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Iris biopsy to investigate feline iris hyperpigmentation

Heidi J. Featherstone[1] | **Emma J. Scurrell**[2] | **Mike Rhodes**[3] | **Rodrigo Pinheiro de Lacerda**[4]

[1]The Ralph Veterinary Referral Centre, The Globe Business Park, Buckinghamshire, UK

[2]CytoPath Ltd, Herefordshire, UK

[3]Focus Referrals, Oxfordshire, UK

[4]Willows Veterinary Centre & Referral Service, West Midlands, UK

Correspondence
Heidi J. Featherstone, The Ralph Veterinary Referral Centre, The Globe Business Park, Buckinghamshire, UK.
Email: heidifeatherstone68@gmail.com

Abstract

Objective: To evaluate the benefit of iris biopsy in cats with iris hyperpigmentation to differentiate melanosis from early feline diffuse iris melanoma (FDIM).

Methods: The medical records of cats with unilateral iris hyperpigmentation that had undergone iris biopsy between February 2013 and September 2016 at Willows Veterinary Centre & Referral Service were reviewed.

Results: Seven cats with unilateral iris hyperpigmentation were included in this retrospective study. The biopsy procedure was performed under general anesthesia (n = 7) with neuromuscular blockade (n = 6) following pre-operative topical miotic therapy (n = 5). One to six biopsy samples per eye were harvested from areas of hyperpigmentation. The samples were partial thickness (n = 4 eyes) and full thickness (n = 3 eyes). Complications were minor: mild intra-operative hemorrhage (n = 4), fibrin clot (n = 2), corneal ulcer (n = 1), post-operative ocular hypertension (n = 1), dyscoria (n = 1), and pseudopolycoria (n = 2). The first biopsy was diagnostic in six cats; a repeat biopsy was necessary in one cat. Histopathology was consistent with melanosis in five cats and with early FDIM in two cats. Screening for signs of metastatic disease (thoracic computed tomography and abdominal ultrasonography) was negative in the two cats with a preliminary diagnosis of early FDIM. Subsequent enucleation and histopathology confirmed the initial diagnosis in both cases.

Conclusions: Iris biopsy in cats with iris hyperpigmentation can be beneficial to differentiate melanosis from early FDIM and thereby help to justify the decision for early enucleation.

KEYWORDS

biopsy, diffuse iris melanoma, melanoma, Melanosis

1 | INTRODUCTION

Hyperpigmentation of the feline iris is a common clinical sign and can be caused by melanosis, diffuse iris melanoma, and anterior uveitis. The clinical appearance of melanosis and early feline diffuse iris melanoma (FDIM) can be indistinguishable as focal or multifocal flat areas of hyperpigmentation on the anterior iris surface, and essentially reflects a spectrum of pathology whereby melanosis appears to be the precursor lesion for FDIM. FDIM originates from the melanocytes that line the anterior iris stroma. The melanocytic proliferation initially restricted to the anterior iris surface is defined as melanosis and may remain as such for months to years.[1] Disease progression is associated with melanocytic invasion into the underlying iris stroma. With progression, iris thickening, dyscoria, reduced pupil mobility, and secondary glaucoma can develop.

Feline diffuse iris melanoma is a malignant neoplasm with a reported metastatic rate between 19% and 66%

involving liver, lung, spleen, lymph nodes, and bone.[2-7] Many cases show slow progression while others are characterized by rapid spread, and this unpredictability in progression rate complicates clinical management. Metastasis can follow a long latency period in some cats (2.3 years has been reported).[3,4,8-11] Prognostic indicators for the risk of metastasis and survival have been described.[2,3,12] Negative indicators included mitotic index,[3,12] invasion of the scleral venous plexus, extrascleral connective tissue, and choroid, tumor necrosis and secondary glaucoma.[2,3,12] Positive prognostic indicators included early disease, with neoplasia confined to the iris surface,[3] or to the iris stroma and trabecular meshwork.[12] The only clinical prognostic indicator for FDIM is the presence of secondary glaucoma[12] although this was not identified in a more recent study.[2] The earlier study showed that the prognosis for survival for cats with early FDIM was similar to that for cats without FDIM[12]—hence the recommendation to consider early enucleation in an otherwise "normal" visual and comfortable eye. These studies highlight the difficulty of clinical prognostication and the decision-making process regarding case management and the appropriate time to enucleate. While some may elect early enucleation of an otherwise visual eye in an attempt to achieve clinical cure, others will argue to enucleate in more advanced disease and when secondary glaucoma is present.

In humans, the clinical diagnosis of iris melanoma is made on the identification of well-defined criteria including lesion diameter and thickness, progression, replacement of iris stroma, prominent blood vessels, ectropion iridis, and secondary cataract and glaucoma.[13,14] Diagnostic techniques include optical coherence tomography, ultrasound biomicroscopy, aqueocentesis (if metastasis suspected), fine needle aspirate biopsy, and multifocal surgical iridectomy biopsy.[15]

Iris biopsy has not been routinely performed in veterinary ophthalmology because of the perceived risks of hemorrhage, non-diagnostic samples, "seeding" of neoplastic cells, difficulty in differentiating "premalignant from benign lesions" and cost.[16]

One of the most difficult problems facing the clinician when presented with feline iris hyperpigmentation is the accurate diagnosis of early FDIM. This paper evaluates a surgical technique for iris biopsy to investigate feline iris hyperpigmentation.

2 | MATERIALS AND METHODS

The medical records of cats with unilateral iris hyperpigmentation investigated by iris biopsy at Willows Veterinary Centre & Referral Service between February 2013 and September 2016 were reviewed. All biopsy samples were examined by the same board-certified pathologist (EJS) at CytoPath Ltd. Information collected from each case included: signalment (breed, age, sex), duration of hyperpigmentation prior to referral, laterality, details of surgical technique, peri-operative medication, histopathological diagnosis, complications, and follow-up.

Melanosis was defined as a proliferation of melanocytes restricted to the anterior surface of the iris, without invasion of the underlying stroma.[1] In accordance with the description by Dubielzig et al,[1] the term melanosis included the proliferation of dysplastic melanocytes, so long as the proliferation was confined to the anterior surface of the iris. FDIM was defined as invasion of the underlying iris stroma by the aforementioned melanocytic proliferation.[1]

3 | RESULTS

All patients were evaluated by one of three board-certified veterinary ophthalmologists (HJF, MR, RPL), and a thorough physical examination was performed prior to surgery. Seven eyes of seven cats underwent iris biopsy to investigate unilateral iris hyperpigmentation. The mean age at diagnosis was 7.3 years (range 6-12 years). Three breeds were affected, including domestic shorthair (n = 5), Maine Coon (n = 1) and Burmese (n = 1). There were four female neutered and three male neutered cats. The iris hyperpigmentation was the sole reason for referral in six cats and was noted during cataract assessment in one cat. Iris hyperpigmentation had been observed prior to referral for one month (n = 3), two months (n = 3), and four years (n = 1, cataract assessment). The hyperpigmentation was unilateral in all cats and was focal (n = 1), multifocal (n = 3), diffuse affecting 30% of the iris (n = 1), and generalized (n = 3) (Figure 1A-D). There was no evidence of increased iris thickness or an apparent change in iris surface texture on slit-lamp biomicroscopy. The cat that presented for cataract assessment was blind because of bilateral mature cataract. A routine ophthalmic examination was otherwise normal in all cats in terms of tear production, intraocular pressure, routine neuro-ophthalmic reflexes, and slit-lamp biomicroscopy; funduscopy was possible in six cats and was unremarkable. A general physical examination revealed no clinically significant findings.

3.1 | Surgical technique

The surgery was performed by one of three board-certified surgeons (HJF, MR, RPL). All patients were anesthetized with a combination of intravenous and gas anesthesia performed by a board-certified anesthesiologist. The biopsy procedure was performed with neuromuscular blockade (n = 6) following pre-operative topical miotic therapy (n = 5). A standard regimen of pre-operative topical medication was followed in five cats and comprised a single drop each of proxymetacaine 0.5% eye drops (Chauvin Pharmaceuticals

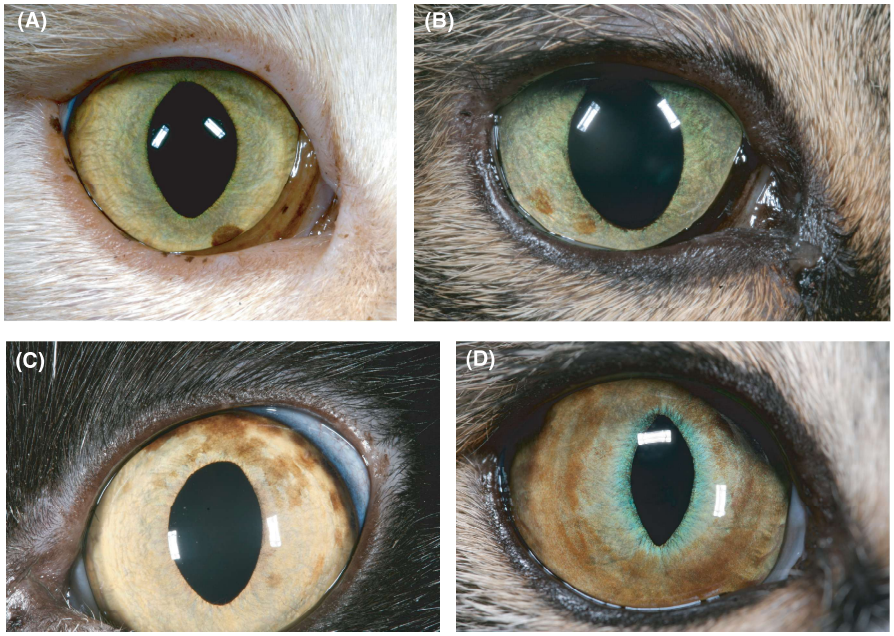

FIGURE 1 A, Focal iris hyperpigmentation in the right eye of Maine Coone (8.5 y, male neutered). The histopathological diagnosis was FDIM. B, Multifocal iris hyperpigmentation in right eye of domestic shorthair cat (12 y, male neutered). The histopathological diagnosis was melanosis. C, Diffuse iris hyperpigmentation in left eye of domestic shorthair cat (7.5 y, female neutered). The histopathological diagnosis was FDIM. D, Generalized iris hyperpigmentation in right eye of domestic shorthair cat (10 y, female neutered). The histopathological diagnosis was melanosis

Ltd.), pilocarpine 0.5% eye drops (Chauvin Pharmaceuticals Ltd.) and travoprost 0.004% eye drops (Travatan®, Novartis Europharm Ltd.) to induce intense miosis (Figure 2A). The iris biopsy was performed immediately prior to bilateral phacoemulsification in one cat; pre-operative topical medication consisted of flurbiprofen 0.03% eye drops (Ocufen®, Allergan Ltd.). Six cats received full neuromuscular blockade with atracurium (Tracium®, Aspen Pharma Trading Ltd.). The cats were positioned in dorsal recumbency, and the eyes were aseptically prepared with dilute povidone-iodine solution (Vetasept® 5% povidone antiseptic solution, AnimalCare). A bimanual approach was followed in all eyes. The number of clear corneal incisions, made with a 2.8 mm keratome, varied from two incisions (n = 5), three incisions (n = 1) to four incisions (n = 1) according to the location of the iris hyperpigmentation and surgeon choice. The anterior chamber was stabilized with 2% hydroxypropyl methylcellulose (CellVet®, AJL Ophthalmic). The major arterial circle was identified and avoided. The iris biopsy was performed by gentle elevation of an area of hyperpigmentation using capsulorhexis forceps (Capsulorhexis Grasping Forceps [curved tips], No. 581 486, Beaver-Visitec International Ltd., Warwickshire, UK) (Figure 2B) and excised with sharp Vannas scissors (Figure 2C). The number of biopsies per eye varied from one (n = 1), two (n = 2), three (n = 3), five (n = 1), and six (n = 1). Partial-thickness biopsies were taken in four cats and full-thickness biopsies in three cats. Following harvest, each biopsy sample was transferred immediately to a pre-sterilised cassette using an insulin needle; the cassette was immediately placed in 10% neutral buffered formalin (NBF). The samples were not moistened with saline and were not allowed to desiccate in room air. Multiple cassettes were necessary in some cases. Anterior chamber irrigation/aspiration was completed with balanced salt solution (Endosol®, Johnson & Johnson) including heparin (0.5 mL, 1000 IU/mL per 500 mL, Wockhardt UK Ltd.) and epinephrine (0.5 mL, 1:1000 per 500 mL, Adrenaline, Macarthy Lab Ltd.). The corneal wounds were sutured with 9/0 polyglactin (Vicryl®; Ethicon, Johnson & Johnson Intl).

3.2 | Post-operative medication

All cats received the same systemic medication including meloxicam 0.1 mg/kg PO SID (Metacam® oral syrup,

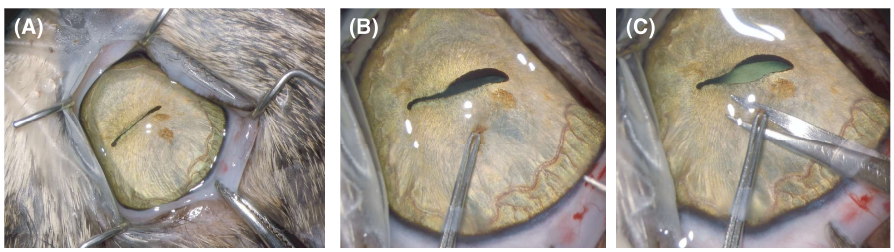

FIGURE 2 A, Intraoperative photograph. Intense miosis (same cat as in Figure 1B). B, Intraoperative photograph. Iris tissue elevation by capsulorhexis forceps (same cat as in Figure 1B). C, Intraoperative photograph. Iris tissue excised by Vannas scissors (same cat as in Figure 1B)

Boehringer Ingelheim, Vetmedica GmbH) and amoxicillin-clavulanate (Synulox®, Zoetis UK Ltd.) 15 mg/kg PO BID, both for one week. Topical anti-inflammatory and antibiotic preparations were used in all cats and included 0.1% dexamethasone/neomycin/polymyxin B eye drops (Maxitrol®, Alcon Laboratories Ltd., Hemel Hempstead) (n = 3), ofloxacin 0.3% eye drops (Exocin®, Allergan Ltd.) (n = 3), chloramphenicol 0.5% eye drops (Martindale Pharma, Martindale Pharmaceuticals) (n = 1), prednisolone acetate 1% eye drops (Pred forte®, Allergan Ltd.) (n = 3), and bromfenac 0.09% eye drops (Yellox®, PharmaSwiss) (n = 1). Mydriatic-cycloplegic therapy was used in three cats: tropicamide 1% eye drops (Tropicamide Minims®, Chauvin Pharmaceuticals Ltd.) (n = 2) and atropine 1% eye drops (Atropine Minims®; Chauvin Pharmaceuticals Ltd.) (n = 1). Topical dorzolamide 2% eye drops (Trusopt®, Star Pharmaceuticals Ltd.) were used in the cat that underwent phacoemulsification.

3.3 | Complications

Minor complications occurred in four eyes. Minor iris hemorrhage at the time of the biopsy occurred in four eyes (Figure 3A); intraocular diathermy was not required and the hemorrhage stopped spontaneously within one minute. A small fibrin clot developed in two eyes and resolved over several days with routine anti-inflammatory medication; intracameral tissue plasminogen activator was not necessary (Figure 3B). A superficial corneal ulcer developed in one eye and resolved within three days. Transient and mild post-operative hypertension developed and resolved in both eyes of the cat that underwent bilateral phacoemulsification. Pupil abnormalities developed in three eyes and included dyscoria (n = 1) (Figure 3C), and pseudopolycoria secondary to full-thickness biopsies (n = 2) (Figure 3D). A nondiagnostic sample was obtained in one cat that did not receive pre-operative miosis and neuromuscular blockade at the surgeon's discretion. The biopsy procedure was repeated following the standard protocol, and the repeat biopsy sample was diagnostic.

3.4 | Histopathology

The majority of biopsy samples were <1 mm in diameter (range of 0.5-0.9 mm) (Figure 4A). The small sample size precluded orientation at the time of embedding, and the biopsy specimens were wax-embedded in their entirety and in the same cassette. Sections of 5 μm were cut and stained with hematoxylin-and-eosin (H&E). Orientation of the partial-thickness biopsies was sometimes difficult on histopathological examination but this was aided by the examination of multiple step levels (see below). Tissue trauma, consistent with forceps use, was evident in some sections (Figure 4B).

A histopathological diagnosis was achieved in all seven eyes, one eye having received a repeat biopsy. A diagnosis of feline iris melanosis was made in five eyes, and a diagnosis of FDIM was made in two eyes (Figure 4C-D). Feline iris melanosis was characterized by a proliferation of dysplastic melanocytes in 1-3 layers restricted to the anterior iris surface and without evidence of stromal invasion. The features of dysplasia included plump, pigmented melanocytes with varying degrees of anisokaryosis and/or hyperchromasia and/or discernible nucleoli. With the exception of the two cases diagnosed as FDIM, where iris invasion by neoplastic melanocytes was identified on the initial

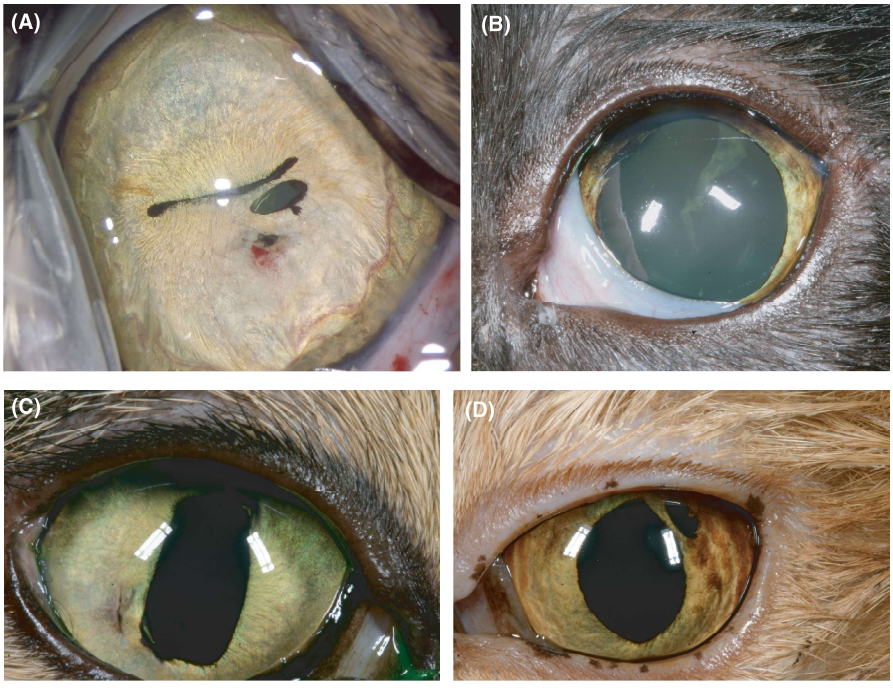

FIGURE 3 A, Intraoperative photograph. Mild hemorrhage from the first of two biopsy sites, resolved spontaneously. Note second full-thickness biopsy site (same cat as in Figure 1B, 2A-C). B, Small fibrin clot following biopsy in left eye of a domestic shorthair cat (6 y, male neutered). The histopathological diagnosis was FDIM. C, Dyscoria following biopsy in right eye of a domestic shorthair cat (same cat as in Figure 1B, 2A-C, 3A). D, Pseudopolycoria following biopsy in left eye of a domestic shorthair cat (8 y, female neutered). The histopathological diagnosis was FDIM

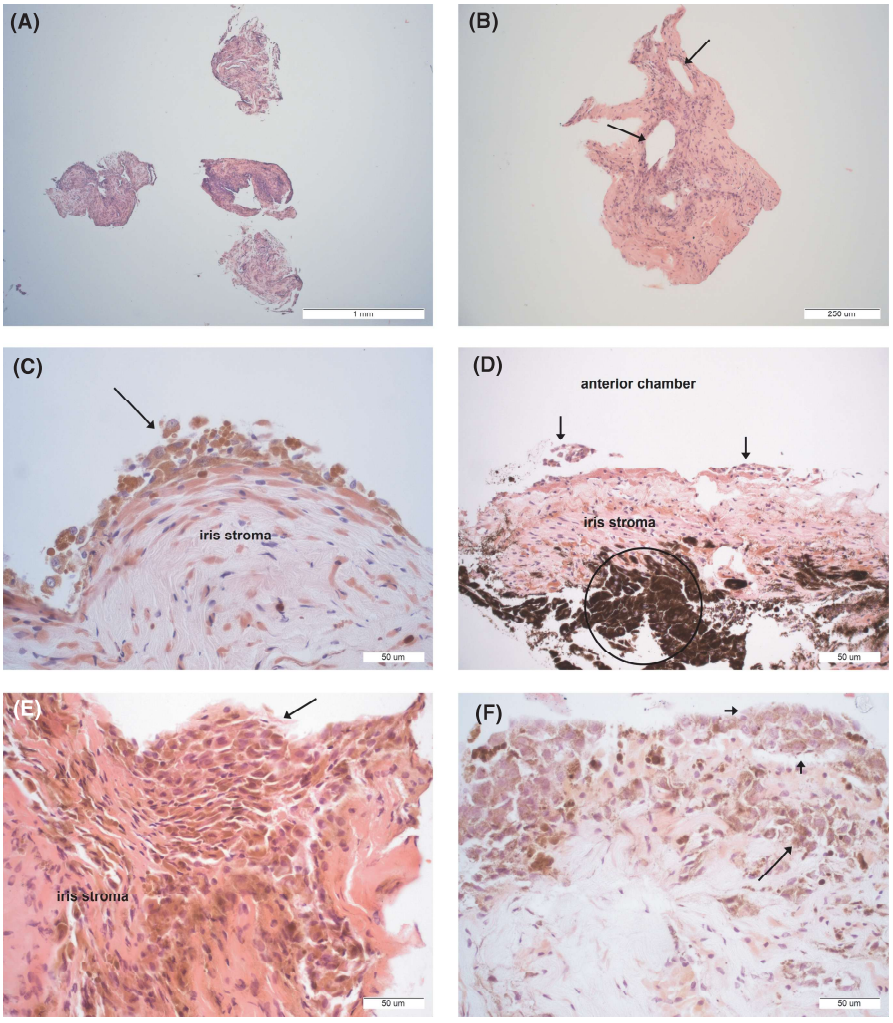

FIGURE 4 A, A histopathology section at low magnification showing four small iris biopsies. H&E. B, An iris biopsy with tissue trauma related to the use of forceps (arrows). H&E. C, A partial-thickness iris biopsy in which the hyperpigmentation was characterized by a proliferation of melanocytes restricted to the anterior surface. The melanocytes are plump and display nuclear atypia characterized by open nuclei with small distinct nucleoli and moderate anisokaryosis. There is no evidence of stromal invasion. The histopathological diagnosis was melanosis. H&E. D, A full-thickness iris biopsy near the pupil. The anterior iris surface is multifocally lined by 1-2 layers melanocytes with nuclear atypia (arrows). There is no evidence of stromal invasion. The pigmented posterior iridal epithelium helps with orientation (circle). The histopathological diagnosis was melanosis. H&E. E, A partial-thickness iris biopsy showing a proliferative atypical melanocytic population at the iris surface (arrow) and the same cell population in the underlying stroma, justifying a diagnosis of FDIM. H&E. F, A partial thickness iris biopsy confirming FDIM. The short arrows delineate a proliferation of atypical melanocytes at the anterior iris surface that infiltrate the underlying iris stroma (long arrow). H&E

sections, a minimum of three step levels of each biopsy were examined (range of 3-5 step levels per submission). The diagnosis of feline iris melanosis was accompanied by a comment that such lesions retain a risk of progression to FDIM but that the rate of progression is unpredictable, if it is to occur at all. FDIM was characterized by invasion of the iris stroma by the melanocytic proliferation described above (Figure 4E-F).

3.5 | Further management

The two cats with early FDIM had screening for metastatic disease by thoracic computed tomography and abdominal ultrasonography; no abnormalities were identified. The affected eyes were removed by routine transconjunctival enucleation and fixed in 10% NBF and submitted for histopathology. Histopathological evaluation of both globes confirmed early FDIM, consistent with the previous iris biopsy findings (Figure 5A-C).

3.6 | Follow-up

The duration of follow-up varied: Two months in the four cats diagnosed with melanosis, five days following enucleation in the two cats diagnosed with FDIM, and three years in the cat that underwent phacoemulsification and had concurrent

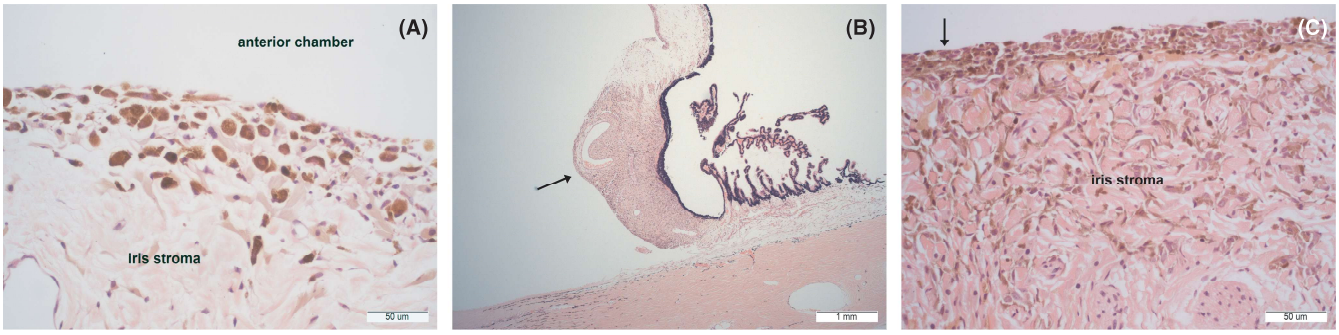

FIGURE 5 A, An enucleation specimen of the same cat in Figure 4E. with early FDIM characterized by melanocytic infiltration of the anterior iris stroma. H&E. B, An enucleation specimen of the same cat in Figure 4F. confirming early FDIM with focally extensive melanocytic infiltration of the iris (arrow). H&E. C, Higher magnification of the FDIM in Figure 5B H&E. The arrow indicates the proliferating melanocytes on the anterior iris surface that have progressed to infiltrate the iris stroma

melanosis. There were no signs of progressive hyperpigmentation in the five cats with the diagnosis of melanosis, and no observed complications from the biopsy procedure (posterior synechiae, hemorrhage).

4 | DISCUSSION

This study shows that iris biopsy in cats with iris hyperpigmentation can be beneficial to differentiate melanosis from early FDIM. It is most helpful when it confirms early FDIM. This helps to facilitate the decision-making process for both the owner and the ophthalmologist when considering early enucleation.

Feline iris hyperpigmentation poses two problems for the clinician. Firstly, the clinical differentiation of early FDIM from melanosis associated with increasing age. Secondly, the biological behavior of FDIM—the actual risk of metastatic disease and the benefit of early enucleation. The rate of progression of iris hyperpigmentation is variable and unpredictable. Slow progression over years, without deleterious effects on the eye, and rapid progression with secondary glaucoma and metastatic disease have both been described.[1,4] The only reported clinical prognostic indicator for FDIM has been the presence of secondary glaucoma.[12] Secondary glaucoma was associated with a reduced survival rate. Four of 19 cats (21%) with FDIM and concurrent secondary glaucoma survived, compared to 11 of 15 cats (73%) with FDIM and without glaucoma. However, a more recent study showed that neither the clinical or histologic evidence of secondary glaucoma was associated with metastatic disease.[2]

The rate of metastatic disease is difficult to document because of long latency periods and the reduced frequency of postmortem examinations on client-owned cats over recent years.[1,8,16] The evaluation of histologic features to help with prognostication is limited by long latency periods between primary and metastatic disease, loss of cases to follow-up, a reduction of postmortems and/or lack of cytologically or histopathologically confirmed metastases.

The rate of metastatic disease has been reported to range from 62.5% in 1988[4] to 19% in 2016.[2] Early literature described cases with very advanced FDIM and metastatic disease was confirmed with postmortem examination.[4] Patnaik & Mooney[4] described intraocular melanoma in 16 cats. The tumor involved the iris and ciliary body in six cats, the iris, ciliary body, sclera, cornea and intraocular compartments in six cats, and "the whole eye" in four cats. Follow-up data were available on fourteen of those sixteen cats; metastatic disease was confirmed on postmortem examination with histopathology in ten cats (10/16, 62.5%). This high risk of metastatic disease, approximately 63%, has been widely quoted by veterinary ophthalmologists over the last three decades, based on ten cats with advanced ocular disease. Later literature describes cases with earlier disease and the metastatic rate was often presumed rather than confirmed by cytology or histopathology. Early enucleation has become more common since the work in 1998 by Kalishman et al[12] Cats with early FDIM confined to the iris had the same survival rates as cats without FDIM. In that study, the cause of death was not documented in most cases; data were suggestive of metastatic disease because of "palpable abdominal masses or weight loss." Wiggans et al[2] published the most recent figure of 19% for the rate of metastatic disease. This was based on confirmed metastases in two cats and suspected metastasis in seven cats (9/47 cats, 19%). "Confirmed" metastatic disease was defined as melanocytic neoplasia identified by histology at a nonocular site, and "suspected" metastatic disease when there was radiographic or ultrasonographic evidence of metastatic disease. Case management has changed over the decades because of increased awareness, and concern about the 62.5% risk of metastasis based on the work from 1988.[4] Early enucleation appears to be more frequently performed because of the work by Kalishman et al[12] 10 years later. Some clinicians recommend enucleation when there are signs of progression and/or involvement of the iridocorneal drainage

angle, and others wait for signs of secondary glaucoma. These issues highlight the difficulty of clinical prognostication and decision-making process regarding the appropriate time to enucleate. In the last three decades, enucleation of visual and normotensive eyes appears to be more frequently performed.

The enucleation of a visual, nonpainful, and nonglaucomatous eye is never undertaken lightly and iris biopsies in such cases may help to facilitate the decision-making process. The proliferation of melanocytes restricted to the iris surface could potentially remain as such for months to years and enucleation of a visual eye at this stage is difficult to justify. Invasion of the melanocytes into the iris stroma indicates disease progression and confirms the diagnosis of FDIM.

Wiggans et al[2] reported three immunohistochemical parameters to help to determine the risk of metastasis of FDIM. Increased label intensity of melan-A and E-cadherin was associated with an increased risk of metastasis, and homogenous labeling of PNL2 was associated with a decreased risk of metastasis. Such validated molecular assays could be used to guide decision-making and help clinicians preserve visual and comfortable eyes.[2,17,18] With further investigations, it is possible that such immunohistochemical markers could be applied to incisional biopsy samples, iris aspirates, and aqueocentesis samples.[2]

The terminology used in cases of feline iris hyperpigmentation can be controversial and confusing, and a proposed review of the current nomenclature is beyond the scope of, and is not the intention of, this article. In brief, however, the term feline iris melanosis is understood by many pathologists to indicate a noninvasive proliferation of melanocytes restricted to the anterior iris surface, regardless of dysplasia. In the authors' experience, the proliferative melanocytes frequently display varying degrees of nuclear atypia and appropriate terminology to reflect this atypia may be more accurate. It may be beneficial to consider comparative pathology as outlined in the WHO classification of tumors of the eye, specifically the term primary acquired melanosis (PAM) of the conjunctiva in human patients.[19] Older terms for this include "pre-cancerous melanosis" and "pre-malignant melanosis." PAM encompasses proliferative melanocytic lesions within the surface conjunctival epithelium ranging from melanosis without atypia to melanosis with mild to severe atypia, or melanoma "in situ." The melanosis in this context is equated with a superficial, noninvasive melanocytic proliferation and melanosis with atypia is considered to reflect a neoplastic melanocytic proliferation. In addition, melanosis with atypia is at increased risk for progression to invasive melanoma. Although the location is different, this is a somewhat similar scenario to the superficial iris melanosis and invasive FDIM seen in cats. If iris biopsies are to be more routinely performed in the investigation of feline iris hyperpigmentation, a review of the current nomenclature could be helpful to more accurately reflect the spectrum of melanocytic proliferation.

The authors wish to highlight some aspects of the surgical technique and handling of the samples to maximize success. Pre-operative miosis and neuromuscular blockade are considered mandatory. These two steps were omitted in one case, which required repeat surgery. *Sharp* Vannas scissors were effective to harvest the iris tissue; vitreoretinal scissors, and used, semi-blunt vannus scissors were ineffective and caused tissue trauma. Intraocular diathermy should be available although it was not required in any of the cases in this study. Multiple biopsies will also help maximize the chances of achieving a fully representative sample of the iris hyperpigmentation, particularly in early stages of FDIM where iris invasion can be patchy.

The small size of the biopsy samples necessitates careful planning, meticulous tissue handling and close collaboration with the pathologist and the laboratory technician(s). Rapid desiccation of the harvested tissue must be prevented. The cassettes to hold the samples should be pre-sterilized and available on the operating trolley; the container(s) with 10% NBF should be available in theater. Delicate transfer of the iris tissue to the cassette is achieved with a 27-gauge needle to "nudge" the sample from the tips of the forceps. Technical staff at the laboratory may need to sieve the 10% NBF to retrieve samples that float loose from the cassettes. The pathologist must be familiar with the architecture of the feline iris. Multiple step levels are typically required.

The study has several limitations. The number of cats is small because this was a pilot study to evaluate the diagnostic potential of iris biopsy. The surgical protocol and peri-operative medications were not identical. The follow-up was variable and short, with the exception of the cat that was monitored for three years following cataract surgery. The two cats that had enucleation were not monitored beyond the 5-day routine post-operative check-up. The short follow-up time of 2 months in the four cats that were diagnosed with melanosis in this study demonstrates suboptimal client communication. The authors feel that the owners' erroneous perception of melanosis was that there was no future risk. It is essential to educate clients about the ongoing potential for melanosis to transform into FDIM and the need for long-term monitoring. A change in terminology to include melanosis with atypia or melanoma "in situ" could perhaps facilitate this communication.

An important limitation of the technique is the possibility of harvesting a nonrepresentative biopsy, which could provide a diagnosis of melanosis in an eye with early FDIM.

5 | CONCLUSIONS

In conclusion, iris biopsy is a beneficial technique to differentiate melanosis from early FDIM in cats with iris

hyperpigmentation. The complications are minor and mostly transient. Careful preparation and close collaboration with the ocular pathologist and laboratory are essential to maximize the chance of obtaining a histopathological diagnosis. Iris biopsy is most helpful when a diagnosis of early FDIM is made. This helps to justify early enucleation and is easier for both the owner and the ophthalmologist to accept. Caution is required when the diagnosis is melanosis. It is essential to educate clients about the ongoing potential for melanosis to transform into FDIM and the need for long-term monitoring and potential repeat biopsy.

ORCID

Heidi J. Featherstone https://orcid.org/0000-0002-4746-4019

Emma J. Scurrell https://orcid.org/0000-0001-5488-1841

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