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Analyses of changes on skin by aging

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Background: This study aimed to evaluate the histological changes occurring in rat skin with increasing age, starting from the intrauterine period.

Methods: Thirty-two healthy female Sprague–Dawley rats were evaluated in four groups: group 1 – intrauterine day 19, group 2 – postpartum day 21, group 3 – postpartum day 60, and group 4 – postpartum month 19. Skin samples from the back, abdomen, head, and upper and lower limbs were obtained from each subject under anesthesia. Tissue specimens were evaluated statistically and morphologically for the thicknesses of the epidermis, dermis, and basement membrane; the number, height, and width of dermal papillae; and the mast cell and pilosebaceous counts per group. The changes in collagen/elastic fibers and glycosaminoglycans were also assessed.

Results: Epidermal thickness was the highest in the intrauterine group; it decreased in the postpartum period and increased again in the aged group. Basal membrane thickness increased steadily with age. The number, height, and width of dermal papillae and dermal thickness increased up to day 60 after birth although these decreased in the aged group. Mast cell count also reached the maximum in the intrauterine group and gradually decreased with age. Pilosebaceous units of the dermis were fewer in intrauterine specimens; they showed an increase during the postpartum period and a decrease in the aged group.

Conclusion: Skin specimens obtained from rats showed striking differences between the intrauterine and postpartum groups. Moreover, the postpartum group showed considerable intra-group differences.

Key words: aging – skin – rat – morphology – intrauterine skin histology – epidermal thickness – mast cell

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Aging is an essential biological process of living organisms and its pace differs markedly among different species and even among individuals of the same species (1). Changes in human skin due to aging are a major concern for both the pharmaceutical and the cosmetic sectors worldwide. A considerable amount of expenses and investments are required for the pharmaceuticals and cosmetics intended to delay or reverse aging.

Skin aging is characterized by certain anatomical, physiological, and histological changes. Aged skin is dry, crusted, and wrinkled. Although these changes are a result of multiple factors, the main reason is the progressive impairment of skin barrier function. It is also suggested that transepidermal water loss, decreased epidermal lipid levels in lamellar bodies, and decreased epidermal filaggrin in aged skin play important roles in drying and wrinkling (2, 3).

Many researchers have divided skin aging into two categories: physiological aging and photoaging. The former is a lifelong process accompanied by certain physiological changes, such as the shortening of telomeres (2, 3), decreased antioxidant enzyme activity, and decreased elastin gene expression. The latter, also referred to as extrinsic aging, is basically linked to sun exposure, although other factors such as inappropriate nutrition and smoking may contribute. It is noteworthy that telomeres do not have a key role in extrinsic aging (2).

Many functions of the skin decrease with aging. Among these are the renewal of cells, chemical cleansing, mechanical protection, immune response, DNA repair, production of sweat and sebum, and vitamin D production (3).

This study aims to evaluate the morphological changes associated with physiological aging in the skin of rats, starting from the intrauterine period, and to provide a preliminary foundation on which further studies can build.

Materials and Method

This study was conducted on 32 healthy female Sprague–Dawley rats obtained from Inonu University Research Animal Resource and Research Center (2010/46), Malatya, Turkey. The animals were kept at 22 ± 2°C (room temperature) and 50 ± 10% humidity with a 12-h light/12-h dark cycle and were fed regular rodent pellets and tap water.

This study was conducted on four different age groups, each group consisting of eight rats, as follows: Group 1: intrauterine (prenatal) day 19; group 2: postpartum (postnatal) day 21; group 3: postpartum (postnatal) day 60; and group 4: postpartum (postnatal) month 19.

Under ketamine/xylazine anesthesia, skin samples from the back, abdomen, head, and upper and lower limbs were obtained from each subject. These samples were fixed with 10% formalin solution and were then subjected to routine tissue processing. Each sample was separately embedded in paraffin, and 5-μm-thick sections were obtained. The sections were stained with Mayer's hematoxylin-eosin (H-E) for the evaluation of epidermal thickness, Masson's trichrome for dermal thickness, periodic acid-Schiff for basal membrane thickness, elastic Van Gieson for the evaluation of elastic fibers, toluidine blue for mast cell count, and Mowry's colloidal iron for the evaluation of glycosaminoglycans (GAGs).

All tissue specimens were analyzed for the keratin layer and other epidermal layers, keratinocytes, basal membrane and underlying papillary and reticular regions, collagen and elastic fibers, and GAGs. Finally, the hypodermis was evaluated and preplanned measurements were conducted. The stained specimens were examined and photographed under a Leica DM LB2 microscope, and measurements were taken using the Leica Q-Win Plus analytical system (Leica Micros Imaging Solutions Ltd, Cambridge, UK).

For all measurements, five different sections were evaluated per specimen. For the determination of epidermal and dermal thicknesses, under ×10 magnification, eight different areas from all five sections were measured. Measurements of the number, height, and width of the dermal papillae were made from five different areas under the ×10 magnification. For the basal membrane thickness, six different areas from all five sections were measured under ×100 magnification. Mast cell counts were obtained from 15 different areas under ×100 magnification and pilosebaceous unit counts were obtained from five different areas under ×20 magnification.

Statistical analysis

For the statistical analysis of all results, 13.0 SPSS for Windows software was used. All data are presented as the mean ± standard deviation (SD). The Shapiro–Wilk normality test proved that our quantitative variables did not exhibit a normal distribution ($P < 0.05$). Comparison of variables in each group with respect to time variables was performed using the Wilcoxon signed-rank test as a non-parametric test. Results were regarded as statistically significant at $P < 0.05$.

Results

The skin samples from the back, abdomen, head, and upper and lower limbs obtained from the intrauterine day 19 group showed a fairly regular stratified squamous epithelium rich in cells. The epidermis, consisting of five or six rows of cells was distinguishable from the underlying dermis and exhibited a thicker structure compared with that in the other groups.

In the specimens obtained from the day 21 group, the epidermis showed a four-layered structure, as observed in all other groups, and consisted of two or three rows of cells. The day 60 group did not show any regional differences with respect to the epidermal layers, cell distribution, and morphology. Specimens from the month 19 group also showed two or three rows of epithelial cells with euchromatic nuclei and nucleoli that were positioned close together (Figs 1–4). All measurement results are shown in the Table 1 and Fig. 5.

Examination of the periodic acid-Schiff-stained specimens showed that the day 19 (intrauterine) group had the thinnest basal membrane, while the thickness gradually increased in the day 21 and day 60 groups. However, the thickest basal membrane was observed in the

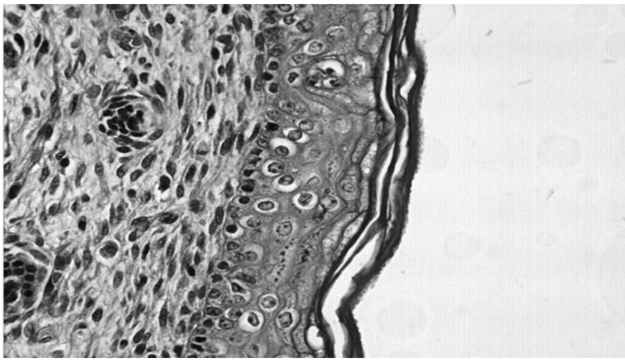

Fig. 1. *Day 19 (intrauterin) group:back skin epidermis. H&E; ×40.*

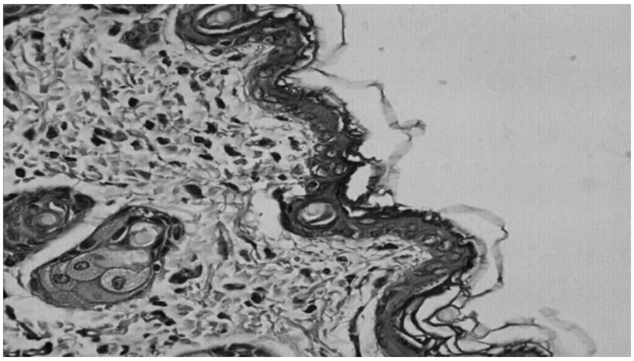

Fig. 2. *Day 21 group upper limb skin epidermis. H&E; ×40.*

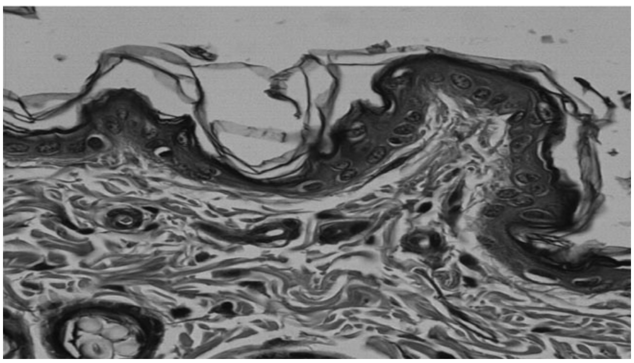

Fig. 3. *Day 60 group head skin epidermis. Sb; Stratum basale H&E; ×40.*

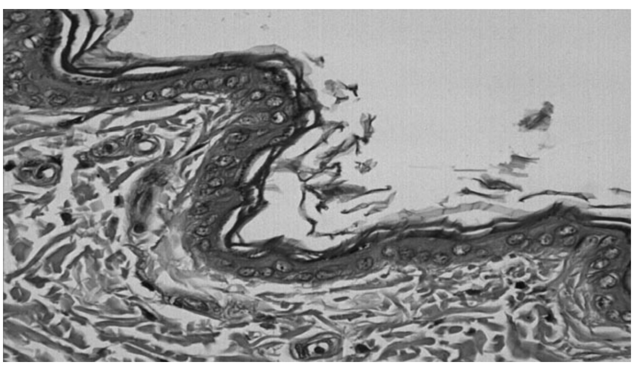

Fig. 4. *Month 19 group abdomen skin epidermis. H&E; ×40.*

day 19 group; it was structurally quite regular and consistent, as observed in the other groups (Figs 6–9). All measurement results are shown in the Table 2 and Fig. 10.

The dermis was examined using elastic Van Gieson (EVG) and Masson's trichrome stains. The prenatal group had the thinnest dermis and was relatively rich in cells. In this group, the collagen in the extracellular matrix had the smallest volume, was the thinnest, and had lowest intensity staining. Elastic fibers could hardly be observed in the intrauterine group, although a small number of weakly stained elastic fibers could be observed close to the hypodermis. Mowry's colloidal iron-stained specimens obtained from this group showed the highest level of GAGs. In the postpartum day 21 group, besides being rich in cells, the fibrils were longer and had a greater diameter than those in the intrauterine group. The hypodermis was also thicker and was easily distinguishable from the overlying dermis. Although reticular fibers could not be observed in this group, collagen fibers were increased in both quantity and thickness in the extracellular matrix; they extended parallel to the dermis and the papillary and reticular dermis could be easily distinguished. In this group, cells in the papillary dermis were abundant, while in the reticular dermis, there were a limited number of cells and a larger number of fibrils. Owing to the thinner collagen structure, the papillary dermis was weakly stained in comparison to the reticular dermis. Upon approaching the hypodermis, the collagen fibers became thicker and started to form anastomoses. Elastic fibers were stained dark black and mostly observed at the dermis within the close vicinity of the hypodermis; they could also be observed within the hypodermis as fibers surrounding the adipocytes. In general, in this group, elastic fibers were more prominent than the intrauterine group and mostly condensed in the middle of the dermis. GAGs were stained light blue and mostly observed between the collagen bundles in the papillary dermis and also around the pilosebaceous units. In the postpartum day 60 group, the dermis appeared strikingly dense and was heavily stained due to the abundance and thickness of fibrils. Furthermore, among all the groups, the thickest dermis was observed in this group. All measurement results are shown in the Table 3 and Fig. 11.

TABLE 1. Epidermis layer thickness for all groups

Groups	BACK ($\bar{x} \pm$ SD)	ABDOMEN ($\bar{x} \pm$ SD)	HEAD ($\bar{x} \pm$ SD)	LOWER LIMB ($\bar{x} \pm$ SD)	UPPER LIMB ($\bar{x} \pm$ SD)
Day 19 (intrauterine)	54.3 ± 5.8	49.2 ± 5.5	37.1 ± 1.7	70.4 ± 3.5	55.9 ± 6.0
Day 21	16.9 ± 0.7*	17.8 ± 1.7*	15.3 ± 0.7*	16.1 ± 0.9*	16.4 ± 1.8*
Day 60	21.5 ± 1.4*,†	18.3 ± 1.1*	16.7 ± 0.8*,†	16.6 ± 1.5*	17.2 ± 1.1*,†
Month 19	28.0 ± 2.3*,†,‡	22.5 ± 2.0*,†,‡	21.5 ± 1.5*,†,‡	20.4 ± 1.6*,†,‡	22.4 ± 2.2*,†,‡

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

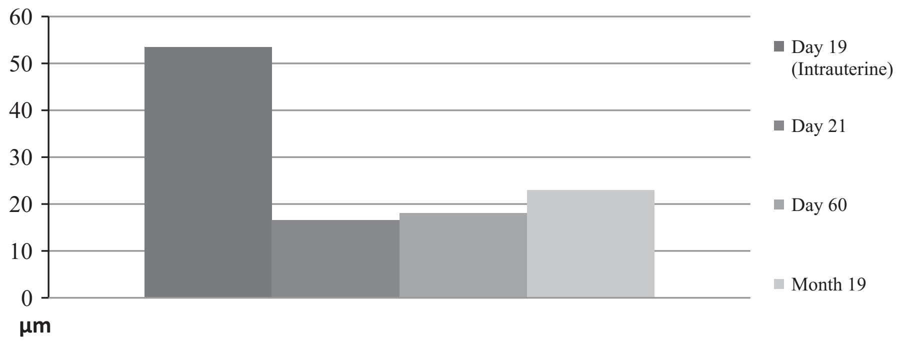

Fig. 5. Epidermis thickness.

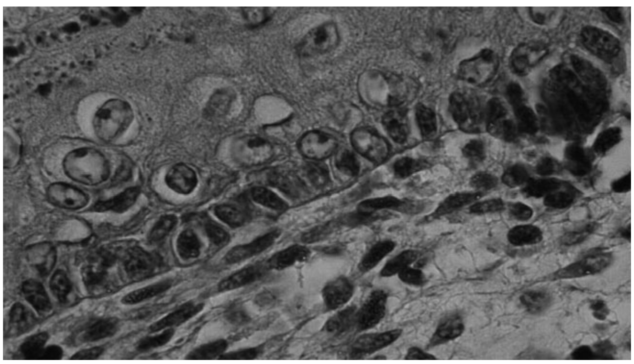

Fig. 6. Day 19 (intrauterin) group basal membrane. PAS; ×100.

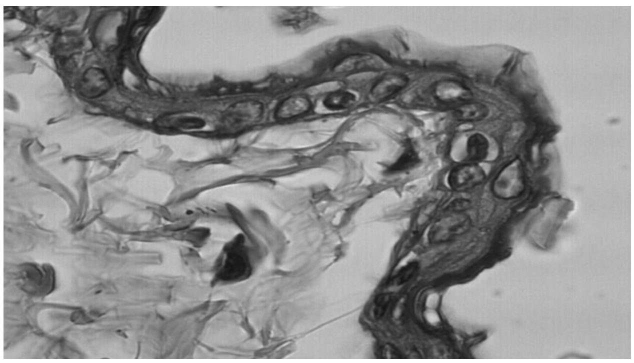

Fig. 8. Day 60 group basal membrane. PAS; ×100.

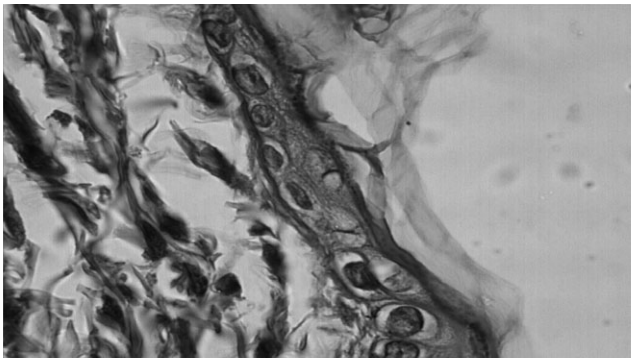

Fig. 7. Day 21 group basal membrane. PAS; ×100.

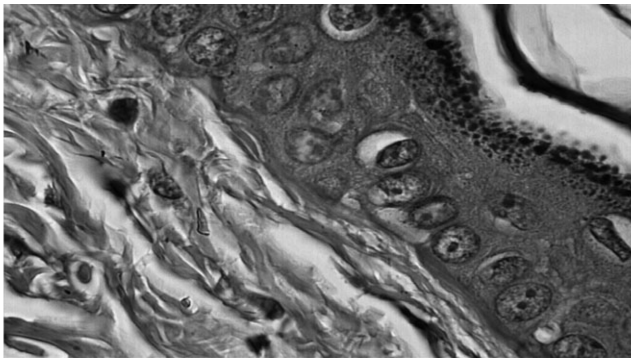

Fig. 9. Month 19 group basal membrane. PAS; ×100.

Observation of the back, abdomen, and head and limb specimens did not show any major differences in the structure of the dermis; the hypodermis was evident and was easily distinguishable from the overlying dermis. Papillary dermis and reticular dermis were also clearly

TABLE 2. Basal membrane thickness for all groups

Groups	BACK ($\bar{x} \pm$ SD)	ABDOMEN ($\bar{x} \pm$ SD)	HEAD ($\bar{x} \pm$ SD)	LOWER LIMB ($\bar{x} \pm$ SD)	UPPER LIMB ($\bar{x} \pm$ SD)
Day 19 (intrauterine)	6.7 ± 0.4	6.3 ± 0.5	4.6 ± 0.7	5.6 ± 0.8	6.7 ± 0.7
Day 21	9.9 ± 0.9*	8.8 ± 0.4*	8.4 ± 0.8*	8.6 ± 0.5*	8.7 ± 0.9*
Day 60	13.23 ± 1.2*,†	11.5 ± 0.5*,†	11.9 ± 0.7*,†	11.4 ± 0.6*,†	11.0 ± 0.5*,†
Month 19	16.9 ± 0.6*,†,‡	14.6 ± 0.8*,†,‡	14.4 ± 1.6*,†,‡	14.4 ± 0.4*,†,‡	13.3 ± 0.9*,†,‡

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

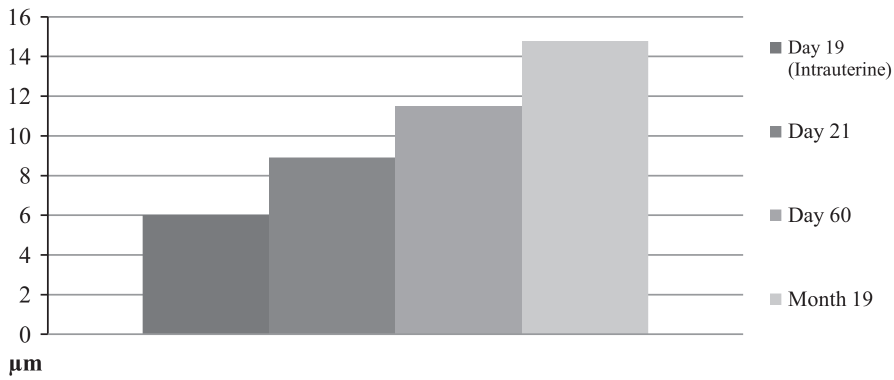

Fig. 10. Basal membrane thickness.

TABLE 3. Dermis thickness for all groups

Groups	BACK ($\bar{x} \pm$ SD)	ABDOMEN ($\bar{x} \pm$ SD)	HEAD ($\bar{x} \pm$ SD)	LOWER LIMB ($\bar{x} \pm$ SD)	UPPER LIMB ($\bar{x} \pm$ SD)
Day 19 (intrauterine)	128.2 ± 5.6	105.4 ± 3.5	93.4 ± 8.4	92.13 ± 5.0	74.1 ± 3.9
Day 21	202.9 ± 7.0*	141.8 ± 7.5*	124.0 ± 10.7*	132.2 ± 7.0*	139.9 ± 8.4*
Day 60	997.1 ± 127.9*,†	522.3 ± 61.0*,†	537.9 ± 57.3*,†	397.8 ± 40.2*,†	387.7 ± 52.0*,†
Month 19	879.3 ± 111.5*,†,‡	312.2 ± 27.8*,†,‡	338.9 ± 49.5*,†,‡	241.6 ± 49.7*,†,‡	229.8 ± 20.4*,†,‡

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

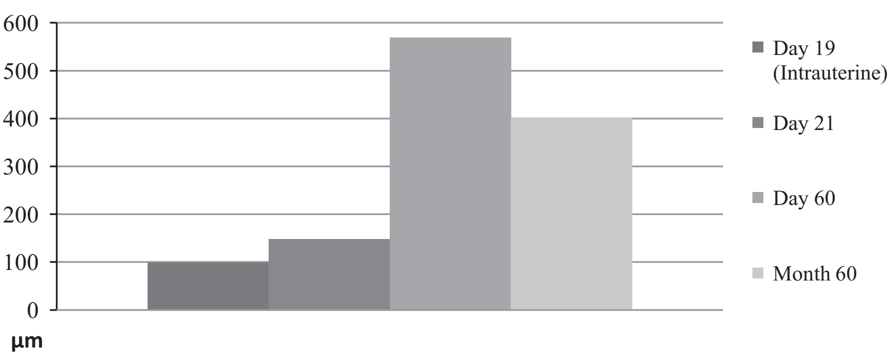

Fig. 11. Dermis thickness.

distinguishable. Dense collagen fibers extended parallel to the dermis and exhibited slightly paler staining in the papillary dermis. Cell content was low in both, although the papillary dermis had more cells than the reticular dermis. On the other hand, the reticular dermis was richer in fibrils, which were densely accumulated parallel to the dermis. Elastic fibers were spread among the collagen fibers and observed as thin, elongated, and branching black fibers. Blue-stained acid

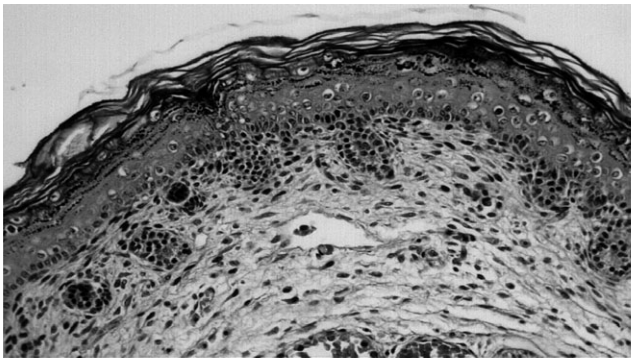

Fig. 12. Day 19 (intrauterin) group dermis layer. EVG; ×20.

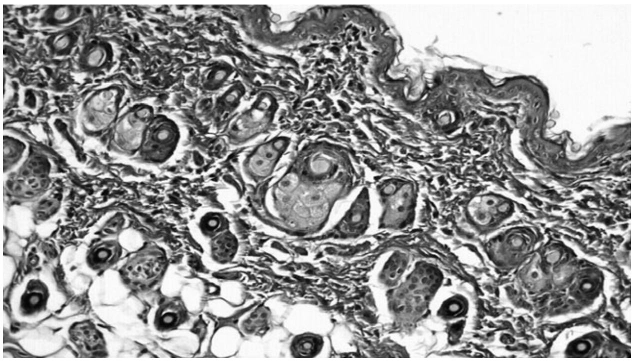

Fig. 13. Day 21 group dermis layer. EVG; ×20.

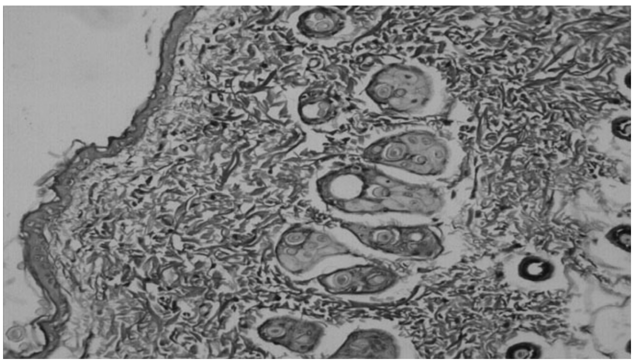

Fig. 14. Day 60 group dermiş layer. EVG; ×20.

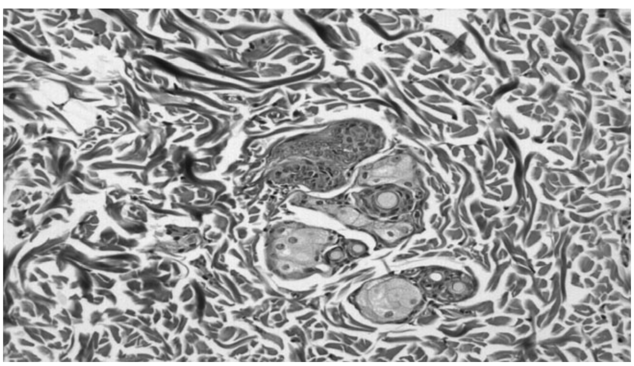

Fig. 15. Month 19 group dermis layer. EVG; ×20.

mucopolysaccharides were generally present around the pilosebaceous units, although they were also scattered within the dermis. In the aged group, the hypodermis was quite evident, while the dermis was more darkly stained and thinner than in the adult group although it presented the same structure in both groups. The papillary dermis and reticular dermis were clearly distinguished in this group. The papillary dermis contained more cells and collagen fibers; although the collagen was densely packed, these fibers were slightly thinner and less intensely stained than the reticular dermis. Collagen and elastic fibers were abundant in the reticular dermis and formed the thickest part of the dermis. In the postpartum month 19 group, the elastic fibers in the dermis were observed to be irregularly distributed. GAGs in this group, as observed in other postnatal groups, were spread around the pilosebaceous units and in-between the collagen bundles within the papillary dermis (Figs 12–15).

In the prenatal group, the dermal papillae, which are projections of the dermis toward the epidermis, were very few in number and also quite small in thickness and height. In the postpartum day 21 group, the number and dimensions of dermal papillae were greater than those in the prenatal group. The number and dimensions of the dermal papillae were the greatest in the postpartum day 60 group. In the aged group, both the number and the dimensions of the dermal papillae decreased compared with those in the postpartum day 21 and adult groups (Figs 16–19). All measurement results are shown in the Tables 4–6 and Figs 20–22.

Mast cell counts are shown in the Table 7 and Fig. 23, and pilosebaceous units counts are shown in the Table 8 and Fig. 24.

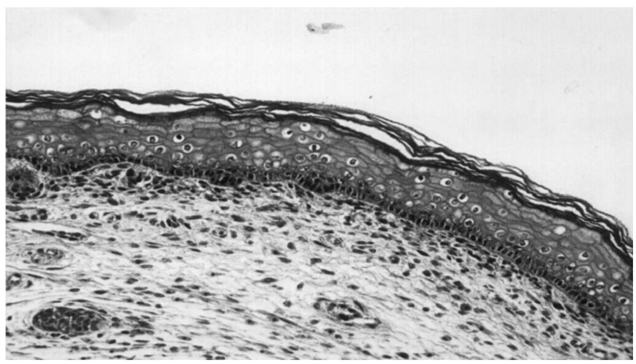

Fig. 16. Day 19 (intrauterin) group dermis layer. Masson's Trichome; ×20.

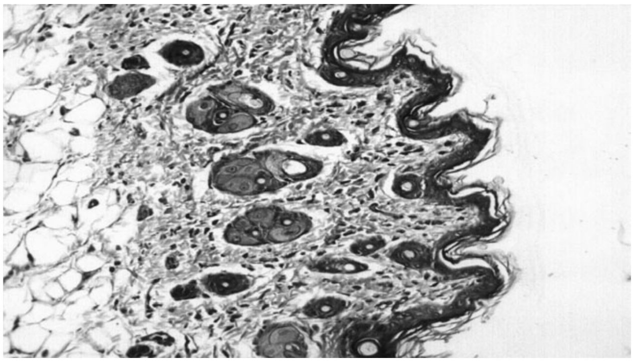

Fig. 17. Day 21 group dermis layer. Masson's Trichome; ×20.

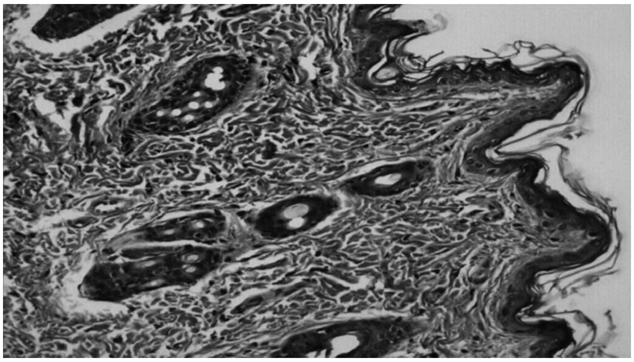

Fig. 18. Day 60 group dermiş layer. Masson's Trichome; ×20.

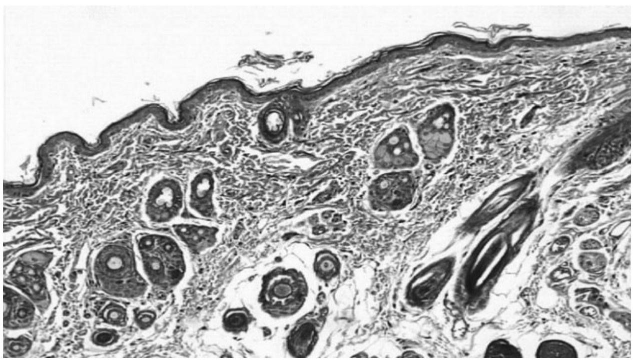

Fig. 19. Month 19 group dermis layer. Masson's Trichome; ×10.

Discussion

This study evaluated the histological structure and changes that occur in rat skin with aging. To our knowledge, no previous morphological studies evaluated the skin changes from the beginning of the intrauterine period by considering parameters such as the thicknesses of the epidermis and dermis, the number of pilosebaceous units and mast cells, and the structures of collagen, elastic fibers, and GAGs.

Opinions differ regarding how the epidermal thickness changes with age. Some researchers have proposed that it does not change with age (4), while others have proposed that it gets thinner (5, 6) or thicker (7, 8). According to the researchers who suggested that the epidermis gets thinner with age, such thinning occurs due to increased apoptosis toward the granular layer, cytological atypia, and increased keratinization (5, 6). On the other hand, those who suggested that the epidermis gets thicker with age explained that this occurs due to the irregularity of the size and form of keratinocytes in the basement and spinous layers (9) as well as an increased surface area and decreased desquamation (8), both of which were found to be more prominent in skin areas exposed to ultraviolet radiation (7, 8).

In this study, the specimens from the intrauterine group showed the thickest epidermis, while the specimens from the early postpartum group had the thinnest epidermis. This suggests that augmented growth of the epidermis occurs during the intrauterine period, followed by a sudden decrease postpartum. On the other hand, comparison of the postpartum specimens indicated that an increase in epidermal thickness occurs toward old age.

It has already been shown that the basement membrane plays an important role in supporting the epidermis. Both the functional and morphological integrity of the basement membrane are essential for the healthy growth of the epidermis. Basement membrane integrity affects

TABLE 4. Dermal papillae's height

Groups	BACK ($\bar{x}$ ± SD)	ABDOMEN ($\bar{x}$ ± SD)	HEAD ($\bar{x}$ ± SD)	LOWER LIMB ($\bar{x}$ ± SD)	UPPER LIMB ($\bar{x}$ ± SD)
Day 19 (intrauterine)	10.27 ± 1.2	9.9 ± 1.4	8.7 ± 1.5	9.6 ± 1.7	7.3 ± 1.2
Day 21	32.0 ± 5.6*	20.1 ± 5.7*	29.7 ± 2.6*	23.9 ± 3.3*	26.8 ± 8.1*
Day 60	61.0 ± 13.0*,†	51.0 ± 8.3*,†	46.0 ± 5.1*,†	43.5 ± 5.6*,†	46.7 ± 10.4*,†
Month 19	33.3 ± 6.7*,‡	22.0 ± 5.7*,‡	30.4 ± 5.0*,‡	30.3 ± 5.9*,†,‡	32.3 ± 8.1*,‡

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

TABLE 5. Dermal papillae's width

Groups	BACK ($\bar{x}$ ± SD)	ABDOMEN ($\bar{x}$ ± SD)	HEAD ($\bar{x}$ ± SD)	LOWER LIMB ($\bar{x}$ ± SD)	UPPER LIMB ($\bar{x}$ ± SD)
Day 19 (intrauterine)	12.1 ± 3.5	11.9 ± 1.6	11.8 ± 1.6	15.2 ± 3.7	10.0 ± 1.5
Day 21	41.97 ± 3.8*	28.5 ± 6.2*	30.8 ± 4.8*	25.4 ± 2.6*	28.4 ± 8.3*
Day 60	88.4 ± 14.5*,†	54.1 ± 7.8*,†	53.9 ± 5.8*,†	46.4 ± 4.1*,†	58.0 ± 9.6*,†
Month 19	43.42 ± 7.5*,‡	30.7 ± 6.1*,‡	41.6 ± 7.5*,†,‡	35.8 ± 6.3*,†,‡	34.5 ± 5.9*,‡

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

TABLE 6. Number of dermal papillaes

Groups	BACK ($\bar{x}$ ± SD)	ABDOMEN ($\bar{x}$ ± SD)	HEAD ($\bar{x}$ ± SD)	LOWER LIMB ($\bar{x}$ ± SD)	UPPER LIMB ($\bar{x}$ ± SD)
Day 19 (intrauterine)	1.7 ± 0.5	1.4 ± 0.2*	0.9 ± 0.2	1.7 ± 0.5	1.4 ± 0.3
Day 21	5.8 ± 0.9*	5.0 ± 1.7*	9.1 ± 2.6*	6.4 ± 1.7*	4.1 ± 1.3*
Day 60	6.3 ± 2.2*	8.1 ± 0.8*,†	7.6 ± 1.4*	7.2 ± 0.8*	6.3 ± 2.2*
Month 19	5.0 ± 0.9*	4.0 ± 1.3*,‡	4.8 ± 1.1*,†,‡	4.4 ± 1.1*,†,‡	5.0 ± 07*

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

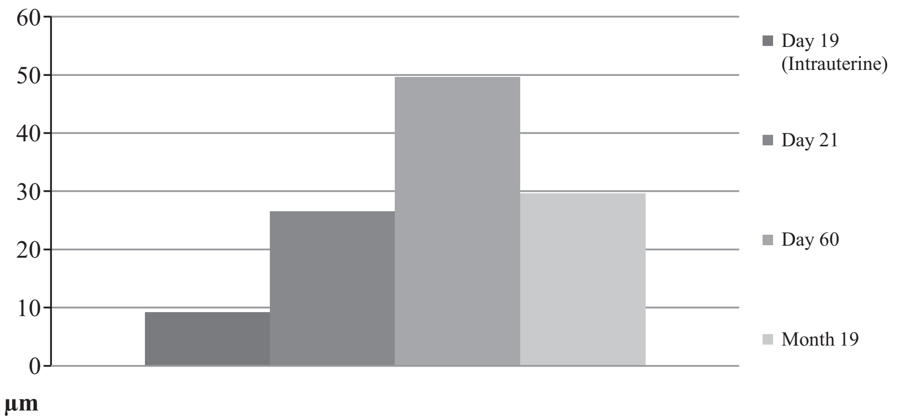

Fig. 20. Dermal papillae's height.

the features and polarization of the cell type as well as cell migration, growth, and morphogenesis during healing (10).

Sauerman et al. (11) observed that the basement membrane in humans becomes thinner with age. They linked this to its irregularity and flattening of the dermo-epidermal junction. Another study conducted in mice showed that basement membrane thickness increases with age, which was probably due to the increase of collagen in the basement membrane and decrease in total tissue turnover (12).

Our study showed an increase in basement membrane thickness with age, which was statistically significant among the postpartum groups, which we consider to be reasonable given the thickening of the epidermis in these groups.

The indentations within the dermo-epidermal junction promote the nutritional status of the skin (10) and protect it from mechanical effects (7). It is generally accepted that a decrease in the number of dermal papillae and flattening of the dermo-epidermal indentations are specific findings of intrinsic aging (7, 13). This is explained by the retraction of epidermal papillae as a result of microindentations of basal cells into the dermis. A decrease in surface area, decreased capillaries in the papillae, and increased skin fragility impair the nutritional status of the skin (5, 11, 14).

Our findings regarding the number, width, and height of the dermal papillae in the postpartum period are consistent with the literature. As has been previously observed in other studies, all our subjects showed decreases in the

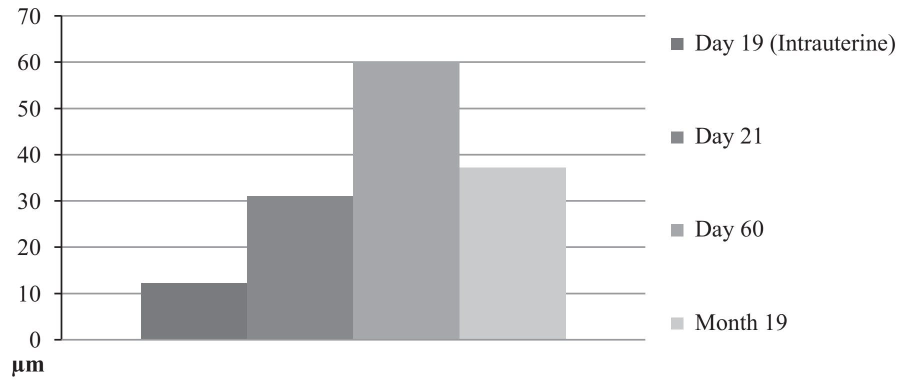

Fig. 21. Dermal papillae's width.

Fig. 22. Number of dermal papillaes.

TABLE 7. Mast cell counts

Groups	BACK ($\bar{x} \pm$ SD)	ABDOMEN ($\bar{x} \pm$ SD)	HEAD ($\bar{x} \pm$ SD)	LOWER LIMB ($\bar{x} \pm$ SD)	UPPER LIMB ($\bar{x} \pm$ SD)
Day 19 (intrauterine)	41.3 ± 1.8	33.8 ± 2.2	42.5 ± 6.0	57.5 ± 2.7	79.8 ± 9.3
Day 21	33.87 ± 1.8*	45.5 ± 7.2*	28.3 ± 3.3*	34.3 ± 2.1*	41.2 ± 4.5*
Day 60	23.6 ± 3.6*,†	23.2 ± 3.1*,†	24.8 ± 2.3*,†	27.2 ± 2.1*,†	27.0 ± 1.1*,†
Month 19	19.5 ± 1.8*,†,‡	20.3 ± 2.4*,†,‡	15.6 ± 1.4*,†,‡	20.6 ± 2.1*,†,‡	21.5 ± 1.8*,†,‡

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

width and height of the dermal papillae with aging, which could be part of the natural process of intrinsic aging.

The connective tissue of the dermis is known to be composed of collagen, elastic fibers, and an amorphous substance, all of which are produced by fibroblasts. According to some studies, aging is associated with an approximately 20% increase in dermal thickness. However, while the young dermis is rich in vessels and contains fewer cells, the aged dermis is relatively poor in vessels but contains numerous cells (2). It is suggested that the main reason for the increase in skin thickness is elastosis, that is, the accumulation of substances containing abnormal elastin, which occurs as a result of photoaging (15). On the other hand, many researchers have found that the thickness of the dermis decreases with age (16, 17). According to them, while the young dermis has a well-organized tight matrix, in aged skin, the loss of matrix proteins occurs, which results in a disorganized and loose matrix. Although skin aging is a complex process, the decreased synthesis of collagen, decreased collagen content, and diminished fibroblast function can explain the dermal atrophy and delayed wound healing in aged skin (13).

The results of our study showed a gradual increase in skin thickness toward adulthood in

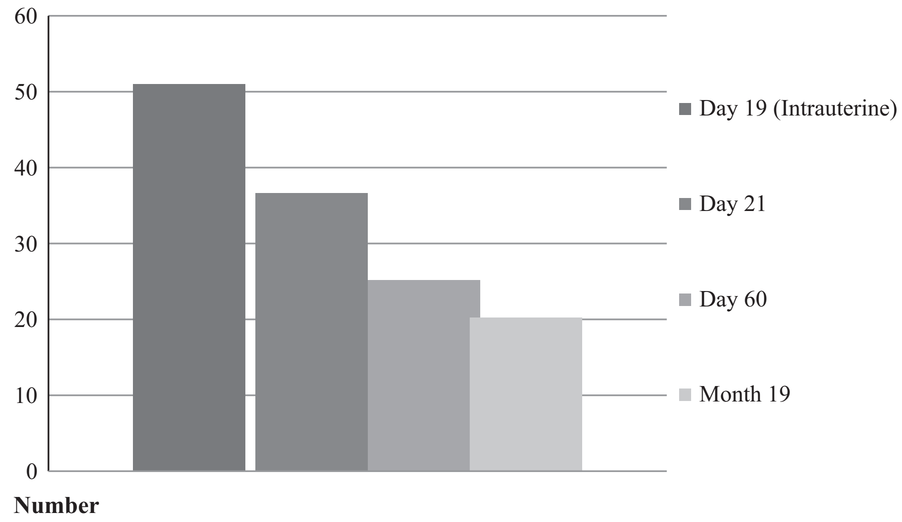

Fig. 23. Number of mast cells.

TABLE 8. Pilosebaseus unit counts

Gruplar	BACK ($\bar{x}$ ± SD)	ABDOMEN ($\bar{x}$ ± SD)	HEAD ($\bar{x}$ ± SD)	LOWER LIMB ($\bar{x}$ ± SD)	UPPER LIMB ($\bar{x}$ ± SD)
Day 19 (intrauterine)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 21	17.8 ± 2.0*	22.1 ± 4.2*	19.8 ± 2.9*	31.0 ± 2.3*	25.8 ± 6.8*
Day 60	19.6 ± 4.0*	25.1 ± 3.0*	23.5 ± 3.0*,†	32.7 ± 2.1*	30.0 ± 3.4*
Month 19	13.8 ± 1.5*,†,‡	16.3 ± 1.9*,†,‡	15.6 ± 1.4*,†,‡	19.5 ± 2.7*,†,‡	19.6 ± 2.6*,‡

*Significantly different from values of day 19 (intrauterine) ($P < 0.05$).
†Significantly different from values of day 21 ($P < 0.05$).
‡Significantly different from values of day 60 ($P < 0.05$).

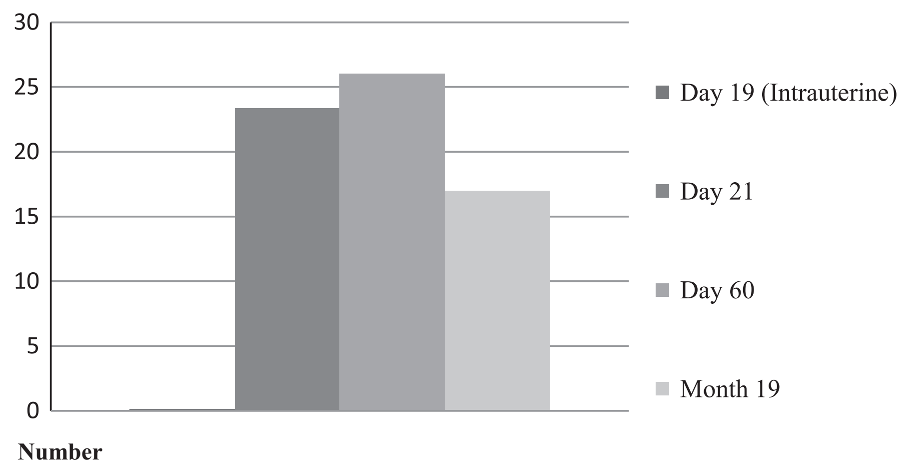

Fig. 24. Number of pilosebase units.

all tissue specimens. In addition, a statistically significant decrease in thickness was observed with increased aging.

In the embryo, the tissue under the epidermis is organized as a fiberless cell network (17, 18), although within a couple of months into the postpartum period, the collagen fibers increase in size and density, and the dermis becomes a fibrous tissue containing dense bundles of fibrils (17). According to some researchers, in aging skin, the amount and thickness of collagen increase (14, 19) and the skin shows a more compact structure due to the diminished space between the collagen fibers (5). However, some researchers showed that aged fibroblasts display a delayed response to mitotic stimuli, so the collagen content of the skin decreases with aging (20).

The results of our study showed that from the intrauterine period toward adulthood, the number and thickness of collagen fibers increases; in contrast, both parameters decrease from adulthood toward month 19. A delayed response of aged fibroblasts to mitotic stimuli might be a good explanation for these decreases in aging skin.

Studies have shown that the development of elastic fibers continues after birth, they mature in young adults, and they become abundant in adults. In other words, the connective tissue of the dermis is mainly formed during the postnatal period (17). Studies have shown that with aging, the number and diameter of elastic fibers decrease in the papillary dermis, while the opposite happens in the reticular dermis, although the latter is accompanied by the disintegration of thickened elastic fibers (21). A comparison of young and adult skin with that of premature infants showed considerable difference in elastic fibers (19); as the space between the elastic fibers is filled with connective tissue, it displays a more compact appearance (5). In accordance with these previous studies, our study also showed that with aging, elastic fibers display a looser and thinner structure.

In addition to collagen and elastic fibrils, the amorphous substance is another component of the dermis. GAGs, which determine the structure of the amorphous substance, also show changes with aging. Specifically, it has been shown that GAGs decrease after birth and during infancy, this decrease then stops until middle age, but once more starts decreasing toward old age (14, 19, 22). In our study, the results for the skin specimens stained with Mowry's colloidal iron that were obtained from the back, abdomen, head, arm, and leg were in accordance with the findings of the aforementioned studies.

The dermis has its own arterial supply and venous drainage (2, 10). Studies have shown that the intensity of dermal vessels also changes with age: young dermis is poor in cells and rich in vessels, while aged skin has relatively few cells and vessels (2). We observed very few vessels in the close vicinity of the hypodermis in samples from various skin areas obtained from the intrauterine group. In the postpartum period, the number of vessels increased and they were most abundant and thick in the day 60 group. However, vessel density and caliber decreased in the month 19 group. These results are in agreement with those of previous studies.

Another important element of the dermis is the mast cells. These cells respond to chemical, physical, and mechanical stimuli by secreting biologically active products and participate in inflammatory conditions (10). Few studies have dealt with changes in the physical, functional, and quantitative properties of mast cells with aging. Previous studies have shown both an increase (23) and a decrease (24) in the number of mast cells with aging. The day 19 group in our study showed the highest number of mast cells, although their number gradually decreased in the postpartum groups.

Pilosebaceous units are located in the dermis and consist of errector pili muscle, hair follicles, and the sebaceous gland complex (10). Although few studies have focused on pilosebaceous units, some studies have suggested that age-related changes also occur in these units. According to some studies, secretion from the sebaceous glands is high in newborns, although it diminishes within a couple of weeks and remains low during childhood. The level of secretion again increases during puberty. Finally, without any quantitative increase, the glands display hyperplasia toward menopause and in the elderly although sebum secretion does not increase. Therefore, secretion does not parallel the size of these glands in the elderly (25). However, the results of another study did not prove any age-related changes in pilosebaceous units (14).

In our study, pilosebaceous units were virtually absent from the specimens obtained from the intrauterine month 19 group. However, there were copious amounts of developing hair follicles. In the postpartum day 21 group, we observed numerous pilosebaceous units in the dermis and even more in the reticular dermis. The quantity of pilosebaceous units peaked in the day 60 group, while there was a considerable decrease in the elderly group. These findings are in agreement with the literature.

It is noteworthy that during the intrauterine period, although the epidermis is significantly thick, the basal lamina is relatively thin, the dermal papillae are flat and relatively few, the pilosebaceous units are virtually absent, and the dermis is at its thinnest. While no studies have specifically focused on this, it was observed in rat skin that keratinization starts within the last 2 days of the intrauterine period, enabling the supply of nutrition to the skin through diffusion until birth (18). Besides this, the fetal skin is well protected against any mechanical trauma from the external environment, which explains why the basal lamina, dermal papillae, and dermis do not fully develop until the postnatal period.

When we compared the postpartum specimens, we observed that the epidermis thickness increased gradually and peaked in the oldest (month 19) group, which also had the thickest basal lamina. On the other hand, the largest and most abundant papillae, as well as the thickest dermis, were observed in the adult group. This suggests that in comparison to the dermis, epidermal nutrition is to a greater extent provided through the basal membrane. Our observations suggest that the increased thickness of the epidermis with aging might be due to decreased renewal, structural impairment of keratinocytes, and increased surface area.

In conclusion, the results of this study showed that the thicknesses of the epidermis, dermis, and basal lamina of rats change with aging. In addition, aging rats exhibit flattening at the dermo-epidermal junction and changes in the composition of collagen, elastic fibers, and GAGs, finally resulting in quantitative changes in pilosebaceous units, vessels, and mast cells. We believe that these findings in rats might be helpful for understanding the skin changes during aging and may provide a foundation for further studies on skin aging.

Conflict of Interest

The all authors declare no potential conflicts of interest with respect to research, authorship and publication of this article.

References

1. Norman RA, Henderson JN. Aging: an overview. Dermatol Ther 2003; 16: 181–185.
2. Baumann L. Skin ageing its treatment. J Pathol 2007; 2112: 241–251.
3. Scharffetter-Kochanek K. Skin aging. Clin Exp Dermatol 2001; 26: 561–568.
4. Whitton JT, Everall JD. The thickness of the epidermis. Br J Dermatol 1973; 89: 467–476.
5. Lavker RM, Zheng PS, Dong S. Aged skin: a study by light, transmission electron, and scanning electron microskopy. J Invest Dermatol 1987; 88: 44–51.
6. Gilhar A, Ulman Y, Karry R, Shalaginov R, Assy B, Serafimovich S, Kalish RS. Aging of human epidermis reversal of aging changes correlates with revensal of keratinocyte fas expression and apoptosis. J Gerentol S A Biol Sci Med Sci 2004; 59: 411–415.
7. Yaar M, Gilchrest BA. Aging and photoaging of keratinocytes and melanoceytes. Clin Exp Dermatol 2001; 26: 583–591.
8. Kligman AM, Zheng P, Lavker RM. The anatomy and pathogenesis of wrinkles. Br J Dermatol 1985; 113: 37.
9. Gilchrest BA, Yaar M. Aging and photoaging of the skin: observations at the molecular level. Br J Dermatol 1992; 127: 25–30.
10. Ross MH, Pawlina W. Histology A Text and Atlas with Correlated Cell and Molecular Biology, 5th edn. Philadelphia PA: Lippincott Williams & Wilkins; 2006: 442–465.
11. Sauerman K, Clemann S, Jaspers S, Gambicher T, Altmeyer P, Hoffmann K, Ennen J. Age related changes of human skin investigated with histomorphometric measurements by confocal laser scanning microscopy in vivo. Skin Res Technol 2002; 8: 52–56.
12. Boyer B, Kern P, Fourtanier A, Jacqeline L-R. Age-dependent variations of the biosyntheses of fibronectin and fibrous collagens in mouse skin. Exp Gerontol 1991; 26: 375–383.
13. Holtkötter O, Schlotmann K, Hofheinz H, Olbrish RR, Petersohn D. Unveiling the molecular basis of intrinsic skin aging. Int J Cosmet Sci 2005; 27: 263–269.
14. Bhattacharrya TK, Merz M, Thomas JR. Modulation of cutaneous aging with calorie restriction in Fischer 344 rats. Arch Facial Plast Surg 2005; 7: 12–16.
15. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultravioletlight. N Engl J Med 1997; 337: 1419–1428.
16. Yaar M, Gilchrest BA. Aging of skin. In: Freedberg MI, Eisen AZ, Wolff K, Austen FK, Goldsmith LA and Katz SL, eds. Fitzpatrick's Dermatology in General Medicine, 6th edn. New York: McGrawe-Hill Book Company; 2003: 1386–1398.
17. Freedberg IM, Eisen AZ, Wolff K, Goldsmith LA, Katz SI. Fitzpatrick's Dermatology in general medicine 6th edition. J Am Acad Dermatol 2004; 2: 325–326.
18. Smith LT, Holbrook KA, Madri JA. Collagen types I, III, and V in human embryonic and fetal skin. Am J Anat 1986; 175: 507–521.
19. Smith JG, Finlayson GR. Dermal connective tissue alterations with age and chronic sun damage. J Soc Cosmet Chem 1965; 16: 527–535.
20. Reenstra WR, Yaar M, Gilchrest BA. Effect of donor age on epidermal growth factor processing in man. Exp Cell Res 1993; 209: 118–122.
21. Gilchrest BA. A review of skin ageing and its medical therapy. Br J Dermatol 1996; 135: 75–87.
22. Breen M, Weinstein HG, Johnson RL, Veis A, Marshall RT. Acidic glycosaminoglycans in human skin during fetal development and adult life. J Invest Dermatol 1970; 201: 54–60.
23. Uvnas B. Chemistery and storage function of mast cell granüles. J Invest Dermatol 1978; 71: 76–80.
24. Montagna W, Carlile K. Structural changes in ageing skin. Br J Dermatol 1990; 122: 61–70.

25. Zouboulis CC, Boshnakow A. Chronological ageing and photo ageing of the human sebaceous gland. Clin Exp Dermatol 2001; 26: 600–608.

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