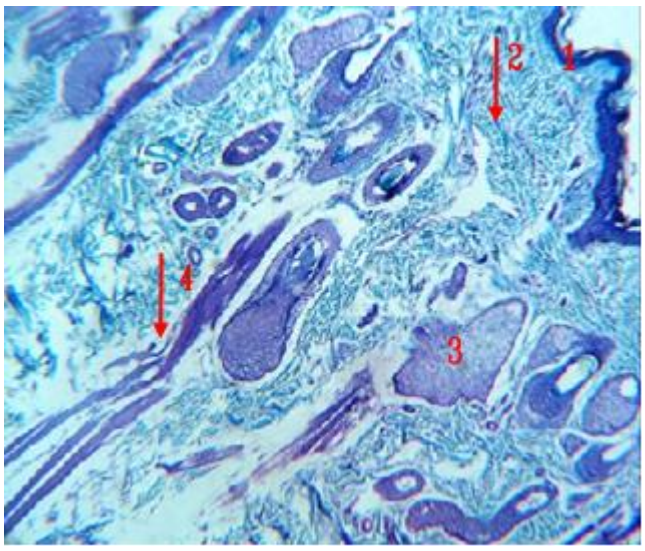

Fig 8: Microscopic section of thigh region skin of hare; epidermis [1], connective tissue [2], smooth muscle [3], skeletal muscle [4], masson trichrome, 100X

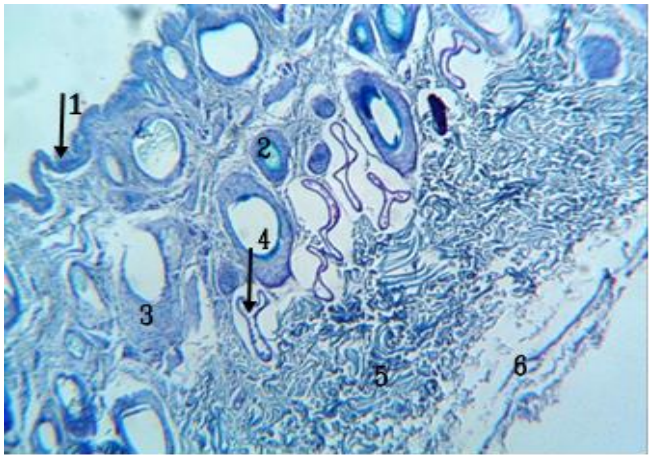

Fig 9: Microscopic section of abdomen region skin of hare; epidermis [1], hair follicle [2], sebaceous gland [3], sweet gland [4], smooth muscle [5], PAS-AB, 100X

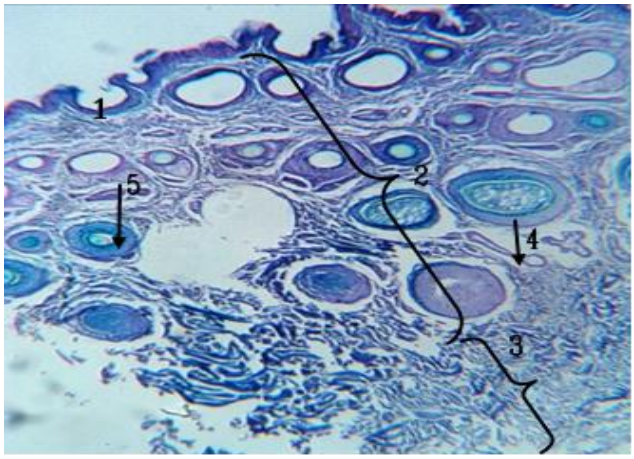

Fig 10: Microscopic section of abdomen region skin of hare**;** epidermis [1], connective tissue [2], reticular layer [3], sweet gland [44], hair follicle [5], PAS-AB, 100X

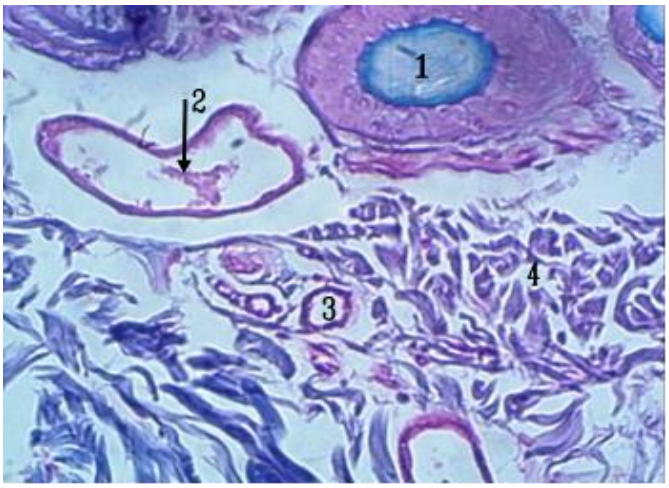

Fig 11: Microscopic section of abdomen region skin of hare; hair follicles [1], duct of sweet gland [2], blood vessel [3], smooth muscle [4], masson trichrome, 100X

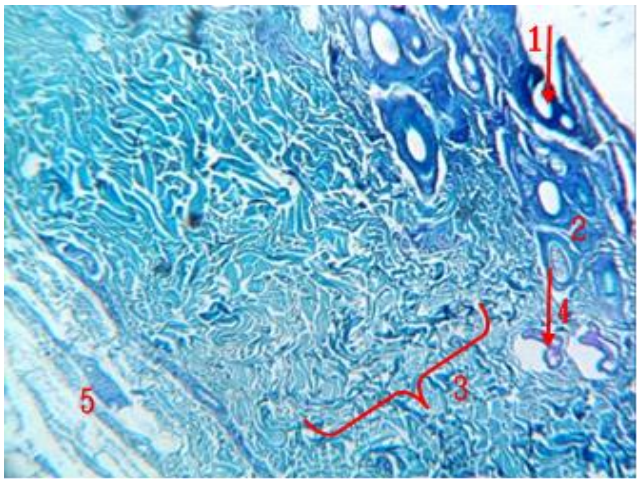

Fig 12: Microscopic section of thigh region skin of rabbit; epithelium [1], hair follicles [2], sweet gland [3], dense connective tissue [4], loose connective tissue [5], AB, 100X

There was difference between study animals in the histological structure and histochemical features of the skin, and knowledge the skin structure between the animals is life in different environment is essential for understanding of the pathology, physiology, and other sciences, and enables for the viewing of the tissue structures and any the distinctive changes which may have occurred. Skins of study animals are not suitable for fur industry since their dermis are thick and connective tissue fibers are loose.

Acknowledgements
The paper was prepared without the financial assistance of any external agency.

Author Contributions: All authors contributed equally.

Conflict of Interest
The authors have no competing interests to declare that are relevant to the content of this article.

Ethics Approval
All applicable were carried out in the accordance by the animal care and use committee, Veterinary Medicine College, Al-Muthanna University, on May 6, 2023.

Funding Statement: This research did not receive external funding. All authors were contributing to supporting this work in a self-supporting manner.

Financial Support
Not available

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How to Cite This Article
Fadhil M. Histomorphometric and histochemical study of skin in cape hare *Lebus cabensis* and domestic rabbit *Oryctolagus cuniculus*, comparative study. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(8):303-308.