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# Analyzes of Changes on Skin by Aging

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## INTRODUCTION

Aging is an essential biological process of living organisms and it space 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 [2,3].

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 intrauterineperiod, and toprovide a preliminary foundation on which further studies can build.

## MATERIALS AND METHOD

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. After routine tissue processing 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). 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.

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 magnifications. Mast cell counts were obtained from 15 different areas under × 100 magnifications and pilosebaceous unit counts were obtained from five different areas under × 20 magnification.

## STATISTICAL ANALYSIS

For the statistical analysis of all results, 13.0SPSS 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. In the specimens obtained from the day 21 group, the epidermis showed a four-layered structure, as observed in all other groups. The day 60 groupdid 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 (**Figure 1, 2).** All measurement results are shown in the **Table 1** and **Graphic 1.** 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 day 19 group (**Figure 3, 4).** All measurement results are shown in the **Table 2** and **Graphic 2.**

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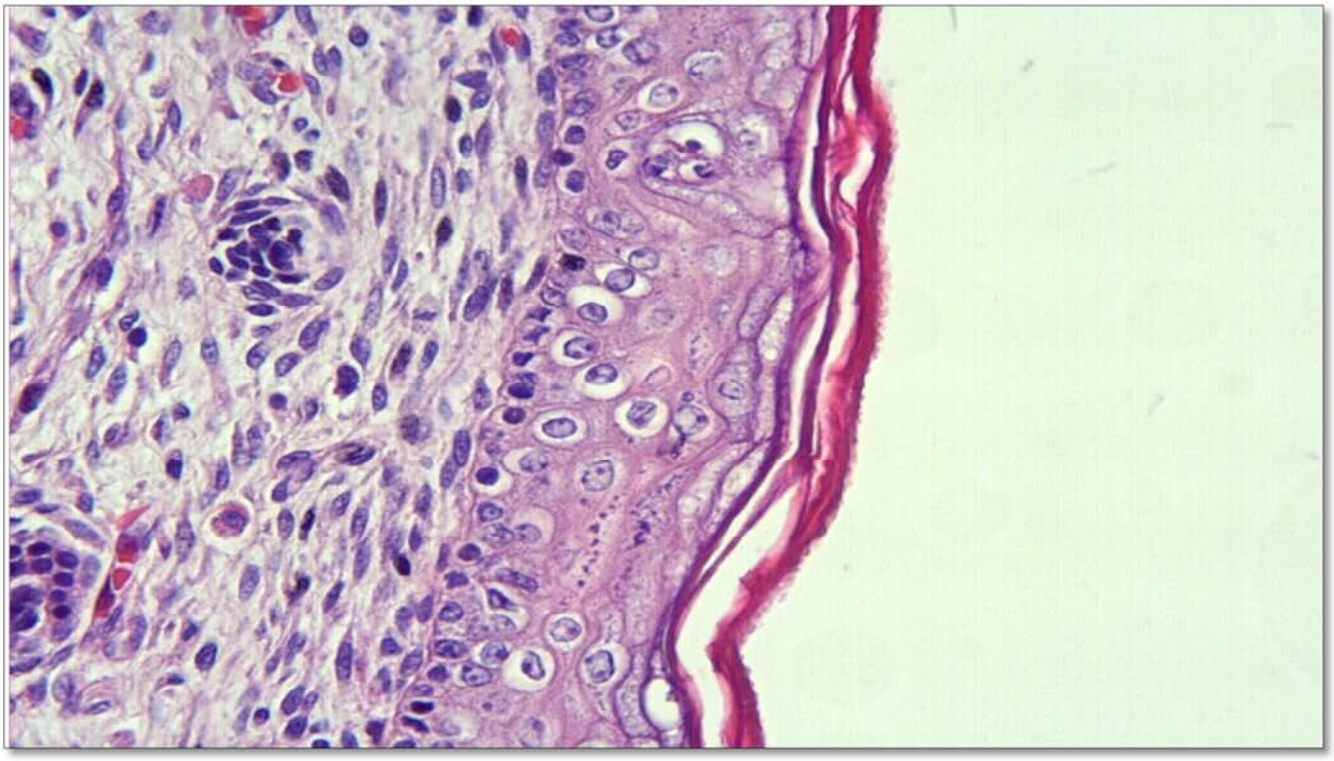


**Figure 1.** Day 19 (intrauterin) group:back skin epidermis. H&E; X40

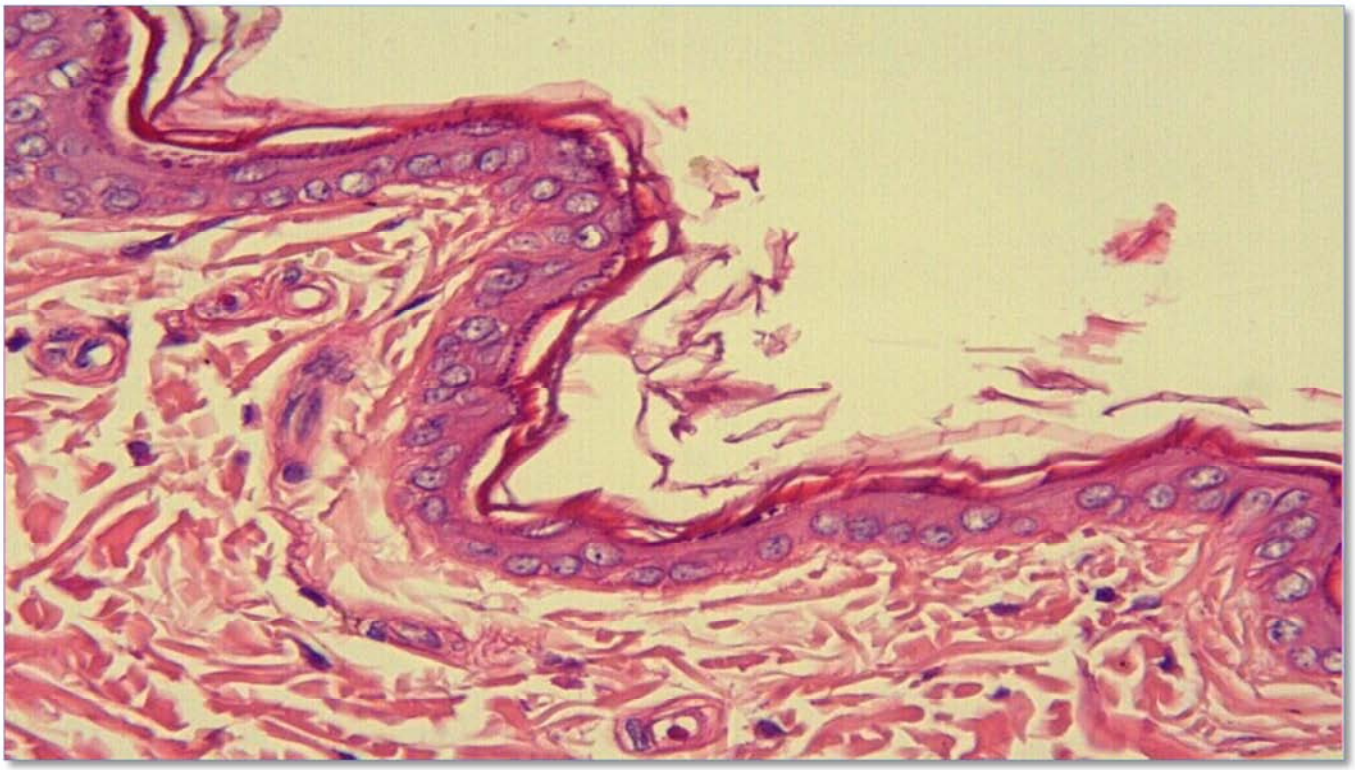


**Figure 2.** Month 19 group abdomen skin epidermis. H&E; X40

**Table 1.** Epidermis layer thickness for all groups

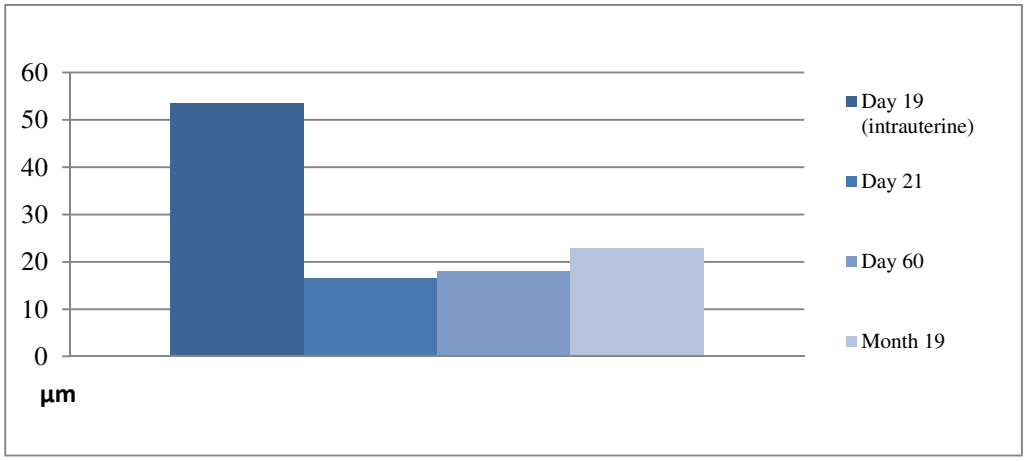
| Groups | BACK ($\bar{X}$ ± SD) | ABDOMEN($\bar{X}$ ± SD) | HEAD ($\bar{X}$ ± SD) | LOWER LIMB ($\bar{X}$ ± SD) | UPPER LIMB ($\bar{X}$ ± SD) |
|---|---|---|---|---|---|
| **Day 19 (intrauterine)** | 54,3 ±5,8 | 49,2±5,5 | 37,1 ± 1,7 | 70,4 ± 3,5 | 55,9±6,0 |
| **Day 21** | 16,9± 0,7[a] | 17,8±1,7[a] | 15,3±0,7[a] | 16,1±0,9[a] | 16,4±1,8[a] |
| **Day 60** | 21,5±1,4[a,b] | 18,3±1,1[a] | 16,7±0,8[a,b] | 16,6±1,5[a] | 17,2±1,1[a,b] |
| **Month 19** | 28,0±2,3[a,b,c] | 22,5±2,0[a,b,c] | 21,5±1,5[a,b,c] | 20,4±1,6[a,b,c] | 22,4±2,2[a,b,c] |

**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).

**b**; Significantly different from values of day 21 ($p < 0.05$).

**c**; Significantly different from values of day 60 ($p < 0.05$).

**Graphic 1**. Epidermis Thickness



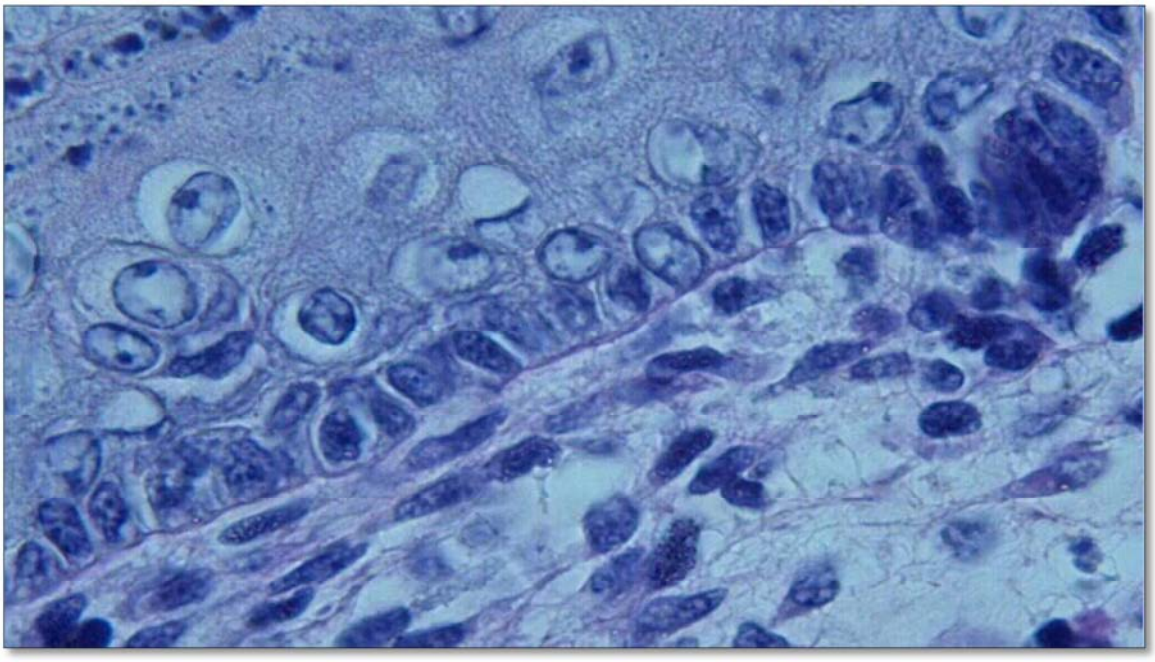



**Figure 3.** Day 19 (intrauterin) group basal membran. PAS; X100.

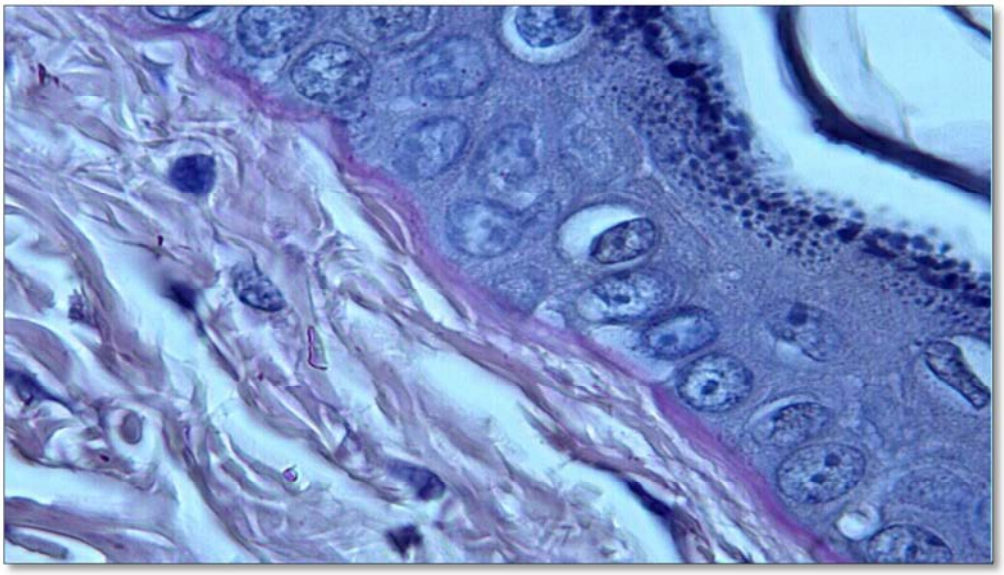


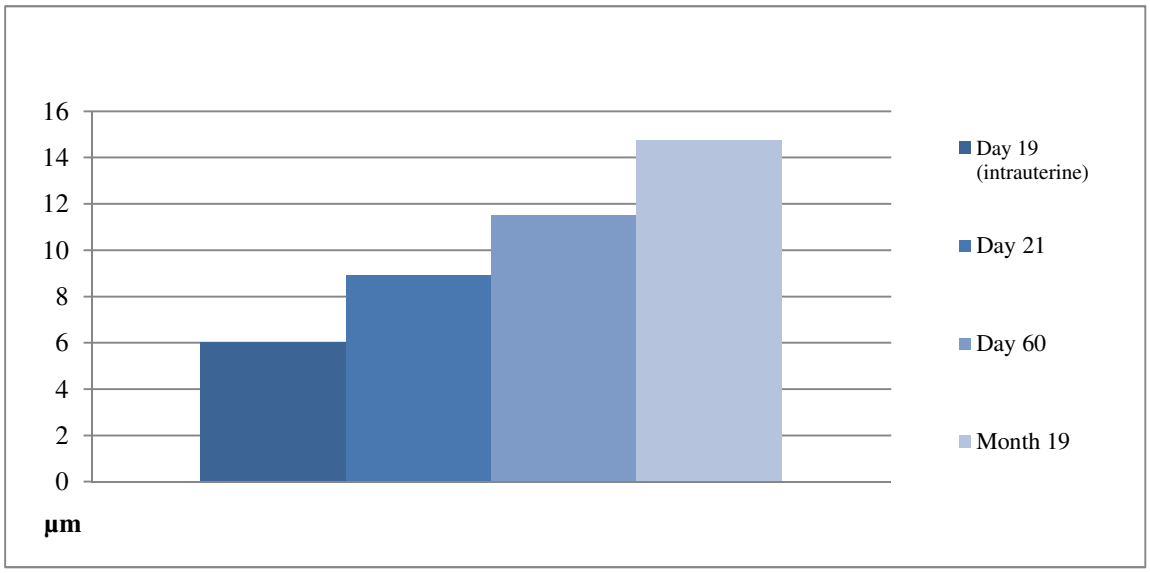
**Figure 4.** Month 19 group basal membran. PAS; X100

**Table 2.** Basal membrane thickness for all groups

| 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)** | 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[a] | 8,8 ± 0,4[a] | 8,4 ± 0,8[a] | 8,6 ± 0,5[a] | 8,7 ± 0,9[a] |
| **Day 60** | 13,23 ± 1,2[a,b] | 11,5 ± 0,5[a,b] | 11,9 ± 0,7[a,b] | 11,4 ± 0,6[a,b] | 11,0 ± 0,5[a,b] |
| **Month 19** | 16,9 ± 0,6[a,b,c] | 14,6 ±0,8[a,b,c] | 14,4 ± 1,6[a,b,c] | 14,4±0,4[a,b,c] | 13,3 ± 0,9[a,b,c] |

**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).
**b**; Significantly different from values of day 21 ($p < 0.05$).
**c**; Significantly different from values of day 60 ($p < 0.05$).

**Graphic 2.** Basal Membrane Thickness



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. Mowry's colloidal iron-stained specimens obtained from this group showed the highest level of GAGs. Inthepostpartumday 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. In general, in this group, elastic fibers were more prominent than the intrauterine group and mostly condensed in the middle of the dermis. 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 (**Figure 5, 6).** All measurement results are shown in the **Table 3** and **Graphic 3.**

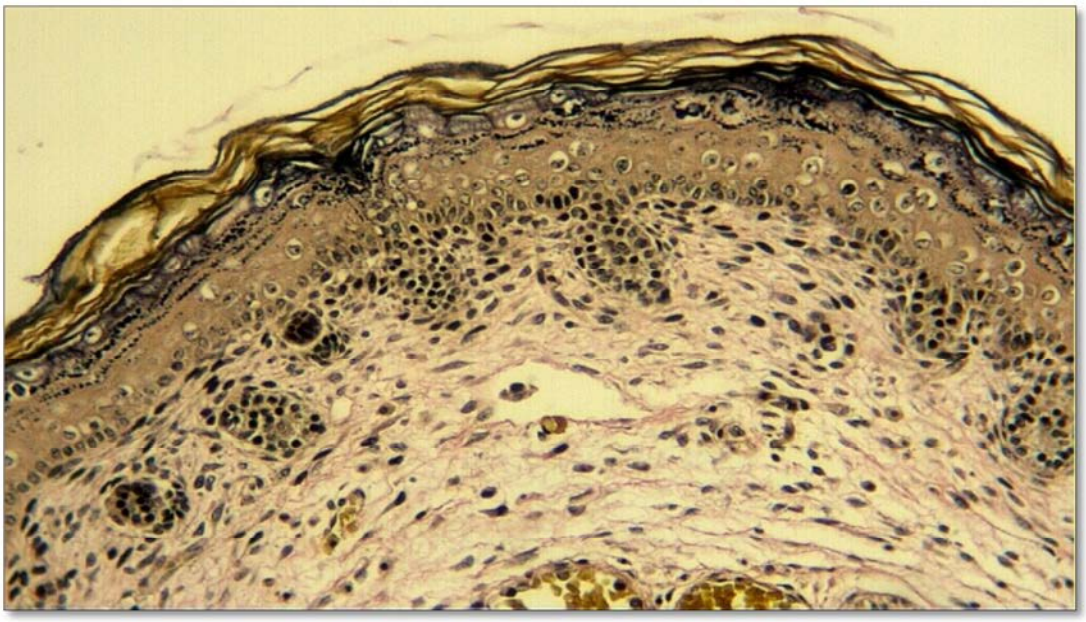


**Figure 5**. Day 19 (intrauterin) group dermis layer. EVG; X20.

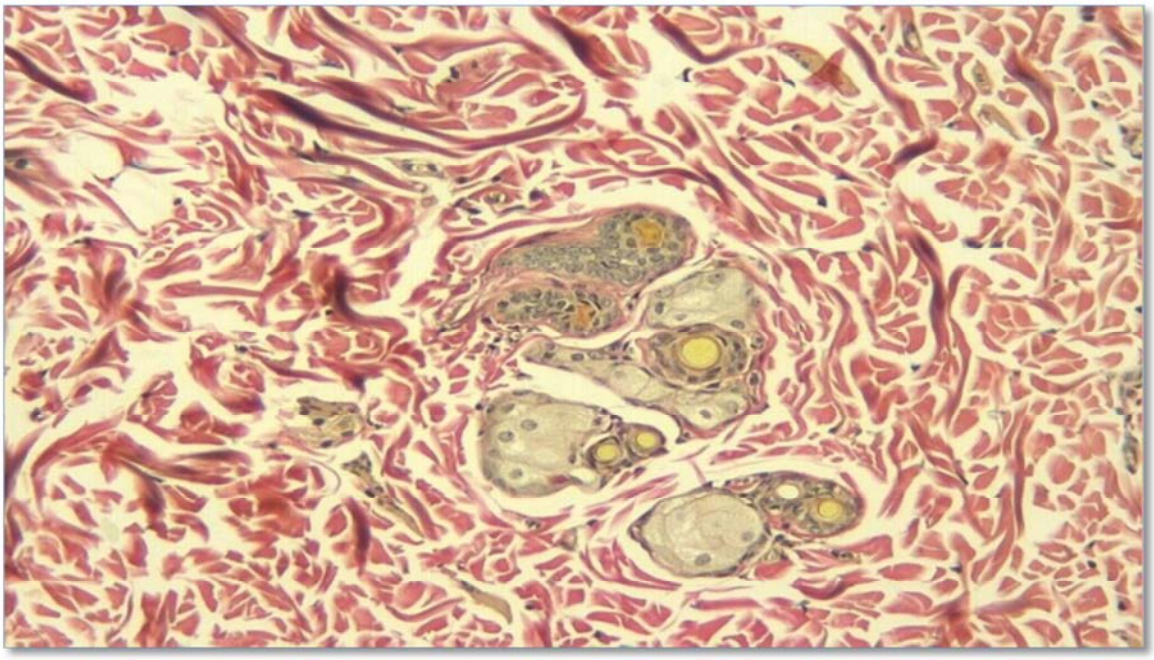


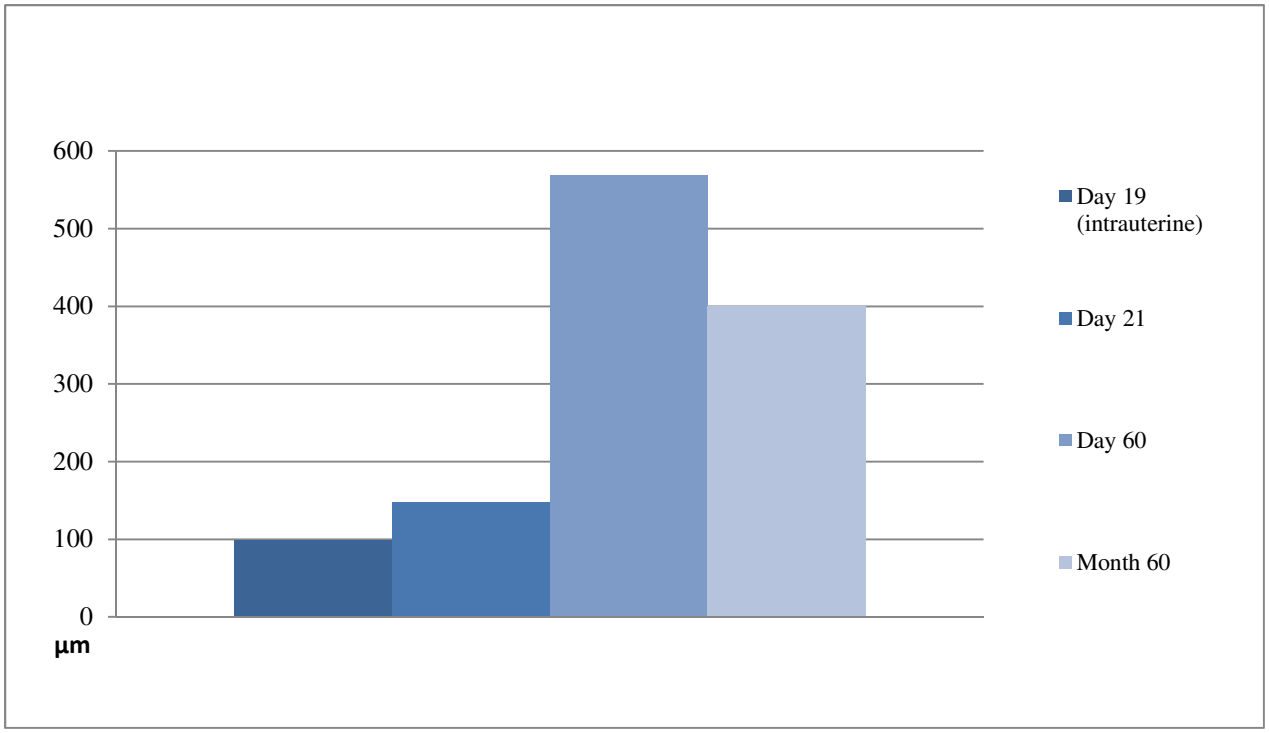
**Figure 6.** Month 19 group dermis layer. EVG; X20.

**Table 3.** Dermis thickness for all groups

| 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)** | 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[a] | 141,8 ± 7,5[a] | 124,0±10,7[a] | 132,2 ± 7,0[a] | 139,9 ± 8,4[a] |
| **Day 60** | 997,1±127,9[a,b] | 522,3±61,0[a,b] | 537,9±57,3[a,b] | 397,8±40,2[a,b] | 387,7±52,0[a,b] |
| **Month 19** | 879,3±111,5[abc] | 312,2±27,8[a,b,c] | 338,9±49,5[a,b,c] | 241,6±49,7[a,b,c] | 229,8±20,4[a,b,c] |

**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).
**b**; Significantly different from values of day 21 ($p < 0.05$).
**c**; Significantly different from values of day 60 ($p < 0.05$).

**Graphic 3.** Dermis Thickness




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 distinguishable. Blue-stained acid 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 (**Figure 7, 8**). 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. All measurement results are shown in the **Tables 4-6** and **Graphics 4-6** Mast cell counts are shown in the **Table 7** and **Graphic 7,** and pilosebaceous units counts are shown in the **Table 8** and **Graphic 8.**

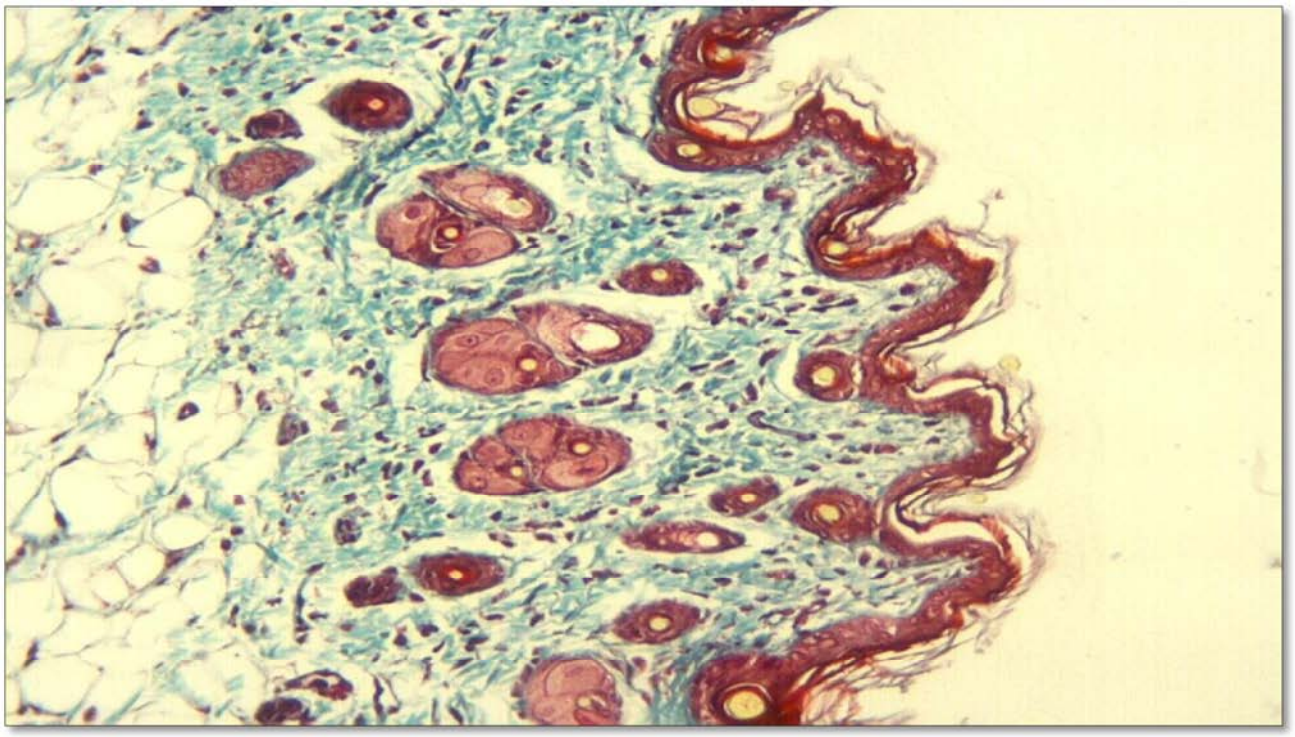


**Figure 7.** Day 19 (intrauterin) group dermis layer. Masson's Trichome; X20.

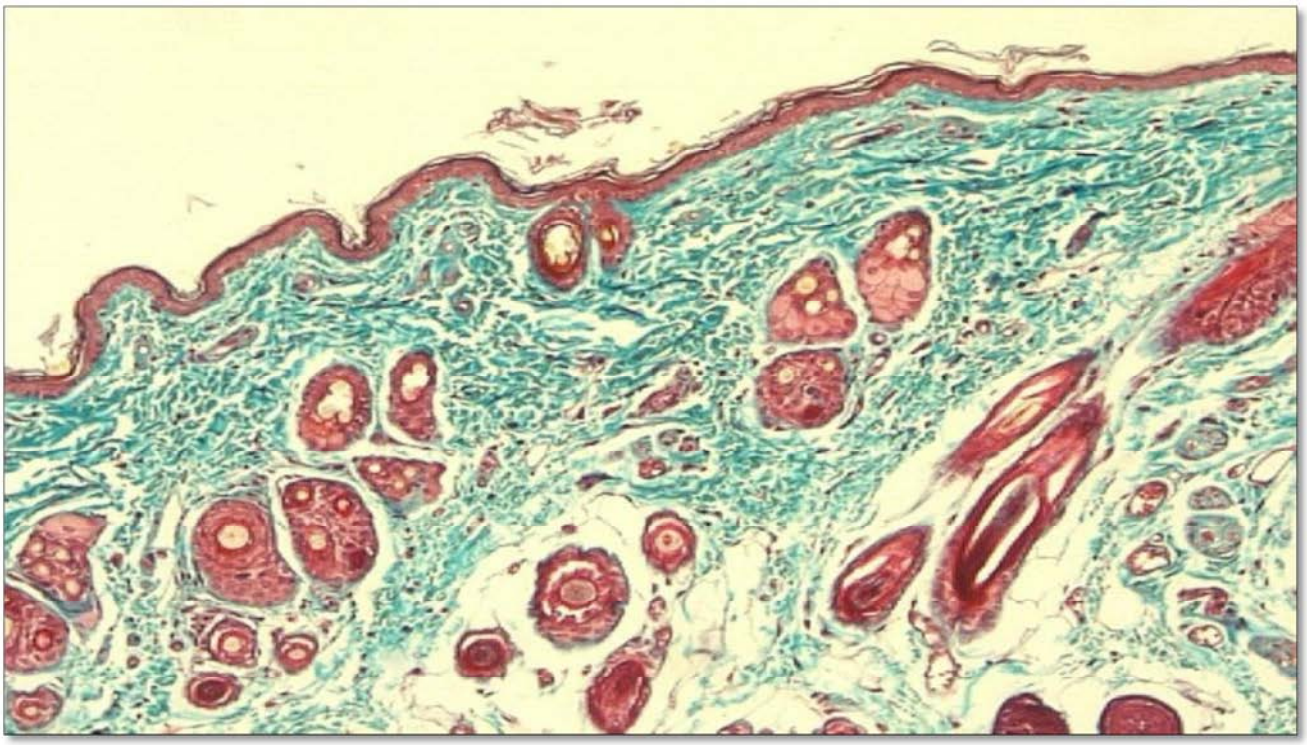


**Figure 8.** Month 19 group dermis layer. Masson's Trichome; X10

**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[a] | 20,1±5,7[a] | 29,7±2,6[a] | 23,9 ± 3,3[a] | 26,8 ± 8,1[a] |
| **Day 60** | 61,0±13,0[a,b] | 51,0±8,3[a,b] | 46,0±5,1[a,b] | 43,5 ± 5,6[a,b] | 46,7 ± 10,4[a,b] |
| **Month 19** | 33,3±6,7[a,c] | 22,0±5,7[a,c] | 30,4±5,0[a,c] | 30,3±5,9[a,b,c] | 32,3 ± 8,1[a,c] |

**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).
**b**; Significantly different from values of day 21 ($p < 0.05$).
**c**; 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[a] | 28,5 ± 6,2[a] | 30,8 ± 4,8[a] | 25,4 ± 2,6[a] | 28,4 ± 8,3[a] |
| **Day 60** | 88,4±14,5[a,b] | 54,1 ± 7,8[a,b] | 53,9 ± 5,8[a,b] | 46,4 ± 4,1[a,b] | 58,0 ± 9,6[a,b] |
| **Month 19** | 43,42±7,5[a,c] | 30,7 ± 6,1[a,c] | 41,6 ± 7,5[a,b,c] | 35,8 ± 6,3[a,b,c] | 34,5 ± 5,9[a,c] |

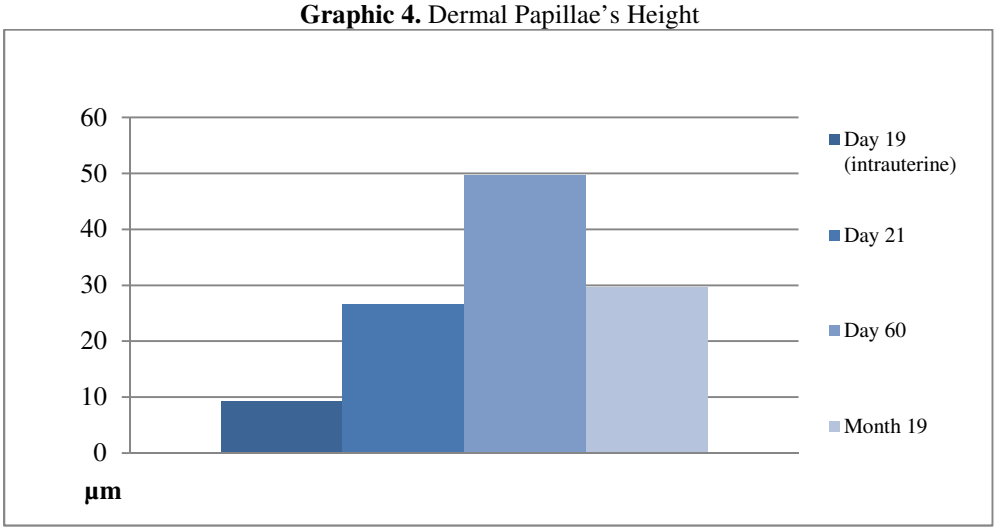
**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).
**b**; Significantly different from values of day 21 ($p < 0.05$).
**c**; Significantly different from values of day 60 ($p < 0.05$).

**Table 6.** Number of Dermal papillaes

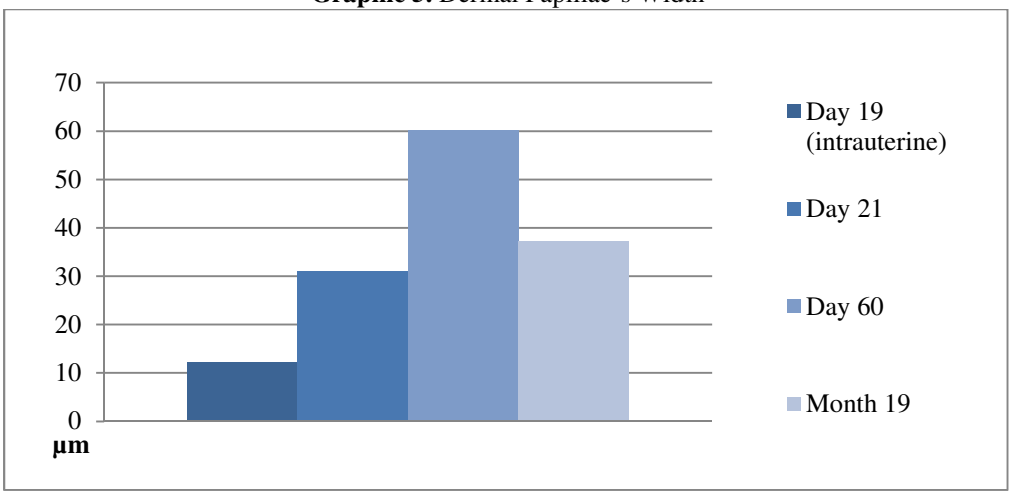
| Groups | BACK ($\bar{X}$ ± SD) | ABDOMEN ($\bar{X}$ ± SD) | HEAD ($\bar{X}$ ± SD) | LOWER LIMB ($\bar{X}$ ± SD) | UPPER LIMB ($\bar{X}$ ± SD) |
|---|---|---|---|---|---|
| **Day 19 (intrauterine)** | 1,7 ± 0,5 | 1,4 ± 0,2a | 0,9 ± 0,2 | 1,7±0,5 | 1,4 ± 0,3 |
| **Day 21** | 5,8 ± 0,9a | 5,0 ± 1,7a | 9,1 ± 2,6a | 6,4 ±1,7a | 4,1 ± 1,3a |
| **Day 60** | 6,3 ± 2,2a | 8,1 ± 0,8a,b | 7,6 ± 1,4a | 7,2 ±0,8a | 6,3 ± 2,2a |
| **Month 19** | 5,0 ± 0,9a | 4,0 ± 1,3a,c | 4,8 ± 1,1a,b,c | 4,4±1,1a,b,c | 5,0 ± 07a |

**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).
**b**; Significantly different from values of day 21 ($p < 0.05$).
**c**; Significantly different from values of day 60 ($p < 0.05$)

**Graphic 4.** Dermal Papillae's Height




**Graphic 5.** Dermal Papillae's Width




**Graphic 6.** Number of Dermal Papillaes

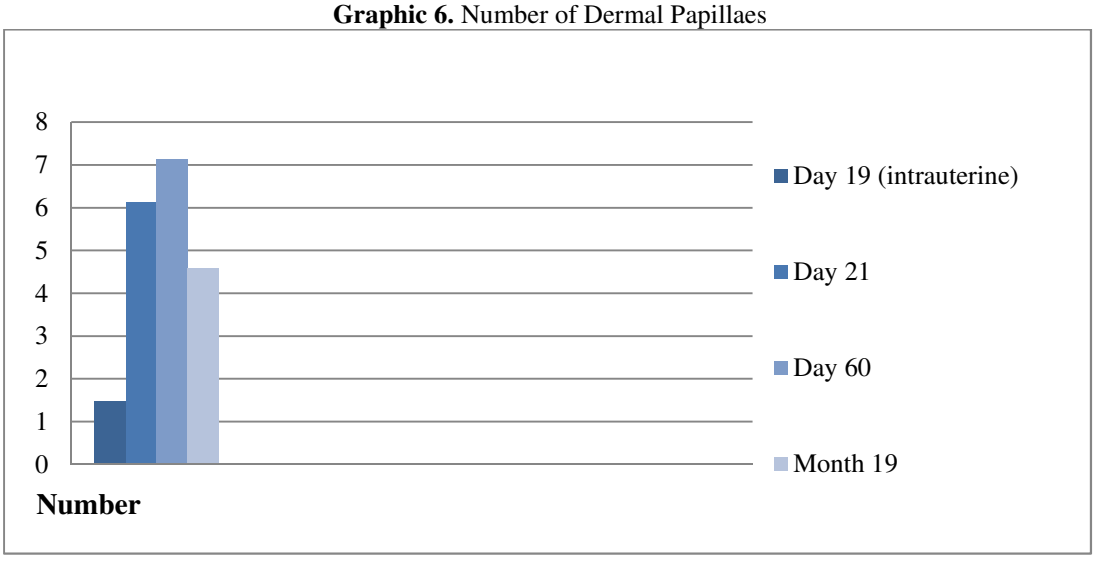


**Table 7.** Mast cell counts

| 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)** | 41,3 ± 1,8 | 33,8±2,2 | 42,5 ± 6,0 | 57,5 ± 2,7 | 79,8 ± 9,3 |
| **Day 21** | 33,87 ± 1,8a | 45,5 ±7,2a | 28,3 ± 3,3a | 34,3 ± 2,1a | 41,2 ± 4,5a |
| **Day 60** | 23,6 ± 3,6a,b | 23,2 ± 3,1a,b | 24,8 ± 2,3a,b | 27,2 ± 2,1a,b | 27,0 ±1,1a,b |
| **Month 19** | 19,5 ± 1,8a,b,c | 20,3 ± 2,4a,b,c | 15,6 ± 1,4a,b,c | 20,6 ±2,1a,b,c | 21,5±1,8a,b,c |

**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).
**b**; Significantly different from values of day 21 ($p < 0.05$).
**c**; Significantly different from values of day 60 ($p < 0.05$).

**Graphic 7.** Number of Mast Cells

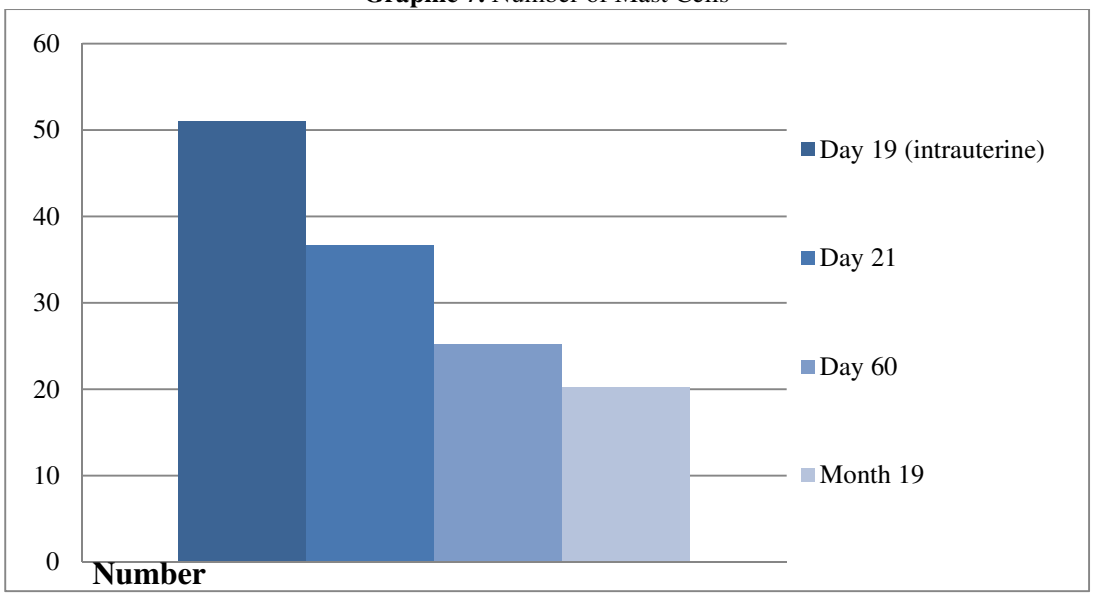
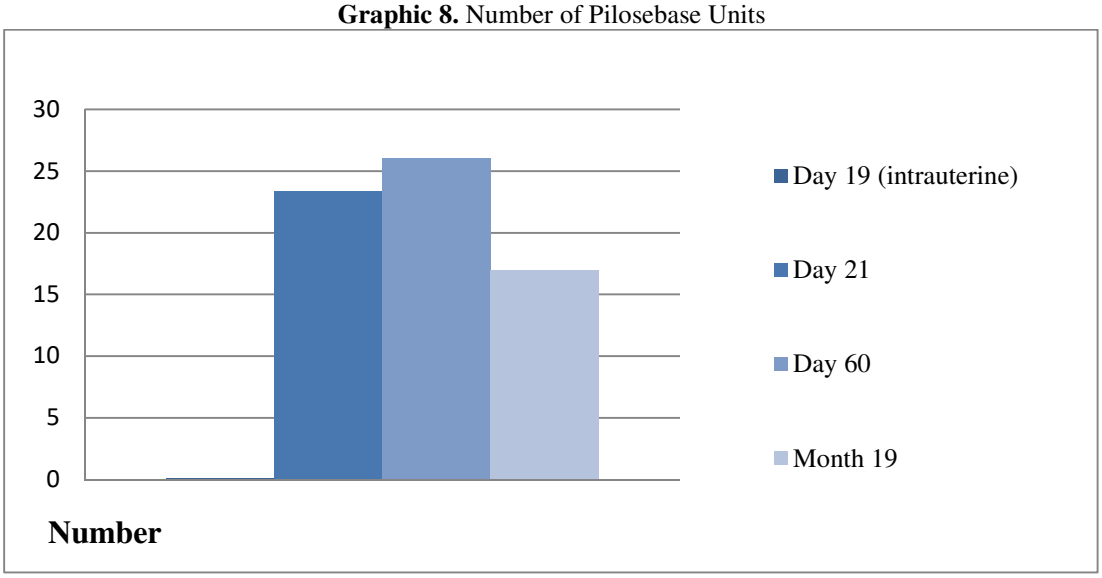



**Table 8.** Pilosebaseus unit counts

| 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[a] | 22,1 ± 4,2[a] | 19,8 ± 2,9[a] | 31,0 ±2,3[a] | 25,8 ± 6,8[a] |
| **Day 60** | 19,6 ± 4,0[a] | 25,1 ± 3,0[a] | 23,5 ±3,0[a,b] | 32,7 ±2,1[a] | 30,0 ± 3,4[a] |
| **Month 19** | 13,8 ± 1,5[a,b,c] | 16,3±1,9[a,b,c] | 15,6 ±1,4[a,b,c] | 19,5 ±2,7[a,b,c] | 19,6 ±2,6[a,c] |

**a**; Significantly different from values of day 19 (intrauterine) ($p < 0.05$).
**b**; Significantly different from values of day 21 ($p < 0.05$).
**c**; Significantly different from values of day 60 ($p < 0.05$).

**Graphic 8.** Number of Pilosebase Units




## CONCLUSION

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 numbers of pilosebaceous units and mast cells, and the structures of collagen, elastic fibers, and GAGs of ratormouse.

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 dermoepidermal 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.

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