WHAT HAPPENS WHEN A DOG LOSES ITS PUPPY COAT? FUNCTIONAL, DEVELOPMENTAL AND BREED-RELATED CHANGES IN THE CANINE HAIR FOLLICLE

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SUMMARY

This study attempts to define anatomic and functional differences in the hair follicle associated with age and breed in a colony of 36 intact, male dogs (12 Siberian Huskies, 12 Miniature Poodles, 12 Labrador Retrievers). Between 10 and 28 weeks of age, the dogs were periodically sampled and follicular morphology was assessed using the following techniques: 1) DermScope examination of the eruptive morphology of the hair shafts; 2) histologic and morphometric examination of site-matched, horizontally-sectioned 6mm skin biopsy samples; and 3) optical-based fibre diameter analyser (OFDA) evaluation to determine the diameter and curvature of hair shafts. Morphometric evaluation of the number of hair follicles and the hair cycle showed that the mean number of hair follicles per follicular unit increased in all breeds. The percentage of follicles in telogen in the Miniature Poodles decreased throughout the study, whereas the number of telogen follicles increased in Siberian Huskies. At the end of the study, both Siberian Huskies and Labrador Retrievers had approximately 50% of their follicles in telogen. In contrast, the Miniature Poodles had only 12% of their follicles in telogen. The OFDA data showed that Labrador Retrievers had the largest mean hair shaft diameter. Siberian Huskies had the greatest mean curvature. Dermscope examination visually confirmed these observations. The results indicate that "loss of the puppy coat" is best thought of as a gain in the adult coat and that this process is extremely breed-specific.

INTRODUCTION

The importance placed on the hair coat by dog owners is evidenced by the diversity of coat types that have evolved over the centuries. With the arguable exception of the contours of a dog's body provided by the musculoskeletal system, the feature that most distinguishes the different canine breeds is the hair coat. Thus, it is no surprise that every breed has standards by which the coat is assessed in the show ring (American Kennel Club, 1997). The hair coat is also important in establishing the animal-human bond; animals are enjoyable to view, but also to "pet". Therefore, diseases associated with hair loss are of concern to most dog owners. Not only do their pets have a less attractive appearance, but the pets are also handled less frequently. These concerns ultimately are transferred to veterinarians who are asked to improve the coat of an alopecic dog.

With the importance that the hair coat plays in establishing the identity of a breed and the human-animal bond, it is surprising that there has been no substantive advancement in defining breed differences in the canine hair coat for over forty years (Brunsch, 1956, Gair, 1928). This lack of knowledge of breed-associated differences in the hair coat is more than just a cosmetic issue. For dermatopathologists, the morphologic assessment of the severity of hair loss is dependent on how the biopsy sample differs from normal. Because little information is available regarding breed-related differences, all biopsy samples are viewed as a generic representative of the species, not of the breed.

We are currently involved in an ongoing study to define breed-related differences in the skin of dogs and to apply this knowledge to better understand the role

factors, such as nutrition, play in maintaining a healthy hair coat. In this report, we describe the pelage differences in three canine breeds (Siberian Huskies, Labrador Retrievers and Miniature Poodles) that occur between the ages of 10 and 28 weeks. During this time, the fine, soft "puppy" coat of many breeds is transformed into the hair coat that characterizes the adult dog, however, little is known about how this process occurs.

MATERIALS AND METHODS

All procedures dealing with animal care, handling and treatment in this study were reviewed, approved and monitored by the Animal Use Committee, Texas A&M University.

This study was performed on a colony of 36 male, purebred dogs consisting of 12 Siberian Huskies, 12 Labrador Retrievers, and 12 Miniature Poodles. These dogs were brought into the colony between the months of June and August 1998 when they were 7-8 weeks old. All dogs were housed indoors in individual runs (Siberian Huskies and Labrador Retrievers) or runs shared by three dogs (Miniature Poodles) maintained at a constant temperature (68° F) with 12-hour light/dark cycles. The dogs were given at least two weeks to adjust to the new environment before any procedures were performed. These dogs were part of a study, the major purpose of which was, to define the effects of diet on the skin. Thus, half of the dogs in each breed were fed a high quality diet and the other half, a lower quality diet. For the purposes of this report, data are included for all 12 dogs in each breed group and diet effects were ignored.

Samples of clipped hair and punch biopsy samples were collected at three week intervals starting when the dogs were 10 weeks old and continuing for 18 weeks. Because of size limitations of the puppies, different sites were used for sample collection (see below).

The assays performed on the dogs were 1) DermScope evaluation; 2) histologic and morphometric assessment of skin biopsy samples; 3) Optical-Based Fibre Diameter Analyser (OFDA) analysis.

The DermScope is a portable, external microscope that produces a high quality digital image. In this study, it was used as an external scanning microscope, allowing us to qualitatively examine the clipped hair coat at magnifications of up to 600X. For each dog, DermScope evaluation was performed on the dorsal lumbar area. To prepare the site for the DermScope evaluation, the area was closely clipped and wiped gently with an alcohol-soaked cotton pad.

Skin biopsy samples were obtained from the middle to caudal dorsolateral thoracic area. Two 6mm skin biopsy samples were used for morphologic and morphometric assessment of the hair follicles. Biopsy samples were obtained under local anesthetic using standard surgical techniques. To avoid inflammation and scarring related to previous biopsy sites, samples were taken a minimum of 4cm apart from other sites. Samples were fixed in 10% neutral buffered formalin and, after fixation, one biopsy sample was trimmed vertically. The other biopsy sample was sectioned horizontally as described by Headington (1984). Samples were routinely processed, embedded cut side down, sectioned at 5um and stained with hematoxylin and eosin. Routine descriptive morphologic evaluation was performed on the vertically sectioned samples. The

horizontally sectioned samples were step-sectioned until the isthmus region of all the hairs in five follicular units could be assessed. A follicular unit was defined as the anatomic aggregation of compound hair follicles that occurs in dogs. Most follicular units consist of three compound follicles adjacent to each other with the middle compound follicle containing the largest (primary) hair shaft. The horizontally sectioned samples were evaluated routinely and morphometrically. The morphometric analysis took two forms: 1) the stage of the hair cycle was defined for each follicle in the five follicular units evaluated and 2) the number of hairs/follicular unit was counted. A hair follicle was considered in anagen when an inner sheath surrounded the hair shaft at the level of the follicular isthmus. A hair follicle was considered in "haired" telogen when trichilemmal cornification surrounded the hair shaft at the isthmic region. A follicle was considered in "hairless" telogen when the hair follicle at the level of the isthmus was devoid of a hair shaft and either had luminal trichilemmal cornification but no hair shaft or was simply an epithelial tube with no luminal cornification (Nixon, 1993). Because catagen hairs are impossible to consistently recognize on horizontal sections and are uncommon, no attempt was made to identify them in this study.

The Optical-Based Fibre Diameter Analyser (OFDA) is a computerized microscope with attached video camera that captures digitized images of a moving sample of cleaned hair shafts that have been cut in 2mm lengths. The hair snippets are randomly spread onto a hinged glass slide using an automatic spreader. No mounting liquid is used, and after measurement the fibres are vacuumed off. The width (diameter) and degree of curvature of each captured fiber image is measured. The advantages of using the OFDA to evaluate hair shafts are in its accuracy (the instrument is capable of a

resolution of 1 micron and can determine diameters in the range of 4 to 300 microns) and speed (> 10,000 fibers can be measured/minute) (Lupton, 1995). For OFDA analysis, the hair in an area approximately 4cm X 4cm over the left scapula was clipped and stored until the hair was ready to be cleaned (Baxter et al., 1992). Because the samples evaluated were from clippings of hair obtained every three weeks, what was actually measured by OFDA analysis was the diameter and curvature of the hair shafts that had erupted during the three-week interval between sampling periods.

RESULTS

The DermScope examination visually defined differences in the number and pattern of hair shafts erupting through the follicular opening. The 200X lens provided sufficient detail to allow for assessment of the cuticular pattern of erupting hair. Differences were noted both between breeds and over time. Siberian Huskies had the largest number of hair shafts with the greatest visible differences between the size of primary and secondary hairs. Miniature Poodles and Labrador Retrievers had approximately the same number of hair shafts per follicular opening, but there was considerable interbreed variability in diameter. The hair shafts of the Miniature Poodles were much finer with smaller diameters and the hair shafts from Labrador Retrievers were coarser with larger diameters.

At the end of the study, when the dogs were 28 weeks old, the pelage of the Siberian Huskies had a marked increase in the number of secondary hairs and a slight and subjective increase in the diameter of primary hairs. The differences in the number of hair shafts were more difficult to discern in Miniature Poodles and Labrador

Retrievers. Miniature Poodles appeared to have a slight increase in the number of hair shafts, but no discernible difference in hair shaft diameter compared to 10 weeks of age. Labrador Retrievers had no discernible increase in the number of hairs, but had a striking increase in the diameter of hair shafts.

The DermScope was also used to qualitatively evaluate the epidermis. In all dogs, the epidermis was non-pigmented and opaque, allowing visualization of the dermis and to some extent, the base of pigmented hairs. Occasionally, tylotrich pads, a localized neuroreceptor organ associated with follicles were identified, especially in Siberian Huskies. Tylotrich pads appeared as slightly raised, white, thickenings of the epidermis.

Histologic and morphometric assessment of vertically sectioned skin biopsy samples revealed that the dermis became somewhat thicker in each breed from the beginning of the study to the end. Sebaceous glands also became larger. The overall follicular morphology was difficult to discern by this method of trimming. However, even here, breed differences were at least suggested that the Siberian Huskies had many more hair shafts in the infundibula and the Labrador Retrievers had fewer and coarser hairs. The Miniature Poodles (the only breed in the group with curly primary hairs) had primary hairs that were vaguely curved. Miniature Poodle primary and secondary hair shafts appeared smaller than the other two breeds and were less numerous than in the Siberian Huskies.

A diagram of the microscopic appearance of a horizontally sectioned skin biopsy sample is presented in Figure 1. The compound follicles had a distinct anatomic orientation with a single, large anterior follicle and multiple smaller posterior follicles.

Because of this orientation, the largest anterior follicle in a compound follicle was called an anatomic primary follicle. All other follicles were considered anatomic secondary follicles. Most follicular units were composed of three compound follicles, but occasionally, follicular units composed of one to five compound follicles were noted. Generally, the more compound follicles in a follicular unit, the larger the central anatomic primary follicle and the hair shaft it produces.

The morphometric evaluation of the number of hair follicles and the hair cycle is presented in Figures 2A and 2B, respectively. For each breed, the mean number of hair follicles per follicular unit increased over the course of the study. The increase was most dramatic for Siberian Huskies and Miniature Poodles (approximately 50%) and less for Labrador Retrievers (approximately 30%). The percentage of follicles in telogen in the Miniature Poodles decreased throughout the study, whereas the number of telogen follicles increased in Siberian Huskies. For Labrador Retrievers, the mean number of telogen follicles decreased from week 10 to week 16 and then the number increased. At the end of the study, both Siberian Huskies and Labrador Retrievers had approximately 50% of their follicles in telogen. In contrast the Miniature Poodles examined had only 12% of their follicles in telogen.

The data obtained from OFDA analysis defined marked breed differences in both diameter (Figure 3A) and curvature of hair shafts (Figure 3B). As was noted on the DermScope images and examination of horizontal histologic sections, Labrador Retrievers had the largest mean diameter with Siberian Huskies having a slightly larger mean diameter than Miniature Poodles. These differences were slightly less noticeable at week 10 than at week 28. For the Labrador Retrievers, the mean diameters increased

from week 10 to 16 and then approached the week 10 level at the end of the study. In contrast, the mean diameters of the Siberian Huskies and Miniature Poodles decreased over the course of the study with a greater rate of decline in the Siberian Huskies.

Of the three breeds studied, hair samples from Siberian Huskies had the greatest mean curvature. In addition, the curvature in this breed increased from weeks 13-19 and then decreased slightly. Miniature Poodle hair had only a slightly greater mean curvature than Labrador Retrievers. Hair samples from the Miniature Poodles did not change substantively during the course of the study. The hair samples from the Labradors decreased in curvature from week 13 to 19, increased by week 22 and then remained constant for the final two measurement periods.

The presence of a relationship between hair diameter and curvature is defined by graphing these data together for each breed (Figures 4A, B and C). For Labrador Retrievers, the increase in diameter correlated with a decrease in curvature; for Siberian Huskies, the hair diameter decreased as the curvature increased; for Miniature Poodles, the differences appeared negligible with only a slight increase in curvature and a slight decrease in diameter.

DISCUSSION

In this study, multiple techniques were used to assess the morphology of the canine hair follicle, the hairs it produced, the number of hairs shafts/follicular unit, the number of follicles in telogen, and the diameter and degree of curvature of these hairs. In combination, these techniques provide a better means of assessing the pelage of the

dog than previously used methods. We applied these techniques in an attempt to understand what happens when a dog "loses" its puppy coat.

Based on our analysis, the maturational changes that occur in the hair follicles are breed-specific and what has been described as the "loss of a puppy coat" is better thought of as the "gain of an adult coat". In the three breeds examined, the period of 10 to 28 weeks of age is associated with an increase in the number of hair follicles and a change in the type of actively growing hairs that are produced, as described below.

In Siberian Huskies, during the 18 week period examined, the growing follicles are predominantly those that produce smaller diameter hair shafts that are highly curved. These data indicate that for Siberian Huskies, the gain of an adult coat is associated with an increase in the number of highly twisted hair shafts that make up the dense, air-filled undercoat of this breed.

A very different pattern of "puppy coat loss" occurs in Labrador Retrievers. At the start of the study, the coat of the Labrador Retrievers had fewer hairs/follicular unit than Siberian Huskies but the diameter and degree of curvature were quite similar. Over the course of the investigation, there was a period in which relatively straight, large diameter hair shafts were produced. This corresponds to the replacement of the fine, soft and moderately curly Labrador Retriever puppy coat with the coarse pelage that defines adult hair coat of the breed.

In Miniature Poodles, only minimal changes were identified in the diameter and curvature of hair shafts over the 18 weeks of investigation. Thus, in this breed, we believe the gain of an adult coat is associated with an increase in the number of follicles but the diameter of these follicles remains fairly constant.

That Siberian Huskies had the largest number of follicles/follicular unit was expected; however, it was surprising that Miniature Poodles and Labrador Retrievers had approximately the same number of follicles/follicular unit, as it was thought that Miniature Poodles would have a denser pelage. It is possible that the number of follicles/follicular unit may not be the only indicator of the density of the hair coat. In this study, we made no attempt to determine how close the follicular units were to each other. If the follicular units of the Miniature Poodle are closer together than the Labrador Retriever, then the coat of Miniature Poodles would have been more dense even though each follicular unit produced the same number of hair shafts. It was also surprising that the mean curvature of the hair from Miniature Poodles was closer to a Labrador than a Siberian Husky. However, on closer inspection, the curvature of undercoat (anatomic secondary follicles) from adolescent Siberian Huskies is curlier than the primary and secondary hair shafts from adolescent Miniature Poodles. However, this is a transient stage of follicular growth. In ongoing studies, we have shown that as Miniature Poodles age, their hair shafts eventually do become curlier than that of even Siberian Huskies (data not shown), as one would expect.

Another unexpected finding was that at the conclusion of the study, approximately 50% of the follicles in Labrador Retrievers and Siberian Huskies were in telogen. This observation has profound implications on the histologic assessment by pathologists who may consider a dog with >50% of its follicles in telogen to be abnormal. In our experience, most adult dogs that do not require hair cuts will have the majority of their follicles in telogen (Credille et al., in preparation). Similarly because

Miniature Poodles have < 10% of their follicles in telogen, observations of > 10% of telogen hairs in this breed may indicate an underlying pathologic condition.

Finally, although all the techniques used in this study were of value in defining the morphology of the canine hair follicle, the single most important was horizontal sectioning of biopsy samples. Although seldom employed by veterinary dermatopathologists, we believe this method of histologic evaluation of the hair follicle is critical for accurate morphologic assessment.

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Figure Captions

Figure 1. The canine follicular unit examined by horizontal sectioning.
Follicular units usually consist of groupings of three compound hair follicles, each with its own follicular opening. In each compound follicle there is a large, anterior primary hair flanked by secondary hairs that become smaller caudally.

Figure 2A. Mean number of hair follicles per follicular unit for Siberian Huskies, Labrador Retrievers and Miniature Poodles.
Each data point represents the mean of 12 dogs +/- standard error of the mean (SEM).

Figure 2B. Percentage of telogen follicles for Siberian Huskies, Labrador Retrievers and Miniature Poodles.

Figure 3A. Optical-based Fibre Diameter Analyzer (OFDA) results of mean hair diameter for Siberian Huskies, Labrador Retrievers and Miniature Poodles.
Each data point represents the mean of 12 dogs +/- SEM.

Figure 3B. Optical-based Fibre Diameter Analyzer (OFDA) results of mean degree of curvature for Siberian Huskies, Labrador Retrievers and Miniature Poodles.
Each data point represents the mean of 12 dogs +/- SEM.

Figure 4A. Optical-based Fibre Diameter Analyzer (OFDA) results of mean hair shaft diameter and degree of curvature for Labrador Retrievers.

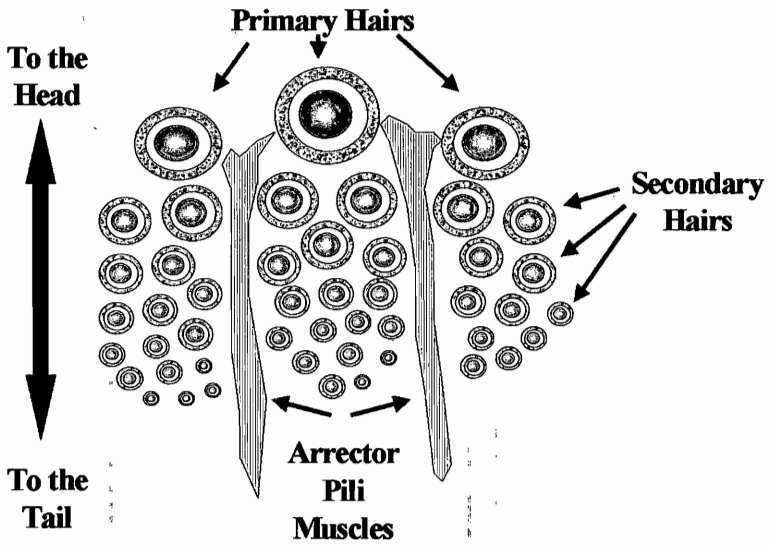
Each data point represents the mean of 12 dogs +/- SEM.

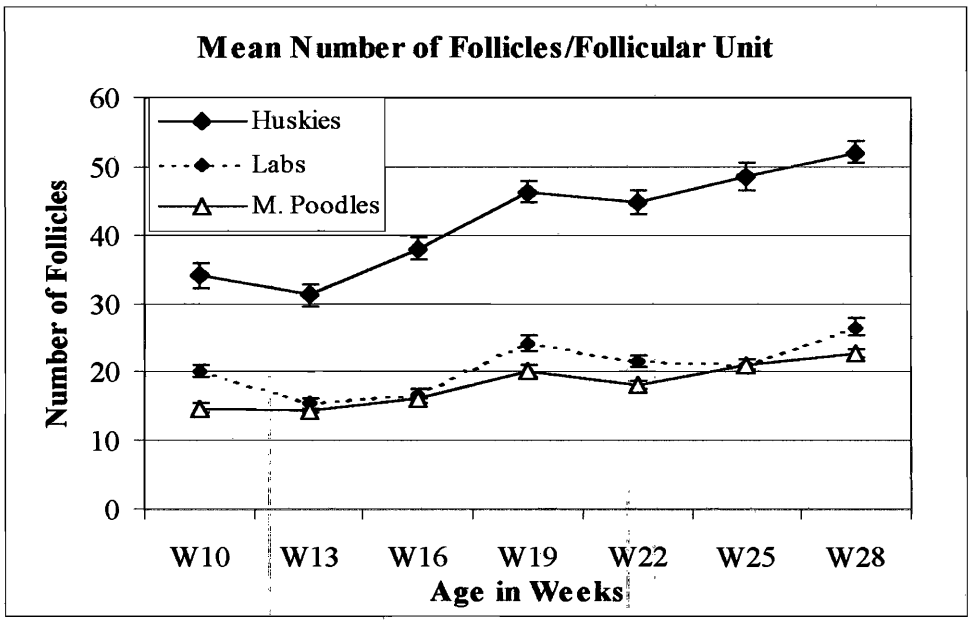
Figure 4B. Optical-based Fibre Diameter Analyzer (OFDA) results of mean hair shaft diameter and degree of curvature for Siberian Huskies.

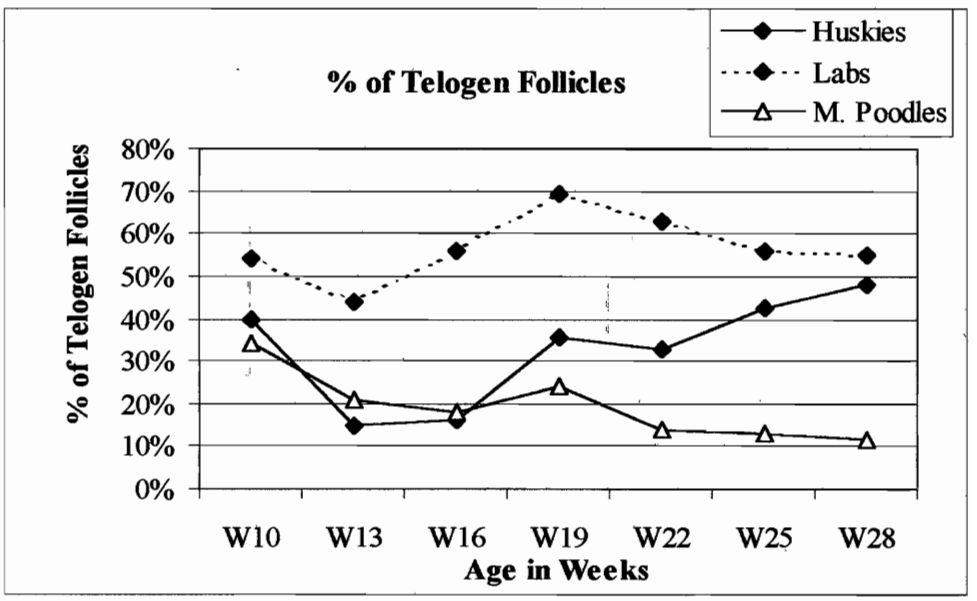
Each data point represents the mean of 12 dogs +/- SEM.

Figure 4C. Optical-based Fibre Diameter Analyzer (OFDA) results of mean hair shaft diameter and degree of curvature for Miniature Poodles.

Each data point represents the mean of 12 dogs +/- SEM.


Primary Hairs
To the Head
Secondary Hairs
To the Tail
Arrector Pili Muscles


Mean Number of Follicles/Follicular Unit
Number of Follicles
60
50
40
30
20
10
0
Huskies
Labs
M. Poodles
W10
W13
W16
W19
W22
W25
W28
Age in Weeks


% of Telogen Follicles
Huskies
Labs
M. Poodles
% of Telogen Follicles
80%
70%
60%
50%
40%
30%
20%
10%
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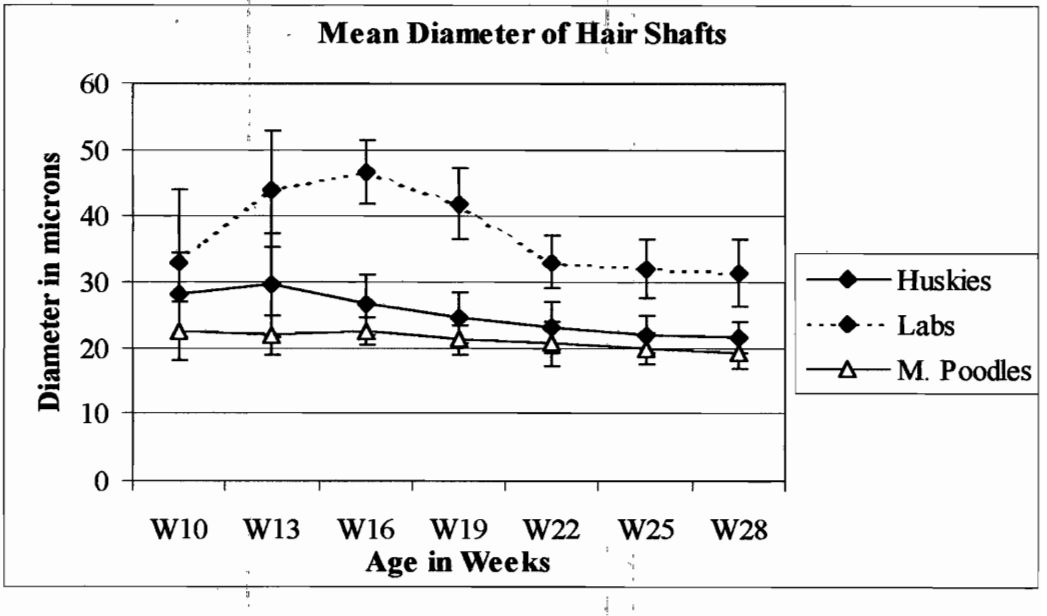
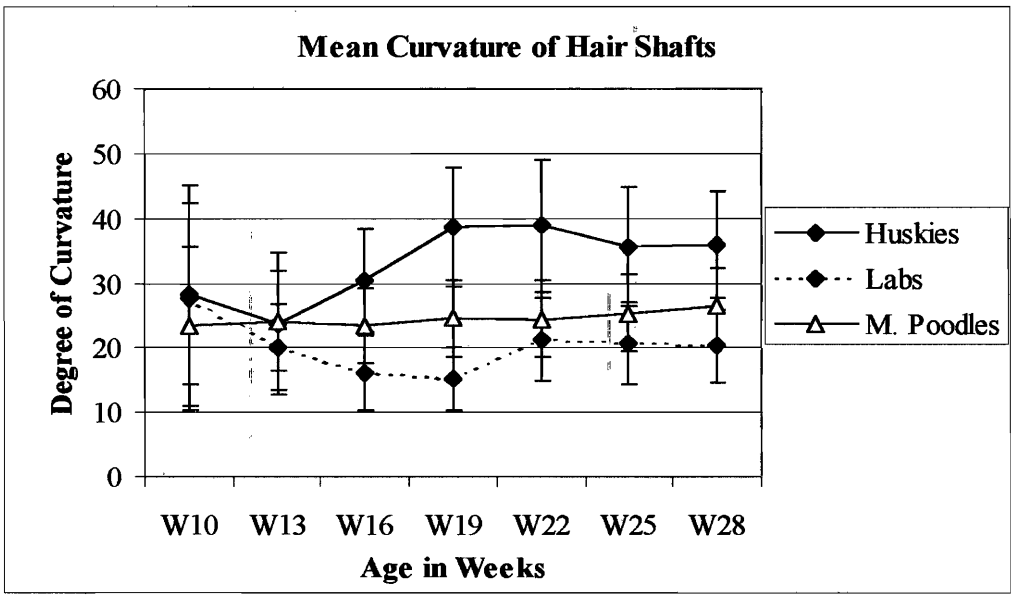
W10
W13
W16
W19
W22
W25
W28
Age in Weeks


Mean Diameter of Hair Shafts
Diameter in microns
60
50
40
30
20
10
0
W10
W13
W16
W19
W22
W25
W28
Age in Weeks
Huskies
Labs
M. Poodles


Mean Curvature of Hair Shafts
Degree of Curvature
60
50
40
30
20
10
0
W10
W13
W16
W19
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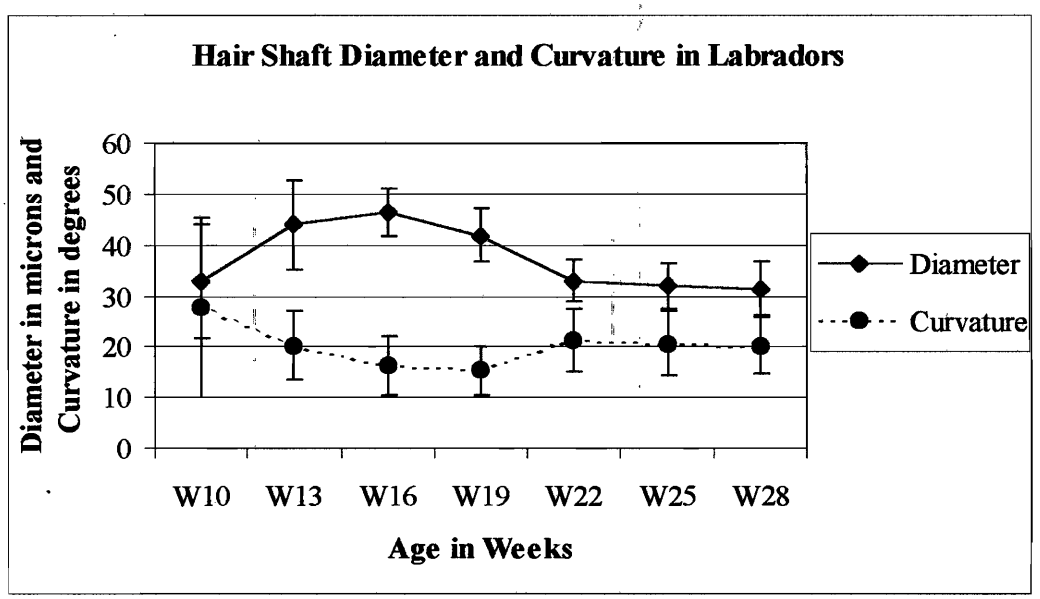
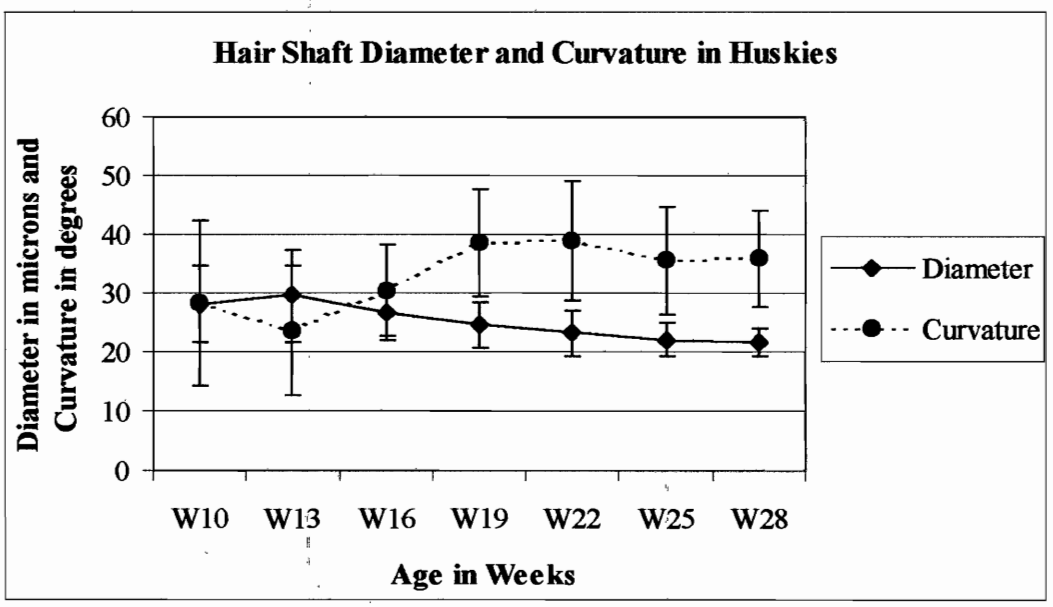
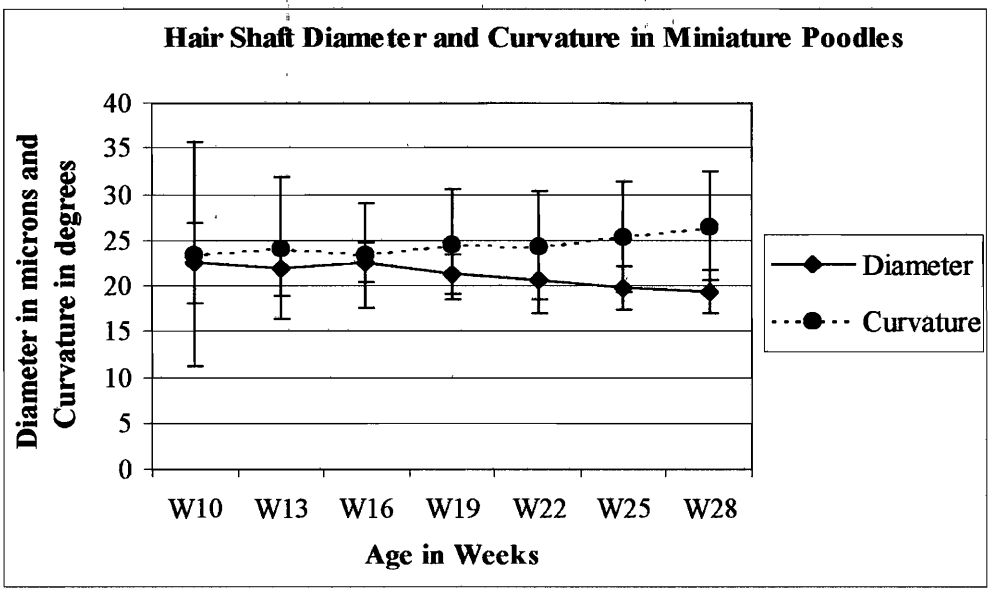
W25
W28
Age in Weeks
Huskies
Labs
M. Poodles


Hair Shaft Diameter and Curvature in Labradors
Diameter in microns and Curvature in degrees
60
50
40
30
20
10
0
W10
W13
W16
W19
W22
W25
W28
Age in Weeks
Diameter
Curvature


Hair Shaft Diameter and Curvature in Huskies
Diameter in microns and Curvature in degrees
60
50
40
30
20
10
0
W10
W13
W16
W19
W22
W25
W28
Age in Weeks
Diameter
Curvature


Hair Shaft Diameter and Curvature in Miniature Poodles
Diameter in microns and Curvature in degrees
40
35
30
25
20
15
10
5
0
W10
W13
W16
W19
W22
W25
W28
Age in Weeks
Diameter
Curvature