Veterinary Ophthalmology (2005) **8**, 5, 311–317

Equine amniotic membrane transplantation for corneal ulceration and keratomalacia in three horses

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Abstract

Purpose Amniotic membrane has antifibrotic, anti-angiogenic and antiprotease properties. This retrospective study was designed to investigate the use of equine amniotic membrane transplants (AMT) at preserving vision, maintaining the structural integrity of the globe and maximizing cosmesis in equine eyes with corneal ulceration and severe keratomalacia.

Methods Equine amnion had previously been aseptically harvested from a 12-year-old Thoroughbred mare during an elective Cesarean section. Sections of amnion were stored at −80 °C and thawed as needed. Records of equine cases at the University of Florida with keratomalacia that received an AMT without adjunctive conjunctival grafting were examined. Clinical description, details of medical and surgical treatment, globe survival and visual outcome were documented. Etiologies were determined by cytology, culture or histology.

Results Three horses with corneal ulceration and severe keratomalacia received an AMT without conjunctival graft between December 2002 and April 2003. *Pseudomonas* spp. were cultured from all three eyes, with evidence of a concurrent fungal infection in two eyes. The three ulcers were 50, 72, and 76% of corneal diameter, and each one worsened in the face of aggressive medical therapy. In all three cases, the AMT sloughed over a 4 to 6-week period. At last follow-up, all three eyes receiving AMT were comfortable and receiving no medication, with light perception and an inconsistent location-dependent menace response. All three horses returned to their prior work.

Conclusions Results of a small number of equine AMT suggest that amnion can be used successfully to preserve both globe structure and limited vision, as well as optimize cosmesis, in horse eyes with corneal ulceration and severe keratomalacia.

Key Words: amniotic membrane, corneal ulceration, horse, keratomalacia

INTRODUCTION

There are numerous reasons why the ocular surface may require reconstruction, including tectonic support, restoration of optical clarity, excision of inflamed, necrotic or infected tissue, and replacement of missing tissue. Corneal stromal melting and necrosis, more commonly called keratomalacia in the veterinary literature, is particularly problematic in terms of ocular surface reconstruction, and provides a considerable challenge because of the poor quality of corneal tissue. Necrotic stroma is often inflamed and infected, and of poor tensile strength for holding sutures. The goal in any ocular surface reconstruction problem is to preserve the integrity of the globe, provide tectonic support, optimize the visual outcome, and minimize the scar. This case series explores the potential of amniotic membrane transplantation to provide a solution to the problem of corneal ulceration and keratomalacia.

Featherstone has provided a set of criteria that a biomaterial should meet to be successful as a corneal transplant.[1] These include optical clarity, support of epithelial migration and adhesion, solute permeability and stability to corneal proteases. Historically, biomaterials used in ocular surface reconstruction have included partial thickness epidermal grafts,[2] oral mucous membrane,[3] vaginal mucous membrane and fetal membrane,[4] rabbit peritoneum,[5] and amnion.[6–10] Veterinary use of biomaterials to reconstruct the ocular surface have included conjunctival grafts,[11] Gore-tex,[12]

split-thickness dermal grafts,[13] equine pericardium,[14] peritoneum,[15] equine amniotic membrane,[16] and equine renal capsule[17] in dogs; porcine small intestinal submucosa in dogs, cats and horses;[1,18,19] and human amniotic membrane in rabbits.[20] Most of these have been controlled experimental studies with artificial wounds created in normal cornea. Melting corneal ulcers that do not respond to medical therapy alone are typically treated with a conjunctival graft, which often leaves a significant scar and may obscure the visual axis.

Several studies have indicated that amniotic membrane can be useful in wound healing in general, demonstrating less proud flesh in distal limb wounds,[21] faster healing in full-thickness trunk wounds in dogs than in other nonadherent bandages,[22] and faster healing of distal limb pinch grafts.[23] Amniotic membrane is a good candidate for use in ocular surface reconstruction because it is avascular, contains anti-angiogenic and anti-inflammatory factors,[24] contains growth factors,[25] and is antifibrotic.[26] In three cases reported here, amniotic membrane transplantation, without adjunctive conjunctival grafting, was used to treat corneal ulceration with severe keratomalacia that was not responsive to medical therapy alone.

MATERIALS AND METHODS

Equine amnion was collected for use in equine corneal wound healing. An equine amnion was aseptically harvested from a 12-year-old Thoroughbred mare during an elective Cesarian section for a term pregnancy following surgical correction of a large colon torsion. Surgery was performed at an equine referral hospital. The mare and colt survived the surgery. The fetal membranes were removed along with the foal under sterile conditions. The allantoamnion was separated from the allantochorion, and was rinsed with sterile saline containing penicillin (Spectrum, Gardena, CA, USA) and streptomycin (Spectrum). Allantoamnion was then shipped on ice to the University of Florida. Once there, the amnion was separated from the allantois by blunt dissection, sectioned, and placed on a nitrocellulose membrane (Biorad Laboratories, Richmond, CA, USA) in Delbecco's modified Eagle's medium (DMEM; Fischer Scientific, Hampton, NH USA) with glycerol (Fischer Scientific), penicillin (Spectrum), streptomycin (Spectrum), neomycin (Spectrum) and amphotericin B (Spectrum). It was then stored for use. Individual sections were thawed as needed. The epithelium of the amnion dies during freezing, leaving only the basement membrane and stroma available for use.

For surgical use of amniotic membrane, an individual section was thawed and rinsed with sterile saline for 30 min to remove any remaining glycerol in which it was stored. Amniotic membrane was left on the nitrocellulose membrane with the allantoic side against the paper until just prior to placement on cornea. Theoretically, orientation of amniotic membrane against the cornea determines whether it will be incorporated into a corneal scar, or sloughed, because the recipient corneal epithelium migrates along the amnion's basement membrane. If the stromal (allantoic) side of the amniotic membrane faces the recipient cornea, the amniotic membrane should be incorporated; but if the basement membrane of the amnion faces the recipient cornea, the amniotic membrane should slough.[27]

Records of equine cases at the University of Florida with keratomalacia that received an amniotic membrane transplant without adjunctive conjunctival grafting were examined. Clinical description, details of medical and surgical treatment, and outcome were documented. Etiologies were determined by cytology, culture or histology.

RESULTS

Three horses with corneal ulceration and severe keratomalacia received an amniotic membrane transplant without conjunctival graft between December 2002 and April 2003. In all three cases, general anesthesia was routinely induced and maintained with the horse in lateral recumbency, the affected eye was routinely prepared for ocular surgery, any intraoperative diagnostic samples were collected, the cornea was debrided, and an amniotic membrane was sutured to the cornea using 8-0 Vicryl (Ethicon; Somerville, NJ, USA). For all three cases, recovery from anesthesia was uneventful. Details of each case, including history, clinical findings, diagnostics, therapy, and outcome, are listed below. Performance prior to corneal ulceration and following surgical repair was used as a measure of success of the surgical procedure.

Case 1

Case 1 was a 4-year-old Thoroughbred mare that had accumulated a significant amount of debris in her left eye (OS) during a race. One week following the race, a corneal abrasion was noted by the referring veterinarian, and was treated with gentamicin ophthalmic ointment (Schering-Plough Animal Health, Kenilworth, NJ, USA). Two weeks following the race, the referring veterinarian collected a corneal scraping OS for cytologic analysis, and detected fungal hyphae in the sample. Treatment with 5% natamycin ophthalmic suspension (Alcon Laboratories, Ft Worth, TX, USA) and triple antibiotic ophthalmic ointment (Pfizer Animal Health, Cambridge, MA, USA) OS was instituted. Three weeks following the race, the ulcer OS worsened in the face of these medications, and treatment was changed by the referring veterinarian to miconazole, tobramycin (Alcon Laboratories), autologous serum, and atropine (Alcon Laboratories). The mare was admitted to the University of Florida Veterinary Medical Teaching Hospital (UF-VMTH) 1 month following the race with a melting corneal ulcer OS.

At admission to UF-VMTH, examination of the mare's left eye revealed severe blepharospasm and epiphora, absent pupillary light reflexes (direct and indirect), an absent menace response, severe corneal edema, neovascularization, 3+ aqueous flare, fibrin in the anterior chamber, and a melting ulcer involving 50% of the corneal diameter (Fig. 1a). Her attitude was quiet but alert and responsive.

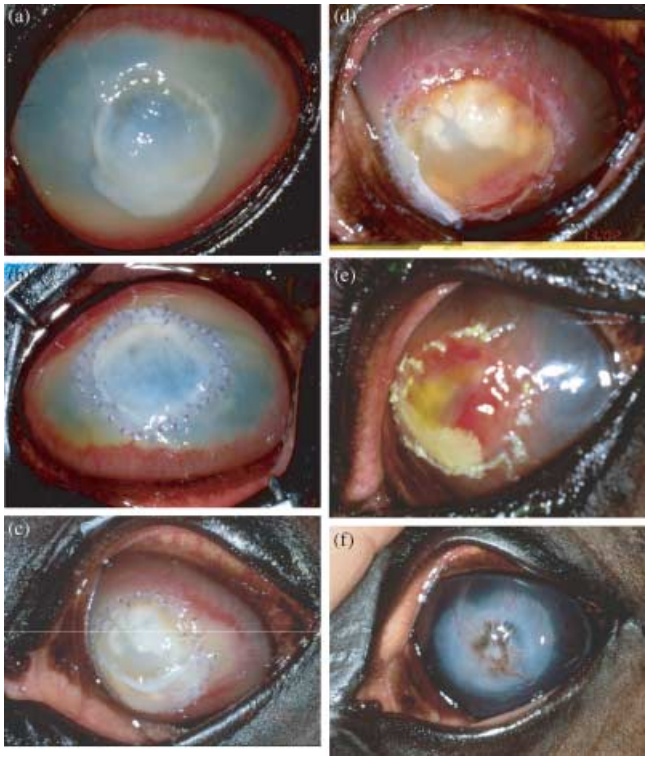

Figure 1. (a) Case 1, OS, day 1 (at admission), with profound blepharospasm, epiphora, large central melting ulcer with deep peripheral neovascularization extending from limbus approximately 8 mm axially, corneal edema, hypopyon, and miosis. (b) Case 1, day 2, at surgery, with double layer AMT sutured in place. (c) Case 1, day 6, 4 days postoperatively, with outer layer AMT sloughing, corneal vascularization advanced dorsally and ventrally to periphery of transplant, and xanthochromia. Anterior chamber is not visible. (d) Case 1, day 14, 12 days postoperatively, with both layers of AMT sloughed, corneal vascularization advanced beyond periphery of ulcer, central corneal xanthochromia, and peripheral corneal clearing. Anterior chamber still not visible. (e) Case 1, day 20, 18 days postoperatively, eye more comfortable, but light reflexes and vision response absent. Lateral cornea clearing such that anterior chamber and peripheral iris visible. Corneal sutures beginning to dissolve. (f) Case 1, 6 months postoperatively. Dazzle and consensual pupillary light reflexes positive, but menace response inconsistent. Eye comfortable, with clear peripheral cornea, central corneal fibrosis and pigmentation, and few faint vessels remaining from limbus to central cornea.

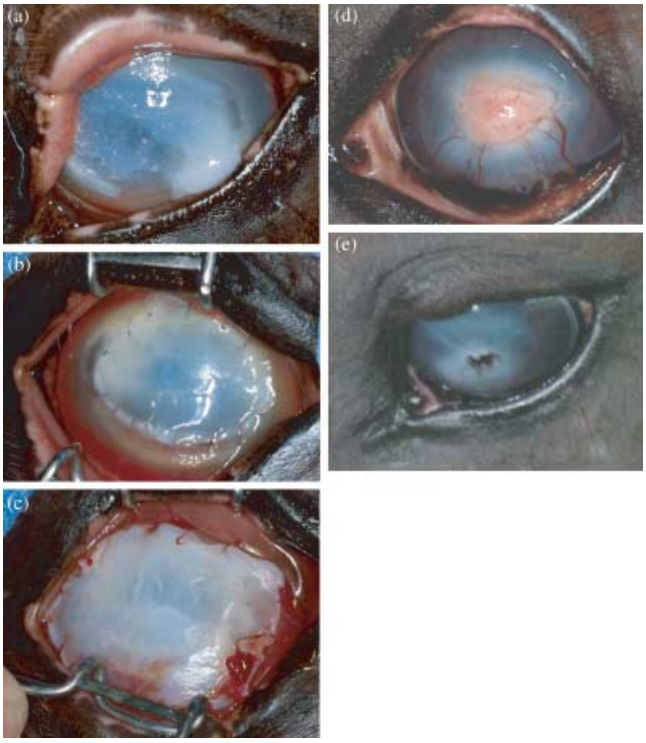

Figure 2. (a) Case 2, OS, at admission, with large deep melting ulcer that developed following dehiscence of upper lid laceration repair. Menace response and dazzle reflex absent but consensual pupillary light reflex positive. Peripheral corneal vascularization extends 2 mm axially from limbus. Cornea is edematous. Anterior chamber not visible. (b) Case 2, day 4, intra-operatively, with AMT sutured in place over melting ulcer. (c) Case 2, intra-operatively, with Acell membrane covering entire cornea, sutured at limbus. (d) Case 2, 2 months postoperatively, with central corneal granulation tissue and peripheral vascularization. Peripheral cornea clearing enough such that anterior chamber and peripheral iris visible. Consensual pupillary light reflex and dazzle reflex positive, but menace response absent. (e) Case 2, 6 months postoperatively, with inconsistent menace, central fibrosis and pigment but peripheral corneal clearing.

Diagnostics Cytology collected on day 1 (the day of admission) revealed Gram-negative rods. Histology collected intra-operatively on day 2 revealed fungal hyphae. Bacterial culture on day 1 showed no growth of infectious agents, but culture taken on day 4 grew a *Pseudomonas* species sensitive to carbenicillin, gentamicin, neomycin, polymyxin, tobramycin, and enrofloxacin (5 mg/kg IV q24 h; Bayer Animal Health, Shawnee Mission, KS, USA).

Treatment Because of suspicion of concurrent fungal infection (based on prevalence of fungal keratitis in melting ulcers at UF-VMTH), along with a Gram-negative bacterial infection suggested by cytology at admission, and severe uveitis, treatment was instituted at admission (following cytologic examination) with itraconazole (3 mg/kg PO q12 h; Wickliffe Pharmaceuticals, Lexington, KY, USA), lufenuron (5 mg/kg PO q6 h; Novartis, Greensboro, NC, USA), doxycycline (10 mg/kg PO q2 h; generic), omeprazole (Gastrogard; 2.26 g PO, QD, Merial, Iselin, NJ, USA), dexamethasone (20 mg PO QD for 1 day, then 10 mg PO QD for 2 days, then 5 mg PO QD for 2 days; Schering-Plough Animal Health) and flunixin (500 mg PO q12 h; Schering-Plough Animal Health), and topical natamycin q4 h, tobramycin q2 h, serum q1 h, and atropine PRN until dilation was achieved, but no more frequently than QID. All topical medications were delivered via a subpalpebral lavage system using 0.2 mL of medication followed by 3 mL of air to flush the tubing. Because the ulcer continued to severely melt over the first 24 h of hospitalization such that there was a significant risk of perforation, even

in the face of aggressive medical therapy, the mare received a double layer AMT on day 2 (Fig. 1b). A temporary tarsorrhaphy was placed OS at the time of surgery and remained in place for recovery and for 2 days postoperatively.

The mare's comfort increased and her attitude brightened throughout the first week postoperatively. Medication frequencies were adjusted as dictated by corneal healing. Her activity was restricted to stall confinement with frequent hand-walking. The dazzle and consensual pupillary light reflex returned within several days of surgery, but a menace response was not present during the mare's hospitalization. By day 6, the outer layer of the AMT had begun to slough, and corneal vessels had advanced from the limbus to reach the periphery of the outer AMT (Fig. 1c). By day 14, the outer AMT had completely sloughed, and the inner layer was beginning to slough, as corneal vascularization reached the periphery of the inner AMT, and the peripheral cornea began to clear (Fig. 1d). By day 20, both layers of the AMT had sloughed, corneal vascularization had reached the central cornea, and the anterior chamber was visible through the peripheral cornea (Fig. 1e).

The mare was discharged from the hospital on day 23. At that time, the left eye was fluorescein negative but there was still no menace response. Treatment at the time of discharge included a decreasing dose of flunixin and topical atropine, such that within 2 weeks of discharge no treatment was being given.

Follow-up The mare returned to training 2 months after discharge from the hospital, had her first start 4 months following discharge, and at follow-up examination 5 months after discharge she had an inconsistent positive menace response and a positive dazzle reflex. She won a race 6 months after discharge, and was considered to have returned to a level of performance comparable to that which she had achieved prior to the injury OS. Six months following discharge, there was central corneal fibrosis with pigmentation and vascularization, but the peripheral cornea was clear (Fig. 1f).

Case 2

Case 2 was a 12-year-old Hanoverian stallion used primarily for dressage. He sustained a lid laceration OS with subsequent cicatricial entropion. A corneal ulcer developed secondary to the entropion 2 weeks following the lid laceration. The stallion was admitted to UF-VMTH several days after development of the corneal ulcer, which at the time of admission was 72% of the corneal diameter in size. At the time of admission, menace response and dazzle reflex were negative OS, but consensual pupillary light reflex OD was positive. There was corneal ulceration with profound keratomalacia, edema and vascularization extending 2 mm from the limbus OS (Fig. 2a). The anterior chamber, lens and fundus were not visible OS. The stallion's attitude remained bright, alert and responsive throughout hospitalization. His activity was restricted to stall confinement with occasional hand-walking.

Diagnostics Cytology collected on day 1 (admission) revealed Gram-negative rods. Culture from day 1 grew *Pseudomonas* species sensitive to carbenicillin, gentamicin, neomycin, polymyxin, and tobramycin. Culture from day 7 grew a Gram-negative rod sensitive to chloramphenicol and enrofloxacin.

Treatment At the time of admission, therapy was instituted with topical natamycin, serum, EDTA, tobramycin, and atropine, and systemic flunixin and gentamicin. As with Case 1, all topical medications were delivered via a subpalpebral lavage system. The corneal melting progressed in the face of medical treatment, and thus an AMT was placed over the ulcerated cornea under general anesthesia on day 4 (Fig. 2b). The AMT was covered with a single layer of A-cell (Acell, Inc., Jessup, MD, USA), an acellular matrix made from porcine urinary bladder designed to provide scaffolding for wound healing (Fig. 2c). A temporary tarsorrhaphy was placed peri-operatively. The A-cell membrane had completely dissolved by the time the temporary tarsorrhaphy was removed 3 days following surgery, so it is unclear what role it played in wound healing.

Follow-up The patient was hospitalized for 10 days, with frequency of medications adjusted as dictated by corneal healing. He was discharged on day 11, with continued treatment at home until the AMT completely sloughed, and there was no fluorescein stain uptake. The stallion gradually returned to work once treatment was stopped. Two months postoperatively the left eye had central corneal granulation tissue with some remaining peripheral vascularization, but the peripheral cornea was clear enough to allow a view of the anterior chamber and peripheral iris (Fig. 2d). At that time, there was a consensual pupillary light reflex OD, a positive dazzle OS, but no menace OS. By 6 months postoperatively, an inconsistent menace had returned, and the appearance of the cornea was similar to that of case 1, with central fibrosis and pigment but peripheral corneal clearing (Fig. 2e).

Case 3

Case 3 was a 3-year-old Thoroughbred colt admitted to UF-VMTH with a 7-day history of a corneal ulcer OS. Prior to development of the ulcer, the colt started several times as a 2-year-old and was in training for his 3-year-old season. Prior treatment by the referring veterinarian included topical tobramycin, atropine, and serum, and systemic flunixin. When the cornea was believed to be free of fluorescein stain uptake, treatment with topical steroids was elected. The ulcer worsened over the 7-day period, leading to referral. At admission to UF-VMTH, the corneal ulcer was 76% of corneal diameter in size, with negligible corneal vascularization and only mild keratomalacia (Fig. 3a). Iris, pupil, lens and fundus were not visible through the corneal edema and menace response was negative OS, but there was a positive dazzle OS and consensual pupillary light reflex OD. The colt was depressed but maintained a good appetite for the first 3 days in hospital.

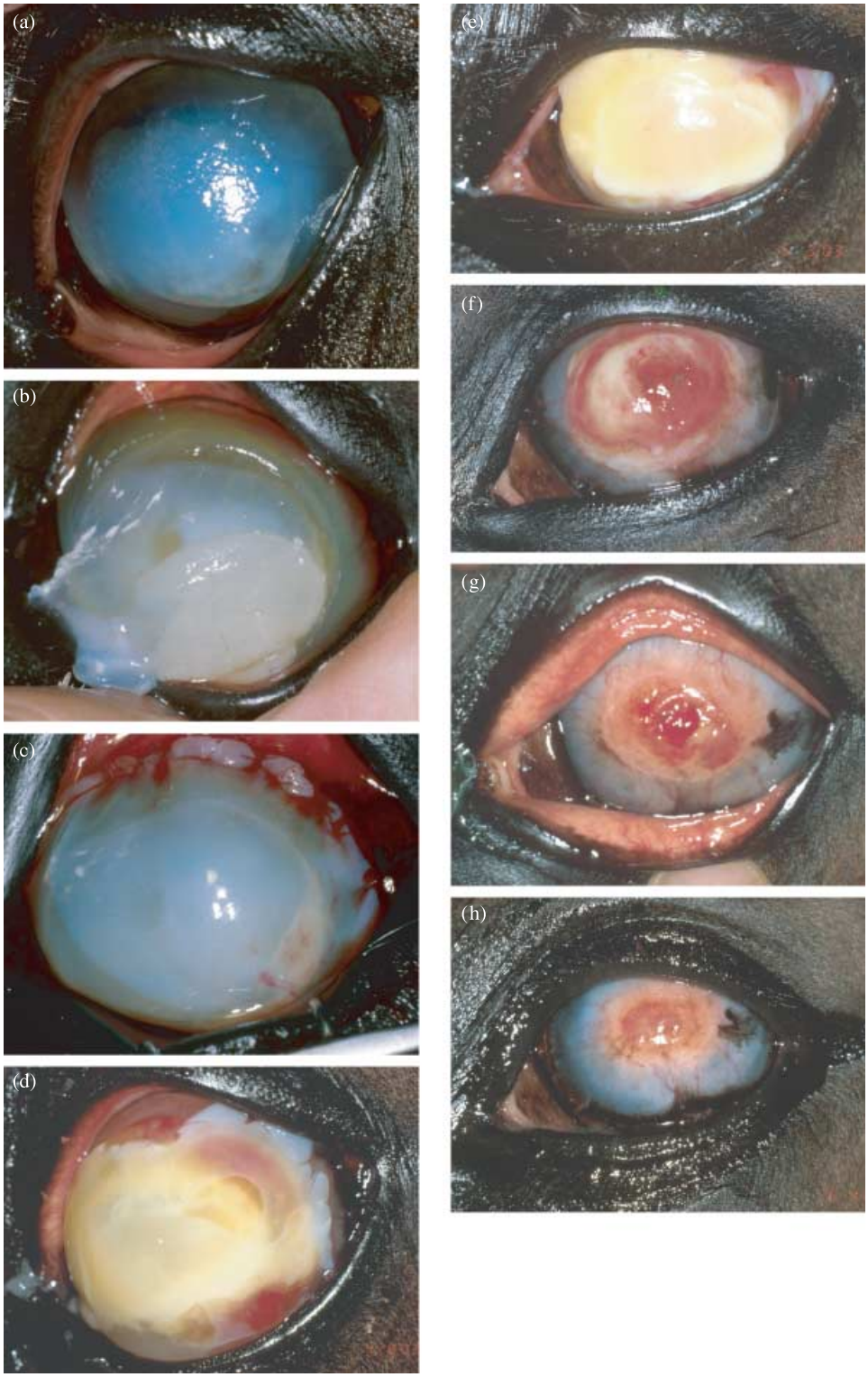

Figure 3. (a) Case 3, OS, at admission, with corneal ulcer 0.76 corneal diameter in size, with negligible corneal vascularization and mild keratomalacia; iris, pupil, lens and fundus not visible through corneal edema; menace response negative, but dazzle reflex and consensual pupillary light reflex positive. (b) Case 3, at surgery on day 5, prior to application of AMT, with profound and rapidly progressive keratomalacia and central corneal necrosis, corneal xanthochromia, and deep corneal vascularization. (c) Case 3, at surgery on day 5, with AMT sutured in place. AMT placed blanket-style, extending from limbus to limbus, and covering entire cornea. (d) Case 3, 2 weeks postoperatively, with peripheral corneal vascularization progressing axially to edge of AMT, which sloughed from periphery but remained adhered centrally. (e) Case 3, 3 weeks postoperatively, demonstrating progressive AMT sloughing and peripheral corneal clearing. (f) Case 3, 4 weeks postoperatively, with irregular raised bed of granulation tissue centrally. (g) Case 3, 6 weeks postoperatively, with granulation tissue flattening and contracting. (h) Case 3, 7 weeks postoperatively, with extensive corneal fibrosis, mild corneal pigmentation, and central plaque of granulation tissue.

Diagnostics Cytology at admission showed fungal hyphae. Culture taken on day 1 grew *Aspergillus* spp., and *Pseudomonas* spp. sensitive to carbenicillin, enrofloxacin, and polymyxin. Repeat culture on day 4 revealed no growth of infectious agents. A repeat culture on day 19 grew *S. zooepidemicus*.

Treatment At admission, treatment with topical natamycin, ciprofloxacin, serum and atropine, along with systemic flunixin, was instituted. Although the colt was at high risk for development of gastric ulcers, treatment with systemic omeprazole was declined. As with cases 1 and 2, topical ophthalmic medications were delivered through a subpalpebral lavage system. Due to rapid progression of corneal melting (Fig. 3b), a blanket-style (limbus to limbus) AMT was performed under general anesthesia on day 5 (Fig. 3c). Systemic enrofloxacin was added to the colt's treatment. The patient experienced a low-grade fever of unknown origin (temperature less than 103°F that was unresponsive to systemic flunixin) during his first week in hospital. Thoracic ultrasound showed no significant abnormalities, and frequent complete blood counts and serum chemistries were within normal limits except for hyperbilirubinemia.

Follow-up Throughout the colt's hospitalization, the iris, pupil, lens and fundus OS were not visible, but a dazzle reflex OS and consensual pupillary light reflex OD remained positive. Ocular ultrasound at several points during hospitalization showed a clear vitreous with no retinal detachment OS. Remarkable considering the extent of corneal pathology was the colt's apparent comfort once the AMT was placed – he routinely held the eye wide open OS. Medication frequency was adjusted, as with cases 1 and 2, as determined by corneal wound healing.

At 2 weeks postoperatively, the peripheral AMT had begun to slough from the underlying cornea, as the peripheral cornea vascularized (Fig. 3d). Cornea under the AMT remained xanthochromic. Because his condition was considered stable, and the colt was becoming more difficult to manage due to his extended stall confinement (even with frequent hand-walking and brief, daily small paddock turnout) he was discharged on day 19. At recheck examination 3 weeks postoperatively there was progressive sloughing of AMT, and clearing in the peripheral cornea (Fig. 3e). Four weeks postoperatively there was an irregular raised bed of granulation tissue occupying the central cornea (Fig. 3f), which had begun to flatten and contract by 6 weeks postoperatively (Fig. 3g). At 7 weeks postoperatively there was extensive corneal fibrosis with mild pigmentation and a central plaque of granulation tissue remaining (Fig. 3h). The colt eventually returned to training but was not raced for the remainder of his 3-year old season, after which he was lost to follow-up.

DISCUSSION

Corneal ulceration and keratomalacia can provide a significant challenge to the combined goals of maintaining the structural integrity of the globe while optimizing corneal clarity in horses. Traditionally, conjunctival grafts are used to treat melting corneal ulcers that do not respond to medical therapy alone. Conjunctival grafts, though, particularly for large-diameter ulcers, cause significant scarring and may impair vision, and do not always provide sufficient tectonic support. Amniotic membrane, which has been used to facilitate nonocular wound healing, as well as to reconstruct diseased cornea, has been suggested as a material useful in meeting both goals of strength and clarity. The amnion is the layer of the fetal membrane that envelops the fetus, and is attached to the rest of the placenta only at the umbilicus. Amnion is composed of an epithelium, a thick basement membrane, and an avascular stroma. Several qualities of amniotic membrane make it a good candidate for use in ocular surface reconstruction. These include that it is avascular and strong, contains anti-angiogenic and anti-inflammatory factors,[24] contains growth factors[25] and is antifibrotic.[26] Basement membranes in general facilitate epithelial migration, reinforce epithelial adhesion, and prevent epithelial apoptosis.[28]

The mechanism of action by which amnion exerts its anti-angiogenic and anti-inflammatory properties is unknown. One hypothesis is that amnion provides oxygen permeability, epithelial hydration, and mechanical protection from the eyelid for the cornea, in effect acting like a bandage contact lens.[29] Other authors have argued that amnion supports corneal wound healing through a combination of mechanical and biologic factors.[27]

The principles of application of amnion are dictated by its composition of a single layer of cuboidal epithelial cells, a basement membrane, which is a network of reticular fibers, and a stroma. Amnion can be applied as a single sheet, or multiple sheets in a blanket-fold. Use of nonabsorbable sutures for controlled removal is recommended by some authors.[27] Orientation of application of amniotic membrane on the cornea, epithelium up (i.e. basement membrane if frozen amnion is used, as epithelium is lost during freezing), or stroma up determines whether the AMT will be incorporated into the recipient cornea or sloughed. This is because regenerating corneal epithelium grows along the AMT basement membrane.[27]

In the three cases of severe corneal ulceration with keratomalacia reported here, all of which involved infection with *Pseudomonas* spp. and two of which showed evidence of concurrent fungal infection, an AMT was used to control corneal melting which progressed in the face of aggressive medical therapy. Each case had as the end result an eye with a positive dazzle reflex, a positive consensual pupillary light reflex, and an inconsistently positive menace response. In cases with a smaller, less severe, melting ulcer, the visual outcome might have been even better. Even with impaired vision, all three patients returned to their prior level of performance.

In these three cases, the AMT appears to have provided adequate support and been stable in the face of proteases, which digested underlying cornea. AMT appeared to have

anti-angiogenic properties (as evidenced by the progression of corneal vascularization only at the periphery of the AMT in each case), and appeared to have been permeable to topical medications (as evidenced by control of corneal infection). The practical use of equine amniotic membrane transplantation, of course, is limited by acquisition and storage of equine amnion.

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