Successful use of porcine small intestinal submucosa grafts for the treatment of a deep melting corneal ulcer in a foal

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Summary: Severe corneal opacity and, thus, persistent visual impairment is the main risk following the treatment of keratomalacia. This report describes the successful use of commercially available porcine small intestinal submucosa (SIS) grafts (BioSIS Plus⁺, Vetrix®) to treat a melting corneal ulcer in a foal. A 1-month-old Hanoverian filly was presented with a deep melting corneal ulcer unresponsive to local medical therapy for one week. A severe ulcerative mycotic keratitis was diagnosed. Due to the high risk of corneal perforation, surgical treatment was initiated the following day. A lamellar keratectomy followed by the placement of two single overlapping multilayer porcine SIS grafts was performed under general anaesthesia. After surgery, a head bandage was placed to close the eyelids for eight days. During hospitalization, increasing transparency of the peripheral cornea was observed. The foal was discharged four weeks postoperatively. A focal pigmentation of the cornea and slight corneal oedema remained visible five months after the surgery.

Keywords: horse, cornea, keratomycosis, intestinal submucosa, graft

Introduction

The cornea and its thin precorneal tear film (PCTF) form a complex and fragile barrier of the visual organ against the external environment. The equine cornea is vulnerable to pathologic agents due to its large size and exposure. Direct trauma and different infectious and non-infectious diseases can destruct the ocular surface, which typically results in temporary or prolonged loss of transparency, impairing visual function (*Brooks* et al. 2017). Ulcerative keratitis is a common and potentially blinding ocular disease in horses, with the risk of corneal perforation within 24 hours (*Strubbe* et al. 2000).

Corneal wound healing is a complex interdependent process of different cellular and acellular components aiming to restore the smoothness, integrity and function of the ocular surface. It depends on the mitosis and centripetal migration of healthy epithelial cells to cover the ulcer site and their subsequent attachment to the injured corneal stroma (*Wilson* et al. 2001). Different proteolytic enzymes and their inhibitors participate in controlling the tissue turnover during health and disease (*Sivak* and *Fini* 2002, *Ollivier* et al. 2007, *Brooks* et al. 2017). These enzymes derive from the PCTF, corneal epithelial cells, keratocytes, infiltrating inflammatory cells and infectious organisms. In the event of corneal damage, they can provoke and enhance proteolytic activity (*Kessler* et al. 1977, *Zhu* et al. 1990, *Matsubara* et al. 1991, *Twining* et al. 1994a, *Twining* et al. 1994b, *Matsumoto* 2000, *Strubbe* et al. 2000). The imbalance in the healing

progression and enhanced proteolytic activity results in the liquefaction and gelatinization of the corneal stroma, which is described by the term "melting ulcer" or "keratomalacia" (*Fini* et al. 1998). This pathologic process was observed in different species but is thought to be most harmful in horses (*Brooks* 1999, *Ollivier* 2005, *Ollivier* et al. 2007). Previous studies showed that the matrix metalloproteinases (MMPs) MMP-2 and MMP-9 and a serine proteinase (neutrophile elastase) play a key role during this pathologic condition (*Fini* et al. 1998, *Strubbe* et al. 2000, *Sivak* and *Fini* 2002, *Ollivier* et al. 2007). A study focusing on proteinases of the equine PCTF showed that median MMP-2, MMP-9 and neutrophil elastase levels were significantly higher in ulcerated eyes compared to normal controls. The MMP-2 levels were even significantly elevated in the PCTF of the contralateral eyes of affected horses (*Strubbe* et al. 2000). Proteolytic activity decreases at the time of ulcer healing. In the case of "melting" ulcers, proteinase levels remain elevated, leading to rapid progression of the ulcer.

The medical treatment of corneal ulcers aims primarily to eliminate provoking agents and decrease collagenolysis and reflex uveitis (*Brooks* et al. 2017). Deep corneal ulcers which affect more than 50 % of the total corneal thickness or inefficiency of medical treatment require consistent surgical intervention (*Nasisse* and *Nelms* 1992, *Brooks* 2008, *Brooks* et al. 2017). The aim of surgical treatment is to provide an immediate supply of fibroblasts, growth factors and protease inhibitors in combination with giving

structural support by using different biomaterial grafts (*Nasisse* and *Nelms* 1992, *Brooks* 2008, *Brooks* et al. 2017). Appropriate graft material has to fulfil different biological and economical requirements for its use in veterinary medicine. The successful use of different biomaterials including equine amnion (*Lassaline* et al. 2005, *Plummer* 2009), porcine urinary bladder (*Mancuso* et al. 2016, *Cichocki* et al. 2017, *Davis* et al. 2019) and porcine small intestinal submucosa (SIS) (*Bussieres* et al. 2004) in the surgical treatment of corneal defects in equine patients has been reported. Transplantation of full or split thickness allogenic corneal grafts is also a valid option to treat corneal defects in horses (*Whittaker* et al. 1997, *Andrew* et al. 2000, *Lassaline* et al. 2005, *Brooks* et al. 2008, *Brooks* 2010, *de Linde Henriksen* et al. 2012) but requires the availability of fresh or frozen cornea from donor horses.

SIS is a non-immunogenic, acellular collagen matrix derived from porcine jejunum (*Lewin* 1999, *Bussieres* et al. 2004, *Featherstone* and *Sansom* 2004, *Vanore* et al. 2007). SIS grafts are composed of the tunica muscularis mucosa, tunica submucosa and the stratum compactum layer of the tunica mucosa. The mesenteric tissue, tunica mucosa, serosa and tunica muscularis are mechanically removed before the remaining tissue is decellularized (*Bussieres* et al. 2004). Former studies describe the experimental and clinical use of SIS grafts as a scaffold medium for regeneration in different types of tissue, such as vessels, fascia, dura, tendons and ligaments (*Badylak* et al. 1989, *Lantz* et al. 1990, *Aiken* et al. 1994, *Cobb* et al. 1999, *Suckow* et al. 1999, *Dejardin* et al. 1999, *Gastel* et al. 2001, *Derwin* et al. 2004, *Fiala* et al. 2007, *Ding* et al. 2015). These SIS grafts have repeatedly been used for the reconstruction of infected or non-infected corneal defects in canine (*Lewin* 1999, *Bussieres* et al. 2004, *Vanore* et al. 2007, *Goulle* 2012) and equine patients (*Bussieres* et al. 2004) with limited complications. In previous reports, SIS grafts were additionally covered with a conjunctival (*Lewin* 1999, *Bussieres* et al. 2004) or third eyelid flap (*Vanore* et al. 2007, *Goulle* 2012) to support tissue integrity.

Case History

A 1-month-old Hanoverian filly was referred to the Clinic for Horses at the University of Veterinary Medicine Hanover with a history of deep corneal ulceration of the left eye. Before admission, the foal had been unresponsive to local treatment with gentamycin, neomycin, polymyxin B and dexamethasone for one week.

Clinical findings

For the examination procedure the foal was sedated with detomidine (¹Cepesedan® 10 µg/kg bwt i.v.), and butorphanol (¹Butorgesic®, 25 µg/kg bwt i.v.). Clinical examination of the left eye revealed severe blepharospasm, epiphora, purulent ocular discharge, photophobia and severe corneal opacification (Fig. 1a). Detailed inspection of the cornea, including slit lamp investigation, showed a central marked rounded area (25 × 15 mm) of deep stromal ulceration. Fluorescein application on the corneal surface resulted in generalized staining

of the defect with decreased intensity at the central area. The entire corneal surface surrounding the defect showed marked oedema with distinct opacification. The limbal area showed a narrow rim of multiple tiny blood vessels. The menace response was questionable and the pupillary light reflex could not be investigated due to the opacification of the whole cornea. Further investigation by direct or indirect visualisation of the globe was ineffective. Severe miosis was the only abnormality observed in the ultrasonographic examination. Cytologic examination showed the existence of fungal hyphae on the corneal surface, microbiological culturing revealed a mild content of *Staphylococcus haemolyticus*, *Weissella confusa* and *Bacillus* species. A mycotic ulcerative keratitis with subsequent keratomalacia was diagnosed.

Treatment

The foal was hospitalised and topical medical treatment was initiated with sodium-EDTA (²Regoculan®), n-Acetylcysteine (³N.A.C. Collyre Cicatrisant®), moxifloxacin (⁴Vigamox®), voriconazole (⁵VFEND®) and atropinsulfate (⁶Atropin-POS®). Initial treatment included systemic anti-inflammatory treatment with flunixin-meglumin (¹Flunidol 5%®, 1.1 mg/kg bwt per os, b.i.d.). Within the first day of treatment the foal was unresponsive to medical therapy and clinical findings worsened significantly. Due to the high risk of corneal perforation associated with ongoing corneal melting, surgical treatment was conducted the following day (Fig. 1b).

Prior to surgery, the foal received trimethoprim-sulfadiazine (⁷Synutrim®, 30 mg/kg bwt per os) and tetanus serum (²Tetanus-Serum®, 5 ml i.m.). The foal was sedated with xylazine (¹Xylavet®, 0.4 mg/kg bwt i.v.) and butorphanol (¹Butorgesic®, 25 µg/kg bwt i.v.) pre anaesthesia. General anaesthesia was induced with ketamin (⁷Narketan®, 2.5 mg/kg bwt i.v.) and diazepam (⁸Ziapam®, 0.1 mg/kg bwt i.v.) and maintained with isoflurane (¹Isofluran CP®) delivered in 100% oxygen, xylazine (¹Xylavet®, 0.6 mg/kg bwt/h CRI i.v.) and dobutamine CRI (⁹Dobutamin-ratiopharm®, 0.6–1.2 µg/kg bwt/min i.v. to effect). Atracurium (¹⁰Atracurium-hameln®, 0.1 mg/kg bwt i.v.), a systemic paralytic muscle relaxant, was administered intraoperatively to facilitate proper globe position. Hence, artificial ventilation was conducted.

A keratectomy of the infected and necrotic parts of the cornea was performed using a #64 Beaver microsurgical blade. The remaining cornea was flushed with 5% N-acetylcysteine (³N.A.C. Collyre Cicatrisant®) and coated with carboxymethyl-glucose-sulfate (¹¹Clerapliq®). Two commercial multilayer SIS grafts (¹²Vetrix® BioSIS Plus⁺ 15 mm Multi-layer Ocular Discs) were fitted adjacent to the corneal defect and placed side by side with a small overlap at the central border. The grafts were sutured to the cornea at their border using multiple simple interrupted sutures (¹³Vicryl®, 8–0 USP) (Fig. 1c). After the placement of the two patches, 0.2 ml of 1% voriconazol (⁵VFEND®) was injected into the anterior chamber of the eye. A subpalpebral lavage catheter was placed to enable topical medication of the cornea postoperatively. The eyelids were closed, covered with moistened swabs and a head bandage was applied using an elastic adhesive bandage (¹⁴Equi-Plast®) to protect the grafts following surgery.

The foal was treated postoperatively with oral trimethoprim-sulfadiazine ([7]Synutrim®, 30 mg/kg bwt per os b.i.d.) and flunixin-meglumin ([1]Flunidol 5%®, 1.1 mg/kg bwt, per os, b.i.d.) for five days. Topical medication of the left eye was continued after surgery with moxifloxacin ([4]Vigamox®), sodium-EDTA ([2]Regoculan®), voriconazol ([5]VFEND®) and autologous serum every three hours and atropinsulfate ([6]Atropin-POS®) twice daily by using the subpalpebral lavage catheter. The foal showed mild diarrhoea at day five after surgery, hence, systemic medication was stopped.

Faecal output showed no further abnormalities in the following days.

The head bandage was changed at day three and six after surgery. At day six post-surgery, subjective evaluation revealed a mild loss of intraocular pressure, additionally one of the grafts showed decreased thickness at the non-overlapping part. All sutures were still in place. At day eight, intraocular pressure seemed unremarkable, but the lower lid showed mild entropion. Coverage by a head bandage was discontinued
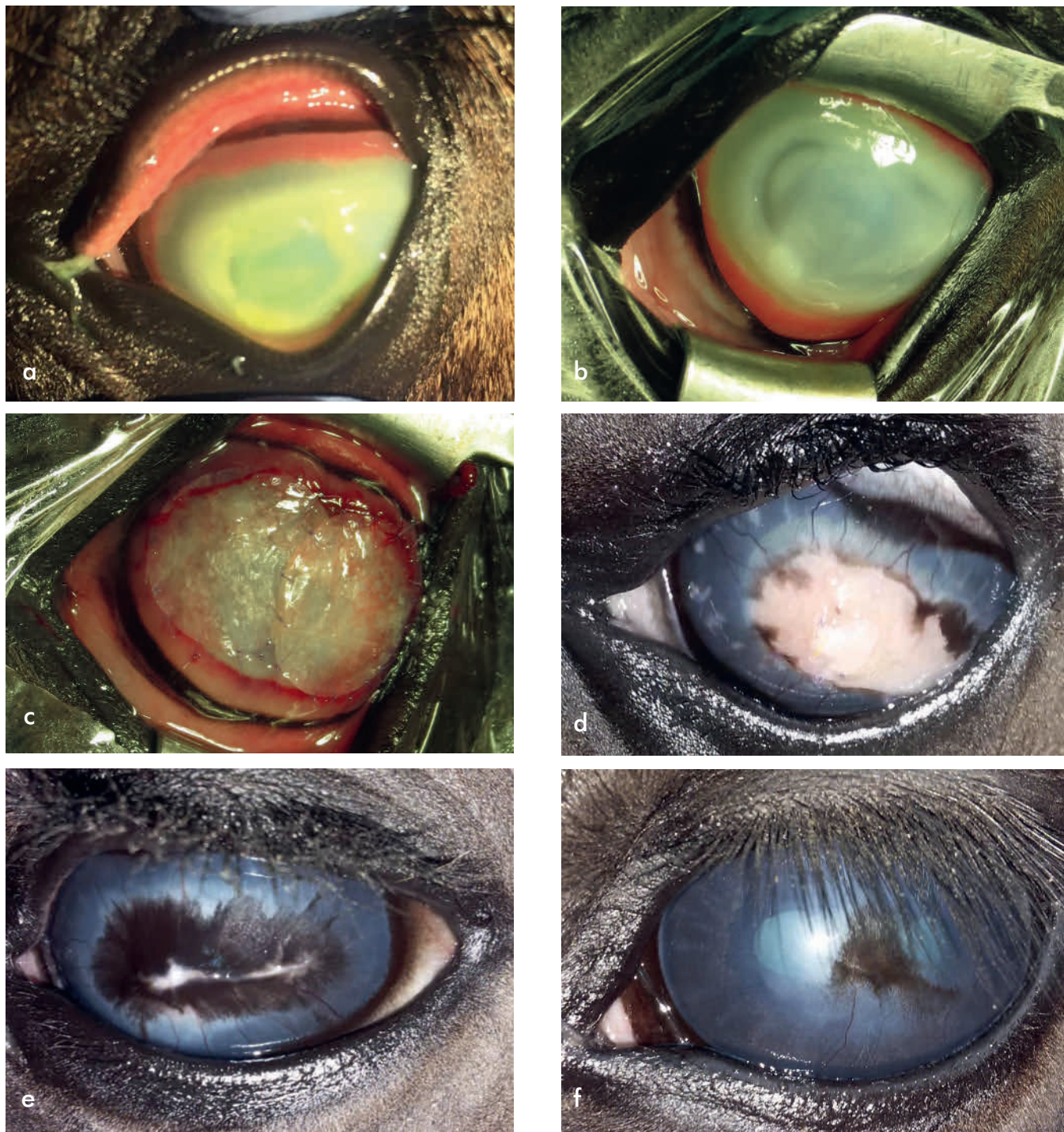

Fig. 1   Photograph of the left eye: a) at the time of presentation; b) and c) at the day of surgery, b) before and c) after surgery; d) four weeks post-surgery; e) six weeks post-surgery; f) five months post-surgery | *Foto des linken Auges: a) zum Zeitpunkt der Vorstellung; b) und c) am Tag der Operation, b) vor und c) nach der Operation; d) vier Wochen nach der Operation; e) sechs Wochen nach der Operation; f) fünf Monate nach der Operation.*

at this time and the entropion was corrected manually several times a day. The subpalpebral lavage system was left in situ for the time of hospitalization. Increasing cell proliferation and peripheral corneal neovascularization was observed from day ten postoperatively. Furthermore, the entropion was no longer present.

At the time of discharge, the SIS graft material was completely integrated into the opaque granulation tissue and multiple marked vessels surrounded the former defect (Fig. 1d). The foal was discharged four weeks postoperatively.

Outcome

Follow-up information is available until five months postoperatively. After discharge, the foal underwent clinical examination at six weeks and five months postoperatively by one of the authors. At six weeks postoperatively, the corneal surface affected was still opaque and substantially darkened (Fig. 1e). However, at five months postoperatively, the transparency of the affected cornea had significantly increased and neovascularization of the cornea had decreased comparably. A small pigmented scar at the central area of the cornea, mild vascular ingrowth and a minor oedema was still visible (Fig. 1f). No ocular discomfort was present at any after discharge. Dazzle reflex was positive at each time of examination.

Discussion

Different infectious and non-infectious agents can cause infiltrative ulcerative keratitis with keratomalacia. Infectious disease primarily includes bacterial and fungal keratitis. Viral keratitis is a rare condition. Non-infectious disease includes foreign body penetration, sterile keratitis and eosinophilic keratoconjunctivitis. Nonulcerative conditions, such as immune-mediated keratitis, stromal abscess formation, calcific band keratopathy or corneal neoplasia also need to be considered (*Brooks* et al. 2017).

In the present case, a mycotic ulcerative keratitis with keratomalacia was diagnosed. Fungal keratitis is one of the most common ophthalmologic disease in horses. Due to the horse's environment different fungi, especially *Aspergillus* and *Fusarium* subspecies, are present on the equine conjunctiva and cornea (*Whitley* et al. 1983, *Samuelson* et al. 1984, *Moore* et al. 1988, *Sherman* et al. 2017). Corneal traumata can enable these fungi to easily enter the deep corneal stroma. Furthermore, defects in the equine corneal immune system in combination with the large exposed corneal surface make horses more susceptible for keratomycosis than other species (*Andrew* et al. 1998, *Galán* et al. 2009, *Brooks* et al. 2013). Before admission, the foal was treated with local antimicrobial and corticosteroid medication. Although the findings of the ophthalmologic examinations before admission are unknown, worsening of the corneal damage due to corticosteroid therapy has to be assumed. Topical corticosteroid therapy is known to interfere with cellular immunity, corneal re-epithelization, vascularization and delay of non-specific inflammatory response (*Andrew* et al. 1998, *Thomas* 2003, *Galán* et al. 2009). Moreover, an association between corticosteroid

therapy and the formation of keratomycosis is assumed because corticosteroids can inhibit growth of nonpathogenic bacterial strains and thus enhance fungal replication (*Coad* et al. 1985, *Brooks* et al. 1998, *Sansom* et al. 2005, *Ledbetter* et al. 2007). However, two studies showed no difference in the outcome of horses suffering from keratomycosis after pretreatment with local corticosteroids (*Galán* et al. 2009, *Sherman* et al. 2017).

Severe "melting" corneal lesions often require surgical therapy due to the high risk of corneal perforation. Additionally, pathogenic fungi appear to have a specific affinity to the posterior stroma and the Descemet's membrane, enhancing the risk for abscess formation (*Brooks* et al. 2017). In the case described, the foal showed severe signs of keratomalacia and was unresponsive to topical medical therapy. Thus, surgical therapy was conducted. Surgical intervention in horses suffering from keratomalacia incorporates debridement of the lesion to remove the necrotic or melted tissue and subsequent reconstruction of the cornea (*Gelatt* et al. 2013). The purpose of corneal reconstruction with different corneal grafts is to enable the migration and differentiation of epithelial cells, reinforcement of cellular adhesion and decrease of the ocular surface inflammation. The main goal is to achieve healing without impairing long-term corneal transparency.

Previous literature describes the use of different biomaterials as corneal grafts in horses (*Bussieres* et al. 2004, *Lassaline* et al. 2005, *Plummer* 2009, *Mancuso* et al. 2016, *Davis* et al. 2019). Conjunctival grafting is the most wide-spread technique for this condition (*Gelatt* et al. 2013). Conjunctival autografts support the corneal healing with the patient's own cellular and acellular components and vascular supply. It provides sufficient tissue to strengthen weakened corneas, but often results in corneal scars that can impair the vision and occasionally perforates due to insufficient mechanical support (*Ollivier* 2005, *Brooks* 2008, *Brooks* et al. 2017). Other biomaterials, such as equine amnion (*Lassaline* et al. 2005, *Plummer* 2009), porcine urinary bladder (*Mancuso* et al. 2016, *Davis* et al. 2019) and porcine small intestinal submucosa (*Bussieres* et al. 2004) caused less scarring and resulted in better corneal transparency in equine patients. Transplantation of allogenic amniotic membranes has been used successfully for ocular surface reconstruction in horses (*Lassaline* et al. 2005, *Plummer* 2009). Amnion consists of a thick basement membrane and an avascular stromal matrix. The relatively acellular and strong tissue makes it suitable as a corneal graft. However, the lack of commercial availability for veterinary medicine and, thus, the necessity of donor horses to acquire fresh or stored graft material limited its use in the recent past (*Plummer* 2009). Porcine SIS and porcine urinary bladder are both extracellular matrix (ECM) products which have resulted in good corneal transparency (*Bussieres* et al. 2004, *Mancuso* et al. 2016, *Davis* et al. 2019). The lack of cellular components is thought to be associated with a reduced immune response, which could affect graft rejection. The ECM forms a scaffold for cellular and vascular ingrowth and provides different endogenous growth factors (*Bussieres* et al. 2004, *Vanore* et al. 2007, *Mancuso* et al. 2016). Both products are currently commercially available for veterinary use.

BioSIS Plus+ is produced from the porcine jejunum. The superficial layers of the tunica mucosa, the tunica muscularis externa and the tunica serosa are removed. The remaining tissue represents the SIS and consists of the tunica submucosa and basilar layers of the tunica mucosa. The tissue is decellularized and consists entirely of ECM (*Vanore* et al. 2007). Each BioSIS Plus+ graft consists of four of these single layer grafts lying upon another (*Barachetti* et al. 2016). The acellular collagen matrix acts as a scaffold medium for fibroblasts which are later replaced by corneal stroma cells (*Vanore* et al. 2007). The ECM product also consists of fibronectin, hyaluronic acid, chondroitin sulphate, heparin sulphate and growth factors (*Vanore* et al. 2007). Among these, transforming growth factor-beta has also been shown to inhibit the synthesis of metalloproteinases (*Roberts* et al. 1990).

Postoperatively, the graft material is prone to desiccation or mechanical irritation by continuous eyelid movement or abrasion due to pruritus. Covering the graft material can prevent implant failure (*Goulle* 2012). Conjunctival flaps (*Bussieres* et al. 2004) or a temporary tarsorrhaphy (*Mancuso* et al. 2016, *Davis* et al. 2019) have been used to cover porcine SIS or urinary bladder grafts in horses in the past. The large size of the defect in this case impeded the use of a conjunctival graft to cover the SIS grafts. Although, conjunctival grafts can lead to an enhanced opacity of the cornea, as described previously. The use of a temporary tarsorrhaphy precludes the clinical evaluation of the affected eye in the postoperative period (*Mancuso* et al. 2016). This is important, as keratomalacia is often related to some degree of iridocyclitis (*Brooks* et al. 2017). Thus, tarsorrhaphy was routinely partially opened at three to five days postoperatively in a previous study to allow the evaluation of globe integrity, peripheral corneal and anterior chamber transparency and pupil size (*Mancuso* et al. 2016). Potential benefits of a prolonged time of temporary tarsorrhaphy may lead to improved corneal comfort, providence of structural support to the surgical site and an increased corneal contact time of topical medications (*Mancuso* et al. 2016).

Compared to previous descriptions, we used porcine SIS grafts (BioSIS Plus+), which consist of four single layers laminated together. The enhanced thickness of this material makes it more resistant to mechanical irritation, which minimises the need for an additional coverage. In the case described, the corneal surface and the adjacent grafts have been protected by closed eyelids, maintained by a head bandage, for eight days. No tarsorrhaphy was performed. This enabled simple investigation of graft integrity at every bandage change. Subsequently, the cornea was left uncovered. At six days postoperatively, a decreased thickness of the graft material was observed, potentially provoked by mechanical irritation. Hypothetically, this could have led to graft failure if a less resistant material, such as single-layer SIS or urinary bladder grafts, were used. Desiccation, direct mechanical irritation or implant failure were not observed at any time. Progressive clearing of the cornea with only very mild impairment of vision was observed during follow-up examinations.

Due to the surgeon's personal experience, post-operatively 1% voriconazole has been injected intracameral. Intracameral injection of voriconazole did not induce a significant gross change in rabbit corneal endothelial cells up to a concentration of 1%. However, risk of microstructural damages might exist with a concentration of $\geq 0.25\%$ (*Han* et al. 2012).

To conclude, the use of multilayer SIS grafts is an appropriate technique for the treatment of horses suffering from mycotic corneal ulcers and accessory keratomalacia. Furthermore, this report shows that covering the grafts with a replaceable head bandage instead of tarsorrhaphy or an additional conjunctival flap can be beneficial in different aspects.

Author's declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Not applicable

Source of funding

No funding received

Authorship

All authors collaborated in case management. Alexander Schwieder prepared the manuscript. All authors gave their final approval of the manuscript.

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