UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

Lamellar keratoplasty for the treatment of feline corneal sequestrum

Dissertação de Mestrado em Medicina Veterinária

Catarina Alexandra Ventura Gonçalves

Orientador: Professor Doutor José Eduardo Teixeira Pereira

Co-Orientador: Professor Doutor Luís Miguel Viana Maltez da Costa

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DESIGNAÇÃO DO MESTRADO: MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

TÍTULO DA DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA: LAMELLAR KERATOPLASTY FOR THE TREATMENT OF FELINE CORNEAL SEQUESTRUM

ORIENTADORES:
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PROFESSOR DOUTOR LUÍS MIGUEL VIANA MALTEZ DA COSTA

ANO DE CONCLUSÃO: 2013

DECLARO QUE ESTA DISSERTAÇÃO DE MESTRADO É RESULTADO DA MINHA PESQUISA E TRABALHO PESSOAL E DAS ORIENTAÇÕES DOS MEUS SUPERVISORES. O SEU CONTEÚDO É ORIGINAL E TODAS AS FONTES CONSULTADAS ESTÃO DEVIDAMENTE MENCIONADAS NO TEXTO, E NA BIBLIOGRAFIA FINAL. DECLARO AINDA QUE ESTE TRABALHO NÃO FOI APRESENTADO EM NENHUMA OUTRA INSTITUIÇÃO PARA OBTENÇÃO DE QUALQUER GRAU ACADÉMICO.

VILA REAL, 6 DE JUNHO DE 2013.
CATARINA ALEXANDRA VENTURA GONÇALVES

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Abstract

Feline corneal sequestrum is a disease characterized by a pigmented corneal lesion. Various degrees of corneal discoloration as well as corneal depth involvement are reported. Corneal sequestrum pathophysiology is not yet completely elucidated; however corneal trauma is generally accepted as an important initiating factor and brachycephalic breeds are the most frequently affected. This is a painful condition which needs to be assessed medically or surgically. Time until healing and final results are not satisfactory when medical treatment is used as a single therapy. Surgically, various techniques are available for sequestrum removal and re-establishment of corneal integrity; however, to completely restore animal's vision ability, keratoplasty is the option of choice. This retrospective study includes 25 corneal sequestra which were diagnosed in 20 cats and managed by lamellar keratoplasty. With this surgical procedure the corneal sequestrum is removed and a fresh or frozen lamellar corneal graft is used to restore corneal integrity and transparency. A total of 27 keratoplasties were performed, since two corneas underwent a second keratoplasty. Probability of graft rejection is reduced by the removal of the graft's endothelial layer and topical immunosuppressive therapy. Recurrence of the sequestrum is uncommon after this technique and in this study only occurred in one case. The information present in this retrospective study was collected during a period of clinical training of 4 months in Hospital Veterinário de Trás-os-Montes and a 3 month period in Fundació Hospital Clínic Veterinari, UAB.

Key-words: feline corneal sequestrum, corneal surgery, lamellar keratoplasty, corneal transparency.

Resumo

O sequestro corneal felino caracteriza-se por uma lesão corneal pigmentada. Esta lesão apresenta várias tonalidades e pode afetar diferentes camadas da córnea. A fisiopatologia do sequestro não é completamente conhecida, contudo o trauma corneal é considerado um importante fator desencadeador e as raças braquicefálicas são mais frequentemente afetadas. O sequestro corneal é uma lesão dolorosa, cujo tratamento pode ser médico ou cirúrgico. Quando o tratamento médico é usado como tratamento único, o tempo de cicatrização e o resultado final não são satisfatórios. Cirurgicamente várias técnicas podem ser usadas na remoção do sequestro e restauro da integridade da córnea, no entanto, a fim de restaurar a capacidade de visão do animal, a queratoplastia é a única opção. Este estudo retrospetivo analisa 25 sequestros corneais diagnosticados em 20 gatos e cujo tratamento foi efetuado por queratoplastia lamelar. Esta técnica implica a remoção da região corneal afetada pelo sequestro e a colocação de um transplante corneal proveniente de um globo ocular fresco ou congelado. Desta forma, a integridade e a transparência da córnea são restauradas. No total foram realizadas 27 queratoplastias lamelares, pois duas córneas foram submetidas a uma segunda queratolastia. A probabilidade de rejeição do transplante é reduzida pela remoção do endotélio deste, bem como através do uso de medicação imunossupressora tópica. A recorrência do sequestro após esta intervenção cirúrgica é pouco comum, e neste estudo ocorreu apenas num caso. A informação contida neste estudo retrospetivo foi recolhida durante um período de estágio de 4 meses no Hospital Veterinário de Trás-os-Montes e outro período de 3 meses no Fundació Hospital Clínic Veterinari, UAB.

Palavras-chave: sequestro corneal felino, cirurgia corneal, queratoplastia lamelar, transparência corneal.

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ATP: adenosine triphosphate

DNA: deoxyribonucleic acid

ELISA: enzyme-linked immunosorbent assay

FGF-2: fibroblast growth factor 2

FHV-1: feline herpesvirus type 1

FeLV: feline leukemia virus

FIV: feline immunodeficiency virus

FV: final vascularization

HGF: hepatocyte growth factor

HVTM: Hospital Veterinário de Trás-os-Montes

IL: interleukins

nPCR: nested polymerase chain reaction

OD: *Oculus dexter* (right eye)

OS: *Oculus sinister* (left eye)

PCR: polymerase chain reaction

PDGF: platelet derived growth factor

PEDF: pigment epithelium derived factor

PO: *per os*

PSIS: porcine small intestinal submucosa

PV: previous vascularization

SCCED: spontaneous chronic corneal epithelial defects

srPCR: single round polymerase chain reaction

TGF β: Transforming growth factor β

TIMP: tissue inhibitors of metalloproteinase

TNF: tumor necrosis factor

UAB: Universitat Autònoma de Barcelona

1. Introduction

1.1. Anatomy of the cornea

The cat cornea measures 15-16 mm vertically and 16-17 mm horizontally, and its thickness is about 0.58 mm, ranging between 0.469 and 0.832 mm (Gelatt and Brooks, 2011; Herring, 2003). Corneal thickness varies with age, weight and gender. In dogs corneas a difference in thickness is observed between central and peripheral cornea. The latter being thicker than central cornea, however this difference may not be present in cats (Herring, 2003).

The cat cornea is roughly elliptical, as in most animals. The horizontal diameter is slightly greater than the vertical diameter. Cat and dog corneas are larger than human corneas. This is probably to help in night vision, as larger corneas are found in animals typically nocturnal, and they allow a higher entrance of light, through the pupil, in reduced light environments (Gelatt and Brooks, 2011).

The cornea and the sclera together constitute the fibrous tunic of the globe. The transition zone between the cornea and the sclera is the limbus (Gelatt and Brooks, 2011).

The cornea is divided into axial (central), para-axial (para-central) and peripheral zones, with the axial area being the most important for vision. The division of the cornea may also be made into quadrants (Gelatt and Brooks, 2011).

Histologically, four individual regions are identified. From external to internal, the cornea is composed of: epithelia with a basal membrane; thick stromal layer; Descemet's membrane; and a posterior single layer of endothelial cells (Figure 1) (Cullen et al, 2005).

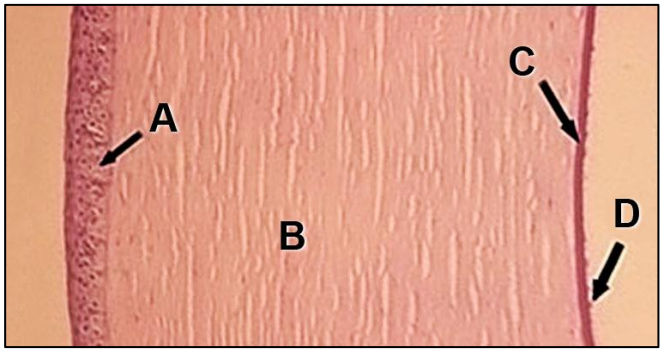

Figure 1. Histological image of a normal cat cornea. It is possible to identify the anatomical layers of the cornea. A: epithelium. B: stroma, C: Descemet's membrane, D: endothelium. H & E, 25x. (Adapted from Gelatt and Brooks, 2011).

The epithelial stratum is formed by 5-7 cell layers and consists of: an external layer of two to three sheets of non-keratinized squamous cells; intermediate two to three layers of polyhedral or wing cells; and a layer of basal columnar cells that is disposed upon a basal membrane. The turnover of corneal epithelium is approximately 7 days (Gelatt and Brooks, 2011).

The basal columnar cells produce the basement membrane, which attaches them, via hemidesmosomes, to the anterior stroma. This basement membrane is a slender acellular layer similar to Bowman's membrane (Cullen et al, 2005).

Although not present in the dog and the cat, Bowman's membrane, a modified anterior region of the corneal stroma, is found in humans and most birds (Gelatt and Brooks, 2011).

The corneal stroma, is also named *substantia propria*, and accounts for about 90% of the corneal thickness (Gelatt and Brooks, 2011). The stroma is formed by collagen fibrils, keratocytes and a matrix of glycosaminoglycans. The collagen fibrils are tightly packed, regularly arranged in parallel beams, forming the collagen lamellae (Herring, 2003). Individual lamellae are oriented in different planes (Cullen et al, 2005; Herring, 2003).

Keratocytes are specialized fibroblasts. They are elongated cells with irregularly shaped nuclei and cytoplasm containing rough endoplasmic reticulum and abundant membrane-bound vesicles. These cells are disposed among the collagen fibrils and the matrix of glycosaminoglycans (Cullen et al, 2005; Gelatt and Brooks, 2011).

Cornea's transparency is achieved by the interaction between the following factors: the tear film components, a non-keratinized anterior epithelium, stroma's collagen lamellae and fibrils organization, a relative dehydration status, and the absence of both pigmentation and blood vessels. These features allow the transparency which characterizes the normal cornea (Herring, 2003; Moore, 2005).

When corneal tissue undergoes damage, the arrangement of collagen fibrils and the matrix of glycosaminoglycans become distorted and corneal opacification is observed (Herring, 2003; Moore, 2005).

Corneal sensory nerves derive from the ophthalmic branch of the trigeminal nerve. They cross through the middle posterior stroma and terminate in subepithelial plexuses, providing free nerve endings to the intermediate layer of the epithelial stratum. Corneal epithelium and the anterior corneal stroma have pain and pressure receptors that came from the ophthalmic branch of the trigeminal nerve. Pain occurs from stimulation of the superficial nerve endings and from axonal reflex (Gelatt and Brooks, 2011).

Below the stromal layer, lays the Descemet's membrane. This is a translucent, rather elastic and relatively thick basement membrane, and its thickness increases with aging. The following layer, the endothelium, is responsible for the production of the Descemet's membrane. Exposure of the Descemet's membrane requires immediate surgical repair, as corneal rupture may follow (Gelatt and Brooks, 2011).

The posterior layer of the cornea is the endothelia. It is a single layer of hexagonal cells, bonded by interdigitations, forming different cell junctions, such as *zonulae occludentes*, *maculae adherents*, and *nexi*. These cells, apart from the production of Descemet's membrane, are also responsible for the relatively dehydration of the cornea. The water is removed via sodium-potassium ATPase pump system, an active pumping mechanism. The anterior corneal epithelium barrier function and corneal endothelium active pumping mechanisms prevent corneal edema, since the corneal stroma is hydrophilic (Herring, 2003).

Corneal edema occurs when the endothelia layer suffers surgical or traumatic damage. Decreased endothelia cell numbers and aging are also factors responsible for corneal edema (Gelatt and Brooks, 2011).

1.2. Feline corneal sequestrum

Feline corneal sequestration was first reported by *Roberts* in 1964. This disease is also known as isolated black lesion (Featherstone and Sansom, 2004), focal degeneration of the cornea (Andrew et al, 2001), corneal necrosis, *corneal nigrum*, corneal mummification, partial mummification, keratitis nigra, primary necrotizing keratitis and chronic ulcerative keratitis (Dalla et al, 2007). This is a common disease on domestic cat (Featherstone et al, 2004), and thought unique of this species (Gimenez and Fariña, 1998), but also described in horses (Håkanson and Dubielzig, 1994; McLellan and Archer, 2000) and, more recently, in a dog (Bouhanna et al, 2008).

The clinical appearance of the disease is characteristic, even pathognomonic according to Nasisse (Nasisse, 1995). It is characterized by an area of coagulation necrosis (Glaze, 2005), always pigmented but the intensity of the discoloration ranges from a diffuse, amber stain to a well-defined black lesion (Figure 2) (Featherstone et al, 2004). The darker region is usually surrounded by an amber yellow halo circled by various degrees of corneal vascularization and edema (Dalla et al, 2007), as well as inflammation (Grahn et al, 2005). Corneal edema and neovascularization only develop with chronicity (Moore, 2005).

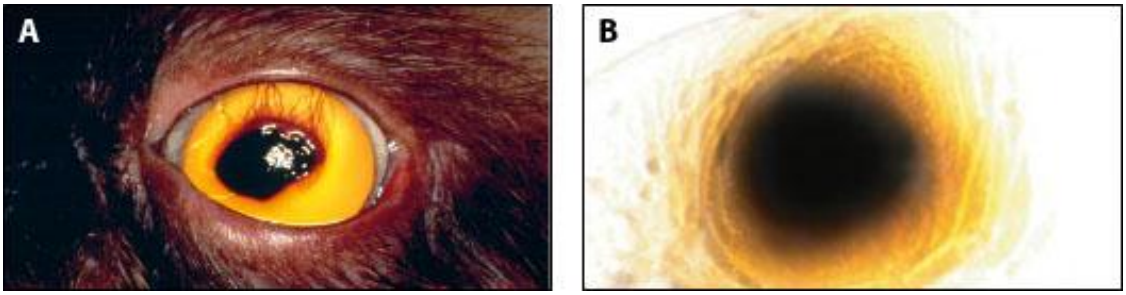

Figure 2. Corneal sequestrum. A: a 6-mm diameter, dark brown sequestrum affecting the corneal epithelium and the superficial stroma. B: Feline corneal sequestrum removed by keratectomy. It shows three characteristic zones of discoloration: a black or dark brown center, encircled by a lighter brown halo, and an amber periphery. (Adapted from Andrew et al, 2001 and Featherstone et al, 2004).

Sequestra may be located centrally or paracentrally in the cornea (Cullen et al, 2005) and range from a small to extensive, circular or oval necrotic lesion (Featherstone and Sansom, 2004). The depth of corneal involvement ranges from the epithelium to the Descemet's membrane (Cullen et al, 2005). It usually affects the epithelium and anterior stroma but often progresses and extends to the deeper stromal layers of the cornea (Gimenez and Fariña, 1998), leading to corneal perforation (Featherstone and Sansom, 2004). In some cases the sequestrum may naturally slough, but the time needed for this process to occur varies from days to several months, and in the meantime the lesion may progress to deeper layers of the cornea (Bouhanna, 2008; Featherstone and Sansom, 2004). If mineralization of the sequestrum occurs, a granular dark yellow protruding lesion is visible (Figure 3) (Gemensky and Wilkie, 2001). The intraocular structures are usually not affected (Dalla et al, 2007).

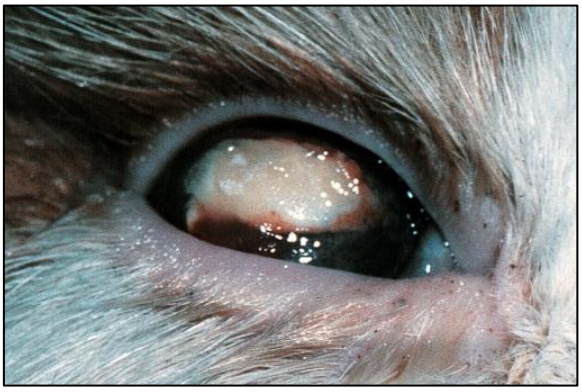

Figure 3. Mineralized corneal sequestrum. It is observed a deposition of an amber colored, granular material, circled by stromal edema and corneal vessels. (Adapted from Gemensky and Wilkie, 2001).

Generally, sequestra do not significantly stain with fluorescein (Mc Lellan and Archer, 2000), so, until recently, it was thought that sequestra are epithelialized or just loose the epithelium surrounding the ulcerated surface (Gemensky and Wilkie, 2001). Nowadays, it is commonly accepted that the overlying epithelium is disrupted, although there are alterations in stromal characteristics which impair fluorescein retention, except by the edges of the sequestrum (Glaze, 2005). The corneal stroma of the sequestrum site is desiccated impairing penetration of fluorescein, which is a hydrophilic stain (Mc Lellan and Archer, 2000).

Sequestra may expand rapidly over a short period of time (days or weeks) or may stabilize for years, as pigmentation may be present for years before sequestrum development (Featherstone and Sansom, 2004).

This condition affects cats aged between 5 months to 17 years, with the majority of diseased cats aged between 2 and 7 years (Featherstone and Sansom, 2004).

There is no sex predisposition, but a breed predisposition is recognized for Persian, Colorpoint, Siamese, Burmese, Himalayan, Domestic shorthaired and other breeds of long-haired cats (Andrew et al, 2001; Featherstone and Sansom, 2004; Townsend et al, 2008).

The sequestrum is more frequently unilateral than bilateral (Andrew et al, 2001; Glaze, 2005). Bilateral sequestra may occur simultaneously or separately in time, and is more common in Persians and other brachycephalic breeds (Featherstone and Sansom, 2004).

Ocular and systemic clinical sings are observed (Dalla et al, 2007). Common clinical signs accompanying this lesion are epiphora, photophobia, blepharospasm, enophthalmos, and protrusion of the third eyelid (Bouhanna et al, 2008; Moore, 2005). These clinical findings indicate discomfort, ocular pain and irritation (Andrew et al, 2001; Nasisse, 1995).

Conjunctivitis with chemosis, conjunctival hyperemia and ocular discharge (Glaze, 2005; Townsend et al, 2008) which varies from brown to black are also observed (Featherstone and Sansom, 2004).

Conjunctival sequestrum is occasionally found concurrently with corneal sequestration (Grahn et al, 2005), which may indicate that the process involved in the discoloration of the cornea may also occur in other tissues, such as the conjunctiva (Featherstone and Sansom, 2004).

Clinical signs observed by slit lamp biomicroscopy include corneal edema, ulceration and vascularization (Bouhanna et al, 2008; Featherstone and Sansom, 2004).

Apathy and loss of appetite are systemic clinical sings observed, usually associated with pain (Dalla et al, 2007).

The cause(s) and pathogenesis of feline corneal sequestration, as well as the mechanism of discoloration are not yet completely elucidated (Bouhanna et al, 2008; Cullen et al, 2005). However, it is accepted that corneal insult is an important initiating factor, and sequestrum may develop as a nonspecific response to substantial corneal damage (Featherstone and Sansom, 2004; La Croix, 2000).

Sequestra formation may be initiated by corneal irritation or damage due to exposure, anatomic defects or infectious causes (Townsend et al, 2008). Many predisposing factors have been proposed: ulcerative keratitis, lagophthalmos, entropion (Figure 4), medial canthal trichiasis, excision of the nictitans, keratoconjunctivitis sicca, tear film abnormalities, heritable genetic defects, epithelial or basement membrane defects, systemic or local metabolic defects, primary corneal dystrophy, altered sebaceous secretion, toxins, defects in catecholamine metabolism and topical corticosteroid therapy (Andrew et al, 2001; Cullen et al, 2005; Featherstone and Sansom, 2004; Townsend et al, 2008).

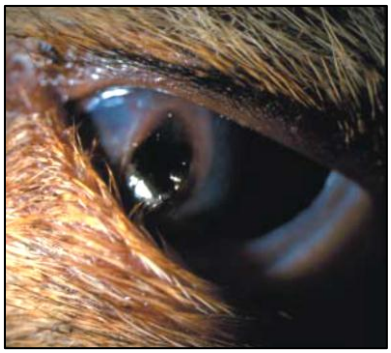

Figure 4. Ventrolateral corneal sequestrum. The sequestrum is associated with a lower eyelid entropion. (Adapted from Featherstone and Sansom, 2004).

Cats with corneal sequestrum, very often, have history of previous disorders of the anterior segment of the eye, dating back months or years (Dalla et al, 2007).

Procedures to stimulate corneal healing in indolent ulceration like debridement, grid keratotomy, phenol cautery and silver nitrate cauterization represent a form of iatrogenic trauma to the cornea and stroma, and appear to facilitate sequestrum formation (Featherstone and Sansom, 2004). La Croix and colleagues observed that 10% (2/21) of nonhealing ulcers treated with debridement and 31% (4/13) treated with a grid keratotomy developed corneal sequestration. This observation supports the hypothesis that corneal injury may predispose to sequestra formation (Townsend et al, 2008). Another standpoint reveals that feline

herpesvirus type 1 (FHV-1) may be present in corneal sequestra, and after the keratotomy procedure, the virus may penetrate deeper into the stroma (Gelatt and Brooks, 2011).

Breed predisposition is associated to brachycephalic and facial conformation with exophthalmos and lagophthalmos that may lead to chronic exposure of the central cornea resulting in exposure keratopathy (Andrew et al, 2001; Barachetti et al, 2010; Cullen et al, 2001; Townsend et al, 2008). The predilection for brachycephalic breeds, with shallow orbits and prominent eyes reflects their susceptibility to corneal exposure, tear film evaporation, and secondary corneal irritation/injury. The mean palpebral fissure length is approximately, 1 mm longer in Persians when compared with other breeds. Therefore, the central cornea of these cats is more predisposed to damage (Glaze, 2005). Moreover, brachycephalic cats tend to develop lagophthalmos and medial canthal entropion, leading to corneal irritation, which may predispose these breeds to sequestrum (Moore, 2005). Entropion is the in-turning of the eyelid, it may be congenital or occur due to chronic ocular pain. Together, these may be the causes for a higher predisposition for the sequestrum in brachycephalic cats (Glaze, 2005).

Blocker and van der Woerdt measured the corneal sensitivity using a Cochet-Bonnet aesthesiometer and compared the corneal touch threshold between domestic shorthaired cats and brachycephalic cats. The aesthesiometer has a nylon filament, which is used to touch the cornea at least three times attempting to cause a blink reflex. If it is not elicited the filament's length is reduced until the reflex occurs. The corneal touch threshold is achieved when more than 50% of the attempts result in a consistent blink reflex. The corneal sensitivity is inversely proportional to corneal touch threshold. Statistical analysis of the results showed that, the central cornea is significantly more sensitive than the peripheral cornea, this may be due to a higher density and greater overlap of nerve fibers in the central cornea. When compared with domestic shorthaired cats, brachycephalic cats have a significantly higher corneal touch threshold and a reduced central corneal sensitivity. There may be a relationship between increased exposure of the globe and decreased corneal sensitivity (Blocker and van der Woerdt, 2001).

The majority of sequestra develop in the central or paracentral cornea, however the presence of entropion influences the site of the sequestrum formation. When entropion is present, the sequestrum faces to the entropion (Figure 4; Figure 5). Thereby, the localization of the sequestra varies in relation to the position of the inciting cause, with the corneal irritation and insult being the initiating factor of corneal necrosis (Bouhanna, 2008; Featherstone and Sansom, 2004).

Figure 5. Corneal sequestra with different locations. A: Central corneal sequestrum, associated with slight neovascularization. B: Central corneal sequestrum, associated with moderate neovascularization; C: Paracentral corneal sequestrum associated with very severe corneal vascularization and edema. (Adapted from Featherstone and Sansom, 2004).

The eyes affected often develop tears and deposits in the margins of the eyelids with the same color of the sequestrum, so this discoloration may result from tear film alterations (Newkirk et al, 2011).

In humans, it was reported the occurrence of black deposits on the conjunctiva and cornea after long-term application of topical epinephrine eye drops. This alteration is assigned to the oxidation of epinephrine to an adrenochrome pigment. This product is unstable and, eventually is converted into melanin (Newkirk et al, 2011). The lesions identified in humans are identical to those seen in the feline corneal sequestra. However these compounds have not been used in cats affected with sequestrum. Notwithstanding, an endogenous defect in the epinephrine metabolism may be involved (Featherstone et al, 2004).

The tear film has been suggested to participate in the brown discoloration of the sequestra. Tear film break up time usually is accelerated in corneas with sequestra when compared with the contralateral eye or aged-matched normal feline corneas. Grahn (2005) found a tear film break-up time of 21±12s for the normal corneas and 14±13s for eyes affected with corneal sequestra. He concluded that there was no significant correlation between qualitative tear film composition of cats with sequestra and normal cats. Although, this study, showed a trend to a decreased tear film break-up time in eyes with sequestra. However, a diminished tear film break up time instead of being an etiology, may be a consequence of the sequestrum (Grahn, 2005).

High-performance lipid chromatography showed that the total lipid content of the tears is significantly lower in eyes with sequestrum when compared to control eyes (eyes from healthy cats) and that there was no significant difference between the diseased and contralateral eye. In other words, the total lipid content of the contralateral eye is also

reduced, predisposing the cornea to injury. The reduction in lipid content verified in the contralateral eye, indicates that it is also, predisposed to development of a sequestrum (Featherstone et al, 2004).

According to Davindson, the difference in protein tear content between affected and normal eyes is not significant (Davidson et al, 1992 cited by Featherstone and Sansom, 2004). However, a spontaneous mucin deficiency cannot be excluded (Cullen et al, 2005).

Newkirk and colleagues hypothesized that a pigmented compound of the tear may contribute to sequestrum formation, and investigated the possible porphyrin accumulation in the lacrimal glands. Porphyrins are produced by the harderian gland in rodents, but are also found in the lacrimal glands. Porphyrins produce free radicals responsible for oxidative damage, leading to corneal sequestra. However, all samples analyzed histologically revealed no evidence of porphyrin accumulation in the lacrimal glands, glands of the third eyelid, normal corneas nor corneas with sequestrum (Newkirk et al, 2011).

Conjunctival goblet cells are the major responsible for the production of mucin, which takes part of the innermost tear film layer. To determine whether the goblet cell atrophy is a cause or effect of sequestra the mean goblet/epithelial cell ratio for each region of the normal and the eyes with sequestra were calculated. The results were, respectively: 0.66, 0.56 for the dorsal nasal fornix, 0.68, 0.57 for the ventral nasal fornix, 0.63, 0.48 for the temporal dorsal fornix, and 0.55, 0.49 for the temporal ventral fornix. No significant difference in goblet cell ratios of normal eyes and eyes with sequestra was verified. However, it was found a tendency for decreased goblet cell/epithelial cell ratios in cats with sequestra in all locations within the fornix. This fact may be associated to corneal and conjunctival inflammation in the eyes with sequestra (Grahn, 2005).

Research with ultraviolet absorbance spectroscopy found that the absorbance spectra for sequestra and unaffected corneas are different. The absorbance spectrum is produced by the compounds presented in the sample. Control corneas have a peak at 385 nm and sequestra either do not have a peak or have a small peak at 280 nm. Therefore, this method revealed a significant difference between the compounds present in sequestra and the ones presented in unaffected corneas. The peak at 280 nm indicates the presence of aromatic groups, chromophores (Featherstone et al, 2004).

Ejima found an iron concentration a hundred times greater in a single sequestrum sample when compared to unaffected corneas. However, a more recent elemental analysis study revealed that there is no single inorganic element present in large amounts. The highest

concentrations found were of oxygen, sodium and chlorine (Ejima et al, 1993 cited by Featherstone et al, 2004). Bellhorn observed granules that were positive with a periodic acid-Schiff reaction (Bellhorn, 1970 cited by Featherstone et al, 2004). Electron-dense coccoid bodies have also been seen in electron microscopy studies. The results of different histochemical stains have been inconclusive (Featherstone et al, 2004).

The role of FHV-1 is still unclear. This is a pathogen exclusive to the cat and a high prevalence of this virus is found on the cat population. Typically, cats are infected in a young age but the virus has the capacity of latency and further reactivation. It replicates in epithelial tissues and is responsible for ocular and upper- respiratory clinical sings. The cornea is a place where this virus may be in the latency phase, as it was found FHV-1 DNA in corneal and conjunctival tissues in a high percentage of clinically normal cats (Stiles, 2003). This virus is known or suspected to be responsible for disease syndromes that affect the eye, such as: conjunctivitis, epithelial keratitis, stromal keratitis and corneal sequestration (Volopich et al, 2005). Sequestra may not develop due to direct viral infection, but as a result of stromal inflammation and chronic corneal ulceration caused by the virus (Gemensky and Wilkie, 2001; Stiles, 2003).

Nasisse found FHV-1 DNA by single round PCR (srPCR) in 86/156 corneal sequestra and revealed a significantly higher prevalence of FHV-1 DNA in cats with corneal sequestration when compared with clinically healthy cats (Nasisse et al, 1998 cited by Volopich et al, 2005). However, applying nested PCR (nPCR), Stiles detected only 5/28 positive corneal specimens, and showed no statistically significant association between FHV-1 and corneal sequestrum (Stiles et al, 1997 cited by Volopich et al, 2005). PCR was used for being more sensitive than other detection methods for identifying FHV-1 in ocular tissues, and it is also reported that nPCR is 9.5 times more sensitive than srPCR (Gemensky and Wilkie, 2001; Volopich et al, 2005).

In a latter research, no significant correlation was found between the detection of FHV-1 DNA and conjunctivitis, stromal keratitis or corneal sequestrum. This study comprised twelve cats with characteristic signs of corneal sequestration. There were one Domestic Shorthair and eleven Persians, ranging from one to eleven years, with average age of 5.5 years. The clinical sings ranged from slight bronze discoloration to an almost black lesion, with or without corneal edema and vascularization. The samples analyzed were corneoconjunctival swabs, and conjunctival and corneal biopsies. Only three of these cats tested positive for FHV-1-specific DNA. Samples of two cats were positive applying srPCR.

One cat had positive conjunctival and corneal biopsies and the other had positive corneal scraping and biopsy in the other, as well as positive conjunctival biopsy using nPCR. In the third cat, the FHV-1 specific DNA was found using nPCR, on the corneal biopsy (Volopich et al, 2005).

A supposition that corneal tissue altered by necrosis makes the detection of viral DNA impossible was made. However, PCR was also applied to nonnecrotic corneal tissue adjacent to the sequestrum (Volopich et al, 2005).

FHV-1 DNA was detected in conjunctival and corneal biopsy samples of cats with sequestra but not in corneoconjunctival swabs of the same cats, implying that too little viral DNA is present in corneoconjunctival swabs (Volopich et al, 2005).

Corticosteroids administration may also be implied in the sequestrum formation. Subconjunctival administration of corticosteroids followed by experimental FHV-1 infection resulted in extensive geographic ulcers and stromal keratitis, which may lead to sequestra formation (Gemensky and Wilkie, 2001). Moreover, it looks like cats on topical or subconjunctival corticosteroids treatment are more susceptible to sequestra development (Stiles, 2003).

Apoptosis of keratocytes was observed in transmission electron microscopy studies of corneal sequestrum samples. When a cell undergoes apoptosis it dies with little release of inflammatory mediators and enzymes that could damage adjacent tissues. Then, damage to the adjacent corneal tissue is reduced. This process may prevent extension of FHV infection, avoiding further stromal damage. However, apoptosis is present even if an infection with FHV 1 is not present (Cullen et al, 2005).

Vawer, found a possible genetic component to corneal sequestration, suggesting that Colorpoint cats may inherit a tendency for sequestra to occur by a recessive gene (Vawer, 1981 cited by Andrew et al, 2001). Another hypothesis is that this condition results from a stromal dystrophy with an inherited basis (Mc Lellan and Archer, 2000).

Bacterial agents such as *Chlamydia psittaci*, and mycotic infections are also possible predisposing factors (Andrew et al, 2001). However, bacterial infection may be secondary to sequestrum formation (Featherstone and Sansom, 2004).

With light-microscopy it is possible to recognize the distinct discoloration of the sequestrum. With this method, it is possible to distinguish three zones of discoloration: a central zone with a dark brown/black lesion, surrounded by a lighter brown zone, adjacent to an amber peripheral zone. The transition between the zones varies from gradual to evident.

The different zones may imply a continuous process progressing in a centripetal or centrifugal direction (Featherstone et al, 2004).

In the inner and outer zones, numerous linear striations are also observed. These may stand for physical scratches or natural striations of the tissue (Featherstone et al, 2004).

There are numerous dark particles on both the anterior and posterior surfaces of the lesions. The particle distribution follows a pattern, decreasing from the central to the peripheral zones. There are particles of different sizes with the largest more concentrated in the central zone, but also present in the peripheral lighter zones. These larger particles may be individual particles or aggregates. Due to their presence in the peripheral zones, they are probably not related with the intensity of the discoloration (Featherstone et al, 2004).

These particles characteristics are consistent with the typical appearance of melanin. Melanin may be present as phaeomelanin (yellow/red particles) or as eumelanin (brown/black particles). This color patterns correspond to those clinically observed in corneal sequestrum (Featherstone et al, 2004).

In humans, the clinical conditions that evolve with the presence of melanin are black adrenochrome deposits associated with topical epinephrine therapy, and yellow contact lens discoloration. The pigment formation associated with the adrenochrome deposits, is only induced in a cornea that had suffered iatrogenic epithelial abrasions. Identically, feline corneal sequestration is associated with previous corneal injuries (Featherstone et al, 2004).

However, the pigmentation was also believed to be hemosiderin pigment. More recently, it was found that the pigment appears to be water-soluble and probably caused by desiccation of the necrotic stroma (Andrew et al, 2001; Townsend et al, 2008).

Histologically, the sequestrum contains ulcerated surfaces with irregular and loose corneal epithelia extending to, or partially overlying, the periphery of the sequestrum (Cullen et al, 2005). The central zone of the sequestrum consists of an acellular area with necrotic stromal lamellae, collagen degeneration, without fibroblasts neither inflammatory cells (Andrew et al, 2001; Cullen et al, 2005; Moore, 2005; Townsend et al, 2008).

In the anterior corneal stroma coagulation necrosis is found, and is thought to create the dark brown or black color associated with the sequestrum (Andrew et al, 2001; Glaze, 2005).

Degenerated fibroblasts and interlamellar inflammatory cells are present surrounding the lesion in the early course of the disease (Moore, 2005). Inflammatory cells, fibroblasts and keratocytes (fibrocytes) encircle the edge of the sequestrum and extend towards the base.

Inflammatory cells are primarily neutrophils with some lymphocytes and plasma cells (Cullen et al, 2005).

Some keratocytes morphology indicate apoptosis, as clumping and margination of chromatin, and shrunken cytoplasm are observed (Cullen et al, 2005).

Histology indicates classic characteristics of a nonspecific, foreign body-type response to degenerated collagen (Featherstone and Sansom, 2004).

1.3. Management of corneal sequestrum

Management of corneal sequestra involves medical therapy, surgical therapy or a combination of both (Featherstone and Sansom, 2004).

Medical treatment involves the direct instillation of drugs on the corneal surface, bandage contact lenses, as well as regular monitoring (Featherstone and Sansom, 2004; Gelatt and Brooks, 2011).

Topical treatment includes solutions, suspensions, and/or ointments. Drugs provided systemically and subconjunctivally may supplement topical treatment when corneal disease is progressing and a high frequency of topical administration is required. The corneal epithelium is lipophilic preventing penetration by most antibiotics. If the corneal epithelium is intact and a therapeutic level of antibiotic is necessary in the cornea and anterior chamber, chloramphenicol is the one of choice. On the other hand, if the corneal epithelium is injured, the epithelial barrier is not functioning properly. Under these circumstances, a broad-spectrum topical antibiotic, like gentamicin, tobramycin, the fluoroquinolones and the combination of neomycin, polymixin B, and bacitracin are recommended (Gelatt and Brooks, 2011).

Other reported topical preparations suggested to have a positive outcome in the medical management of sequestra are: topical mucinomimetic therapy with sodium hyaluronate or carbomer 940 gel, every six hours (Cullen et al, 1999) and topical interferon α -2b, 1000 units/mL, every six hours. A dose of 3000 units/mL, every six hours of topical interferon α -2b, has been suggested to diminish the corneal discoloration associated with the sequestrum (Featherstone and Sansom, 2004).

Medical therapy alone may be considered based upon the degree of ocular pain, the depth and the extension of the lesion, as well as owner factors, such as commitment and budgetary constraints. If the lesion is small and superficial on slit lamp biomicroscopy examination and little or no ocular pain is found, medical approach may be tried (Dalla et al, 2007; Featherstone and Sansom, 2004).

In a group of 37 cats, 40 eyes were diagnosed with corneal sequestration. The lesions dimension ranged from 1 to 3 mm and corneal edema and neovascularization were present in different degrees. The lesions appeared to be subacute and chronic. Clinical signs of ocular pain were present, such as blepharospasm, enophthalmos and protrusion of the third eyelid. The therapeutic protocol used consisted of: topical broad-spectrum antibiotic applied to both eyes every 6 hours for ten days, artificial tears applied to both eyes daily, acetylcysteine collyrium eye drops administered to the diseased eye every 8 hours, dietary supplementation with vitamins, minerals and proteins and placement of an Elizabethan collar. Every medication was administered until after the recovery, except for the topical broad-spectrum antibiotic. Time until recovery was 1 month for 31.8 % of cases, 2 months for 27.3 % of cases, 5 months for 13.7 % of cases, 7–10 months for 4.5 % of the cases and 12 months for 18.2 % of cases. However, medical treatment in all of these patients resulted in a leukoma of various dimensions, according to the dimension of the original lesion (Dalla et al, 2007).

Nevertheless, sequestra may spontaneously slough over, but it may require weeks or months. In the meantime, the administration of prophylactic topical antibiotics or artificial tears may demonstrate successful results (Glaze, 2005, Nasisse, 1995).

The cornea, after suffering trauma or ulceration, requires several days to initiate satisfactory inflammatory and healing responses. Meanwhile, infectious agents, proteases, and collagenases produced by bacteria and damaged corneal cells, cause rapid degradation of the cornea, and threaten the maintenance of vision. Surgical treatment may accelerate the start of the healing process, being the treatment of choice when ocular pain or discomfort is obvious (Glaze, 2005; Gelatt and Brooks, 2011). The surgical management has many advantages as it increases patient quality of life as the pain is decreased, diminishes the chance of progression to deeper corneal tissues, decreases the probability of recurrence and shortens the recovery time (Andrew et al, 2001; Glaze, 2005). In a clinical study, it was found that the average resolution time with conservative therapy was 11.2 weeks and 3.8 weeks with keratectomy as the surgical option (Glaze, 2005).

Surgical management options include: keratectomy (with a graft and without a graft placement), transposition, or a keratoplasty procedure (Gelatt and Brooks, 2011).

Keratectomy may be superficial, partial lamellar and deep. Following keratectomy a graft may or may not be placed. The graft material used may be conjunctiva, porcine small intestinal submucosa (PSIS), or equine amniotic membrane (Barachetti et al, 2010). When the graft material used is corneal tissues, the procedure is named keratoplasty.

Conjunctival grafts are classified as 360° conjunctival graft, hood conjunctival graft, bridge, pedicle and island grafts. Other options to apply are: nictitans flap, application of a bandage contact lens, cyanoacrylate adhesives or temporary tarsorrhaphy (Townsend et al, 2008).

The transposition procedure can be corneoconjunctival (Barachetti et al, 2010) or corneoscleral (Gelatt and Brooks, 2011).

Keratoplasty may be lamellar (Gimenez and Fariña, 1998) or penetrating (Townsend et al, 2008), depending on the depth of the corneal graft.

The choice of the surgical procedure is determined according to the depth of the keratectomy required, character of the cat, and surgeon's experience and preference (Featherstone and Sansom, 2004).

1.4. Surgical management of corneal sequestrum

1.4.1. Corneal healing

The cornea has two important functions, one mechanical and one optical. Cornea, along with the sclera, is responsible for the maintenance of the physical integrity of the eye. Optically, the cornea has to supply a clear passage for light transmission up to the retina, refracting and bending the light to help in focusing (Herring, 2003).

The goals of corneal surgical procedures are directed to preserve or restore one of these roles. The most important goal is to maintain the physical integrity of the cornea. The secondary goal is to maintain corneal transparency. Although, maintenance of a normal corneal shape or curvature is of the most importance in human medicine, it is not in domestic species. Astigmatism, an alteration in the radius of corneal curvature, does not cause clinical vision problems in dogs and cats (Herring, 2003).

Corneal transparency is affected by numerous alterations, decreasing its ability of light transmission. However, cat corneas have the capability of repair and restore transparency. Possible causes for an altered corneal transparency are: invasion of the cornea by blood vessels, edema, migration of inflammatory cells, infiltration with pigment cells from the limbus, conjunctiva, and anterior synechiae; and deposition of lipids and calcium in the cornea (Herring, 2003).

Due to the physiologic absence of blood vessels, corneal tissues are dependent of tear film, limbal vasculature and aqueous humor, for nutrients and oxygen, as well as for

elimination of metabolic products (Gelatt and Brooks, 2011;Herring, 2003). Quantitative and qualitative alterations in tear film, have a negative effect on corneal health and postsurgical healing. Normal eyelid anatomy and function are of the uttermost importance for the physical protection of the cornea, as well as for tear-film distribution and maintenance (Gelatt and Brooks, 2011).

After damage, corneal epithelial cells undergo mitosis and new wing cells slide over the injured cornea. While re-epitheliazation is a quick process, solid adhesion between new epithelial cells, by hemidesmosomes, may require several weeks (Gelatt and Brooks, 2011).

Proteases, collagenases and other enzymes are produced by degenerated corneal cells, inflammatory cells and bacteria. The metabolic products affect collagen fibrils and glycosaminoglycans leading to their degeneration and progression of the lesion (Gelatt and Brooks, 2011).

Corneal stroma needs more time to repair than the epithelium stratum. Usually it is invaded by blood vessels and inflammatory cells. New collagen fibrils are produced by fibroblasts, converted from keratocytes and histiocytes. Slowly, a new glycosaminoglycans matrix is also produced. This process needs several weeks or months (Gelatt and Brooks, 2011).

After a deep keratectomy procedure, recovery of corneal stroma normal thickness may take months, or even never occur. The new collagen fibrils, formed after the surgical procedure, may not line up with adjacent lamellae, resulting in variable levels of scarring.

Corneal blood vessels remain present after stromal repair, giving rise to ghost vessels which can be visualized by biomicroscopy, even years later (Gelatt and Brooks, 2011).

When Descemet's membrane is injured, it curls and retracts. A fibrin clot is formed and adjacent endothelial cells migrate and produce a new Descemet's membrane. This process requires several weeks (Gelatt and Brooks, 2011).

Endothelial regeneration, although little understood, is dependent of animal species and age. Endothelial cells undergo mitosis to cover endothelial defects in young animals. In older animals, these defects are covered primarily by endothelial cell enlargement (Gelatt and Brooks, 2011).

1.4.2. Corneal surgical instruments

For surgical management of corneal sequestrum, microsurgical ophthalmic instruments and magnification are needed. An ophthalmic microscope provides the necessary magnification for the surgical procedures used for sequestrum management (Herring, 2003; Gelatt, 2011).

Ophthalmic surgical instruments include: forceps, needle holder, knife blades and handles, scissors, eyelid speculum, corneal desiccators and trephines, and cannulas (Herring, 2003; Gelatt, 2011).

An eyelid speculum is used to retract the eyelids during the surgical procedure (Figure 6) (Herring, 2003).

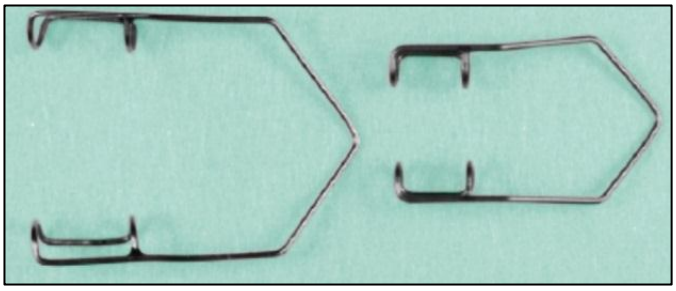

Figure 6. Eyelid specula of two sizes: adult and pediatric. (Adapted from Gelatt, 2011).

A wide variety of forceps are available. Bishop-Harmon tissue forceps have 1x2 right-angle teeth and are adequate to handle the eyelids, nictitans and conjunctiva. Castroviejo fixation forceps has 1x2 protuberant teeth appropriate for stabilization of the globe and handling of the cornea. Colibri style forceps have angled tips with 1x2 protuberant teeth and usually are used for corneal and conjunctival handling (Figure 7). Tying forceps have a suture-tying platform appropriate for tying small suture material (6/0 USP or less) (Herring, 2003).

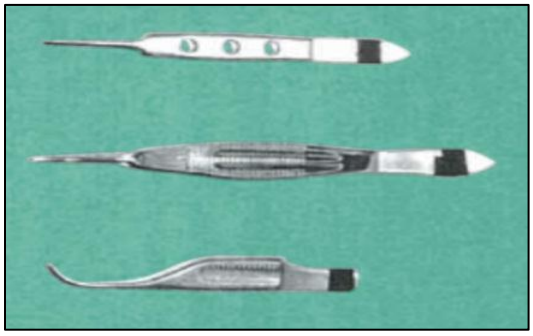

Figure 7. Forceps used in ophthalmic surgery. Up: Bishop-Harmon tissue forceps. Middle: Castroviejo fixation forceps. Bottom: Colibri-style corneal forceps. (Adapted from Maggs, 2002).

Needle holders have straight or curved tips, and may be nonlocking or locking. Usually, a nonlocking needle holder with curved tips is preferred for corneal surgery (Figure 8) (Herring, 2003).

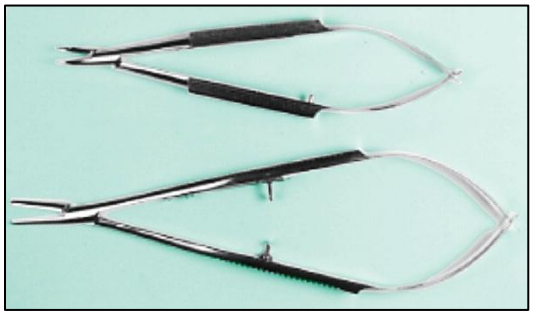

Figure 8. Needle holders used in ophthalmic surgery, both with curved tips. Top: Small microsurgical needle holder with no lock. Bottom: Castroviejo needle holder with lock. (Adapted from Gelatt, 2011).

For corneal sectioning, Beaver handles and blades are the most used. The #64 Beaver blades are preferred to perform the initial corneal incision and the #65 is chosen to penetrate the anterior chamber, when needed (Figure 9) (Herring, 2003).

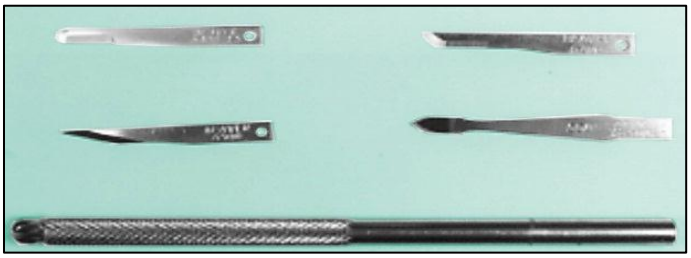

Figure 9. Beaver microsurgical blades used in corneal surgery. Left: top, #64 Beaver blade; middle, #65 Beaver blade. Right: top, #67 Beaver blade; middle, keratome. Bottom: Beaver scalpel handle. (Adapted from Gelatt, 2011).

A variety of scissors is available. Tenotomy scissors are used to perform conjunctival graft procedures, in an attempt to dissect the Tenon's capsule from the conjunctiva. There are two varieties of these scissors: the Stevens scissors and the Westcott scissors. The Stevens has a ring handle and the Westcott has a spring handle (Figure 10). Corneal transplant scissors are used to cut corneal buttons when performing a keratoplasty. Vannas scissors are very fine and used essentially for gentle corneal cutting (Figure 11) (Herring, 2003).

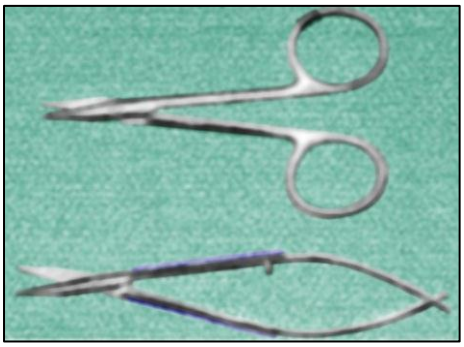

Figure 10. Corneal transplant scissors. Top: Stevens tenotomy scissors; Bottom: Westcott tenotomy scissors. (Adapted from Maggs, 2002).

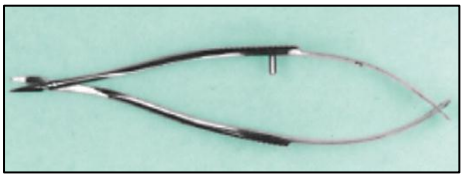

Figure 11. Vannas scissors. (Adapted from Maggs, 2002).

Corneal dissectors, like the Martinez corneal dissector, are used to perform intralamellar stromal dissection (Figure 12) (Herring, 2003).

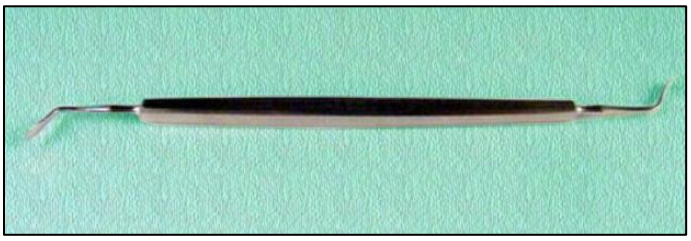

Figure 12. Martinez corneal dissector (Adapted from Medetzsurgical, 2012).

Corneal trephines are needed to outline the corneal sequestrum and cut to an adjustable depth (Figure 13). They may also be used to collect corneal grafts (Herring, 2003).

Figure 13. Corneal trephines. (Adapted from Surgicaltools, 2013).

Finally, cannulas are used for irrigation of the tissues, as well as for the intracameral injection of solutions and viscoelastic material (Figure 14) (Herring, 2003).

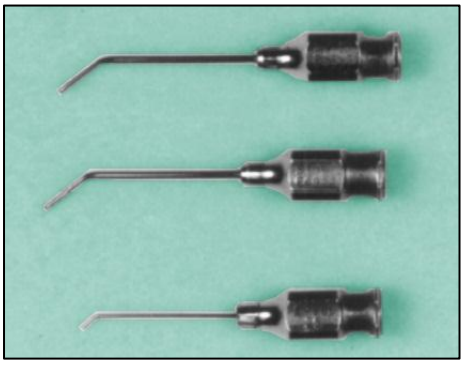

Figure 14. Ophthalmic cannulas. Top: air injection cannula; Middle: Castroviejo cannula, used to inject solutions; Bottom: Bracken cannula, used to inject solutions. (Adapted from Maggs, 2002).

Ophthalmic surgical sutures may be absorbable or non-absorbable. Non-absorbable sutures cause less tissue reaction but have to be removed. Monofilament nylon is the non-absorbable suture more frequently used. Available absorbable sutures include: polyglicolic acid, polyglactin 910 and polydioxanone (Herring, 2003).

The caliber of the suture material ranges from 7/0 USP to 10/0 USP. Needles should have spatulated tips to allow a more accurate placement with minimal stromal disruption (Gelatt, 2011; Herring, 2003).

1.4.3. Preoperative considerations

A careful slit lamp examination should be performed before any surgical procedure, in order to access corneal integrity and thickness, as well as, the lesion's depth (Herring, 2003).

Corneal sequestrum underlying causes should be assessed. The presence of entropion and ectopic cilia might be associated with sequestrum, and are addressed surgically at the same time to avoid surgical failure or recurrence (Herring, 2003).

Cultures should be obtained routinely in cases of corneal perforation and deep corneal ulceration, preferably before application of topical antibiotics. Preoperative broad-spectrum topical antibiotic administration is recommended, as it is an effective measure to decrease the local bacteria. It should begin 24 hours before surgery (Herring, 2003). Most frequently used topical antibiotics are: chloramphenicol, gentamicin, tobramycin, and the combination of neomycin, polymixin B, and bacitracin. Bacteria often found in corneal ulcers cultures are *Staphylococcus and Streptococcus spp*., agents susceptible to most antibiotics (Gelatt, 2011).

The use of systemic antibiotics, such as amoxicillin or cephalexin, is reserved for cases where the integrity of the globe is threatened (Gelatt, 2011).

Due to corneal inflammation, secondary involvement of the anterior uvea is common. Topical iridocycloplegics decrease ocular pain, and the probability of posterior synechiae and cataract formation. A moderately dilated pupil is achieved, but still some iris movement is allowed, avoiding posterior synechiae formation (Gelatt, 2011).

A margin of 2 to 3 cm around the eye should be cleaned and clipped, however if a very deep lesion or a corneal perforation is present minimal clipping with scissors is preferable to avoid corneal or intraocular contamination (Herring, 2003). Following, a diluted solution of povidone-iodine (1:10 to 1:50), is applied to the eyelids, conjunctiva and cornea (Herring, 2003).

The patient should be positioned in dorsal recumbency, with the head turned slight to the opposite side of the prepared eye. Therefore, the corneal surface and the operating table should be parallel (Herring, 2003).

The anesthesia leads to ventromedial eye rotation. To avoid this situation a non-depolarizing neuromuscular blocking agent is used. Atracurium is, usually, the agent of choice (0.2 mg/kg IV). It produces the paralysis of all striated muscles, including the extraocular muscles, so the eye remains in the anatomic position. However, the muscles related with breathing are also paralyzed, as so artificial ventilation is required to these patients while under the effect of atracurium (Gelatt, 2011).

The use of a paralyzing agent also decreases intraocular tissue displacement when the anterior chamber is entered (Gelatt, 2011).

Hemorrhage is predictable, especially when handling conjunctival tissues. It may be managed with application of cellulose sponges until clotting, topical administration of a dilute solution of epinephrine (1:10000) or cautery (Herring, 2003).

Corneal tissue dries quickly during general anesthesia, due to reduced tear production, and loss of the blinking reflex (Herring, 2003).

To avoid extension of the lesions, the corneal surface is intermittently irrigated with lactated Ringer's solution or balanced saline solution, during surgical intervention (Gelatt, 2011).

After the surgical procedure, a temporary lateral tarsorrhaphy may be placed for protection of the surgical area and improvement of epithelial healing. If the medial aspect of the eyelids is left opened, topical medication can be applied normally (Herring, 2003).

1.4.4. Postoperative considerations

An Elizabethan collar must be placed to avoid damage to the surgical site by self-trauma. In all cases a topical broad-spectrum antibiotic is recommended. However, the use of topical and systemic anti-inflammatories, topical mydriatics or cycloplegics, and systemic antibiotics is determined in an individual basis, as well as by the surgical procedure performed (Gelatt, 2011).

1.5. Surgical techniques for corneal sequestrum management

1.5.1. Superficial keratectomy

Keratectomy procedures involve the removal of total or partial corneal thickness resulting in superficial, deep and full thickness keratectomies. Superficial keratectomy is the removal of the epithelia and anterior one-half of corneal stroma; deep keratectomy is the ablation of the epithelia and corneal stroma until its posterior one-half; and full-thickness when the entire depth of the cornea is removed. The detail surgical technique will be discussed later in Materials and Methods.

For the treatment of corneal sequestrum, superficial keratectomy is the only keratectomy procedure used alone (Featherstone and Sansom, 2004; Gelatt and Brooks, 2011). The removal of the necrotic tissue speeds healing, minimizes scarring, decreases the stimulus for keratitis and iridocyclitis, relieves discomfort and prevents progression of the sequestrum to deeper corneal layers (Featherstone and Sansom, 2004; Gelatt and Brooks, 2011).

Removal of the entire pigmented lesion is believed to reduce the recurrence of the sequestrum (Bouhanna et al, 2008). Featherstone and Sansom (2004) found no recurrence in the eyes where a complete excision of the corneal discoloration was accomplished, contrasting with a 38% recurrence rate in the corneas where an incomplete keratectomy was performed, suggesting that recurrence is likely if complete excision of the pigment is not realized (Featherstone and Sansom, 2004). However, Gimenez and Fariña (1998) reported no long-term consequence in the two cases of incomplete excision of deep stromal pigment (Gimenez and Fariña, 1998).

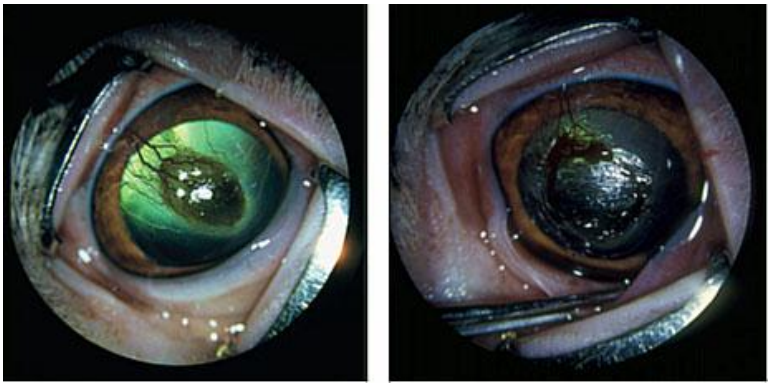

Figure 15. Corneal sequestrum managed by a superficial keratectomy.(Adapted from Gelatt and Brooks, 2011).

Following superficial keratectomy, the remaining wound is treated medically, like an ulcer (Figure 15) (Gelatt and Brooks, 2011).

Recommended post-operative treatment include topical broad-spectrum antibiotics, applied four to six times a day and topical 1% atropine one drop a day or each 48 hours. Atropine is used to promote a moderately dilated pupil, preventing synechia formation. However, this drug also decreases tear formation, extending corneal re-epithelialization period (Gelatt and Brooks, 2011).

Re-epithelialization should start within 48 hours. The keratectomy site is evaluated every day or each 2 days with and without topical fluorescein. The newly formed epithelium stains faintly with topical fluorescein and adhere incompletely. As re-epithelialization continues the area retaining fluorescein decreases (Gelatt and Brooks, 2011).

If re-epithelialization is slow or ceases, a topical solution of 0.5% povidone-iodine may be applied in the wound edges. This procedure will stimulate the epithelial cells activity (Gelatt and Brooks, 2011).

After superficial keratectomy epithelia regeneration is commonly achieved, but the recovery of normal stromal thickness is questionable. Following the performance of three keratectomies in a life time, the stroma appears to have about one-half to two-thirds of normal thickness. Therefore, it is recommended a maximum of three superficial keratectomies (Gelatt and Brooks, 2011).

Once the keratectomy wound is fluorescein negative, re-epithelialization is completed. At this time, topical antibiotics are continued for a few more days and topical corticosteroids are added to the treatment protocol. Topical corticosteroids used are 0.25–0.5% prednisolone or 2.5% hydrocortisone, administered two to four times a day (Gelatt and Brooks, 2011).

To minimize corneal scarring cyclosporine A is administered once a day (Gelatt and Brooks, 2011).

Corneal healing after sequestrum removal is slow and often results in corneal scarring. This corneal scarring is responsible for opacity, but usually is not very obvious in cats and dogs. Final corneal transparency is achieved when corneal stroma becomes reorganized, which may delay several weeks (Gelatt and Brooks, 2011).

Postoperative complications include bacterial infection and recurrence of the sequestrum. Bacterial infection is rare, as appropriate topical antibiotics are administered. Recurrence of the sequestrum is enhanced if predisposing factors, such as lagophthalmos, nasal fold trichiasis, or tear film disorder, are not addressed at the time of superficial keratectomy (Gelatt and Brooks, 2011).

1.5.2. Cyanoacrylate adhesives

Cyanoacrylates are composed of esters of cyanocrylic acid and an alkyl chain. When in contact with a weak base and at room temperature, it undergoes anionic polymerization, by which it solidifies and adheres to the surrounding tissue. During this conversion of cyanoacrylate from liquid to solid, minimal heat is released (Watté et al, 2004).

The size of the alkyl chain is related to cyanoacrylate toxicity. Higher alkyl chains have lower tissue toxicity and polymerize quicker (Watté et al, 2004).

Following a keratectomy procedure, the cornea is dried with a cellulose sponge and is ready for the cyanoacrylate deposition. A layer of adhesive is applied with a 25-27 gauge needle and a 1 ml syringe. This layer has to be thin and uniformly placed. Within a few seconds the adhesive is solidified and the cornea is cleaned with saline solution (Figure 16) (Watté et al, 2004).

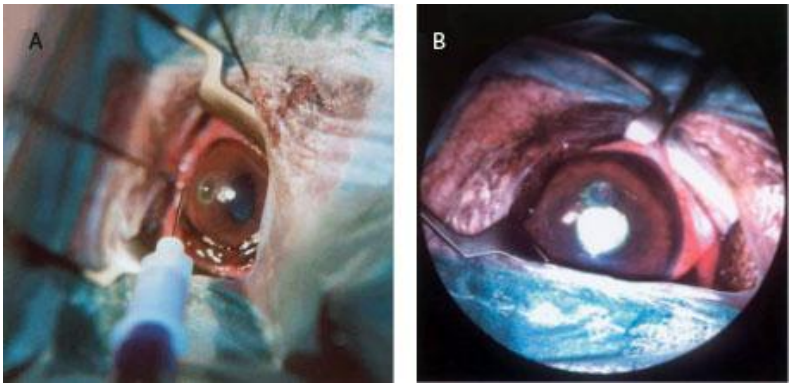

Figure 16. Cyanoacrylate adhesive. A: application of cyanoacrylate adhesive in a corneal descemetocele. B: Image of a cyanoacrylate adhesive after polymerization. (Adapted from Watté et al, 2004).

Postoperative therapy includes topical and systemic antibiotics, systemic nonsteroidal anti-inflammatory drugs and topical atropine (Watté et al, 2004).

Depending on the time that corneal stroma needs to recover, the cyanoacrylate adhesive may remain in place for several weeks, and slough over spontaneously or be removed with forceps (Watté et al, 2004).

Postsurgical neovascularization and pigmentation are the most frequent complications. Cyanoacrylate may induce an inflammatory process which leads to angiogenesis (Watté et al, 2004).

This procedure is not advised for very deep, infected or large corneal defects. Another drawback is the possibility of premature glue sloughing (Hansen and Guandalini, 1999).

However, cyanoacrylate adhesive is considered a safe and less expensive therapy. The surgical time is reduced and the cosmetic outcome is good, when compared to conjunctival and corneoscleral grafts (Hansen and Guandalini, 1999; Watté et al, 2004).

1.5.3. Conjunctival grafts

Conjunctival grafts or flaps are classified as pedicle grafts, hood conjunctival grafts, and 360° conjunctival grafts. Conjunctival grafts are also named conjunctival autografts, as they originate from the patient itself (Townsend et al, 2008).

Conjunctival grafts consist of bulbar or palpebral conjunctival epithelium, mucosa and connective tissue (fibroblasts, blood vessels, and lymphatics) (Gelatt and Brooks, 2011).

These grafts facilitate healing in several ways, as they provide tectonic support and tissue to fill the keratectomy site, and a direct blood supply to the lesion (Andrew et al, 2001;

Moore, 2005). After keratectomies deeper than one half of the corneal depth, conjunctival graft placement is advised (Moore, 2005). With these grafts there is no risk of host rejection (Gelatt and Brooks, 2011).

When harvested from the limbus, the transplanted conjunctival epithelium is capable of generation and transition into corneal epithelium (Gelatt and Brooks, 2011).

As conjunctival autografts contain blood vessels and lymphatics, systemic antibiotics leukocytes, antibodies, and alfa-2-macroglobulins, arrive directly to the lesion. The deeper layer of the conjunctival transplant carries fibroblasts and collagen that will help corneal stroma regeneration (Barachetti et al, 2010; Gelatt and Brooks, 2011).

Grafts from bulbar or palpebral conjunctiva should not include Tenon's capsule or the bulbar fascia. Tenon's capsule creates a thicker graft, leading to tissue contraction and tension on the transplanted conjunctiva. Grafts from transpalpebral conjunctiva may contain portions of the fibrous tarsal layer. This is needed to maintain the graft base attached to the deeper aspects of the eyelid. Usually, if the lesion is closer to the central cornea, the graft procedure will be more difficult (Gelatt and Brooks, 2011; Herring, 2003).

Featherstone and Sansom (2004) found no significant difference in the rate of recurrence between corneas receiving a graft (17%) and those not (25%) (Featherstone and Sansom, 2004). However, placement of a conjunctival graft has been reported to lessen the recurrence rate of corneal sequestrum (Gimenez and Fariña, 1998). These studies refer to pedicle conjunctival grafts, and concluded that, in case of a sequestrum, it is prudent to leave the conjunctival pedicle intact permanently (Gimenez and Fariña, 1998; Featherstone and Sansom, 2004).

Conjunctival autografts provide sufficient tissue to strengthen the weakened corneas. Although providing tectonic support, conjunctival grafts are not as strong as corneal grafts.

Postoperatively, they result in corneal scarring or leukoma of various sizes and depths and corneal neovascularization (Barachetti et al, 2010; Gelatt and Brooks, 2011; Nam et al, 2012; Vanore et al, 2007).

The density of this leukoma is reported to decrease with time, especially in the feline cornea, but clinically detectable corneal fibrosis is permanent (Gimenez and Fariña, 1998). This residual corneal leukoma may impair vision, especially when located axially (Andrew et al 2001; Bussieres, 2004; Goulle, 2012).

1.5.3.1. Complete (360º) bulbar conjunctival graft (Gundersen type)

In the complete bulbar conjunctival graft almost all the bulbar conjunctiva is separated from the Tenon's capsule and pulled over the entire cornea. The graft covers the keratectomy site and is not directly sutured to the cornea (Gelatt and Brooks, 2011).

In the beginning of the procedure the dorsal bulbar conjunctiva is elevated by fine teeth thumb forceps and incised by scissors at the limbus. Following, the bulbar conjunctiva is detached from the Tenon's capsule by blunt and sharp dissection with small tenotomy scissors. To obtain a thin conjunctival graft, the surgeon should be able to observe the scissors' tips through the mucosa, during the dissection. The conjunctival graft has to be thin in an attempt to reduce traction and pressure on the sutures postoperatively (Gelatt and Brooks, 2011).

Once dissected, the loosened edges of the conjunctiva should be placed on the central cornea and not retract spontaneously to the limbus. These edges are then sutured horizontally with 5/0 USP to 7/0 USP absorbable simple interrupted or simple mattress sutures. The conjunctival edges are sutured with four to six sutures. If the graft is thicker or traction on the suture line is anticipated it is recommended the use of simple interrupted mattress sutures. Therefore the transposed conjunctival mucosa is not sutured directly to the corneal defect (Figure 17) (Gelatt and Brooks, 2011).

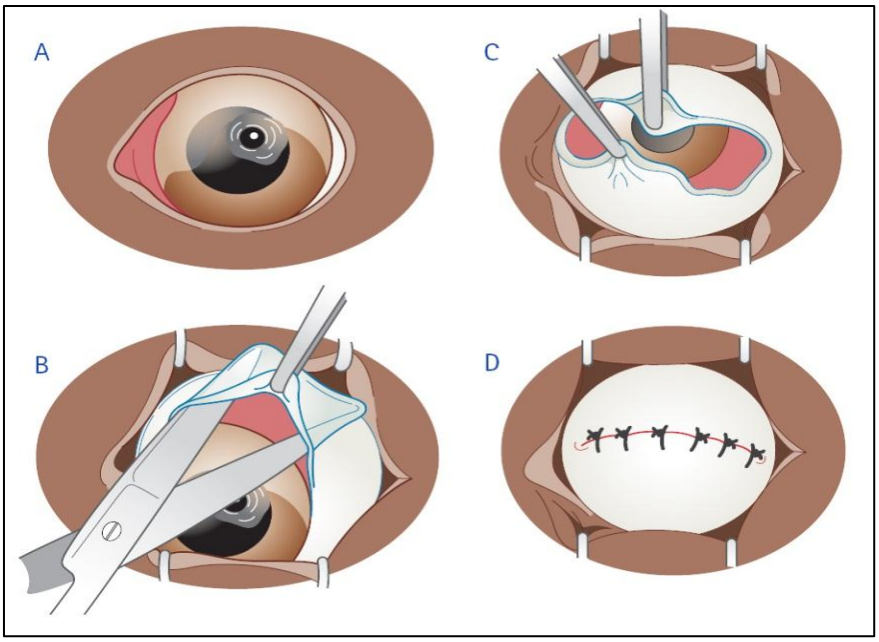

Figure 17. Complete bulbar conjunctival graft. A: Central corneal sequestrum. B: The dorsal bulbar conjunctiva is incised by scissors at the limbus. C: Dissection is performed to detach the Tenon's capsule from the bulbar conjunctiva. D: Following, the conjunctiva is pulled over the cornea and sutured. (Adapted from Martin, 2010).

The disadvantages of this procedure include impaired vision, a more challenging eye examination and decreased intraocular penetration of topical medicines. However, this type of conjunctival graft is the one that provides the greatest support to the cornea (Gelatt and Brooks, 2011).

1.5.3.2. Modified complete (360º) bulbar conjunctival graft with two suture lines

The postsurgical complications of the previous procedure are the premature retraction of the conjunctival graft and suture dehiscence. To avoid these problems, in this technique a 'relief incision' on the dorsal bulbar conjunctiva is performed to lessen the tension on the sutures apposing the edges of the graft (Gelatt and Brooks, 2011).

A band of 10–15 mm from dorsal bulbar conjunctiva is prepared by two incisions. One dorsal to the limbus for 180º, and another parallel to the first. With the second incision, additional bulbar conjunctival mucosa is allowed to slide ventrally towards the limbus. Both edges of the band of dorsal bulbar conjunctiva are sutured with 5/0 to 7/0 USP simple interrupted absorbable sutures. The ventral edge of the dorsal bulbar conjunctiva is also sutured with 5/0 to 7/0 USP simple interrupted absorbable sutures to the edge of the ventral bulbar conjunctiva (Figure 18) (Gelatt and Brooks, 2011).

The double suture line reduces the likelihood of suture dehiscence by reducing suture tension (Gelatt and Brooks, 2011).

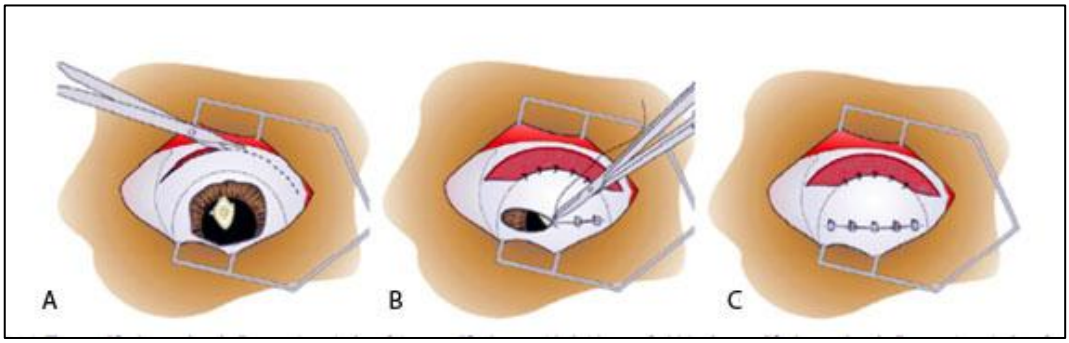

Figure 18. Modified complete (360º) bulbar conjunctival graft. A: A band of 10-15 mm from dorsal bulbar conjunctiva is constructed. B: The dorsal aspect of the bulbar conjunctival graft slides ventrally and is sutured to the limbus. C: The ventral aspect of the dorsal bulbar conjunctiva is apposed to the ventral bulbar conjunctiva. (Adapted from Gelatt and Brooks, 2011).

1.5.3.3. Advancement (hood or 180º) bulbar conjunctival graft

In the advancement (hood or 180º) bulbar conjunctival graft the dorsal or lateral bulbar conjunctiva are transposed into the cornea. This method is most useful for dorsal and lateral paracentral and peripheral sequestra (Gelatt and Brooks, 2011).

This type of graft allows corneal and intraocular drug penetration, postoperative intraocular examinations and does not completely prevent vision (Gelatt and Brooks, 2011).

Initially, an incision is made, by small curved tenotomy scissors, on the dorsal or lateral bulbar conjunctiva at the limbus. This incision is continued for 180-200º. The conjunctiva is dissected towards the conjunctival fornix for about 10-12 mm (Gelatt and Brooks, 2011).

The bulbar conjunctiva is separated from the underlying Tenon's capsule, and the graft should be thin, as described for the previous surgeries (Gelatt and Brooks, 2011).

The graft is placed on the top of the corneal defect, and tension should not be felt. The entire length of the graft is apposed to the cornea with 5/0 to 7/0 USP simple interrupted absorbable sutures. The conjunctival graft will only adhere to the keratectomy site; however these sutures provide support for the graft and decrease the likelihood of suture failure at the keratectomy site (Figure 19) (Gelatt and Brooks, 2011).

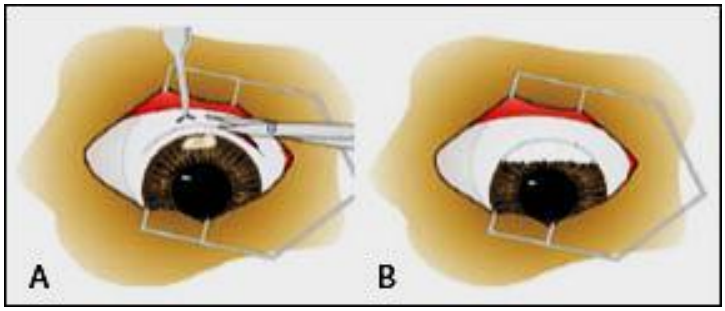

Figure 19. Advancement (hood or 180º) bulbar conjunctival graft. A: An incision is performed on the dorsal bulbar conjunctiva, and it is separated from the Tenon's capsule for 180º. B: The bulbar conjunctival graft is advanced ventrally to cover the keratectomy site, and its edges are sutured to the corneal defect and to the adjacent tissues. (Adapted from Gelatt and Brooks, 2011).

If the lesion is just in the peripheral or paracentral cornea the graft has limited or no impact in the animal's vision (Gelatt and Brooks, 2011).

1.5.3.4. Bridge bulbar conjunctival graft

The bridge bulbar conjunctival graft is another modification of the complete bulbar conjunctival graft with two suture lines. This graft is also named bipedicle bulbar conjunctival graft (Gelatt and Brooks, 2011).

With a small curved tenotomy scissors, two parallel bulbar conjunctival incisions are realized at the limbus. The incisions are advanced 10-12 mm towards the conjunctival fornix. The resulting graft should be thin and its edges are apposed to the corneal defect and to the normal cornea with 5/0 to 7/0 USP simple interrupted absorbable sutures. The bridge graft should have a width of 10 mm or more to further viability (Figure 20) (Gelatt and Brooks, 2011).

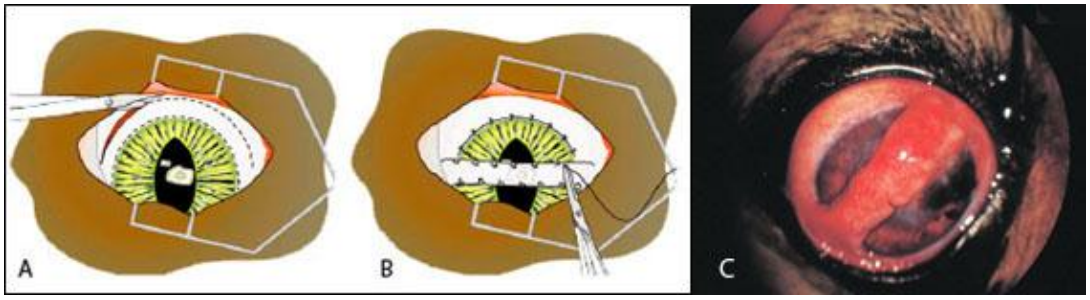

Figure 20. Bridge conjunctival graft. A: A strip of thin dorsal conjunctiva is dessicated with small tenotomy scissors, and moved over the corneal defect. B: The bridge bulbar conjunctival graft is apposed to the corneal defect and to the adjacent corneal tissue. C: Post-surgical appearance of a bridge conjunctival graft. (Adapted from Gelatt and Brooks, 2011).

The bridge graft has the advantages of being perfused at both edges, reducing the likelihood of graft ischemia; does not completely impair patients vision, allow intraocular postoperative examination and has a reduced impact in corneal and intraocular drug penetration (Gelatt and Brooks, 2011).

Along with this procedure, a temporary partial tarsorrhaphy may be performed, to preventing lid trauma to the graft and its sutures (Gelatt and Brooks, 2011).

1.5.3.5. Pedicle bulbar conjunctival graft

Pedicle bulbar conjunctival grafts are very common in small animal ophthalmology (Gelatt and Brooks, 2011).

After a careful keratectomy, with the excision of all necrotic material, the pedicle bulbar conjunctival graft is prepared. With tenotomy or Stevens scissors, the bulbar conjunctiva is incised. Meanwhile, the underlying Tenon's capsule is separated by alternating

blunt and sharp dissection. The pedicle base should be slightly wider than its tip to guarantee adequate blood supply to the entire pedicle (Gelatt and Brooks, 2011).

Following, the conjunctival pedicle graft is placed above the cornea and its tip clipped to cover the corneal defect. The width of the pedicle should be approximately 1-2 mm larger than the diameter of the corneal defect. Excessive tension is avoided and the graft should be rotated less than 45º from the vertical (Gelatt and Brooks, 2011).

To assure contact between the keratectomy site and the pedicle graft, a single suture between the long axis of the pedicle graft and the dorsal corneal defect edge is realized. The remaining tip and edges of the pedicle graft are apposed with 5/0 to 7/0 USP simple interrupted absorbable sutures (Figure 21) (Gelatt and Brooks, 2011).

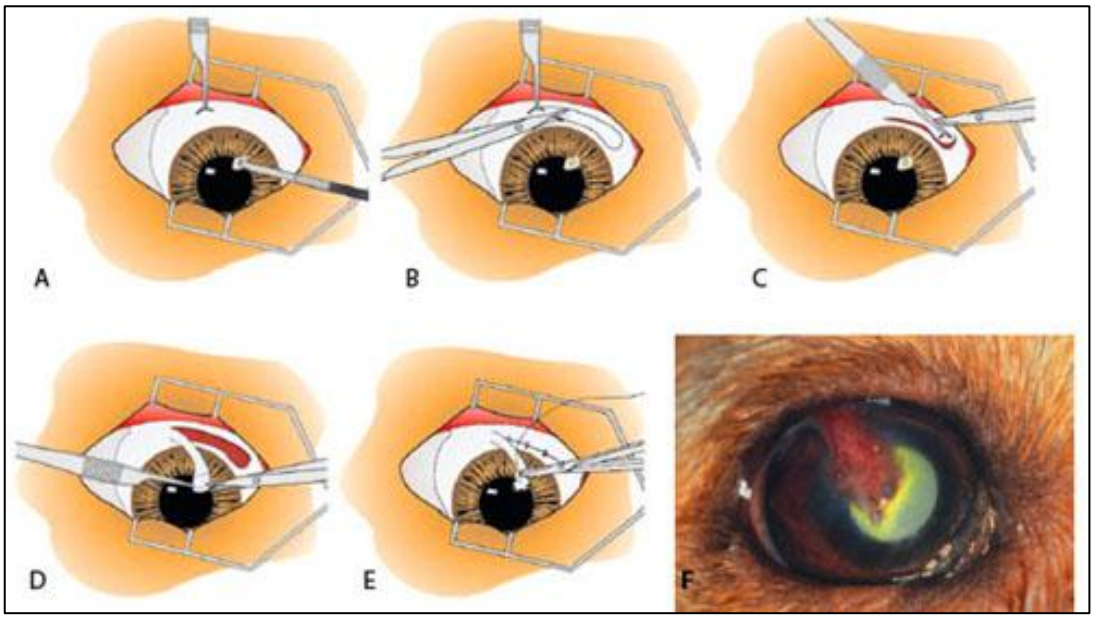

Figure 21. Pedicle conjunctival graft. A: Removal of the corneal sequestrum. B: The pedicle graft is outlined on the dorsolateral bulbar conjunctiva. C: The conjunctival graft is separated from the adjacent tissues. D: The tip of the pedicle graft is trimmed according to the keratectomy site diameter. E: The graft is sutured to the corneal bed. F. Post-surgical appearance of a pedicle conjunctival graft. (Adapted from Gelatt and Brooks, 2011).

This type of graft is appropriate for peripheral, paracentral and central sequestra. Although they create minimal vision impairment, intraocular postoperative examination and drug penetration in the cornea and anterior segment are achievable (Gelatt and Brooks, 2011). Nevertheless, conjunctival pedicle grafts are practical, versatile and, also suggested to reduce the incidence of sequestrum recurrence, when compared with keratectomy alone (Andrew et al, 2001, Gimenez and Fariña, 1998).

Postoperative complications of conjunctival pedicle graft placement include displacement of the graft, and loss of graft viability (Bussieres, 2004; Featherstone and Sansom, 2004).

1.5.3.6. Island conjunctival graft

A free or island conjunctival graft is a modified conjunctival graft without vascular support. The conjunctiva may be harvested from the tarsal or bulbar conjunctiva (Gelatt and Brooks, 2011).

A circular section of transpalpebral or bulbar conjunctiva is harvested. As shrinkage of the conjunctival graft is possible, the graft should be 10% larger than the corneal defect. The island conjunctival graft is transposed to the corneal defect, and trimmed according to its diameter. The graft is sutured to the corneal lesion edges with either 8/0 or 9/0 USP nylon with simple interrupted absorbable sutures (Figure 22) (Gelatt and Brooks, 2011).

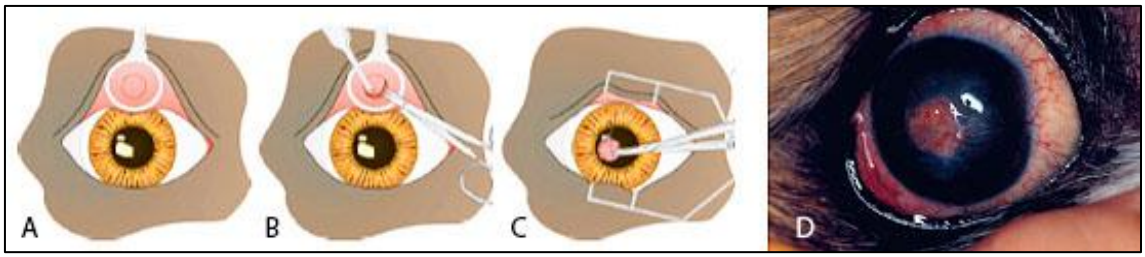

Figure 22. Island or free conjunctival graft. A: A circular shaped section of palpebral conjunctiva, 0.5 to 1 mm larger than the corneal defect, is incised from the upper eyelid. B: The graft is detached from the adjacent tissue by careful dissection with scissors. C: The island conjunctival graft is carefully sutured to the corneal bed. D: Post-surgical appearance of an island conjunctival graft. (Adapted from Gelatt and Brooks, 2011).

Free island grafts are used for central or paracentral sequestra (Gelatt and Brooks, 2011).

This type of graft allows patient's vision, postoperative eye examination and drug penetration into the cornea and anterior segment (Gelatt and Brooks, 2011).

However, as there is no blood supply, graft viability is reduced and the susceptibility of infection is higher. Therefore, it is not recommended if infection is present (Gelatt and Brooks, 2011).

The adjacent corneal stroma, aqueous humor, and tears are responsible for graft's nutrition (Gelatt and Brooks, 2011).

1.5.4. Porcine small intestinal submucosa grafts

The porcine small intestinal submucosa (PSIS) is a biomaterial composed of an acellular collagen matrix (collagen type I, III and VI), glycoaminoglycans (hyaluronic acid, chondroitin sulfate A and B, heparin, and heparan sulfate), proteoglycans, and glycoproteins (fibronectin), which are known to have important roles in host tissue repair and remodeling (Bussieres, 2004; Gelatt and Brooks, 2011; Goulle, 2012). The PSIS extracellular matrix is remodeled into host tissue acquiring the specific structural and functional properties of the host (Bussieres, 2004).

The growth factors present in the small intestinal submucosa are: transforming growth factor β (TGFβ) and fibroblast growth factor (FGF-2). These factors promote tissue development and differentiation (Bussieres, 2004; Featherstone et al, 2001).

Three different transforming growth factors β are recognized: TGF-β1, -β2 and -β3. Depending on the cell type and conditions, the cellular response modulated by TGF βs can be stimulatory or inhibitory (Featherstone et al, 2001; Vanore et al, 2007). TGF βs are inhibitors of matrix metalloproteinase synthesis (Goulle, 2012), and appear to regulate tissue remodeling. They stimulate the deposition of extracellular matrix and inhibit the matrix degradation, as they suppress the synthesis of matrix-degrading proteinases such as collagenase, and stimulate the synthesis of proteinase inhibitors (Featherstone et al, 2001). FGFs-2 induce mitosis in epithelial, endothelial and stromal cells, also increasing proliferation and migration of the latter. FGFs-2 are naturally present in the endothelial basement membrane, inducing endothelial cell migration during wound healing (Featherstone et al, 2001; Vanore et al, 2007).

PSIS is a biomaterial derived from porcine jejunum. It is formed by three layers: the tunica muscularis mucosa, tunica submucosa, and the stratum compactum layer of the tunica mucosa (Bussieres, 2004).

After mechanical debridement of all the mesenteric tissues, tunica mucosa, serosa and tunica muscularis, the PSIS is washed with a hypotonic solution to remove the remaining endothelial cells and fibrocytes. The remaining sheet of collagen is approximately 100 μm thick, with two surfaces. The ‘rough surface’ is the tunica muscularis mucosa and the ‘smooth surface’ is the stratum compactum surface of the tunica mucosa. Lyophilization is the following process, intending to stabilize the product. Then it is sterilized with ethylene oxide. Commercially, the product is supplied as 10 x 7 cm sheets, or as round ophthalmic discs with 10 to 15mm of diameter (Bussieres, 2004; Gelatt and Brooks, 2011; Vanore et al, 2007).

Following a keratectomy procedure, the PSIS graft placement is realized. The graft is cut from the commercial available sheets, with either a trephine or micro-scissors. The trephine is used when the corneal defect is circular and the micro-scissors are used when the defect is irregular-shaped. Alternatively, a Stiefel biopsy punch of appropriate diameter may be used. The PSIS material is trimmed according to the shape of the corneal defect, but 1-2 mm larger, and rehydrated with sterile saline for 1-3 minutes. When the PSIS transplant is ready, it is apposed over the surgical defect, with its rough surface downwards. Depending on the depth of the defect, some layers of PSIS may be placed on the defect, one layer on top of the other. The graft is then sutured to the cornea using 8/0, 9/0 or 10/0 absorbable monofilament sutures. Four sutures are placed at the four cardinal points to anchor the graft, followed by a simple interrupted and/or a simple continuous suture pattern which, firmly fixes, the transplant to the cornea (Figure 23) (Bussieres, 2004; Featherstone et al, 2001; Goulle, 2012; Vanore et al, 2007).

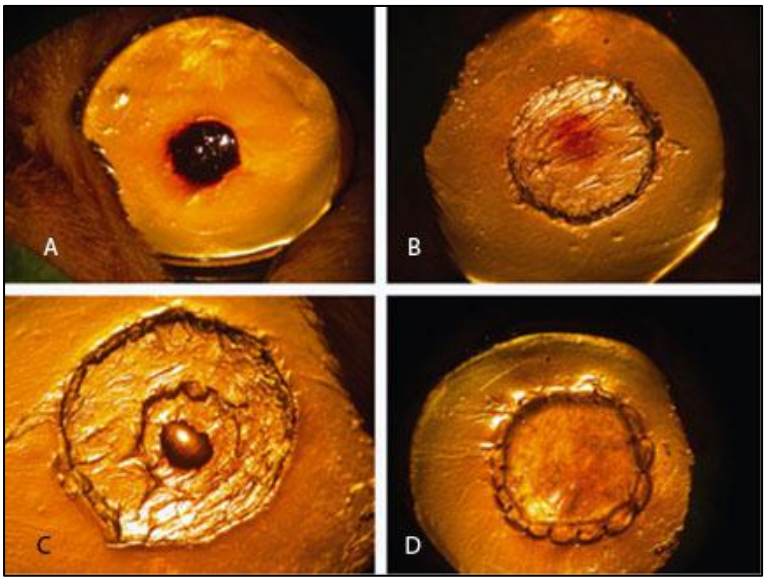

Figure 23. Surgical procedure for PSIS graft placement. A: Deep corneal sequestrum in the left eye of a five-year-old Persian. B: The sequestrum was partially removed by a keratectomy procedure, however deep corneal pigmentation persists. C: A second keratectomy was performed in an attempt to remove the deep corneal pigment, and it resulted in a corneal perforation. D: The keratectomy site was covered by a PSIS graft. (Adapted from Goulle, 2012).

Postoperative medication includes topical and systemic antibiotics and topical atropine. Topical antiviral drugs, such as idoxuridine are also recommended (Featherstone et al, 2001). Systemic antibiotics include marbofloxacin (2 mg/kg/day) for three weeks (Goulle, 2012).

For topical antibiotic therapy either gentamicin eye drops applied twice a day (Goulle, 2012) or 0.5% chloramphenicol eye drops three times a day (Featherstone et al, 2001) are recommended.

A topical suspension of dexamethasone 0.1% and tobramycin 0.3% may be used in an attempt to reduce corneal neovascularization and scarring. The administration of topical dexamethasone may be, at first once a day or once every other day for two weeks, and then twice a day depending on the intensity of scarring and corneal neovascularization. However, it should not be used in cats suspected to have a herpetic infection. On the other hand, 50% of clinically normal cats have FHV1-DNA in the cornea, and the use of topical corticosteroids increases the risk of latent herpesvirus activation (Goulle, 2012).

PSIS integration on the healing cornea is divided in three stages: corneal neovascularization, proliferation of the corneal epithelium and stroma, and remodeling of the extracellular matrix to obtain a transparent cornea, and preserve corneal integrity. Corneal neovascularization is common at the time of presentation. This is probably amplified during PSIS integration by the stimulation produced by the growth factors within the PSIS. Following, PSIS is invaded by fibroblasts that use its protein matrix. The final step is the replacement of fibroblasts for corneal stromal cells, resulting in good corneal transparency (Vanore et al, 2007).

PSIS grafting has been reported in dogs, cats, rabbits, and horses, and used in the regeneration of various tissues (Featherstone and Sansom, 2004; Featherstone et al, 2001). PSIS has been used as a dural substitute, intra-articular ligamentous graft material, a large-diameter vascular graft, a substitute for large fascial defects, for bladder regeneration, and in promoting meniscal regeneration in dogs. In these cases, the PSIS material was remodeled into host tissue, achieving its specific structural and functional proprieties (Bussieres, 2004; Vanore et al, 2007).

In a study conducted by Featherstone and Sansom (2004), PSIS was used in six cases of corneal sequestrum. The results showed one corneal scar without pigment, two corneal scars with faint pigment and three cases of brown discoloration of the PSIS. The brown discoloration disappeared in two cases and a leukoma resulted at 5-10 months postoperative. In the other case, the sequestrum recurred, requiring a new surgical procedure (Featherstone and Sansom, 2004).

In a research performed by Goulle (2012), PSIS was used in 34 eyes with deep corneal sequestrum, involving more than the half of corneal thickness, including descemetocele and

corneal perforations. The author used a third eyelid flap for a period of three weeks. By the time of the third eyelid flap removal, centripetal corneal neovascularization was observed. To reduce scarring and neovascularization topical 0.1% dexamethasone was administered once a day or once every other day for less than a week. Recurrence of the sequestrum was observed in five eyes, as a mild stromal pigmentation, three months after surgery (Goulle, 2012).

PSIS is a biomaterial inexpensive, easy-to-handle that acts like a scaffold for repair and also provides tectonic support to the cornea. It is very easy to store because it is delivered in individual packages, including ophthalmic discs that can be opened separately (Bussieres, 2004).

As PSIS acquires the specialized properties of the tissue into which it is grafted, healing occurs by regeneration rather than scar formation (Featherstone et al, 2001).

Due to its transparency, it causes minimal vision impairment comparatively to conjunctival and other opaque tissues (Featherstone et al, 2001). Good corneal transparency, preservation of corneal integrity and maintenance of vision are achievable with PSIS (Figure 24) (Vanore et al, 2007).

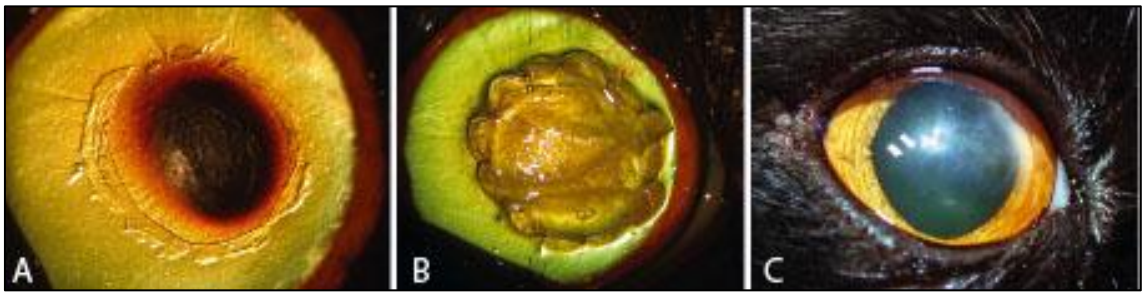

Figure 24. PSIS graft. A: Deep corneal sequestrum in the right eye of a Persian cat with 15 months. B. The removal of the sequestrum was followed by the apposition of a PSIS graft. C: Same eye three months after the procedure, althoug a slight edema is observed, a good corneal transparency allows vision. (Adapted from Goulle, 2012).

PSIS is porous, therefore allowing topical ophthalmic medications absorption (Featherstone et al, 2001).

With this material potential virus transmission (FHV-1 and FeLV) is reduced, however interspecies transmission of retrovirus during xenotransplantation has been demonstrated with in vitro studies of porcine aortic endothelial cells. Nevertheless, PSIS material is disinfected with peracetic acid and sterilized with ethylene oxide, reducing the risk of the presence of virus and bacteria (Featherstone et al, 2001).

Post-surgical complications after PSIS graft placement are: aqueous humor leakage, PSIS laceration, dehiscence or discoloration of the graft, chronic uveitis, hyphema and corneal scar formation (Vanore et al, 2007).

1.5.5. Equine amniotic membrane transplantation

Chorion, allantois and amnion are the three layers that form the fetal membrane. The amniotic membrane is the inner layer. It is composed of epithelium, basement membrane and stroma (Figure 25). The epithelium consists of a single layer of cuboidal or columnar cells. The basement membrane connects the epithelium to the stroma. The stroma is composed by large amounts of collagen. This last two layers contain cytokines, proteoglycans, collagen type I, III, IV, V and VII, laminin and fibronectin (Barachetti et al, 2010).

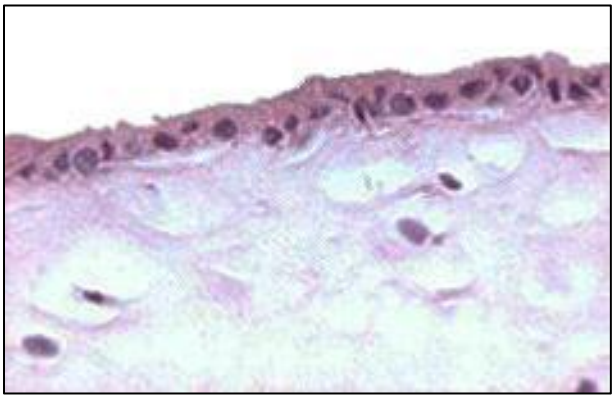

Figure 25. Histological section of equine amniotic membrane. The three layers are observed: the epithelium, formed by a single layer of cuboidal or columnar cells, the basement membrane and stroma, consisting of mostly of collagen.(Adapted from Barachetti et al, 2010).

A wide range of properties have been attributed to the amniotic membrane. When transplanted to a lesioned area, it helps migration and differentiation of epithelial cells, promotes cellular adhesion between basal epithelial cells and prevents apoptosis (Barachetti et al, 2010; Lassaline et al, 2005).

Amniotic membrane also produces multiple growth factors: FGF-2, hepatocyte growth factor (HGF) and TGFβ. It has also anti-inflammatory, anti-angiogenic, antifibrotic, antibacterial and antiviral proprieties. The anti-inflammatory action is carried out by: down-regulation through TGFβ-signaling system, hindrance of inflammatory cell infiltration and suppression of pro-inflammatory cytokines, such as interleukins (IL) α, 2 and 8, interferon λ, tumor necrosis factors (TNF) β and α, FGF-2 and platelet derived growth factor (PDGF). The

anti-angiogenic effect is achieved by anti-inflammatory action combined with the release of anti-angiogenic factors by the epithelial and stromal cells of the amniotic membrane, including IL-1 receptor antagonist, tissue inhibitors of metalloproteinase (TIMP), IL-10, thrombospondin-1 and pigment epithelium-derived factor (PEDF). Antifibrotic proprieties lead to less scar formation. This is possible due to the stromal matrix of the amniotic membrane which suppresses proliferation and differentiation of normal corneal, limbal and conjunctival fibroblasts. Antibacterial and antiviral activities are produced by the presence of interleukins, interferons, TNF-α, activin A, inhibin A, pre-Bcell colony-enhancing factor and leukemia inhibitory factor (Arcelli et al, 2005; Barachetti et al, 2010; Gelatt and Brooks, 2011; Lassaline et al, 2005).

The amniotic membrane is harvested from the placenta of mares undergoing cesarean section. The chorion is removed from the amniotic membrane. The amniotic membrane is then washed with saline solution first, then with solution of 0.1% povidone iodine and with a gentamicin solution 0.2%. This process is repeated three times. With the epithelial surface up, the membranes are placed on a nitrocellulose paper, cut into 5x5 cm pieces and stored in 98% glycerin at room temperature (Barachetti et al, 2010).

Another option is to store frozen. Once placed on the nitrocellulose paper, the amniotic membrane is stored in Delbecco's modified Eagle's medium, which contains glycerol, penicillin, streptomycin, neomycin and amphotericin B. When necessary, single pieces are thawed and washed with sterile saline for 30 min in an attempt to remove any glycerol from the preservation medium. Due to freezing, the epithelium of the amniotic membrane dies and only the basement membrane and stroma are available for use (Lassaline et al, 2005).

Previously, the sequestrum is removed by a keratectomy procedure. Then, the equine amniotic membrane graft is cut to the size of the corneal defect. The keratectomy site is covered by the graft, with the amniotic membrane stroma placed against the exposed corneal stroma. Following the graft is sutured to the margin of the keratectomy, with 8/0 to 10/0 USP polygalactin or nylon, in an interrupted or continuous pattern (Figure 26) (Arcelli et al, 2009; Barachetti et al, 2010).

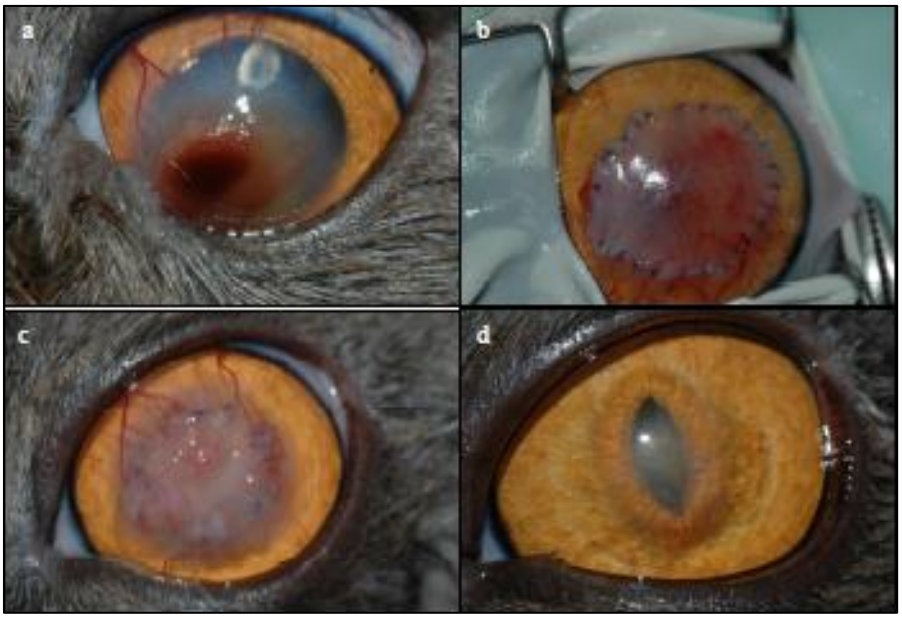

Figure 26. Equine amniotic membrane transplantation. (a) Paracentral corneal sequestrum. (b) Postsurgical appearance after a keratectomy followed by an amniotic membrane transplant. (c) The same eye, three weeks after the surgery. The development of corneal granulation tissue above the amnitotic membrane is observed. (d) The same eye 12 weeks after the amniotic memebrane placement. The cornea still has some opacity, but a substantial clearing of the graft is noted, as well as a reduction on the vascularization. (Adapted from Barachetti et al, 2010).

Amniotic membrane may be used as a graft (inlay), a patch (overlay) or in multiple layers. In the inlay technique, the amniotic membrane is trimmed to the size of the defect, and placed with its basement membrane up, allowing migration of the surrounding epithelial cells (Arcelli et al, 2009).

In the overlay technique, the amniotic membrane is used like to a biological bandage contact lens. The goal is to protect the healing surface from inflammatory cells and proteins of the tear film. In this technique the epithelial side faces the keratectomy site, but as time passes it falls or is removed. In other words, if the stromal side faces the corneal stroma, the amniotic membrane is incorporated in the healing cornea. On the other hand, if the basement membrane faces the corneal stroma, the amniotic membrane should slough (Arcelli et al, 2009; Gelatt and Brooks, 2011; Lassaline et al, 2005).

When using multiple layers the entire depth of the keratectomy site is filled with amniotic membrane portions, cut according to the size of the defect. The orientation of the layers is not important except for the most superficial. The last layer is placed with the basement membrane side up and sutured as an inlay graft, allowing corneal epithelium to grow above it (Barachetti et al, 2010).

Postoperative treatment after this surgery consists of topical and systemic antibiotics. The use of tobramycin or ofloxacin, three times daily, until healing and oral doxycycline 10 mg/kg, once a day for 10 days, has been reported (Arcelli et al, 2009; Barachetti et al, 2010;). The use of non-steroidal anti-inflammatory drugs, three times a day is also recommended (Arcelli et al, 2009).

In a study performed by Barachetti and colleagues, amniotic membrane grafts were used in seven eyes, after sequestra removal. Five of these corneas healed without complications, corneal transparency was achieved and no sequestrum recurrence was detected. However two cases had problems. There was a partial necrosis of the amniotic membrane attributed to bacterial contamination and inflammatory cells invasion of the sequestrum at the time of the surgery. This leukocyte activity increased the proteinase release that was not controlled by the amniotic membrane graft. In another case, a corneal perforation occurred underneath the amniotic membrane graft. This was attributed to the inhibiting influence of amniotic membrane on corneal fibroblasts. The suppression of the myofibroblast differentiation may otherwise increase the risk of corneal thickness decrease, leading to perforation. Moreover, this sequestrum was deep and not vascularized which led to increased levels of matrix metalloproteinase-9 that may also be responsible for the corneal perforation (Barachetti et al, 2010).

Amniotic membrane transplantation is an effective treatment for ocular surface reconstruction as it stimulates basal epithelial cells differentiation and adhesion, reduces apoptosis and antiproteinase activity, as well as, results in a good corneal transparency. However, it is not recommended for deep and not vascularized sequestra (Barachetti et al, 2010; Lassaline et al, 2005).

1.5.6. Corneoscleral transposition

Corneoscleral transposition transfers the peripheral cornea to the central corneal defect, moving the adjacent sclera into the peripheral cornea. Thereby, axial cornea becomes clear, allowing vision. The transposed sclera will cause opacity of the corneal periphery (Gelatt and Brooks, 2011).

After the keratectomy procedure, the corneoscleral graft is prepared. With a Beaver #64 microsurgical blade, two slightly diverging incisions of approximately one-half of the stromal thickness are initiated. These corneal incisions extend from the keratectomy site to the limbus (Gelatt and Brooks, 2011).

At the limbus, the bulbar conjunctiva and Tenon's capsule are incised by tenotomy scissors for about 15-20 mm and reflected caudally to expose the sclera (Gelatt and Brooks, 2011).

The previous corneal incisions are extended into the sclera. The length of the scleral incisions is the same of the keratectomy site. These incisions should be about 0.2-0.3 mm deep. During this process hemorrhage is possible (Gelatt and Brooks, 2011).

With 1x2 teeth thumb forceps, the edge of the corneoscleral graft is elevated and, with a corneal dissector, it is separated from the corneal stroma until the end of the scleral incisions. The length and width of the tip of the corneoscleral graft should be 1-2 mm larger than the corneal ulcer bed (Gelatt and Brooks, 2011).

The corneoscleral graft is sutured to the corneal lesion with 7/0 to 9/0 USP simple interrupted polyglactin 910 sutures (Figure 27) (Gelatt and Brooks, 2011).

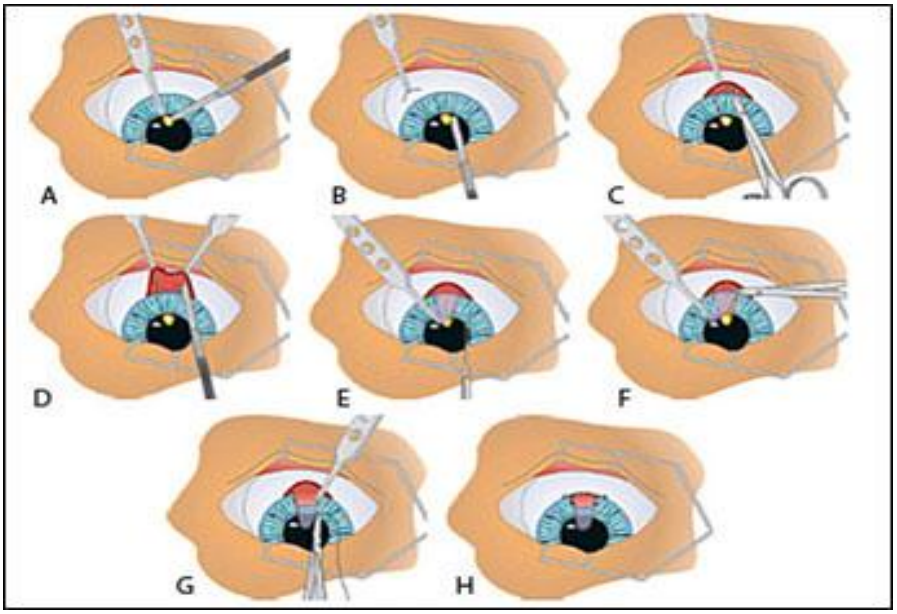

Figure 27. Corneoscleral transposition. A: The corneal sequestra is removed by a keratectomy procedure. B: With a #64 Beaver microsurgical blade, two diverging incisions are performed. They start on the edge of the corneal ulcer and continue up to the limbus. C: At the limbus, the conjunctiva and Tenon's capsule are incised with a Stevens scissors and the sclera is exposed. D: With a #64 Beaver microsurgical blade the corneal incisions are extended into the sclera. E: The corneoscleral graft is separated from the adjacent tissues with a 1x2 teeth thumb forceps and a corneal dissector. F: With a Stevens scissors, the base of the graft is sectioned. G: The graft is trimmed according to the size of the keratectomy site, making sure that it is 0.5 to 1mm larger than the corneal defect. The corneoscleral graft is sutured to the adjacent cornea. H: Finally, the conjunctiva is sutured to the limbus. (Adapted from Gelatt and Brooks, 2011).

Postoperative treatment is achieved with topical and systemic broad-spectrum antibiotics and topical mydriatics in an attempt to maintain a moderately dilated and mobile

pupil. After 7–10 days, topical treatment with corticosteroids is initiated to reduce corneal scarring. Success rate of corneoscleral transpositions is 75-80% (Gelatt and Brooks, 2011).

Complications after this surgery include infection of the graft, suture loss, and abscess formation (Gelatt and Brooks, 2011).

Corneoscleral transposition decreases corneal scaring, providing a clear cornea. It is suitable for the treatment of feline corneal sequestra involving the deeper stroma. However, this procedure damages normal and healthy corneal and scleral tissues and is a time-consuming technique (Gelatt and Brooks, 2011).

1.5.7. Corneoconjunctival transposition

The corneoconjunctival graft is the transposition of the adjacent peripheral cornea and the attached conjunctiva into the central corneal defect (Gelatt and Brooks, 2011). It is similar to the corneoscleral transposition, but in this case the conjunctiva is transposed, instead of scleral tissue (Andrew et al, 2001).

With a Beaver #64 microsurgical blade, the sequestrum is outlined in a square or rectangular shape. The incision depth's is dependent of the depth of the sequestrum. Following, two slightly diverging and linear corneal incisions are realized. These incisions include one half to two thirds of the corneal thickness and extend until the limbus. With a Martinez corneal dissector, the corneal graft is separated from the remaining corneal stroma. Once in the limbus, the incisions progress with Stevens tenotomy scissors or by a scalpel blade, incising the conjunctiva. In the subconjunctival space, the dissection is performed with scissors, separating the conjunctiva from the Tenon's capsule. Once the conjunctiva is dissected, the entire corneoconjunctival graft is advanced to the keratectomy site. During this advancement no tension should be felt by the surgeon. Once in the keratectomy site, the graft's edges are trimmed according to the measure of the corneal defect. The graft should be 0.5–1 mm wider than the keratectomy site, as it will shrink. Following, the graft is sutured to the cornea with 7/0 or 8/0 polygalactin suture in a simple interrupted pattern (Figure 28) (Andrew et al, 2001; Gelatt and Brooks, 2011).

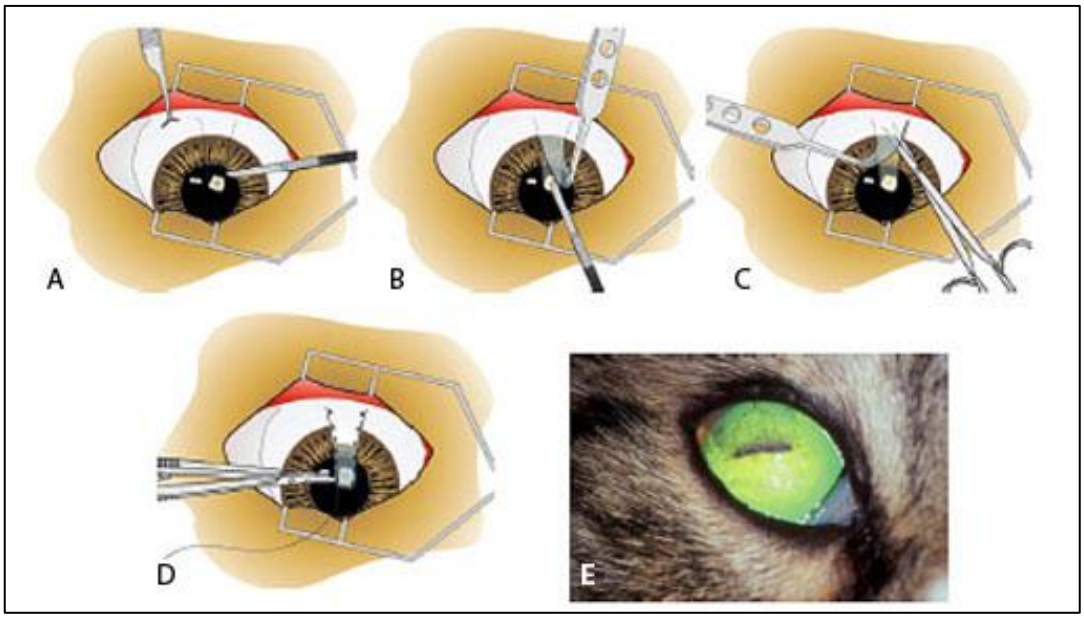

Figure 28. Corneoconjunctival transposition. A. The corneal sequestrum is previously removed by a keratectomy procedure. With a #64 beaver blade, two diverging corneal incisions are performed and extended to the limbus. B: The graft corneal stroma and epithelium are detached from the adjacent stroma. C: Once in the limbus, the dissection is continued with scissors between the bulbar conjunctiva and Tenon's capsule. D: The corneoconjunctival graft slides over the corneal defect and is apposed with the adjacent cornea and bulbar conjunctiva. E: Post-surgical appearance, three weeks after the sequestrum removal followed by a corneoconjunctival graft placement. (Adapted from Gelatt and Brooks, 2011).

Topical and systemic broad-spectrum antibiotics are administered postoperatively, as well as topical atropine to achieve a moderately dilated pupil and cycloplegia. An Elizabethan collar is also recommended. Re-evaluations should be performed weekly for 3-4 weeks (Andrew et al, 2001).

This procedure is an effective treatment for corneal sequestra affecting the deep stroma (Andrew et al, 2001; Bouhanna et al, 2008; Gelatt and Brooks, 2011; Moore, 2005).

It provides structural support and blood flow, increases animal comfort, promotes healing and reduces the likelihood of recurrence (Gelatt and Brooks, 2011, Gimenez and Fariña, 1998). As it is an autograft, the donor tissue is readily available and no rejection is observed (Bussieres, 2004).

This procedure results in less central scarring, when compared with conjunctival grafts, providing a clear visual axis. However, a peripheral linear opacity may remain from the transposed limbus (Andrew et al, 2001; Gimenez and Fariña, 1998). Other drawbacks are the damage caused to healthy corneal tissue and the time needed to accomplish the technique (Goulle, 2012).

Large corneal sequestra may not be managed with this technique because it needs a great extension of healthy surrounding cornea for the repair (Bussieres, 2004).

The conjunctival epithelium once loses its blood supply, can transdifferentiate into corneal epithelium. As a result of this differentiation, a better corneal transparency is achieved (Andrew et al, 2001).

In a study realized by Andrew (2001), this procedure was performed in 17 eyes, resulting in a mean time to healing of 34 days, minimal scarring from the transposed limbus and no recurrence was observed till seven years of follow-up time (Andrew et al, 2001).

1.5.8. Corneal grafts/keratoplasty

1.5.8.1. Basic concepts

The first veterinary corneal graft procedure was performed in 1837, by Bigger who described a successful homograft in a gazelle (Gelatt and Brooks, 2011).

Keratoplasties are classified by the source of the donor cornea and the depth of the graft (Gelatt and Brooks, 2011).

When the corneal graft belongs to an animal from other species it is named heterologous grafts, heteroplastic, xenograft or heterografts. On the other hand, if the donor cornea is provided by an animal of the same specie it is called homologous graft, allogeneic, homoplastic, homografts (Gelatt and Brooks, 2011).

As to the graft depth, keratoplasties are divided in: anterior lamellar (corneal epithelium and anterior stroma), full-thickness or penetrating, and posterior lamellar (posterior stroma). In an anterior lamellar keratoplasty, only the corneal epithelium and the anterior stroma are involved. The posterior lamellar also includes the posterior stroma. Otherwise, full-thickness or penetrating involves the entire thickness of the cornea (Gelatt and Brooks, 2011; Gimenez and Fariña, 1998; Townsend et al, 2008).

Before the keratoplasty, corneal donor graft has to be carefully harvested and storaged. Donor material may be harvested from dogs or cats. The donor animal should be free from clinical systemic diseases, as well as screened for infectious diseases potentially transmitted by the corneal graft. In the case of the donor animal being a cat, it should be screened for feline leukemia virus and feline immunodeficiency virus (Gelatt and Brooks, 2011; Gimenez and Fariña, 1998). Corneal endothelial cell numbers are associated with age. As the animal age increases, the number of these cells decreases. Also, during penetrating keratoplasty, about 20-30% of the endothelial cells are lost. Then, for increasing the probabilities of a

successful transplantation the donor animal should be young, if a penetrating keratoplasty is to be performed (Gelatt and Brooks, 2011).

The preparation of donor corneas and grafts will be later discussed in Materials and Methods.

1.5.8.2. Homologous and heterologous lamellar corneal grafts

Lamellar corneal grafts have no viable cells, but are sources of stromal collagen. This graft provides the support to where the recipient's endothelial cells, keratocytes and epithelial cells will migrate (Gelatt and Brooks, 2011).

After a keratectomy procedure, the corneal lamellar graft is prepared. When the cornea and the attached 1-2 mm of sclera is used, the tissue is positioned on the Teflon W block with epithelial surface down. The cornea is trephined with a corneal trephine 0.5 mm larger than the surgical site (Figure 29). Then, the graft is washed with lactated Ringer's solution (Gelatt and Brooks, 2011).

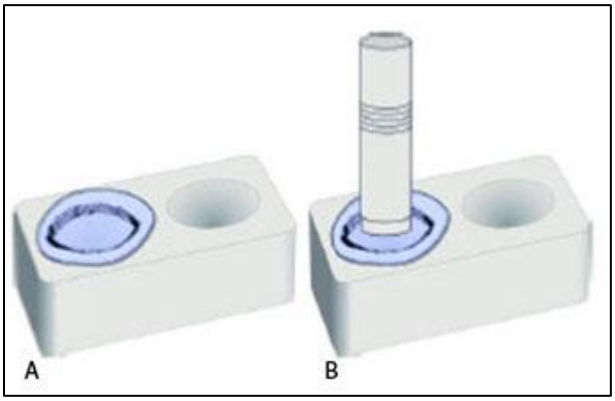

Figure 29. Preparation of donor corneal graft. A: The entire cornea is incised posterior to the limbus and transferred to a Teflon W block. B: With a corneal trephine 0.5 mm larger than the recipient's bed, the corneal graft is perpendicularly cut. The graft is then ready for the keratoplasty procedure. (Adapted from Gelatt and Brooks, 2011).

The detailed surgical technique for lamellar keratoplasty, as shown in figure 30 will be later discussed in Materials and Methods.

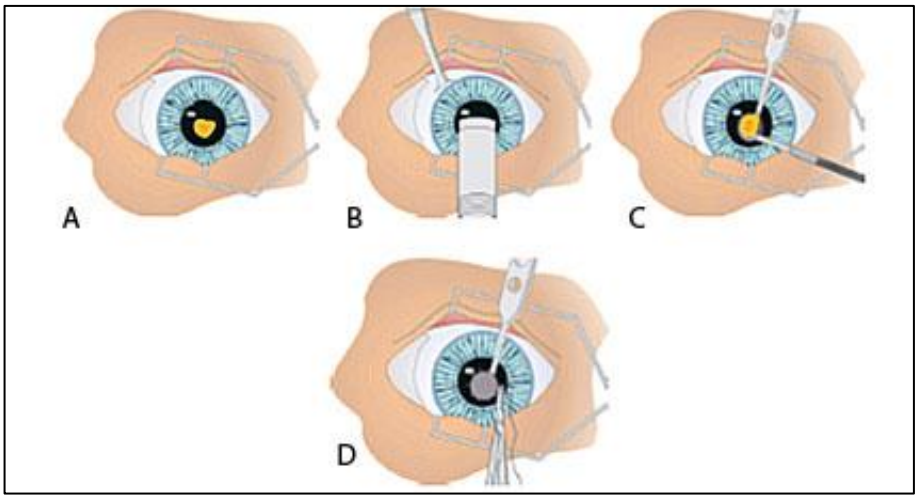

Figure 30. Lamellar corneal keratoplasty. A. Lamelar corneal keratoplasties are used for large superficial corneal sequestra. B: The large superficial sequestrum is incised with a corneal trephine. C: The corneal defect is lifted with 1x2 teeth thumb forceps and removed from the adjacent tissue, using a #64 beaver microsurgical blade or a Martinez corneal dissector. D: A lamellar graft 0.5 mm larger than the corneal defect is placed onto the corneal bed and sutured to the adjacent cornea. (Adapted from Gelatt and Brooks, 2011).

Lamellar grafts often result in translucent grafts. The objective is to provide a graft of collagen to support and/or replace severely damaged corneas (Gelatt and Brooks, 2011; Hansen and Guandalini, 1999).

According to Gelatt and Brooks (2011) and Hansen and Guandalini (1999), the corneal grafts should be covered with a conjunctival graft or nictitans flap. This procedure may reduce the probability of graft infection, collagenolysis, and suture dehiscence, as it protects against blinking movements and maintains pressure on the graft surface (Gelatt and Brooks, 2011; Hansen and Guandalini, 1999).

However, Gimenez and Fariña (1998), found no recurrence of the sequestra in 6 eyes treated with lamellar corneal graft without coverage with a conjunctival graft (Gimenez and Fariña, 1998).

1.5.8.3. Homologous and heterologous full-thickness/penetrating corneal grafts

Firstly, a keratectomy is performed. With a corneal trephine held perpendicular to the corneal surface, the incision is performed through rotation movements until 80% of the stroma had been incised. With a #65 microsurgical beaver blade, a 5 mm incision is extended using the trephine incision into the anterior chamber. It is not recommended to penetrate de anterior chamber with a corneal trephine as the likelihood of focal detachment of Descemet's membrane and endothelia increases (Townsend et al, 2008).

Once the anterior chamber is incised, the aqueous humor exits and the anterior chamber collapses. To re-establish the anterior chamber, a viscoelastic agent is used. With directional corneal section scissors the incision is completed and the diseased cornea removed (Townsend et al, 2008).

The donor corneal graft is prepared as described for the sequestrum removal. It should be carefully harvested to be 0.5 mm larger than the surgical site (Townsend et al, 2008). The posterior aspect of the corneal graft, with the single layer of endothelial cells, is the most susceptible to damage, and this side should not touch any surface or instruments (Gelatt and Brooks, 2011).

The corneal graft is placed with the epithelial surface upright. Then, it is secured with four cardinal sutures with 8/0 USP simple interrupted nylon, placed at the corner of each quadrant to stabilize the transplant. Additional 10-12 simple interrupted sutures are placed, providing slight tension to the graft to limit postoperative optical anomalies. To reinforce this suture, an 8/0 to 10/0 USP simple or saw-toothed continuous nylon suture is placed (Figure 31). Although sutures should be placed deep in the stroma, care should be taken to prevent penetration of Descemet's membrane and the endothelia when suturing (Townsend et al, 2008).

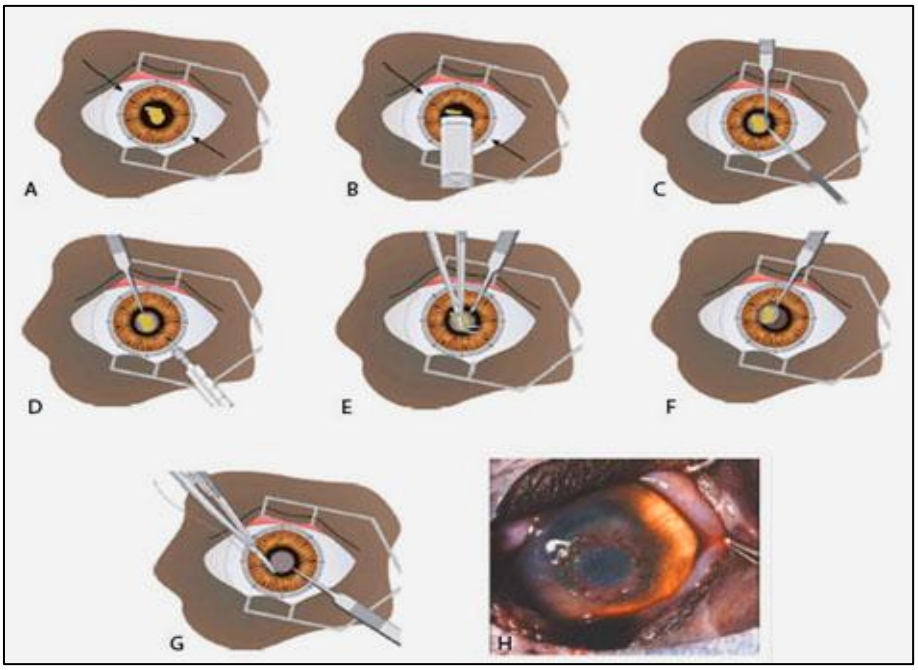

Figure 31. In penetrating keratoplasty, a full-thickness corneal graft with viable endothelial cells is used. A: Penetrating keratoplasties are used for sequestra affecting the deep stroma. B: The corneal lesion is outlined and an incision of 80% of corneal thickness is performed with a trephine. C: With a #65 beaver microsurgical blade, the anterior chamber is incised. D: The anterior chamber collapses once it is incised, to reform and maintain it is filled with viscoelastic agent. E: The incision is completed with keratoplasty scissors (universal or right and left handed scissors). F: The full- thickness corneal button is removed along with the corneal sequestrum. G. The corneal graft, 0.5 mm larger than the corneal defect, is positioned and sutured to the adjacent corneal tissue. H: Post-surgical appearance after a penetrating keratoplasty procedure. (Adapted from Gelatt and Brooks, 2011; Townsend et al, 2008).

The viscoelastic agent may be removed from the anterior chamber and replaced by lactated Ringer's solution (Gelatt and Brooks, 2011). Otherwise, if the viscoelastic agent is not removed, it increases the intraocular pressure but it is associated with a reduced probability of graft rejection (Townsend et al, 2008).

1.5.8.4. Postoperative treatment after keratoplasty

When the anterior chamber is penetrated, acetylcholine 1% is injected into the anterior chamber to prevent iris incarceration while suturing. Before the last suture a recombinant tissue plasminogen activator is also injected to stimulate fibrin reabsorption (Brunette, 2011).

Postoperative treatment after any type of keratoplasty consists of topical and systemic broad-spectrum antibiotics and topical mydriatics or cycloplegics (Gelatt and Brooks, 2011; Hansen and Guandalini, 1999; Townsend et al, 2008).

Topical 1% atropine, once a day for 5 days is used to obtain mydriasis (Gimenez and Fariña, 1998). Tobramycin four times a day for 6 weeks is an option for topical antibiotic treatment (Gimenez and Fariña, 1998). Systemic antibiotic therapy may be achieved with amoxicillin–clavulanic acid, 13 mg/kg PO, twice a day (Moore, 2005). Butorphanol 0.35 mg/kg, intravenously, is administered as needed for analgesia (Townsend et al, 2008).

A subconjunctival injection of dexamethasone (1.5 mg in 0.3 mL), tobramycin (10 mg in 0.25 mL) and cefazolin (50 mg in 0.25 mL) in the inferior fornix, has also been reported (Brunette et al, 2011).

Topical and systemic corticosteroid therapy should not be started until healing of the corneal wound, which takes place 2-3 weeks postoperatively (Gelatt and Brooks, 2011). However, for lamellar keratoplasty, the use of topical 0.1% dexamethasone three times a day, beginning immediately after the surgery, is described (Gimenez and Fariña, 1998).

To reduce the likelihood of graft rejection, 0.2% to 2% topical cyclosporine A is used. In the case of a lamellar keratoplasty, the immunosuppressive treatment may start immediately following the surgery, twice a day for 12 weeks (Gimenez and Fariña, 1998).

However, after a penetrating keratoplasty topical cyclosporine A may be initiated 2–4 weeks postoperatively and continued for 3-6 months, depending on the clearance of the cornea (Gelatt and Brooks, 2011).

An Elizabethan collar should be placed to prevent self-trauma, for three weeks (Brunette, 2011; Gimenez and Fariña, 1998; Townsend et al, 2008).

The corneal sutures should be removed at two different times, reducing the blood vessels formed in reaction to the suture material. Half of the sutures are removed at 2-3 weeks postoperatively and the remainder at 4 or 6 weeks, depending if a penetrating or a lamellar keratoplasty was performed (Gelatt and Brooks, 2011; Gimenez and Fariña, 1998). In lamellar keratoplasties corneal healing is slower than in penetrating keratoplasties (Gimenez and Fariña, 1998).

1.5.8.5. Postsurgical complications

Postsurgical complications include corneal ulcerations, suture dehiscence and graft rejection. If penetration of the anterior chamber occurs other complications may develop, such as fibrin and flare in the anterior chamber, retrocorneal membranes, anterior and posterior synechiae and secondary cataract formation (Brunette, 2011; Gelatt and Brooks, 2011). Long-

term graft vascularization, pigmentation, and opacification may occur, which indicate graft rejection (Gelatt and Brooks, 2011).

Donor corneas may become septic during tissue harvesting, handling, and storage leading to infectious agent's transmission to the recipient animal. Transmission of viral diseases by the donor cornea is also possible (Herring, 2003). However, the majority of this complications are prevented with careful patient selection, adequate pre-, peri-, and postoperative treatments, as well as surgical experience (Gelatt and Brooks, 2011).

Usually, in cats the intensity of corneal inflammation as well as, anterior uveal inflammation is reduced (Gelatt and Brooks, 2011).

Gimenez and Fariña (1998) reported no difference in peripheral corneal vascularization, corneal edema, or final transparency between homologous and heterologous corneal grafts. However, the major target of immunologic recognition is corneal endothelium and epithelium. Therefore, full thickness heterologous grafts pose a greater risk of rejection (Gimenez and Fariña, 1998; Townsend et al, 2008).

When corneal graft rejection occurs, it is characterized by the presence of subepithelial infiltrates, keratic precipitates on the endothelial surface, and a rejection line in the corneal endothelia. Vascularization, edema, inflammation, and opacification of the transplanted corneal tissue are obvious signs of corneal rejection (Gelatt and Brooks, 2011).

The factor most commonly associated with an increased risk of graft rejection is the pre-existing corneal blood vessels (Townsend et al, 2008).

Graft rejection may be reversed by intensive topical immunosuppressive therapy, achieved with topical corticosteroids and cyclosporine A. Immunosuppressive therapy may be administered for 4-6 months or more and until after corneal vascularization and inflammation have disappeared (Gelatt and Brooks, 2011).

In the environment, FeLV is a fragile virus, easily inactivated by heat, desiccation and ultraviolet light. However, recent reports found that, once in an appropriate media, like blood, serum or tissue culture medium, it remains infective for 48 hours at 37ºC and if frozen the virus remains infective indefinitely. Even when the cornea is stored and frozen, the conditions are adequate for maintenance of viral infectivity. The probability of iatrogenic transmission of infection when infected corneal tissue is transplanted is dependent of pre-existing blood vessels on the recipient's cornea and viral load of the donor corneal epithelium (Herring et al, 2001).

Patient selection is very important for a successful keratoplasty. Concurrent diseases such as chronic keratoconjunctivitis sicca, acute-to-chronic iridocyclitis, and glaucoma, often prevent keratoplasty (Gelatt and Brooks, 2011).

The axial or central cornea is of the most importance for animal's vision and this is the local where sequestra are most frequently observed. Alternative surgical procedures include conjunctival grafts, corneoconjunctival and corneoscleral transpositions. However, these procedures always produce corneal opacity of various sizes (Gelatt and Brooks, 2011; Gimenez and Fariña, 1998; Hansen and Guandalini, 1999).

A successful keratoplasty leads to a clear visual axis which allows vision (Hansen and Guandalini, 1999; Townsend et al, 2008).

2. Purpose

The purpose of this study is to describe the surgical management of feline corneal sequestration by means of lamellar keratoplasty, using frozen and fresh corneal grafts.

3. Materials and Methods

The cases 1 to 13, presented in this study, were gently provided by the Servei d'Oftalmologia Veterinària, Fundació Hospital Clínic Veterinari, Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Spain.

In the period between 2003 and 2013, 18 corneal sequestra were managed by means of keratoplasty in this hospital, in a total of 13 cats.

The medical records of these cats were reviewed and the following data collected: previous history and clinical features, gender, breed, age, affected eye, previous therapy, previous neovascularization, chronicity, diameter and depth of the sequestrum, surgical and post-surgical management, time to heal, follow-up time, complications observed, aesthetic outcome, postsurgical neovascularization and opacity, and recurrence.

The cases 14 to 20 were gently provided by Dr. Paulo Pimenta from Hospital Veterinário de Trás-os-Montes (HVTM). For these cases the following information was collected: gender, breed, age, affected eye, previous neovascularization, chronicity, diameter and depth of the sequestrum, surgical and post-surgical management, complications observed, aesthetic outcome, postsurgical neovascularization and opacity.

Corneal sequestrum is the observation of corneal discoloration or pigmentation. For this study the sequestra were classified by the depth of the affected cornea in: superficial (25%), median (25-50%), deep (50-75%) and descemetocele (75-100%).

The presence of ophthalmic findings that may be implied on the sequestrum pathophysiology were observed. Those findings included corneal ulceration and entropion.

Neovascularization was evaluated previously and after the surgery, and it refers to the blood vessels present in the cornea.

Opacity refers to the immunologic recognition that leads to graft rejection (Gimenez and Fariña, 1998).

Both, opacity and neovascularization, were accessed on a scale from 0 to 4: 0 stands no none, 1 to slight, 2 to moderate, 3 to severe and 4 to very severe.

Each surgical area was prepared by clipping an appropriate margin around the eye, and cleaning the eyelids and conjunctiva with a diluted solution of povidone-iodine.

The anesthetic protocol was defined according to each animal, and surgeon’s and anesthetist’s preference.

The patient was positioned in dorsal recumbency, with the head turned to the non-prepared eye.

For each animal, a superficial keratectomy was performed as described by Gelatt and Brooks, 2011 to remove the pigment related to the sequestrum.

Instruments usually necessary for this procedure are: eyelid speculum, smooth and 1x2 teeth tissue forceps (Bishop–Harmon or Colibri), Beaver scalpel handle and #64 microsurgical blade or diamond knife with a micrometer (which limits the depth of the corneal incision), a Martinez dissector, a corneal trephine whose depth can be preset to 0.2-0.3 mm, Westcott tenotomy scissors, and small cannula. For better exposure of the cornea, a lateral canthotomy is performed as needed (Gelatt and Brooks, 2011).

With a Beaver scalpel handle and a #64 microsurgical blade, an incision involving the periphery of the diseased area is performed. According to surgeons preference this step may be performed using a diamond knife with the micrometer set at 0.15 or 0.25 mm, or a preset corneal trephine (0.15-0.25 mm).

Twenty six keratectomies from this study were performed with a corneal trephine. The shape of the corneal sequestrum of case 20 did not allow the use of this instrument, and this step was performed with a microsurgical blade.

Besides outlining the lesion, this incision establishes the depth of the cornea to be removed. This should be enough to remove the lesion's entire depth (Gelatt and Brooks, 2011).

The walls of keratectomy should be perpendicular to the corneal surface if a graft or flap is to be placed and angled if not, to allow for more rapid epithelialization of the defect. This process is dependent of the angle at which the blade is held relative to the corneal surface (Herring, 2003).

Following, the edge of the superficial keratectomy section was grasped carefully with 1x2 teeth tissue forceps allowing the separation of the diseased cornea from the underlying stroma, and lamellar dissection was performed with a surgical blade. The dissection plane was maintained in the same parallel lamellae.

This process is easier when performed with a Martinez dissector. On the other hand, if the Beaver scalpel is used, it must be kept parallel to the corneal stroma to avoid progressive deeper penetration into the stroma. The dissecting instrument is moved with lateral movements and pressure is held forward (Gelatt and Brooks, 2011; Herring, 2003).

The corneal incision and stromal dissection are hampered in the presence of a low intraocular pressure and when stromal architecture is modified (Herring, 2003).

After dissection the diseased portion was lifted from the remained cornea. If needed, Westcott tenotomy scissors is used to carefully cut the flap (Barachetti et al, 2010). This was the option for all the cases of this study.

In the event of complete excision of the sequestrum has not yet been achieved, the procedure may be repeated (Gimenez and Fariña, 1998). This was true for case 19.

At the time of the surgery the cornea is probably vascularized. During the incision a local hemorrhage may occur. A continuous stream of 0.9% sterile saline is used to preclude blood from occluding the surgical field (Gelatt and Brooks, 2011). In the cases 14-20 a 0.01% adrenaline in 0.9% NaCl solution was used to minimize bleeding.

A corneal graft, with the same thickness of the removed cornea, was then placed on the keratectomy site, following the lamellar keratoplasty procedure described by Gelatt and Brooks, 2011.

For these cases, donor eyes were collected from cats, dogs and pigs as described in table 3. Donor animals presented general and ocular examinations without remarks, however no further diagnostic tests were performed.

The collection of the donor globes was performed aseptically, under sterile conditions as described by Gimenez and Fariña, 1998**.**

The donor eye was placed in a moist chamber with the cornea upward; this chamber was stored in a refrigerator. Alternatively, only the cornea attached to a 2 mm scleral rim may be harvested (Gelatt and Brooks, 2011).

When a penetrating keratoplasty is to be performed the donor cornea should be stored in Optisol-GS at 4ºC for up to 10 days for canine tissue and 15 days for feline corneas (Andrew et al, 1999; Arndt et al, 2001). On the other hand, donor corneas for lamellar grafts are kept frozen, and ready for use. In this case the viability of the cells is not important, so the age of the donor animal is not important. The donor eyes are removed and placed in a sterile container filled with an ophthalmic antibiotic solution. If preferred the cornea alone may be harvested and stored, entire or divided in halves or quarters. The containers are then frozen at -20º for a period of one month to one year. When needed, the containers and donor cornea are thawed at room temperature for 30-60 minutes, before harvesting the graft (Gimenez and Fariña, 1998; Hansen and Guandalini, 1999).

For this study, the last preservation method was chosen, as endothelial cell viability is not a concern when a lamellar keratoplasty is the technique to be performed. At the time of the surgery, a vial was removed from the freezer and the corneal graft was harvested by

means of keratectomy. All animals received frozen grafts except cats 9 and 16, where fresh corneal grafts were chosen. In these two cases, the epithelium was removed before transposition to the recipient cornea.

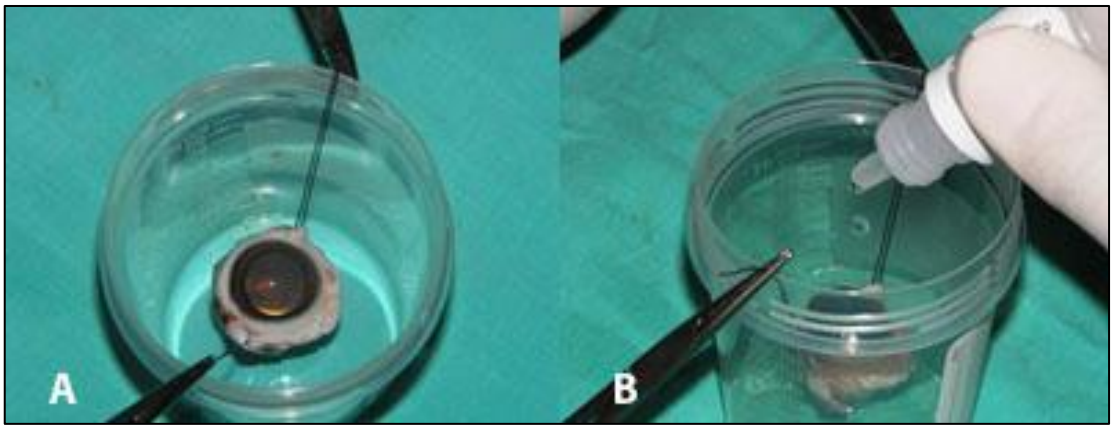

Figure 32. Collection and storage of donor eyes. A: The donor globe is placed in an individual recipient. B: An antibiotic solution is placed over the globe. (Courtesy of HVTM).

For the lamellar keratoplasty the Descemet's membrane and the endothelium were removed with Colibri forceps and scissors, by blunt dissection. Once this procedure was performed, the graft was placed over the keratectomy site and trimmed according to the diameter of the keratectomy site. At this time corneal thickness was restored to the normal. Four cardinal sutures were firstly positioned to align and affix the graft. Then, simple interrupted absorbable/non-absorbable, 8/0-10/0 USP sutures were apposed, as described in table 3.

4. Results

A total of 25 corneal sequestra from 20 animals, were managed by lamellar keratoplasty. A total of 27 procedures were realized, since a second keratoplasty was performed in two eyes. The results are displayed in table 1, table 2, table 3 and table 4.

This group of animals was composed of 15 males and 5 females. Eleven males were intact and the others neutered (n=4), there were a higher number of intact (n=3) than spayed females (n=2). The breeds present were Persian (n=15), American Persian (n=1) and Exotic Shorthair (n=1), Himalayan (n=1), Sphinx (n=1) and Domestic shorthair (n=1).

The age at time of referral ranged between 8 months and 8 years.

Six animals developed bilateral sequestra. Bilateral condition was observed in 4 animals at time of referral, while one animal developed a sequestrum in the contralateral eye, almost one year after the first surgical treatment. Another cat had a sequestrum diagnosed on the contralateral eye, which was managed by the referring veterinary. All animals with bilateral sequestra were Persians (n=6).

The sequestrum affected the right cornea in 12 eyes and the left cornea in 13 eyes.

Previous neovascularization was present in 22 eyes and ranged from slight (n=3) to very severe (n=5). Severe and moderate neovascularization were present in 6 and 8 cases, respectively.

Ophthalmic conditions that may be involved with the sequestra formation were found in seven cases: previous ulceration (n=4) and entropion (n=5). Four of the entropion cases were surgically managed at the time of the keratoplasty. Case 1 was diagnosed with spontaneous chronic corneal epithelial defects which were managed by a superficial keratectomy procedure and a bandage contact lens placement, three months before the appearance of the sequestrum.

Sequestra diameter ranged from 3 mm to 11.5 mm. They were classified in superficial (n=6), median (n=7), deep (n=9) and descemetocele (n=5). From the descemetocele sequestra, one eye presented a corneal perforation.

Regarding the source of the corneal graft, it was homologous (n=11) and heterologous (n=16). Two of the heterologous grafts were of porcine origin.

In the cases from Hospital Clínic Veterinari UAB the following post-surgical treatment was applied. Post-operative medical treatment included topical antibiotic in all eyes. For this purpose it was administered: terramycin (n=6), tobramycin (n=3), chloramphenicol (n=4), aureomycin (n=5), and a combined solution of polymixin B, neomycin and gramicidin

(n=2). Topic mydriatics and cycloplegics were administered in 18 eyes: cyclopentolate hydrochloride (n=9), atropine sulphate (n=7) and tropicamide (n=2). Topical corticosteroid treatment were achieved with dexamethasone (n=17). Artificial tear was used in 14 eyes. For this propose, carbomer (n=12) and sodium hyaluronate (n=2) were applied. Topical immunosuppressive treatment was performed with cyclosporine A (n=16). Systemic anti-inflammatory treatment was needed in 4 patients: meloxicam (n=2) and flubiprofen (n=2). Systemic antibiotic treatment, tilosin, was administered in one animal.

Post-surgical treatment protocol, applied in every animal in HVTM included the topical administration of: fusidic acid (antibiotic), cyclopentolate hydrochloride and tropicamide, flubiprofen sodium (non-steroidal anti-inflammatory agent), dexamethasone and cyclosporine A.

In these study a total of 27 lamellar keratoplasties were performed and in some post-surgical complications were observed. Appearance of pigmentation in 18,5% (n=5), suture reaction in 14,8% (n=4), suture dehiscence 7,4% (n=2), retraction of the graft 3,7% (n=1), synechia formation 3,7% (n=1) and perforation 7,4% (n=2), lifting of a tip of the corneal transplant 7,4% (n=2) and corneal lipid deposition in 3,7% (n=1) of the surgical procedures.

During the follow-up time, two cats underwent a new lamellar keratoplasty. Case 4 experienced a recurrence of the sequestrum. On the other hand, the graft on case 5 was opaque and detached from the corneal bed, leading to a perforation.

Case 2, developed a corneal sequestrum that was previously managed by a superficial keratectomy alone, nine months before. However, the corneal discoloration re-appeared and at this time it was managed by a keratoplasty procedure (Figure 33).

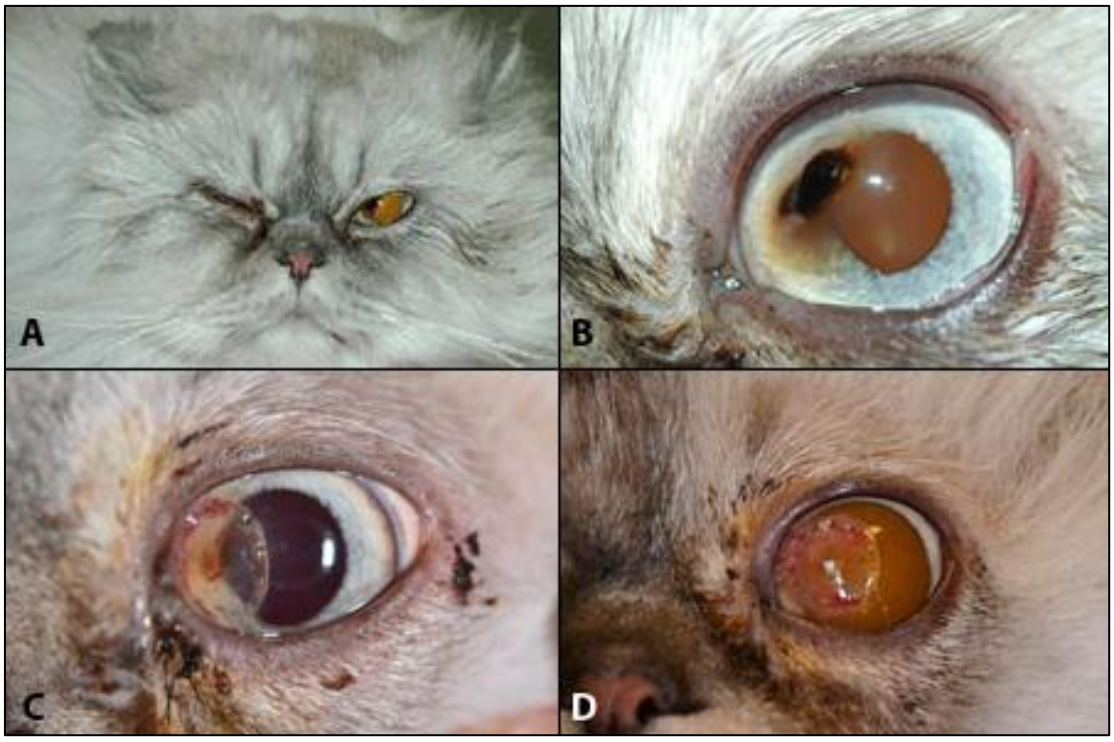

Figure 33. Corneal sequestrum in the left eye of a 6,8-year-old Persian (Case 2). A: An inferior eyelid medial entropion is observed. B: The sequestrum has a diameter of 9 mm, affects the deep corneal stroma and presents a slight neovascularization. C: Immediate post-surgical appearance of the eye after the lamellar keratoplasty and entropion correction. D: Post-surgical appearance of the eye three weeks after the lamellar keratoplasty and entropion correction. (Courtesy of Fundació Hospital Clínic Veterinari, UAB)

In case 4, the sequestrum firstly appeared on the left cornea and was surgically resolved by the referring veterinarian with a keratectomy procedure alone, but it re-occurred two months after and a keratoplasty was required. Almost one year after the left cornea transplantation, a sequestrum developed on the right cornea and a keratoplasty procedure was performed. Unfortunately, a right corneal sequestrum developed again, requiring new surgical management.

Case 5 was diagnosed with left corneal sequestrum which was treated by a lamellar keratoplasty. During the postsurgical management, perforation of the cornea occurred and a new keratoplasty was performed.

One month after the keratoplasty, a tip of the graft lifted, in case 15. In this case a new surgical procedure should be performed, due to the size of the graft that lifted. By means of a superficial keratectomy, that portion would be removed, however it was not authorized by the owner.

Case 14 and case 18 presented a concurrent bilateral entropion, which were managed at the same time of the keratoplasty procedure, by a Celsus-Holtz technique (Figure 34).

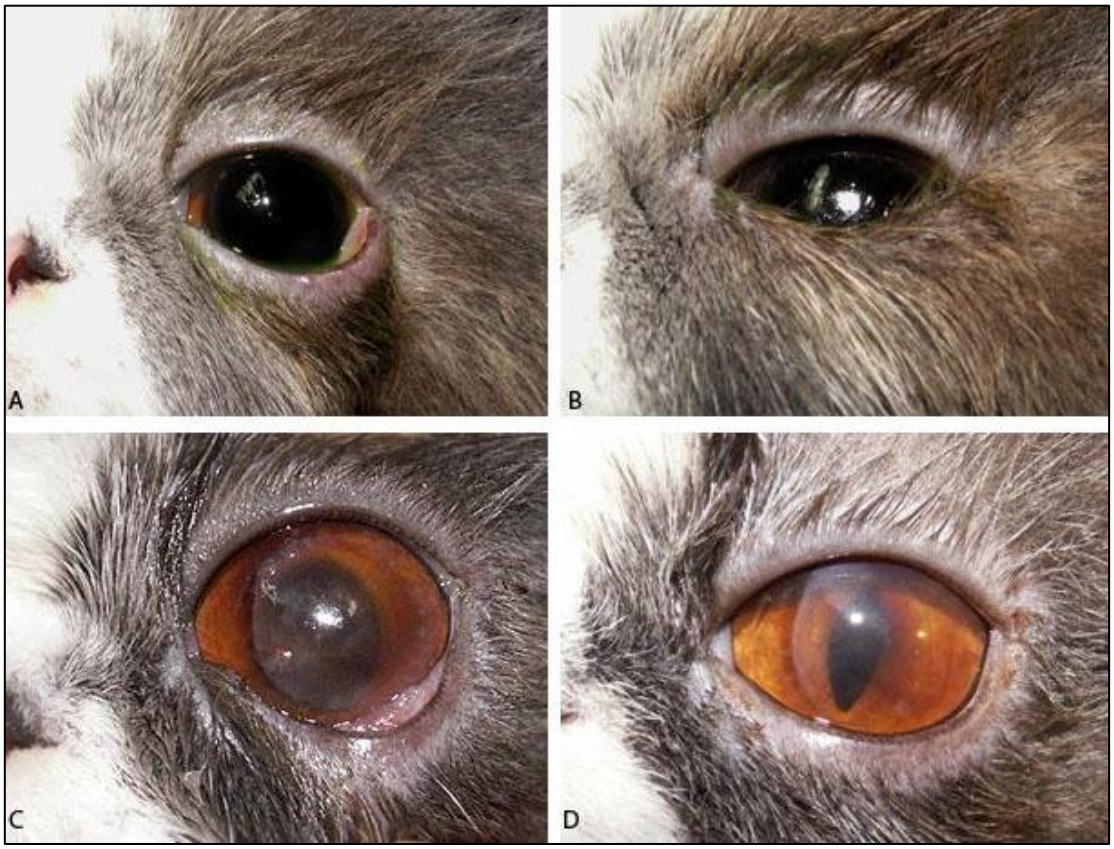

Figure 34. Corneal sequestrum in a 8 month old Persian (Case 14). A: A 11 mm diameter sequestra is present on the left eye. B: It is observed an inferior eyelid entropion probably associated to the sequestrum pathophysiology. C: Post-surgical appearance 3 weeks after the lamellar keratoplasty procedure. D: Post-surgical appearance 5 weeks after the lamellar keratoplasty procedure. The transparency of the cornea was re-established, allowing a clear visual axis. (Courtesy of HVTM).

After the keratoplasty procedure, case 18, developed corneal lipid deposits (Figure 35). This condition is known as lipid keratopathy or degeneration. It may be associated with systemic metabolic conditions (hyperlipidemia), previous corneal diseases or local metabolic alterations. It is also referred that the use of topical corticosteroid therapy predisposes patients to lipid corneal deposition (Martin, 2010). In this case the use of topical corticosteroids was discontinued and the lipid deposits disappeared.

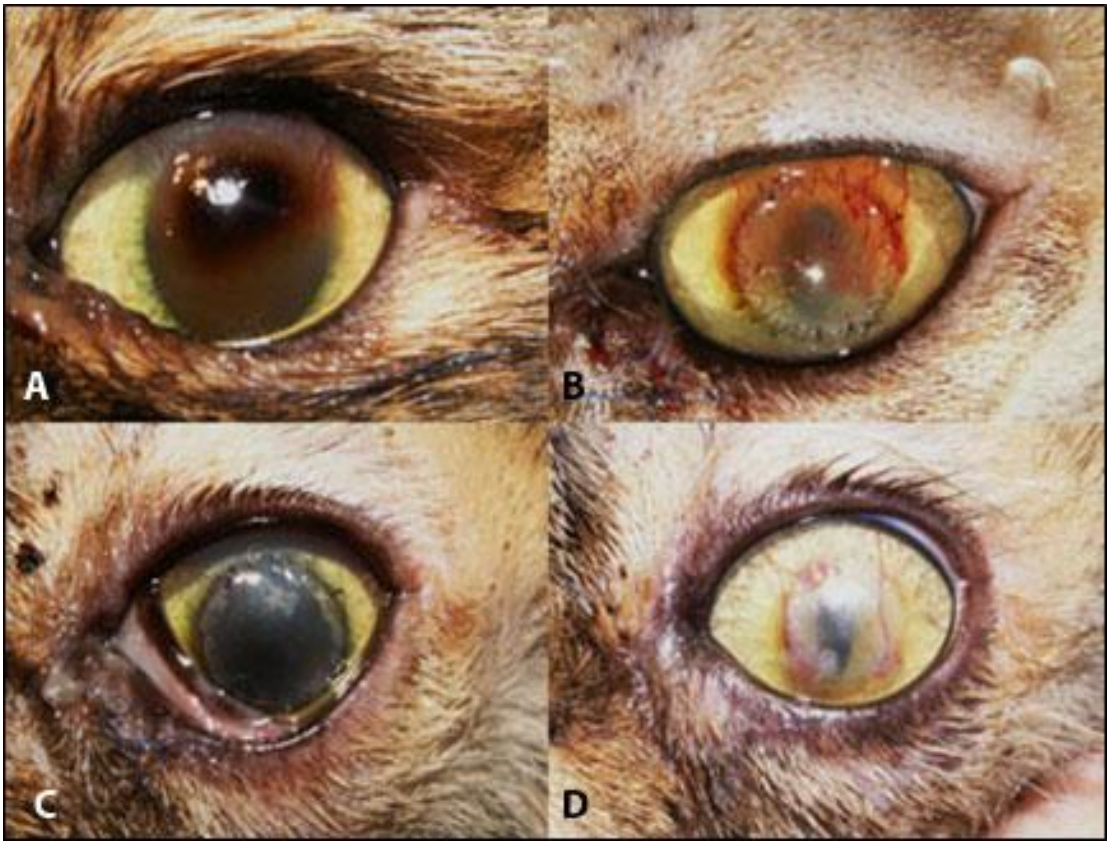

Figure 35. Corneal sequestrum in a 4-year-old Persian (Case 18). A: A 6 mm diameter sequestrum and an inferior eyelid entropion is present on the left eye. B: Immediate post-surgical appearance after the lamellar keratoplasty. C: Post-surgical appearance 3 weeks after the lamellar keratoplasty procedure. D: Post-surgical appearance 5 weeks after the lamellar keratoplasty procedure. Corneal lipid deposits are observed. (Courtesy of HVTM**).**

In a previous attempt to remove the corneal sequestrum, a grid keratectomy was executed in case 19, by the referring veterinarian. Unfortunately, the sequestrum re-appeared, and a keratoplasty was performed (Figure 36).

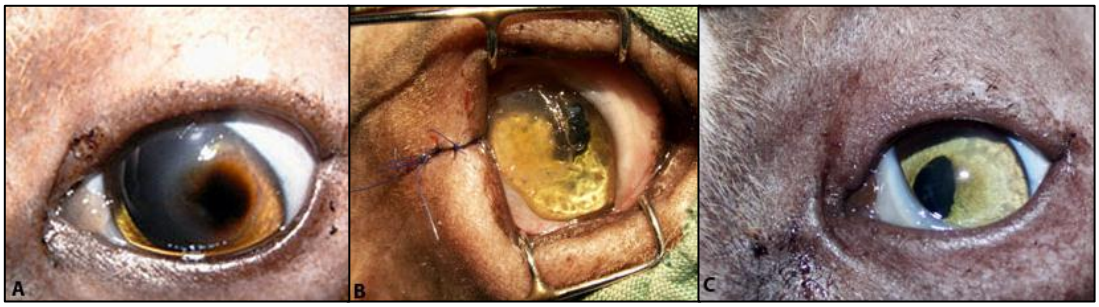

Figure 36. Recurrence of a corneal sequestrum after a grid keratectomy (Case 19). B: Immediate post-surgical aspect of the previous eye after the lamellar keratoplasty procedure. C: Post-surgical appearance 3 months after the lamellar keratoplasty procedure. (Courtesy of HVTM).

Case 20 had previously developed a corneal sequestrum on the left eye which was managed by the referring veterinarian (Figures 37).

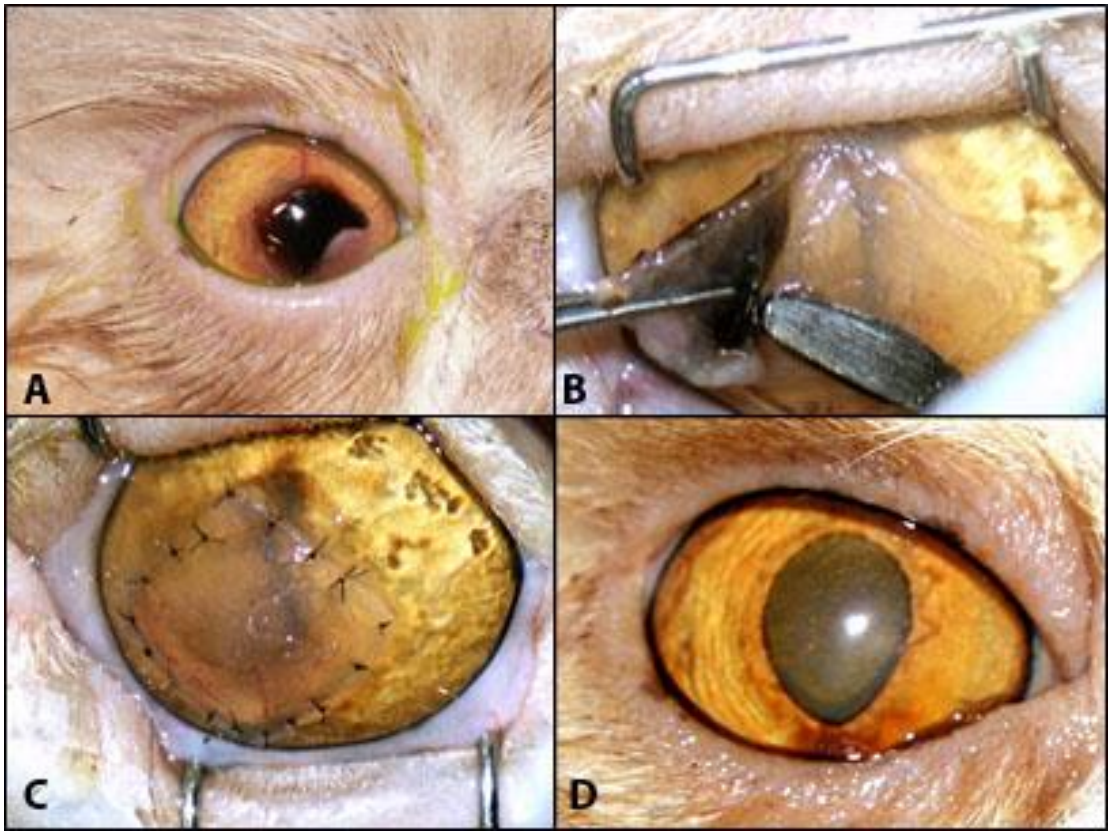

Figure 37. Corneal sequestrum with a diameter of 8 mm in the right eye of a Persian cat (Case 20). A: A very severe neovascularization is observed. B: Intraoperative image of the lamellar keratoplasty. B: Post-surgical appearance of the previous eye immediately after a lamellar keratoplasty procedure. D: The previous eye 9 months after the lamellar keratoplasty procedure. The corneal transparency was restored resulting in a clear visual axis. (Courtesy of HVTM).

Overall, the aesthetic result was good, with light post-surgical vascularization and slight to mild opacity. Final neovascularization ranged from none (n=6) to moderate (n=6), with 13 eyes showing slight neovascularization. Post-surgical opacity, ranged from none (n=4) to severe (n=1), ten eyes presented just a slight opacity and a moderate degree appeared in another 10 eyes.

The success of keratoplasty in this study was around 88.9% (24/27). Three eyes required a new surgical procedure as the sequestrum reoccurred (Case 4, OD), a perforation developed in the corneal graft (Case 5, OS) or a large portion of the graft lifted (Case 15). Only the first two animals underwent a second surgery.

Case nº	Gender	Breed	Age	Eye
1	M	Persian	1,6	OD
2	M	Persian	6,8	OS
3 (OD)	M	Persian	1,5	OD
3 (OS)	M	Persian	1,5	OS
4 (OD)	Mn	Persian	5	OD
4* (OD)	Mn	Persian	5	OD
4 (OS)	Mn	Persian	4,2	OS
5 (OD)	M	Persian	5,5	OD
5 (OS)	M	Persian	5	OS
5* (OS)	M	Persian	5,5	OS
6	Fn	Exotic Shorthair	1,1	OD
7	Fn	American Persian	5,8	OD
8	M	Persian	2,5	OD
9 (OD)	F	Persian	1	OD
9 (OS)	F	Persian	1	OS
10 (OD)	Mn	Persian	3,4	OD
10 (OS)	Mn	Persian	3,4	OS
11	Mn	Persian	5,5	OD
12	Mn	Persian	5	OS
13	F	Persian	2,1	OS
14	M	Persian	0,7	OS
15	M	Domestic Shorthair	8	OS
16	F	Persian	4	OD
17	M	Himalayan	0,8	OS
18	M	Persian	4	OS
19	M	Sphinx	3	OS
20	M	Persian	1,5	OD

Table 1 Characterization of the animals.
F: female; Fn: neutered female; M: male; Mn: neutered male; OD: right eye; OS: left eye; *: second surgery.

Case nº	Sequestrum Depth	⌀ (mm)	PV	Chronicity
1	Superficial	8	2	SCCED
2	Deep	9	2	Sequestrum for 3 months, inferior lid medial entropion
3 (OD)	Descemetocele	10	3	4 months
3 (OS)	Descemetocele	10	3	4 months
4 (OD)	Superficial	3	0	20 days
4* (OD)	Superficial	11	2	N/A, 2nd surgery
4 (OS)	Median	8,5	0	2 months, previous surgery by the referral vet
5 (OD)	Deep	4	1	Sequestrum develop during contralateral treatment
5 (OS)	Median	6	1	2 months
5* (OS)	Deep	9	1	N/A, 2nd surgery
6	Median	6	2	Ulceration with 1.5 months
7	Deep	4	3	Ulceration with 2 months
8	Descemetocele	7	3	Recidivating ulceration with 2 months
9 (OD)	Superficial	11,5	3	1 month
9 (OS)	Descemetocele	11,5	3	2 months, previous surgery by the referral veterinary
10 (OD)	Descemetocele, perforation	8,5	2	3 months
10 (OS)	Median	11	2	3 months
11	Deep	6	0	1 month
12	Deep	9	0	Ulceration with 2 months, entropion
13	Median	6	0	1 month, entropion
14	Median	6	4	3 months
15	Deep	7	2	8 months
16	Deep	4	4	3 months
17	Deep	4	4	3 months
18	Superficial	6	4	3 months
19	Superficial	6	2	2 months
20	Median	8	4	1 year

Table 2. Characterization of the sequestra.
N/A: not applicable; OD: right eye; OS: left eye; PV: previous vascularization; SCCED: spontaneous chronic corneal epithelial defects, ⌀: diameter; *: second surgery.

Case nº	Graft Origin	Concurrent Surgery	Suture	Frozen/ Fresh Graft
1	Heterologous, porcine	No	Dafilon® 9/0	Frozen
2	Heterologous	Entropion, Celsus- Holtz	Dafilon® 9/0	Frozen
3 (OD)	Heterologous	No	Dafilon® 9/0	Frozen
3 (OS)	Heterologous	No	Dafilon® 9/0	Frozen
4 (OD)	Heterologous, porcine	No	Dafilon® 9/0	Frozen
4 *(OD)	Heterologous	No	Dafilon® 9/0	Frozen
4 (OS)	Homologous	No	Dafilon® 9/0	Frozen
5 (OD)	Heterologous	No	Vicryl® 9/0	Frozen
5 (OS)	Heterologous	No	Vicryl® 9/0	Frozen
5* (OS)	Heterologous	No	Vicryl® 9/0	Frozen
6	Homologous	No	Dafilon® 9/0	Frozen
7	Homologous	No	Dafilon® 10/0	Frozen
8	Homologous	No	Dafilon® 9/0	Frozen
9 (OD)	Homologous	No	Dafilon® 9/0	Fresh
9 (OS)	Homologous	No	Dafilon® 9/0	Fresh
10 (OD)	Heterologous	No	Dafilon® 9/0	Frozen
10 (OS)	Heterologous	No	Dafilon® 9/0	Frozen
11	Homologous	No	Dafilon® 9/0	Frozen
12	Homologous	Entropion, Celsus- Holtz	Dafilon® 9/0	Frozen
13	Heterologous	No	Dafilon® 9/0	Frozen
14	Heterologous	Entropion, Celsus-Holtz	Dafilon® 9/0	Frozen
15	Homologous	No	Dafilon® 8/0	Frozen
16	Homologous	No	Dafilon® 8/0	Fresh
17	Heterologous	No	Safil® 9/0	Frozen
18	Homologous	Entropion, Celsus-Holtz	Dafilon® 8/0	Frozen
19	Heterologous	No	Safil® 9/0	Frozen
20	Heterologous	No	Dafilon® 8/0	Frozen

Table 3. Characterization of the surgical procedure.
OD: right eye; OS: left eye; *: second surgery.

Case nº	Complications	Aesthetic Outcome	FV	Opacity
1	None	Good	1	1
2	None	Good	1	2
3 (OD)	None	Good	1	2
3 (OS)	Remaining area of pigment	Good	1	2
4 (OD)	Recurrence of the sequestrum	N/A	N/A	N/A
4* (OD)	Reaction towards the sutures, faint pigment	Good (fibrosis and slight vascularization)	2	2
4 (OS)	None	Good (slight fibrosis)	1	2
5 (OD)	Pigment formation dorsal to the graft that disappeared	Good	1	2
5 (OS)	Perforation	N/A	N/A	N/A
5* (OS)	Graft retraction	Good	1	3
6	Suture dehiscence and pigment formation which disappeared	Good (slight vascularization and pigmentation)	1	2
7	None	Good	0	1
8	Slight reaction towards the sutures	Good	1	0
9 (OD)	None	Good	0	1
9 (OS)	Synechia formation	Good	1	1
10 (OD)	None	Good	2	2
10 (OS)	Suture dehiscence	Good	2	2
11	Perforation	Good	0	1
12	Reaction towards the sutures, graft initially thicker	Good	1	1
13	One month after surgery, the graft lifts and a cellular infiltration is observed, the sutures are removed and the inflammation reduces	Good	2	1
14	Slight edema and neovascularization	Good	0	0
15	One tip of the graft lifted.	Good	1	1
16	None	Good	2	1
17	Remaining area of pigment. One tip of the graft lifted one month after the surgery.	Good	2	2
18	Deposition of lipids	Good	1	1
19	None	Good	0	0
20	None	Good	0	0

Table 4. Postsurgical outcome.
FV: final vascularization; N/A: not applicable ; OD: right eye; OS: left eye; *: second surgery.

5. Discussion

Pure breeds of brachycephalic cats have been reported to have a high incidence of corneal sequestra. The highest incidence is found in Persians, followed by other breeds of brachycephalic cats (Featherstone and Sansom, 2004). In this study, the most prevalent breed was also the Persian, however it only includes the sequestra managed by lamellar keratoplasty. Many other cats were diagnosed in the referred veterinary hospitals but, due to owners financial restrains and ophthalmologist's preference, a lamellar keratoplasty was not performed.

Sequestrum is known to affect cats of all ages, except neonates (Andrew et al, 2001). According to previous reports, cats are diagnosed with sequestrum between 5 months and 17 years, with the majority being 2 to 7 years (Featherstone and Sansom, 2004). In this retrospective study cats age ranged from 8 months to 8 years, with 60% (12/20) of cats between 2 to 7 years and 35% (7/20) less than two years. Only one animal was older than 7 years at the time of diagnosis.

No sex prevalence or predisposition had been identified in previous reports. Although a higher number of males was found, it may be due to the very short number of cases included.

Unilateral sequestrum was more frequently observed than bilateral, which is a common observation in previous reports (Andrew et al, 2001; Featherstone and Sansom, 2004). Previous reviews reported that sequestrum usually is unilateral except in predisposed breeds. Bilateral sequestrum occurs more frequently in breeds of brachycephalic conformation, however it has been diagnosed in Domestic Shorthair cats (Featherstone and Sansom, 2004). Bilateral sequestrum, either presented at the time of referral or developing with time, was only found in Persian cats. However, breed prevalence in this study may be related to the short number of cases and the inclusion of cats whose sequestrum was managed by lamellar keratoplasty.

Previous neovascularization was apparently not related to the chronicity of the process. Usually, vascularization and pigment cell migration to the cornea is a response to chronic corneal irritation. In other words, vascularization increases with the length of the time that the lesion is present. However, inconsistent vascularization behavior is characteristic of feline corneal sequestrum, and similar results were previously showed (Featherstone and Sansom, 2004).

Pre-existing corneal vascularization reduced after the lamellar keratoplasty in sixteen cases, remained constant in 6 cases and increased in 3 cases. It was expected a reduction of corneal vascularization in the postoperative period (Barachetti et al, 2010; Gimenez and Fariña, 1998), as it was observed in sixteen cases. However, blood vessels formation as a reaction to the sutures has been reported previously (Gimenez and Fariña, 1998) and occurred in two of these cases.

Homologous or heterologous grafts were used according to the availability of donor material at the time of the surgery. Heterologous grafts derived either from dogs or pigs. The two failed keratoplasties were performed with grafts of heterologous origin. However, no obvious difference in final neovascularization and opacification between feline and dog/pig grafts was found. In a previous review, reporting corneal lamellar keratoplasty, no difference in the final features was found between homologous and heterologous corneal grafts (from canine donors) (Gimenez and Fariña, 1998).

With keratoplasty procedure, transmission of infectious diseases to the graft recipient is possible (Herring et al, 2001).

Donor animals received a complete physical and ocular examination previously to euthanasia. These animals did not show obvious clinical signs of ocular disease nor systemic infectious disease. However, diagnostic screening tests were not performed.

In humans, transmission of viral agents harbored in the donor cornea has been reported (Gimenez and Fariña, 1998; Herring et al, 2001).

Viral agents detected in human corneas include: rabies, human immunodeficiency virus, hepatitis B, herpes simplex, cytomegalovirus, Epstein Barr virus and varicella zoster. To avoid viral agent transmission to the graft recipient, restrict screening protocols are realized in human corneal banks (Herring et al, 2001).

FHV-1 is commonly transmitted by direct o close contact, fomites and respiratory and ocular secretions. However corneal transplantation may be a route of transmission, as the virus is present in its latency or quiescent form in the corneas of clinically normal cats (Maggs, 2005; Stiles, 2003). On the other hand, cytolytic action of FHV-1 does not always produce loss of the entire depth of corneal epithelium, preventing stromal exposure. If the stroma is not exposed, the application of fluorescein stain will not allow the visualization of the ulcer at this stage. Then, it will fail to notice the viral active replication, which is only possible, at this point, with Bengal stain. Bengal stains degenerative and dead corneal cells (Maggs, 2005).

Fluorescein stain is most frequently used during ophthalmic examination than Bengal stain. This may prevent the identification of these FHV-1 infected corneas.

Feline leukemia virus is also present in corneal tissues of some infected cats, without presenting ocular clinical signs, as it was demonstrated by the presence of FeLV proviral DNA and FeLV antigens in these tissues. The ocular examination turns out normal, as FeLV is non-cytophathic under normal conditions. Moreover, storage conditions of graft material, allow maintenance of FeLV infectivity (Herring et al, 2001).

Ideally, donor cats should have corneal samples tested by PCR (Bussieres, 2004) to exclude FHV-1 infection, as well as, an ELISA test for FeLV in an attempt to exclude or significantly reduce the probability of viral transmission by an infected graft (Herring et al, 2001). FIV has not been reported to be present in corneal tissues, however, it is advised to test donor cats for this virus (Herring et al, 2001).

It is also recommended to test donor dogs for endogenous diseases such as Leishmaniasis (in Europe) and rabies (almost every part of the world) (Gimenez and Fariña, 1998).

To avoid penetration of the anterior chamber, a small amount of pigment may not be excised, as happened in cases 3(OS), 4 (OD) and 17. This residual corneal pigment was previously reported, and disappeared within 4 weeks (Gimenez and Fariña, 1998). However, another review found that the probability of recurrence is higher when a complete excision of the pigment is not performed (Featherstone and Sansom, 2004). In the cases where remaining pigment was found, no sequestrum recurrence occurred.

Formation of new pigment was found in two eyes, but continuing post-surgical treatment led to its disappearance.

Inflammatory reaction towards the sutures occurred in four cases and resolved once the sutures were removed.

Retraction of the graft is a normal reaction after this surgical procedure. To compensate for this reduction of the graft diameter, it is harvested with a diameter, at least 0.5 mm larger than the corneal bed (Gelatt and Brooks, 2011).

Case 9 (OS) developed anterior synechia. After surgical penetration of the anterior chamber, iridocyclitis may result. Inflammation of the iris predisposes to its adhesion to other intraocular tissues, leading to the formation of temporary or permanent adhesions. Anterior synechiae result from the adhesion of the iris to the posterior cornea, and may lead to corneal scars and edema (Gelatt and Brooks, 2011).

Previous corneal neovascularization increases the probability of immunologic recognition of the graft material, leading to graft rejection. This process is visualized by the opacity of the graft (Gimenez and Fariña, 1998). Recognition by the recipient of the graft is mainly directed to the graft's endothelium and epithelium, which were removed from the grafts used. To further decrease the likelihood of graft rejection, topical corticosteroids and cyclosporine A were used routinely. As a result, a visual corneal axis was achieved in every case, with slight to moderate opacity. Case 5 (OS) showed severe opacity, probably related to the graft retraction.

6. Conclusion

Management of feline corneal sequestrum is better achieved by means of a surgical procedure. Usually a keratectomy is performed to remove the pigment associated with the sequestrum. Then, depending on the lesion's depth and surgeon's experience a graft procedure may take place.

Grafting techniques readily available are those which use the patient own tissue (autografts), and include: conjunctival grafts, corneoscleral and corneoconjunctival transpositions. Although these grafts are always available and do not pose a risk of rejection, they always lead to an opaque scar that do not allow vision through it. Even if an island conjunctival graft is used, a visual axis may not be achieved as corneal sequestra are mainly located in central and paracentral cornea.

Porcine small intestinal submucosa may be easy available, as it is commercially provided and easily stored. It allows healing by regeneration, leading to a clear visual axis. However, PSIS laceration and discoloration had occurred, resulting in scar formation.

Equine amniotic membrane is not easily accessible in practice. It increases basal epithelial cells differentiation and adhesion, and reduces apoptosis and antiproteinase activity. It allows a clear visual axis, but is not suitable for deep sequestra.

Cyanoacrylate adhesives are cheap and easy to perform, allowing a clear visual axis. However it does not provide tectonic support to large corneal defects, which may result from the excision of larger sequestra.

Penetrating keratoplasty requires specific storage media and techniques not easily accessible in practice. Although they provide tectonic support and visual axis, they also represent a greater risk of immunologic recognition due to the presence of the endothelial layer.

Lamellar keratoplasty, using fresh or frozen corneal grafts, restores corneal integrity after removal of corneal sequestra of various depth and diameter. It does not require expensive and limited storaged of donor corneal tissue. It may be performed with either heterologous (canine and porcine) or homologous (feline) grafts, with similar results. Although transient rejection of the graft causes its opacity, it decreases with the careful use of topical immunosuppressive therapy. This technique results in better corneal transparency when compared to other graft procedures and decreases the likelihood of immunologic recognition when compared to penetrating keratoplasty.

The treatment of feline corneal sequestration is successfully achieved with removal of the sequestrum followed by a keratoplasty. It is associated with a reduced recurrence rate and good corneal transparency. The removal of the sequestrum and a clear visual axis improve the quality of life of the affected cats, as the ocular pain stimulus is removed and vision possible.

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