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## Morphometry of skin changes in Newfoundland dogs following coat clipping

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## ABSTRACT

Dog breeds are unique in their coat conformation and quality. Newfoundland dogs have a long and fine hair coat, and clipping may induce changes in newly grown hair. This study examined structural changes in the skin of Newfoundland dogs following clipping. Dogs included in the study had visible coat changes following clipping that appeared as loss of gloss, increased scaling and textural changes. The control groups consisted of two groups of dogs that had never been clipped: Newfoundland dogs served as within-breed controls, and long-haired dogs of other breeds served as between-breed controls. All dogs were healthy with no history of dermatological problems. Two skin biopsies were taken from each dog and evaluated for predetermined parameters.

A total of 41 samples were examined: 11 from clipped Newfoundland dogs, 16 from unclipped ones, and 14 from dogs of other breeds. By histopathology, the clipped dogs had a thicker cornified layer ( $P = 0.006$ ) and smaller sebocytes ( $P = 0.022$ ) than the unclipped ones. Newfoundlands had larger and more epitrichial sweat glands than other breeds ( $P = 0.0002$ ,  $P = 0.036$ , respectively), and those were not affected by clipping. These results suggest that hyperkeratosis and decreased sebocyte size may explain the observed coat changes following clipping in Newfoundland dogs.

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## Introduction

Breed variation in dogs is very pronounced and one of the most prominent is hair coat appearance. Hair is usually classified as short, intermediate or normal and long, and is further subdivided into fine and coarse (Scott et al., 2001b). Hair regrowth following clipping may be impaired in some breeds, especially those with a long, fine hair coat, causing concern to owners and requiring frequent veterinary care (Scott et al., 2001a). Owners are also concerned about changes in the quality of hair coat that occur after clipping or spaying.

Several studies have been conducted to address problems associated with hair regrowth, in particular evaluating hormonal influence on hair cycle. Histopathology of the skin of dogs that experienced delay in hair regrowth post-clipping showed hair follicle arrest, which is also reported in endocrine alopecia (Frank, 2005; Gross et al., 2005). Other factors found to influence hair regrowth and replacement were body site, environmental changes, age, sex hormones and breed variation (Al-Bagdadi et al., 1977, 1979; Butler and Wright, 1981; Hale, 1982; Gunaratnam and Wilkinson, 1983; Dunstan et al., 2001). Reichler et al. (2008) reported coat changes in 20% of a cohort of spayed bitches, but could not identify the pathological mechanism underlying these changes.

Other changes in hair quality following clipping have yet to be addressed. The predominant changes in coat quality reported by owners are loss of gloss, increased scaling and texture changes. The aim of the present study was to investigate possible changes in skin structure in Newfoundland dogs with coat quality changes following hair clipping and to compare them with dogs that had never been clipped.

## Materials and methods

## Study animals and samples collection

The work was designed as a case-control study and the study group included skin samples from Newfoundland dogs that had a history of clipping of the entire body or large parts of it, and whose owners had noticed changes in hair quality demonstrated by loss of gloss, increased scaling and texture changes following clipping. This group is referred to as clipped Newfoundlands (C-NF). The hot and humid environment in which the dogs lived was the only reason for clipping. It is important to note that the owners of the clipped Newfoundlands did not seek veterinary care for the problem as their dogs did not have medical problems other than the skin and coat changes.

The within-breed control group included skin samples from pure bred Newfoundland dogs that had never been clipped, and is referred to as unclipped Newfoundlands (N-NF). The between-breed control group included skin samples from dogs of other breeds with fine long hair coat that had never been clipped, and is referred to as other breeds (OB). All dogs were healthy according to their records and the physical examination performed by the first author (GZ) and had no history of skin problems. The dogs did not receive any topical or systemic treatments for at least 1 month prior to obtaining the skin samples.

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From each dog two samples were taken for histological evaluation: one from the chest and one from the back. The chest was chosen because Newfoundlands have a heavy coat and more dogs were clipped only at this site. The back was chosen because the coat and skin changes were easily demonstrated there and for the purpose of having samples from another site. The chest sample was taken in the medial line between the forelimbs and the back sample was taken 2–3 cm lateral to the spine at the line of the last rib. All samples were taken by one author (KR). The biopsies were taken during the months March and April 2007 and 2009; however, no attempt was made to take the samples from the clipped dogs at a particular time after clipping.

For skin biopsy, the area was gently shaved, smeared with local anesthetic cream containing lidocaine 2.5% and prilocaine 2.5% (Emla, AstraZeneca), and after 20–30 min the area was injected subcutaneously (SC) with a local anesthesia solution containing lignocaine–HCl 1% (Esracain, Rafa). The skin biopsies were obtained using a 6 mm biopsy punch and stored in 10% (v/v) neutral buffered formalin until processing for histological examination.

All owners signed an informed consent form and all procedures in the study were approved by the Ethical Committee for Clinical Trials of the Koret School of Veterinary Medicine (Approval Number: KSVM-VTH 07/2007).

#### Histology and tissue examination

Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). From each sample, 3 µm serial sections were put on a slide and the most intact ones were examined microscopically. The following parameters were evaluated: (1) the thicknesses of the epidermis and of the cornified layer and the ratio between them; (2) the number of follicular units in a section (a follicular unit was defined as a group of primary and secondary hair follicles with the sebaceous gland connected to them. These structures are associated also with the arrector pili muscle and are known as a pilosebaceous unit); (3) the number of hair follicles and sebaceous glands per follicular unit and the number of epitrachial sweat glands; (4) in some follicular units the largest sebaceous gland was measured (in µm) by choosing the longest longitudinal and horizontal lines; (5) sizes of sebocytes; numbers of sebocytes in a gland, number of reserve cells, and the ratio between them repeated for 3–4 sebaceous glands in every section.

The reserve cells are deeply basophilic basal cells located at the basement membrane zone which bordered the sebaceous lobules. These cells become sebocytes when progressively accumulating lipids, and then disintegrate to form sebum towards the center of the lobule. The measurements of the epitrachial sweat glands were made as described for sebaceous glands. The epitrachial sweat glands evaluated were chosen arbitrarily in the section. The measurements were done using a special in-lens calibrator (Olympus) in which 10 calibrated lines at a magnification  $\times 100$  is equal to 100 µm. Histological examinations and measurements were performed blindly by all the authors.

#### Statistical analysis

Chi-Square Fisher's exact test was applied for assessing the associations between two categorical variables, and included testing the associations between groups for genders. One-way ANOVA was used to compare the ages of the groups. The Kruskal–Wallis analysis of variance was used to compare quantitative variables between the groups. This included testing the associations between groups, between and within body sites for all the measurable histological variables in skin samples. The Mann–Whitney test was used to examine associations between the gender and histological variables.

In cases where statistical significances were found, analysis of variance was applied to detect which of the two variables (group or gender) had a greater effect on the results. Pearson's correlation coefficient was applied for all histological variables and ages. In cases where the differences between groups were statistically significant and the correlation was found high, analysis of covariance was applied for examining the effect of each variable on the results. All tests applied were two-tailed, and  $P \leq 0.05$  was considered statistically significant.

For associations where statistical significances reached 0.05 or less, multiple pairwise comparisons using the Mann–Whitney test were done, and the significance was set at a value of 0.017. The Bonferroni correction was applied for the three paired comparisons: C-NF and N-NF, C-NF and OB, N-NF and OB.

## Results

#### Study animals

The C-NF group comprised of 11 samples from seven dogs: eight samples from four dogs with whole body clipping, of which two were intact females, one was a neutered female and one a castrated male. Three more samples were from dogs that were clipped only on their ventral coat, consisting of two females and one male. The

age range was 5–10 years with a median of 7 years (standard deviation (SD) = 1.72).

The N-NF group included 16 samples from 10 dogs, 12 from five intact females and one intact male; another sample was taken from one intact female in which only the chest was sampled and three more samples were taken from two intact females and one intact male for which only the back samples were included in this group (these dogs were clipped only on their chests and their chest samples were included in the C-NF group, as described above). The dogs were 2–8 years old with a median of 4 years (SD = 2.12).

The OB group included 14 samples from seven dogs, five males, one of which was castrated, and two intact females. One dog was a Saint Bernard, one a Border collie, and the rest mixed breeds with fine, long hair coats. The age range in this group was 1–8 years old with a median of 4 years (SD = 2.32).

The dogs in the C-NF group were significantly older than the other groups ( $P = 0.003$ ) and there were more neutered dogs in this group than in the other groups ( $P = 0.017$ ), but the number of neutered dogs was small. In the OB group there were more males than in the other groups ( $P = 0.01$ ).

#### Tissue examination

A total of 41 biopsies were examined: 11, 16 and 14 from the C-NF, N-NF and OB groups, respectively. Overall, 21 samples were taken from the chest (seven samples from each group) and 20 samples were taken from the back (four from the C-NF group, nine from the N-NF group, and seven from the OB group). The morphometric data are presented in Table 1.

The cornified layer was significantly thicker in the C-NF group than in the other groups ( $P = 0.011$ ) (Figs. 1–3), and especially thicker than in the N-NF group ( $P = 0.006$  in the pairwise comparisons). The C-NF group had a basket-weave diffuse, orthokeratotic hyperkeratosis (Fig. 1). The epidermis was also thicker (regular mild hyperplasia) in the C-NF group than in the other groups and in both Newfoundland groups than in the OB ( $P = 0.011$ ) (Figs. 1–3). In pairwise comparisons the two Newfoundland groups were similar ( $P = 0.195$ ), but a very statistically significant difference was found between the C-NF and the OB groups ( $P = 0.0002$ ) and to a lesser extent between the N-NF and the OB groups ( $P = 0.004$ ).

The ratio cornified layer/epidermis was significantly higher in the C-NF and OB groups than in the N-NF group ( $P = 0.011$ ). In multiple pairwise comparisons, only the difference between the N-NF and OB groups remained statistically significant ( $P = 0.002$ ).

The number of follicular units in the sections was similar among the groups. The number of hair follicles per follicular unit was similar in the two Newfoundland groups, with a median of 5, but lower in the OB group with a median of 4 ( $P = 0.0434$ ). In multiple pairwise comparisons, however, no significant differences were found between groups.

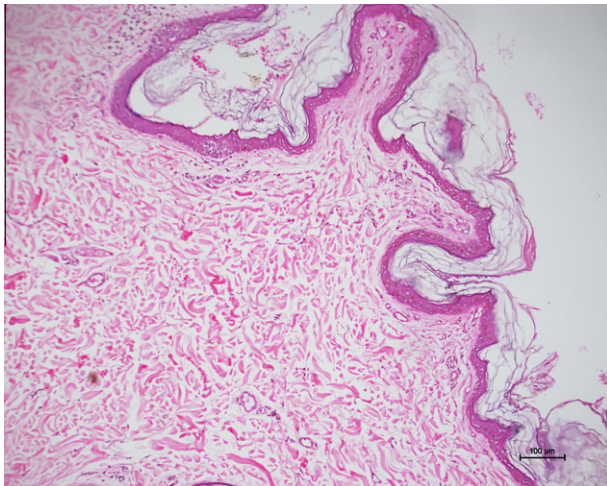
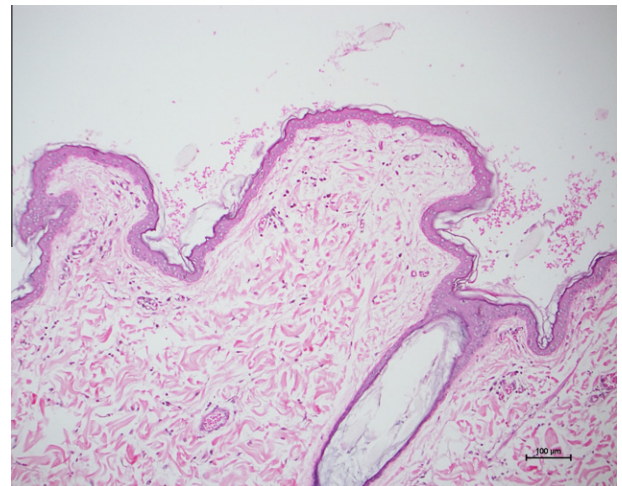
The number of sebaceous glands in a follicular unit was significantly higher in the C-NF group ( $P = 0.0399$ , median 6 vs. 3 and 3.5 in the N-NF and OB groups, respectively). In multiple pairwise comparisons, the significance of the differences between the C-NF and OB groups was  $P = 0.021$ , between the C-NF and the N-NF groups was  $P = 0.030$ , while the two unclipped groups were similar ( $P = 0.79$ ). The size of sebaceous glands and the number of sebocytes per gland were not statistically different between groups (Table 1) ( $P = 0.930$  and  $0.083$  respectively). The C-NF group had the smallest sebocytes and the OB group the largest (medians 35 µm and 45.5 µm respectively ( $P = 0.0222$ ; multiple pairwise comparisons  $P = 0.003$ ). The number of reserve cells in the sebaceous glands was smallest in the C-NF group ( $P = 0.07$ ), with medians of 14, 25 and 35.5 in the C-NF, N-NF and OB groups, respectively.

The ratios between reserve cells and sebocytes were similar between the groups. Newfoundland dogs had more sweat glands per

**Table 1**

Morphometry of the skin of the dogs according to group.

Study Group	Epidermis size (μm)	Cornified layer size (μm)	Cornified layer:epidermis ratio	Number of sebaceous glands in a follicular unit	Size of sebaceous gland (mm <sup>2</sup> )	Size of sebocyte (μm)	Number of reserve cells in sebaceous glands	Reserve cells/sebocytes ratio	Number of sweat glands in a follicular unit	Size of sweat gland (mm <sup>2</sup> )	Number of hair follicles in a follicular unit	Number of epithelial cell layers in a follicle
<i>C-NF<sup>a</sup> (n = 11)</i>												
Mean	26.82	73.64	3.177	7.27	0.0154	36.91	17.64	1.2496	2.09	0.0348	5.82	2.27
Median	30	70	3.000	6	0.0145	35	14	1.11	2	0.025	5	2
Minimum	15	30	0.8	2	0.0060	28	6	0.26	0	0.0021	2	1
Maximum	40	150	6.7	14	0.0279	49	37	3	4	0.1058	8	4
Std. deviation	11.24	37.76	1.93	4.10	0.0062	7.063	10.9	13.54	1.044	0.0317	1.779	1.009
<i>N-NF<sup>b</sup> (n = 16)</i>												
Mean	20.63	38.75	2.119	4.06	0.0174	41.69	37.31	1.2821	3.25	0.0313	4.88	1.94
Median	20	30	1.5	3	0.0164	42	25	1.1289	2	0.0279	5	2
Minimum	5	15	0.5	1	0.0020	14	6	0.35	0	0	2	1
Maximum	40	130	5.2	11	0.0390	56	145	3.25	10	0.1029	9	4
Std. deviation	8.73	29.01	1.45	2.62	0.0107	13.022	34.6	0.766	2.933	0.0287	2.125	0.998
<i>OB<sup>c</sup> (n = 14)</i>												
Mean	11.79	47.14	4.929	3.64	0.0195	48.5	34.71	1.023	1.07	0.0048	4.14	1.93
Median	10	50	4.5	3.5	0.0140	45.5	30.5	0.89	1	0.0029	4	2
Minimum	5	10	1.5	1	0.0052	35	9	0.33	0	0	3	1
Maximum	25	80	16	8	0.0495	63	67	2.8	3	0.0246	8	3
Std. deviation	5.75	19.78	3.75	2.30	0.0150	8.88	20.368	0.6187	0.730	0.0668	1.46	0.475
<i>P<sup>d</sup></i>	<b>0.011(OB)</b>	<b>0.011(C-NF)</b>	<b>0.011(N-NF)</b>	<b>0.0399(C-NF)</b>	0.930	<b>0.0222(C-NF)</b>	0.07	0.558	<b>0.0036(OB)</b>	<b>0.0002(OB)</b>	<b>0.043(OB)</b>	0.564

*P* values in bold indicate statistical significance.<sup>a</sup> Clipped Newfoundland dogs.<sup>b</sup> Unclipped Newfoundland dogs.<sup>c</sup> Other breed dogs.<sup>d</sup> *P* values of Kruskal–Wallis analysis of variance of one group (in parenthesis) versus the other two groups.**Fig. 1.** Photomicrograph of the skin of a Newfoundland dog with skin and coat changes following clipping. Note the basket-weave, diffuse hyperkeratosis and the mild epidermal hyperplasia. Bar, 100 μm.**Fig. 2.** Photomicrograph of the skin of a never been clipped Newfoundland dog. The epidermis is thicker than in the sample from a mixed breed dog, but there is no hyperkeratosis like in clipped Newfoundland dogs. Bar, 100 μm.

follicular units than the 0.011(OB) group (median, 2 vs. 1) ( $P = 0.0036$ ). In multiple pairwise comparisons, the two Newfoundland dog groups were similar ( $P = 0.512$ ) and both groups were significantly different from the OB group ( $P = 0.009$ ,  $P = 0.003$  for the C-NF and N-NF groups, respectively). The Newfoundland dogs also had larger epitrichial sweat glands than the OB group ( $P = 0.0002$ ; median sizes, 0.025 mm<sup>2</sup>, 0.028 mm<sup>2</sup>, and 0.0029 mm<sup>2</sup> in the C-NF, N-NF and OB groups, respectively). In paired associations, the *P* value for the differences between the OB and each of the two Newfoundland groups was  $<0.001$ , whereas the two Newfoundland groups were similar ( $P = 0.716$ ).

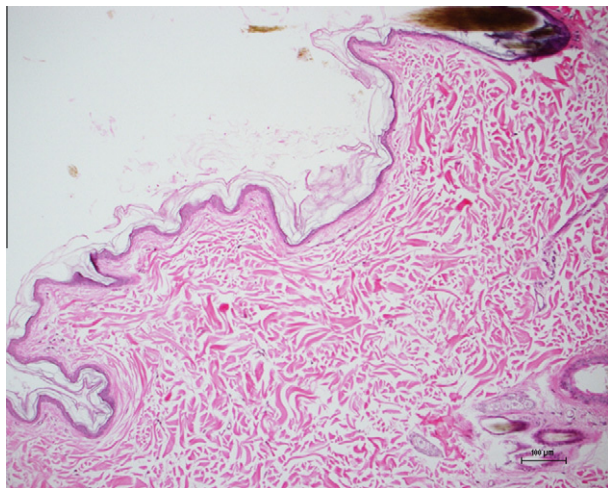
#### Site of sampling

No statistically significant differences were found for any of the examined parameters in any of the groups when samples from the

back were compared with samples from the chest except from the epidermis which was thicker in the skin from the chest in the Newfoundland dogs ( $P = 0.042$  and  $0.031$  in the C-NF and N-NF groups, respectively). This caused the difference between groups to be only marginally significant for the back samples ( $P = 0.06$ ) in contrast to the chest samples ( $P = 0.005$ ; in multiple pairwise comparisons,  $P = 0.002$  between the C-NF and OB groups, and  $P = 0.017$  between N-NF and OB groups).

Although for each group all the other histopathology parameters were similar between the two body sites, when samples of each site were compared separately between groups there were also other differences in the total sample comparisons. The C-NF group had the smallest sebocytes and the difference between the C-NF and OB groups in multiple pairwise comparisons was highly significant ( $P = 0.006$ ).





**Fig. 3.** Photomicrograph of the skin of a mixed breed dog that had never been clipped, showing a thin epidermis. Bar, 100  $\mu$ m.

The number of sweat glands was higher in the N-NF group (median, 3) and lower in the OB groups (median, 1) ( $P = 0.047$ ). The OB group had the smallest sweat glands ( $P = 0.017$ ; multiple pairwise comparisons  $P = 0.012$  for the difference between the OB group and each Newfoundland group).

The OB group had fewer and smaller sweat glands ( $P = 0.005$  and  $0.016$ , respectively). In multiple pairwise comparisons a highly significant difference was found between the OB and C-NF groups for the number of glands ( $P = 0.002$ ) and between the OB and N-NF groups for the size of the glands ( $P = 0.007$ ).

#### Associations between signalment and histology parameters

These analyses were performed because the groups were not equal in their age (the C-NF dogs were older) and gender (the OB group had more males and the C-NF group more neutered dogs). The correlations between age and the various histological parameters are listed in Table 2.

Among the parameters that were found as significantly different between groups, only the number of sebaceous glands per follicular unit was also highly correlated with age. Analysis of covariance for the number of sebaceous gland indicated  $P = 0.475$  for the age and  $P = 0.082$  for the group, meaning that the age had no effect on this parameter although it reduced the effect of the group. The size of the sebaceous gland which was negatively corre-

lated with age was found by analysis of covariance to be more influenced by the age than by the group ( $P = 0.087$  and  $0.133$ , respectively).

Males had a thinner epidermis ( $P = 0.007$ ) than females, smaller sweat glands ( $P = 0.00003$ ) and fewer follicles per follicular unit ( $P = 0.0282$ ). Because the OB group with significantly more males was also found to have a thinner epidermis, smaller sweat glands and fewer follicular units than the Newfoundland dogs, the influence of the gender vs. the influence of the group was examined by two-way ANOVA. Results showed that the differences between groups for the size of the epidermis and the size of sweat glands were due to the group ( $P = 0.005$  and  $0.043$  for epidermis and sweat glands, respectively) and not to the gender ( $P = 0.138$  and  $0.355$ , respectively). However, gender was more important for the number of follicles within a follicular unit ( $P = 0.243$  and  $0.056$  for group and gender, respectively).

#### Discussion

In this study we examined histologically skin biopsies of Newfoundland dogs that had coat quality changes following clipping. These dogs did not exhibit any impaired hair regrowth, as is noted in cases of post-clipping alopecia (Gross et al., 2005). Instead, the new hair and skin were less glossy and became scallier. Various elements in skin morphometry were compared between clipped dogs and those that had never been clipped. Two control groups were used: Newfoundland dogs as within-breed control and other breeds of dogs with a fine, long haired-coat that had never been clipped as between-breed control.

The differences in skin structure between the groups could explain the coat quality changes that were seen externally. The increased cornified layer and the ratio between the cornified layer and the epidermis could explain the increased scaliness and decrease in coat and skin gloss. In this respect, a shiny skin in feline paraneoplastic alopecia has been considered to be due to a reduction in the thickness of the cornified layer (Pascal-Tenorio et al., 1991). Furthermore, the only other differences (besides clipping) between the groups were the average age of the dogs and their neutered status. The age was found not to affect these results, and since there were only three neutered dogs no statistical evaluation was possible.

Hyperkeratosis and epidermal hyperplasia are usually associated with chronic dermatitis. However, the skin samples from the C-NF group did not show any inflammatory change in any skin element, which implies that these changes can be attributed to clipping only. These results show that clipping causes an increase in cornified layer production in Newfoundland dogs. It would be interesting to see whether such changes are also applicable to other breeds with a similar or different coat type in addition to breed variation in skin structure as previously reported (Dunstan et al., 2000).

Another important skin element that contributes to hair coat quality is the sebaceous gland. In the C-NF group, the sebocytes were significantly smaller. This difference was more pronounced between the C-NF and OB groups. In the current study, we did not measure sebum production to verify if the clipped dogs actually produced less sebum, but the dull appearance of the coat could be due to low sebum production, especially in light of the reduction in gland components. Sebum production differs among breeds and changes with age (Dunstan et al., 2000). However, in the current study, age was found to influence the total size of the sebaceous gland, while neither age nor gender was found to have any effect on the differences in the other sebaceous gland parameters. Sebum production has been found to vary between measurements (Groux and Bensignor, 2000), so the differences in sebaceous gland

**Table 2**  
Correlations between skin morphometry and age of the dogs.

Histopathology parameter	Correlation with age <sup>a</sup>
Cornified layer size	0.431
Epidermis size	0.224
Cornified layer/epidermis ratio	−0.006
Number of follicular units per section	−0.219
Number of sebaceous glands per follicular unit	0.342
Size of a sebaceous gland	−0.529
Number of sebocytes per sebaceous gland	−0.477
Size of sebocyte	−0.183
Number of reserve cells per sebaceous gland	−0.335
Reserve cells/sebocytes ratio	0.335
Number of sweat glands in a follicular unit	0.079
Size of sweat gland	0.29
Number of hair follicles in a follicular unit	0.146
Number of epithelial cell layers in a follicle	0.131

<sup>a</sup> Pearson correlation coefficient.

morphology found here were most likely valid and might explain the coat quality changes seen in the C-NF group.

Another interesting finding was the difference in sweat glands between groups. Newfoundland dogs had more and larger sweat glands than the other breeds with similar coat type, and that did not seem to be affected by clipping. One possible explanation is that Newfoundlands could be more susceptible to the hot and humid conditions in which this study was conducted. These climatic conditions are very different from the breed's original location, and this could have resulted in higher transepidermal water loss and lower skin hydration, leading to increased scaliness and decreased coat and skin gloss.

We assumed when selecting the dogs for the OB group that they would have similar coat characteristics. Our observations confirmed that the follicular parameters were similar among the groups, and the differences found among groups were attributed to gender and not to breed variations or clipping.

The major limitation of our study is the small number of dogs in each group, especially when taking into consideration the use of histomorphometric techniques that are highly sensitive to bias. Because the study was based on examining skin biopsies, and the medical, particularly the dermatological history of the dogs, was very important, it was very difficult, both technically and ethically, to include more dogs. Biopsies were taken from two different sites of the body, because of possible variations in skin structure at different body sites (Scott et al., 2001b). However, the parameters we examined were similar between sampling sites so enabling pooling for statistical analysis, and provided a larger database with a higher statistical power. Furthermore, most of the differences between groups remained significant, even when the statistical evaluations were performed on each skin site separately. However, it will be important to confirm these results by using larger cohorts of dogs and also to examine dogs before and at specific time intervals after clipping.

## Conclusions

Newfoundland dogs located in Israel have certain differences in skin features distinguishing them from other breeds, such as a thinner cornified layer and smaller sebocyte size and numbers. Repeated clippings of these dogs cause hyperkeratosis, reduced skin and coat gloss and increased scaliness. Newfoundland dogs also have more and larger sweat glands that are not affected by clipping.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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