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Hypotrichosis congenita (KRT71 mutation) in Hereford cattle in Uruguay[1]

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ABSTRACT.- Romero-Benavente A., Briano-Rodriguez C. & Dutra-Quintela F. 2023. **Hypotrichosis congenita (KRT71 mutation) in Hereford cattle in Uruguay.** *Pesquisa Veterinária Brasileira 43:e07327, 2023.* División de Laboratorios Veterinarios "Miguel C. Rubino", Laboratorio Regional Este, Avelino Miranda 2045, CP 33000, Treinta y Tres, Uruguay. E-mail: fdutra@mgap.gub.uy

Hypotrichosis congenita is a significant disease in Hereford cattle in Uruguay and has been reported worldwide. However, the causal mutation KRT71 has only been recently identified. This communication describes the clinical, histopathological, trichographic, and genetic findings of KRT71-hypotrichosis congenita observed in Hereford calves from two commercial farms. Five affected newborn calves, born in a herd of 15 purebred Polled HF cows, were examined in Farm 1, and one weaned calf in Farm 2. Skin biopsies for histopathology, hair samples for trichogram, and blood samples for genotyping were obtained from affected and control calves and the sire bull and mother cows. Affected animals exhibited a light brown coat with sparse, thin, curly or woolly haircoat. Hypotrichotic skin was dry, erythematous, and scaly. Trichogram analysis revealed thin, fragmented, curly hairs with irregular macro-melanosome groups. The main histopathological findings included marked follicular dysplasia with vacuolation, abnormally large trichohyaline granules in Huxley's layer, and multiple melanin aggregates in hair fragments, matrix cells, and dysplastic follicles. There were no histological lesions of dermatitis. DNA analysis confirmed that hypotrichotic calves were homozygous for the KRT71 mutation, while one control calf, the bull, and cows in Farm 2 were heterozygous carriers. In conclusion, hypotrichosis congenita in Hereford cattle due to the KRT71 mutation is a color dilution follicular dysplasia.

INDEX TERMS: Hypotrichosis congenita, coat-color dilution, Hereford cattle, semi-hairlessness, KRT71 mutation.

RESUMO.- [**Hipotricose congênita (mutação KRT71) em gado Hereford no Uruguai.**] A hipotricose congênita é uma doença significativa em bovinos Hereford no Uruguai e tem sido relatada em todo o mundo. No entanto, a mutação causal KRT71 foi identificada apenas recentemente. Esta comunicação descreve os achados clínicos, histopatológicos, tricográficos e genéticos da KRT71-hipotricose congênita observada em bezerros Hereford de duas fazendas comerciais diferentes. Cinco bezerros recém-nascidos afetados, nascidos em um rebanho de 15 vacas puras Polled HF, foram examinados na Fazenda 1, e um bezerro desmamado na Fazenda 2. Biópsias de pele para histopatologia, amostras de pelo para tricograma e amostras de sangue para genotipagem foram obtidas de bezerros afetados e controle, bem como do touro e das vacas. Animais afetados apresentaram uma pelagem marrom clara com pelos esparsos, finos, crespos ou lanosos. A pele hipotricótica era seca, eritematosa e escamosa. A análise do tricograma revelou pelos finos, fragmentados e crespos, com grupos irregulares de macro-melanossomas. Os principais achados histopatológicos incluíram displasia folicular acentuada com vacuolização e grânulos tricohialinos anormalmente grandes na camada de Huxley e múltiplos agregados de melanina em fragmentos de cabelo, células da matriz e folículos displásicos. Não foram observadas lesões histológicas de dermatite. A análise de DNA confirmou que os bezerros hipotricóticos eram homozigotos para a mutação KRT71, enquanto um bezerro controle, o touro e as vacas da Fazenda 2 eram portadores heterozigotos. Em conclusão, a hipotricose congênita em bovinos Hereford devido à mutação KRT71 é uma displasia folicular de diluição de cor.

TERMOS DE INDEXAÇÃO: Hipotricose congênita, diluição de cor da pelagem, gado Hereford, semipelagem, mutação KRT71.

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INTRODUCTION

In Uruguay, the Hereford breed (HF) was introduced in 1864 and, over the years, expanded to become the main genetic basis for commercial beef cattle production in the country. Uruguay has the largest purebred HF herd in the world, with approximately 5-6 million heads (SNIG 2015). Several inherited disorders have been detected in HF cattle in the country, including cardiomyopathy and wooly haircoat, maple syrup urine disease, mandibulofacial dysostosis, epidermolysis bullosa simplex, hypotrichosis congenita, among many others (Dutra et al. 2011, Kelly et al. 2012).

Hypotrichosis congenita (HY) (OMIA: 002114-9913), also known as semi-hairlessness, is a non-lethal defect of the HF breed, with a simple autosomal recessive mode of inheritance (Craft & Blizzard 1934, Hutt 1963, Olson et al. 1985). Although the disease has been recognized in the Hereford breed for many years, it is only recently that the responsible gene was identified (Markey et al. 2010), and its specific dermatopathology was described (Jacinto et al. 2021). As a result, no other cases of the KRT71 mutation have been reported worldwide. The cause of HY is a deletion of 8 bp in exon 1 of the keratin 71 (KRT71) gene (Markey et al. 2010). This gene encodes a specific protein of hair follicle (K71) that participates in the formation of structurally important keratin intermediate filaments within the inner root sheath of the hair follicle (Jacinto et al. 2021). Mutations in the KRT71 gene also underlie non-syndromic wavy/curly coat phenotypes in dogs (Salmela et al. 2019), cats (Gandolfi et al. 2010), mice (Kikkawa et al. 2003) and a woolly hair/hypotrichosis disorder in humans (Fujimoto et al. 2012).

In Uruguay, cases of HF-hypotrichosis have been recognized for decades, with records in the laboratory database dating back to 1981, yet this genetic trait has never been thoroughly researched. Affected calves are born with partial or complete absence of hair. They are more vulnerable to cold stress and skin infections and have a reduced value as breeding stock (Olson et al. 1985).

This study reports for the first time the clinical, histopathological, and trichogram findings of hypotrichosis congenita due to KRT71 mutation in Polled Hereford calves.

MATERIALS AND METHODS

The diagnosis was made at the "Laboratorio Regional Este" of the DILAVE "Miguel C. Rubino", Treinta y Tres, Uruguay. Newborn and weaned Polled HF calves with hypotrichosis congenita were investigated in two commercial farms in eastern Uruguay. Hair plucks for trichograms and skin biopsies for histological studies were obtained from the affected weaned calf and the control. Local anesthesia (2% lidocaine) was used for the skin biopsies. Both white- and colored-haired skin were sampled. Hair plucks were placed on a glass slide, covered with mineral oil, and examined under a microscope. Biopsies were fixed in 4% neutral buffered formalin, processed routinely, and stained with hematoxylin and eosin (HE).

Blood samples from four affected calves, three cows and the HF bull in Farm 1, and the affected calf and six normal peers in Farm 2, were collected for genotyping. Sampled cows and bulls were the parents of affected calves. The DNA was extracted using a commercial kit (MagMAXTM-96DNA Multi-Sample Kit) and submitted to Neogen GeneSeek, Lincoln, Nebraska, USA, for genotyping using the GeneSeek Genomic Profiler (GGP) Bovine 50K Bead Chip. This test uses Illumina Infinium chemistry.

RESULTS

The first cases occurred in 2017 in a breeding farm (Department of Lavalleja, latitude 33.875 S, longitude 55.121 W), where five calves with generalized hypotrichosis, sired by a recently acquired Polled HF bull, were born in a herd of 15 Polled HF cows. Similar cases have been observed previously on this farm but were never associated with a particular bull or herd. No pedigree registry of the bulls was available. The affected calves were similar in size or somewhat smaller than normal newborns. Body haircoat was light yellow-orange (instead of normal rust brown to deep rich red), and hairs were sparse, thin, and curly, whereas the white hair coat of the head and the ventral side of the body was markedly wooly, and the skin was dry and erythematous (Fig.1 and 2).

The second case occurred in 2018 in a rearing farm (Department of Treinta y Tres, latitude 33.117 S, longitude 54.392 W), where one calf in a lot of 66 recently acquired, weaned calves (6–8 months of age) was affected. The calf had a minor body condition, color dilution, and symmetrical hypotrichosis (Fig.3). Both white- and colored-haired regions were affected. The hair was fine and brittle, appeared wavy or curly, and was easily epilated. The white switch of the tail was shortened and coiled. The coat was covered by flakes of keratin debris and loose dirt, and skin erosions and crusts were present in fetlocks and pasterns. No hematological or biochemical abnormalities were identified. Similar cases bought from the same farm had been observed previously. The calves were weighed upon entering and biweekly after that. The affected calf had a significantly lower body weight at the entrance (72×120kg, SD=19.3, Z-Score=-2.507, $P<0.01$) and when selling for finishing, at 10–12 months of age (195×246kg, SD=24.3, Z-Score=-2.061, $P<0.05$). It remained isolated and separated from the pen mates, suffering repeated aggression most of the time, probably due to its smaller size and different coat appearance. In midwinter (<10°C), a calf jacket was used to protect the animal from apparent cold stress.

Trichography revealed thin hairs, irregular in thickness, both along the same hair shaft and among different hairs, and with abnormal clumps of melanin distorting and fragmenting hair shafts (Fig.4). Histologically, the epidermis showed moderate to marked orthokeratotic hyperkeratosis in both white and colored skin. Sebaceous glands showed variable atrophy, and the sweat glands in the deep dermis were dilated (cystic degeneration). Hair follicle density was normal, but most were in the catagen/telogen phase and devoid of hair shafts. They appeared variably distorted or misshapen, or frankly dysplastic, with an irregular outer contour and variable thickness and surrounded by a thick dermal sheath (Fig.5). In the supra bulbar region, the inner root sheet appeared disorganized. It showed large, round, ellipsoidal, eosinophilic trichohyalin granules in Henle's and Huxley's layer cells (Fig.6). In colored skin only, scattered melanin aggregates were observed in hair matrix cells (Fig.6). Normal control skin and suprabulbar region are shown (Fig.7 and 8). In contrast, several tortuous hair follicles were filled with fragmented and pigmented keratin debris (Fig.9-10). No melanin aggregates were found in the epidermis.

Ectoparasites and bacterial and fungal infections were ruled out since there were no histological lesions of dermatitis and because of the absence of these agents in trichograms. Clumping of melanin and dysplastic hair shafts are specific

for follicular dysplasia and different from endocrine or nutritional hypotrichosis.

All four affected newborn calves from Farm 1 and the weaned calf from Farm 2 were homozygous for the KRT71 mutation (HYA – Affected). The cows, the sire bull of Farm 1, and one of six normal weaned calves from Farm 2 were heterozygous carriers (HYC – Carrier).

DISCUSSION

This report describes hypotrichosis congenita in Polled HF cattle due to KRT71 mutation. This seems to be the first report detailing the clinical and pathological features of KRT71 follicular dysplasia in HF cattle in Uruguay. Genotyping confirmed homozygous recessive KRT71 mutation in affected calves and demonstrated the existence of heterozygous carriers in commercial farms. Genetic studies are necessary to determine the prevalence of KRT71 carriers, particularly among HF-stud bulls. This data is crucial for developing effective strategies to manage and control the disease within the broader commercial farming Hereford (HF) cattle population.

While various forms of syndromic and non-syndromic congenital hypotrichosis exist in HF cattle (Hutt 1963), there is limited information regarding trichography and histopathological descriptions. Therefore, a precise subclassification of these entities is currently difficult. In the present study, HY-affected HF calves exhibited a range of distinctive phenotypic characteristics, including generalized partial alopecia, fine and brittle hair, and color-dilute coat color.

As previously reported, the condition was not progressive and nonlethal (Olson et al. 1985). Characteristically, the woolly coat predominated in the white-haired areas and the wavy or curly pattern in the colored coat. Affected calves were about average in size at birth but significantly lighter at weaning, and they did not grow as well as those with normal hair during the growing period. On weaning, they were sold for growing and then for finishing. This poor growing ability and failure to thrive is probably secondary to the semi-hairless

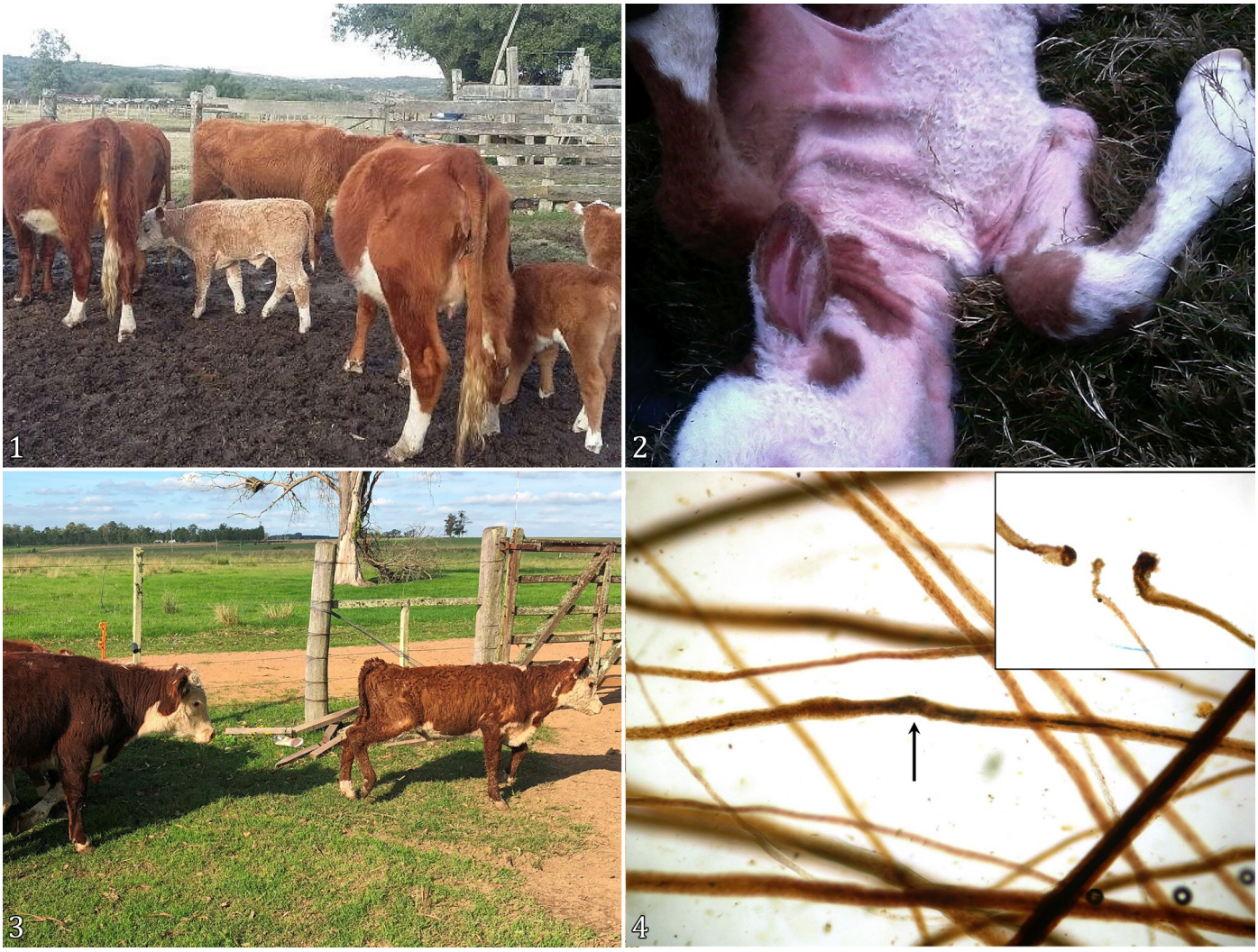

Fig.1-4. Hypotrichosis congenita (KRT71) in Hereford calves. (**1**) Affected newborn calf at the center with a light yellow-orange haircoat, which appears diluted when compared to the rust-brown color of the cows and the normal calf on the right. (**2**) Ventral side of the body and head of a second hypotrichotic newborn calf, showing markedly wooly white haircoat and an erythematous skin. (**3**) Hypotrichotic weaned calf. Note its smaller size and lighter brown color than its healthy peers (behind). (**4**) Trichogram showing pigment clumping (arrow) and a broken hair shaft (inset).

phenotype, making calves more susceptible to bullying, secondary skin infections, heat stress or cold weather, among other environmental factors, thus reducing viability. The disease is thus different from other congenital, HF-specific haircoat disorders, such as "alopecia associated to erythroid hyperplasia and dyserythropoiesis" (Steffen et al. 1991), in which the hypotrichosis is progressive, and "cardiomyopathy and woolly haircoat syndrome", in which the tightly woolly haircoat is generalized (Dutra et al. 2011). Coat-color dilution linked to mutations in the PMel17 gene has been reported in HF crossbred calves, but the hypotrichosis is restricted to the color-haired regions only (Jolly et al. 2008).

Histopathological findings of follicular dysplasia, with the presence of vacuolar-like changes and irregular eosinophilic globules in Huxley's layer, as were found in the present cases, are reported as the more distinctive changes of KRT71-congenital hypotrichosis in Hereford cattle (Jayasekara et al. 1979, Bracho et al. 1984, Jacinto et al. 2021). In the present study, clumps of melanin were found within the pilary canal, in hair fragments and, occasionally, in follicular epithelium. At

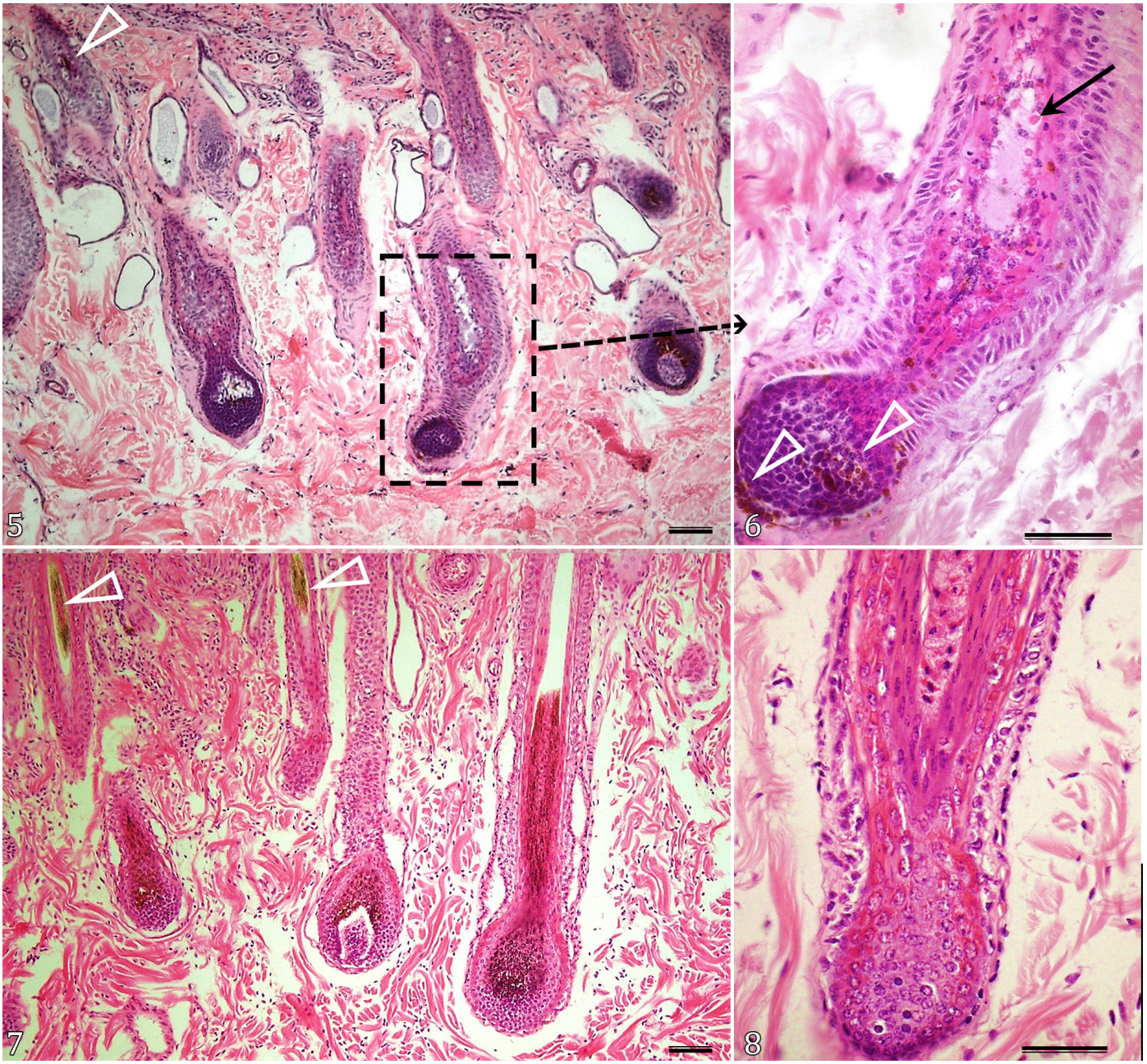

Fig.5-8. Dermatopathology of a KRT71-hypotrichotic Hereford calf. Colored skin biopsies of (**5** and **6**) affected and (**7** and **8**) control calves. (**5**) Dysplastic follicles (indicated by squares) and melanin clumping (arrowhead); note dilated sweat glands and absence of skin inflammation. (**6**) Suprabulbar region of a dysplastic follicle demonstrating large trichohyalin granules in Henle's and Huxley's layer cells (arrow) and melanin aggregates in the hair bulb (arrowheads). (**7**) Control skin biopsy showing follicles with normal supra bulbar regions, sweat glands, and hair shafts (arrowheads). (**8**) Normal follicle exhibiting a regular inner root sheet. (**5-8**) HE, bar = 100µ.

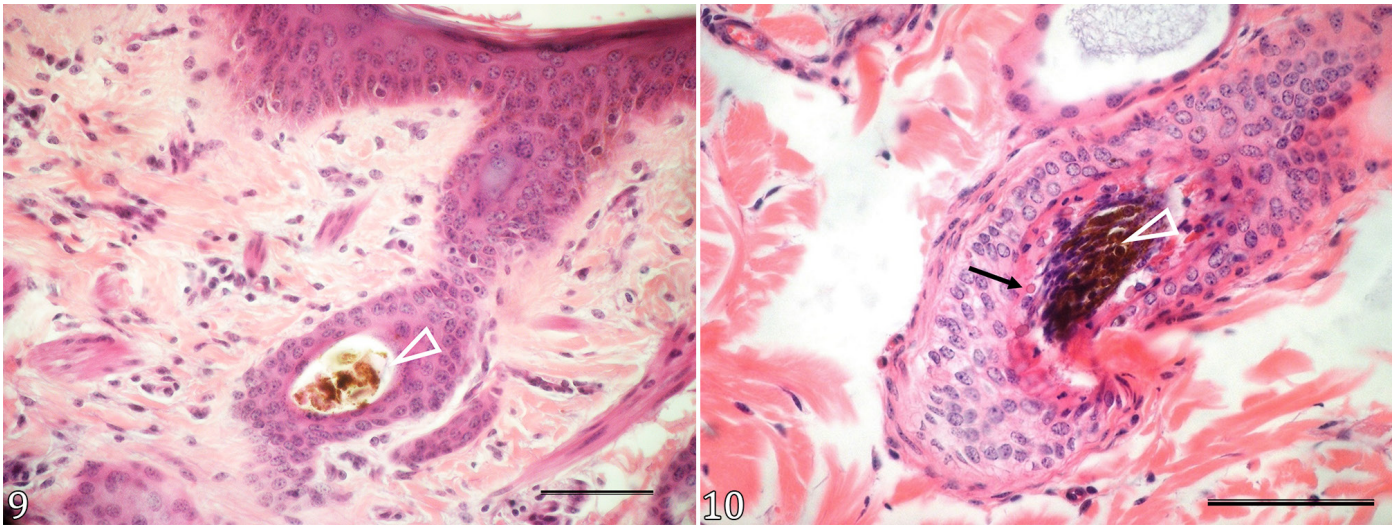

Fig.9-10. Melanin clumping in color-diluted KRT71-hypotrichotic Hereford calf. (9) Hair fragments and melanin aggregates within the pilary canal; note the hyperplasic epidermis and follicle infundibulum. (10) Intraluminal melanin aggregates (arrowhead) and trichohyalin granules (arrow) in the inner root sheath of a dysplastic (oblique section). (9-10) HE, bar = 100µ.

the same time, the trichogram, which is rarely used in cattle, revealed thin, fragile hairs with irregular pigment clumping. Hair fibers with irregular pigmentation are prone to fracture (Rothstein et al. 1998). These features are similar to color dilution alopecia, as described in a wide range of canine breeds (Rothstein et al. 1998) and horses (Henson & Stidworthy 2003), suggesting that KRT71-congenital hypotrichosis of HF cattle is also a color-related genetic defect, except that the degree of pigmentary alteration seems to be milder.

CONCLUSION

KRT71-hypotrichosis congenita is an important disease in Hereford cattle in Uruguay. The dilution of coat color and follicular dysplasia with pigmentary alteration are significant findings of the disease. A trichogram is an easy and inexpensive technique that provides important information in diagnosing the disease, but histopathology and molecular analysis are mandatory for the final diagnosis.

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