Catastrophic Ocular Surface Failure in the Horse

Dennis E. Brooks, DVM, PhD, Diplomate ACVO

I was taught >30 yr ago that the horse eye heals poorly. What I have learned through much trial and failure, and some science, is that the horse eye has very powerful ocular healing capabilities. The uniqueness of the horse seems to be that the diseases that attack the horse eye are some of the strongest, most complicated, and most debilitating conditions in all of ophthalmology. We continue to attempt to understand and use the horse's innate ocular healing capabilities to not inhibit this endogenous healing ability with our "treatments." My goal is finding new ways to treat corneal ulcers so we have fewer blind horses. Author's address: College of Veterinary Medicine, University of Florida, 2015 SW 16 Avenue, Gainesville, Florida 32608, e-mail: brooksd@vetmed.ufl.edu. © 2010 AAEP.

1. Anatomy of the Ocular Surface

The ocular surface and its associated adnexal structures form an integrated functional unit that is essential for vision and ocular health. The optically specialized ocular surface consists of the conjunctiva, limbus, and cornea. Normal eyelid function and a healthy precorneal tear film are also vital components of the ocular surface.

Conjunctiva

The conjunctiva is a non-keratinized, vascularized mucosal epithelium interspersed with mucin-producing goblet cells and lymphoid follicles and a connective tissue stroma that extends from the eyelid margin to the corneo-scleral limbus. The conjunctival attachment to the underlying stroma provides a slight physical barrier to movement of antibodies, inflammatory cells, infectious agents, and ophthalmic medications. The conjunctival epithelium is continuous with the corneal epithelium. Conjunctival epithelial cells secrete a glycocalyx of mucopolysaccharides that coat the conjunctival and corneal surfaces to evenly distribute the precorneal tear film located anterior to the epithelial layers (Fig. 1).

Conjunctiva-associated lymphoid tissue is critical for ocular surface defense and includes lymphocytes, macrophages, mast cells, immunoglobulin G (IgG), IgA, and IgM. The conjunctival goblet cells produce the hydrophobic inner layer of the tear film, the mucin layer, which is responsible for precorneal tear film adherence and stability.

Precorneal Tear Film

The ocular surface is covered by a precorneal tear film (PTF). It lubricates the roughened corneal surface, provides oxygen and nutrition to the avascular cornea, enables epithelial cell proliferation, maturation, and movement over the ocular surface, and participates in ocular immunologic defenses. The PTF averages 7 µm in thickness (range, 6–20 µm) in humans. The thickness of the PTF of the horse has not been determined. The architecture of the PTF is a complex, layered, mucosal aqueous gradient gel. The anterior most layer is the lipid or oily layer derived from secretions of the eyelid margin meibomian glands. The lipid layer is 0.1–0.5 µm thick and prevents premature evaporation of the PTF and ocular surface failure. The aqueous lacri-
The total thickness of the equine cornea is 0.8–1.0 mm (Figs. 2 and 3). The thickness of the equine equatorial sclera 15 mm posterior to the limbus is 0.53 ± 0.01 μm. The normal equine corneal epithelium consists of 8–10 cell layers and is ~60 μm thick. It has a thin periodic acid-Schiff (PAS)-positive basement membrane that attaches the basal cuboidal epithelial cells through hemidesmosomes, collagen fibrils, and laminin to the stroma. Microvilli of the superficial apical corneal epithelial cells are coated with a mucopolysaccharide glyocalyx that provides a scaffold for the attachment of the PTF to the cornea to produce an optically smooth corneal surface. The thickest corneal layer (~700 μm thick in the horse), the stroma, is composed of a three-dimensional mosaic of interconnected keratocytes in an extracellular matrix (ECM) of proteoglycans and small-diameter collagen fibrils. These fibrils are combined into highly organized, stratified squamous non-keratinized tissue consisting of differentiated cells on a basement membrane. The avascular cornea derives nutrition anteriorly from the tear film and internally from the aqueous humor. Corneal vascularization causes irregularities of the ocular surface that interferes with light refraction and increases the vulnerability of the ocular surface to immunological attack.
ordered, sheet-like lamellae that lie essentially parallel to the corneal surface. The horse stroma has regional biochemical differences of the proteoglycans chondroitin 6 sulfate (C6S) and chondroitin 4 sulfate (C4S), with higher levels of C6S in the superficial and superficial central layers and more C4S in the deeper areas and deeper peripheral layers. The C4S has less water-holding capacity than the C6S such that corneal edema tends to clinically become more prominent in the superficial cornea. The swelling properties of the superficial anterior stroma may affect epithelial function and tear film adhesion in the horse with marked corneal edema. The small, consistent diameter of the stromal fibrils and their highly ordered spatial arrangement are partially responsible for corneal transparency. The endothelium of the horse cornea is a 7-μm-thick cellular monolayer with a thick collagenous basement membrane (Descemet’s membrane; 21–42 μm thick). The PAS-positive Descemet’s membrane of the horse is quite elastic and the thickness increases with age. The density of horse endothelial cells is 3155 cells/mm². The mitotic potential of the horse corneal endothelium is probably limited but is not known. Clinically, equine corneal endothelial cells seem to be resilient and able to restore and maintain corneal transparency despite persistent insult. The number of equine endothelial cells decreases with age.

The cornea of the adult horse is very sensitive, with the central cornea being the most sensitive region and the dorsal region being the least sensitive. Corneal sensitivity determined by evaluating the corneal touch threshold (CTT) with a Cochet-Bonnet esthesiometer was 2.12 ± 0.62 cm (central) in one study and 4.82 ± 0.87 cm in a second study. The CTT of sick foals was less than healthy foals and adults, being 3.21 ± 0.24 and 5.01 ± 0.61 cm, respectively.

The Limbus

The corneoscleral limbus is the transition zone between the cornea and conjunctival epithelium. Limbal stem cells amplify, proliferate, and differentiate into the corneal epithelium and are critical for corneal ulcer healing. Limbal stem cell deficiency after severe corneal inflammation leads to invasion of conjunctival epithelial cells and blood vessels onto the corneal surface to result in a slightly opaque, irregular, and unstable “corneal” epithelium (“conjunctivalization”) that is prone to damage after minor injury.

2. Ocular Surface Failure

Failure of the ocular surface is precipitated by trauma, inflammation, and infection. Eyelid abnormalities, tear film problems, and altered corneal sensation exacerbate the failure of the ocular surface. Ocular surface failure is most often manifested as corneal ulceration in the horse. The core principal for managing ocular surface failure in the horse is promoting new growth of corneal epithelium. The therapeutic approach for ocular surface failure is to eliminate infection, reduce PTF protease activity, and suppress iridocyclitis. This is achieved by topical antimicrobial and antiprotease (e.g. serum) therapy, contact lenses, surgical tarsorrhaphy, corneal transplantation, amniotic membrane transplantation (AMT), and now limbal epithelial stem cell transplantation.

3. Corneal Wound Healing: General Concepts

Healing of corneal wounds such as ulcers is a complicated process that depends on the centripetal migration of healthy corneal and conjunctival epithelial cells to cover the ulcer site and their subsequent attachment to the injured corneal stroma (Fig. 2). Corneal wound healing involves intercellular signaling between epithelial and stromal cells from cytokines, neuroptides, growth factors, and chemokines, activity of matrix metalloproteinases, fibroblast transformation, protection of corneal tissue from free radical damage, and corneal vascularization.

Polymorphonuclear cells (PMNs) are the first cells to migrate into the injured cornea and release cytokines, growth factors, and proteases to initiate the cellular interactions necessary to heal the corneal wound. Fibronectin is a plasma glycoprotein that functions as an attachment factor and promoter of cell migration. Early in the repair process, fibronectin and fibrin are deposited on exposed epithelial basement membrane (EBM) and/or stroma and then disappear as the epithelial cells cover the defect. Excessive numbers of PMNs are associated with catastrophic increases in tear and stromal protease levels.

Uninjured epithelial cells at the margin of the wound loosen their intercellular and basal attachments and begin to migrate en masse toward the ulcerate region. Migrating epithelial cells derived from the limbal stem cells will cover the ulcer. The new epithelium in healing corneas will be much thinner than normal until mitotic division re-establishes the normal corneal thickness. Reformation of EBM may not be detectable for several weeks after injury. A healed superficial corneal epithelial injury with no stromal involvement is clinically transparent.

Healing of corneal injuries with stromal involvement generally results in some degree of corneal opacification. The anterior stroma has elastic properties that tend to pull the anterior margins of a wound apart. Collagen lamellae retract when cut, causing the corneal laceration wound to gape. The deeper the stromal wound, the more the wound gapes. If the extent of the incision is limited, the gaping may be countered by swelling of corneal stroma exposed to aqueous media. Exposure of the relatively dehydrated corneal stroma to tears will cause swelling of the corneal stroma that tends to close the wound.
Corneal injuries involving at least one third of the stroma are covered initially by proliferating surface epithelium. The epithelial cells may extend for a considerable distance into the stroma. The extent of corneal epithelial migration is controlled at least in part by contact inhibition of migrating corneal cells. As the stroma heals, new collagen is produced by keratocytes or transformed monocytes. The new repair collagen is different from the native collagen in size and in orientation and causes the healed wound to be opaque and obvious clinically.

In corneal wounds and ulcers with deeper stromal involvement, the epithelium is hyperplastic, anterior stroma is absent, and the corneal stroma is thinned. A stromal scar is characterized by an increased number and haphazard arrangement of keratocytes.

Full-thickness wounds of the cornea are associated with retraction of Descemet’s membrane and separation of the posterior aspect of the wound. Fibrin-filled aqueous humor may help seal the wound posteriorly until healthy surrounding endothelial cells can spread and migrate into the injured area. Fistulas to the anterior chamber may develop to hinder corneal wound healing. Migrating corneal endothelial cells will produce new Descemet’s membrane.

The portions of Descemet’s membrane displaced into the stroma are not resorbed but remain as a histological marker of the site of injury. The anterior cellular surface of full-thickness corneal perforations is repaired by the migration and mitosis of epithelial cells, similar to that described for more superficial corneal wounds.

4. Corneal Healing in the Horse

Disruption of corneal epithelial layer integrity to cause corneal ulceration is a common occurrence in the horse. Epithelial cell layer disruption may be limited to a few epithelial cell layers and is termed a superficial abrasion. Erosions are abrasions with loss of all epithelial layers but an intact epithelial basement membrane. The term erosion is generally used to refer to persistent or recurrent abrasions with an underlying disorder of epithelial adherence to the EBM. Superficial abrasions and erosions stain faintly with fluorescein dye. Complete disruption of the epithelial cell layers and varying amounts of stromal damage is called an ulcer. Ulcers stain brightly with fluorescein dye (Fig. 4). Absence or instability of the mucin dominated PTF will also result in rose bengal retention of abrasions, erosions, and ulcers (Fig. 5).

Ulcers can range in depth from simple, superficial ulcers to full-thickness corneal perforations with iridodonesis (Figs. 4–25). Ulcers in horses are associated with excessive proteinase activity of the tear film and stroma and may be infected or sterile (Figs. 3, 6, 7, 10, 11, 14, 16–18, and 20). Equine corneal ulceration is a potentially sight-threatening disease requiring early clinical diagnosis, laboratory confirmation as to the presence of infection, and appropriate medical and surgical therapy. Corneal infection, iridodonesis, and a lack of regulation of tear film protease activity are always major concerns in even the slightest corneal ulcerations in horses.

Healing of the avascular cornea of horses and other animals is dependent on interrelationships on the tear film, conjunctiva, limbus, stroma, endothelium, and aqueous humor for nutrients, humoral molecules, cytokines, growth factors, and leukocytes. Mitosis and migration of basal epithelial cells covers epithelial defects. Thickness increases from restoration of the typical epithelial layers occurs by mitosis. Metabolite and energy levels in healing epithelial cells are elevated. A few fibroblast-like keratocytes replace damaged stromal keratocytes. Tear film proteases are elevated by two to four times normal levels and must be reduced to baseline levels before healing is complete. The normal equine corneal epithelium increases to 10–15 cell layers with hypertrophy of the basal epithelial cells after corneal trephination injury. Epithelial cells migrate at a rate of 0.6–1.2 mm/day in non-infected horse corneas. The EBM is not
completely formed 6 wk after such an injury in the horse, despite the epithelium completely covering the ulcer site. Corneal vascularization occurs at a rate of ~1 mm/day in the horse. Neutrophils migrate in the cornea at ~8 mm/day. Neutrophils migrate in the cornea at ~8 mm/day. Neutrophils migrate in the cornea at ~8 mm/day. Neutrophils migrate in the cornea at ~8 mm/day.

Healing of infected stroma in horses is typically manifested by the massive infiltration of PMN leukocytes, delayed epithelialization, and slight to severe neovascularization. The immense leucotactic activity of microbes is a primary contributor to ulceration in the horse. Ulcers can, however, continue to deteriorate despite sterilization of the ulcer site because of excessive protease activity.

The corneal endothelium does not seem to undergo mitotic repair after endothelial cell injury. Endothelial cells hypertrophy and migrate to form a functional monolayer after injury. The endothelial cells of most species do not divide.

Epidermal growth factor (EGF) receptors are found on epithelial, endothelial, and stromal fibroblasts. EGF is derived from lacrimal gland and is present in tears. It increases epithelial cell proliferation, migration, adhesion, and differentiation, in-
creases the number of stromal fibroblasts, and increases endothelial cell mitosis and density. Platelet-derived growth factor (PDGF) stimulates production of fibronectin, collagenase, and hyaluronic acid and increases wound strength.\textsuperscript{30} Transforming growth factor-\(\alpha\) (TGF-\(\alpha\)) is found in aqueous and tears and increases wound healing.\textsuperscript{30} TGF-\(\beta\) antagonizes EGF. It stimulates fibronectin production and scar formation.\textsuperscript{30} PDGF and EGF stimulate, whereas TGF-\(\beta1\) inhibits, the proliferation of equine corneal epithelial cells and keratocytes in vitro.\textsuperscript{30} EGF did not speed healing and was associated with more intense keratitis, neovascularization, melanization, and scarring at high dosage levels in an in vivo study in the horse.\textsuperscript{30} Opioid growth factor (OGF) and its receptor are present in large quantities in the horse corneal epithelium and absent in the stroma.\textsuperscript{31} Blocking OGF receptors could enhance corneal healing because OGF acts as a negative growth factor during corneal re-epithelialization.

5. Pathogenesis of Ocular Surface Failure Manifested as Infectious Corneal Ulceration in the Horse

The failure of corneal epithelial wound healing to repair damage to the ocular surface results in corneal ulceration.\textsuperscript{10,14} The formation of plasmin from plasminogen by plasminogen activator (PA) is an early step in ulceration. Plasmin produced in the damaged cornea causes production of matrix metalloproteinase-1 (MMP-1). Prostaglandins, leukotrienes, and cytokines are released from epithelial cells and subepithelial keratocytes in response to injury.\textsuperscript{10,14} Interleukin-1 (IL-1) enhances MMP synthesis and activity in humans with corneal injury but has not been found to be elevated in the tears of horses with ulcers. Leukocyte infiltration of ulcerated stroma results in a change from clear to turbid white to yellow. Neutrophils eliminate bacteria by phagocytosis and are too small to phagocytize fungi. Leucocytes can release massive quantities of degradative proteases such as lysosomal enzymes, MMP-2 and MMP-9, neutrophil elastase, su-

Fig. 8. (A) A superficial ulcer with ventral corneal vascularization and cellular infiltration is present after traumatic facial nerve paralysis. (B) Fluorescein dye delimits the ulcer size. (C) Granulation tissue is present 3 wk after placement of a third eyelid flap. (D) The cornea has remodeled to leave a small corneal scar 7 wk later.

Fig. 9. (A) A corneal ulcer with extensive stromal abscess formation and linear areas of stromal neutrophil infiltration. (B) Fluorescein delimits the ulcer. (C) A penetrating keratoplasty remains covered by a conjunctival pedicle flap is healed 5 wk later.
peroxide radicals, and peroxides to damage the predominantly cell free corneal stroma to cause stromal collagen and stromal ECM breakdown or corneal “melting.”\textsuperscript{10,14} Leukocyte infiltration of the cornea is thus highly undesirable if present in excessive numbers.\textsuperscript{10,14} Antibiotic therapy can rapidly sterilize corneal ulcers, but the large numbers of neutrophils chemotactically attracted to the ulcer remain to cause severe corneal stromal pathology. If the excessive protease activity can be controlled, extensive fibroblastic collagen deposition and corneal scarring generally follow the stromal damage.\textsuperscript{7,8,10,14,24,26,32–34} The horse cornea does, however, have the often unrecognized amazing ability to slowly remodel itself to diminish the size of large corneal scars (Fig. 8).\textsuperscript{10,24,34} The environment of the horse is such that the conjunctiva and cornea are constantly exposed to bacteria and fungi. The conjunctival microbial flora of the horse varies depending on the age of the horse, season of year, housing situation, and the geographic area.\textsuperscript{10,35–42} Many bacterial and fungal organisms normally found in the equine ocular flora

Fig. 10. (A) Large diameter melting ulcer. (B) Four days later, some necrotic tissue has sloughed to reveal a deep ulcer, white stromal cellular infiltrate, and red corneal vascularization ventrally despite aggressive medical therapy. (C) A thin area of granulation tissue remains after 5 wk of medical therapy.

Fig. 11. (A) This catastrophic ulcer has melting of the entire cornea and a very thin center. (B) A conjunctival hood flap was placed. (C) The scarring of the large diameter hood flap was so extensive at 5 wk postoperatively that no vision was present. (D) A positive Seidel’s test in this fungal ulcer indicates a corneal fistula. Aqueous humor leaking through the fistula changes the fluorescein color from orange to green. (E) A moistened fluorescein strip is applied to the topically anesthetized cornea to perform the Seidel’s test. (F) The change in fluorescein color from orange to green indicates aqueous humor leaking around a suture in a positive Seidel’s test.
Fusarium and Aspergillus sp. are both common causes of ulcerative fungal keratitis among horses in the United States. Young horses seem to be at risk for gram-negative and fungal infections. Gram-negative bacteria known to be associated with equine corneal ulcers include Pseudomonas sp. and assorted coliform bacteria, Staphylococcus sp., Listeria sp., and Streptococcus sp. and other gram-positive bacteria can cause infectious equine keratitis. Mixed bacterial and fungal infections can also be present. The microbial population of a horse with an ulcer can change during the treatment of an ulcer with antimicrobials.

The defense mechanisms of the cornea include the protective effects of the eyelashes, the mechanical washing effect of the tear film and blinking, the coating of the cornea with ocular mucin, and antimicrobial substances such as lysozyme, lactoferrin, betalysin, and immunoglobulin A antibodies in tears. If bacteria and fungi can resist the natural corneal defense mechanisms that suppress microbial growth and attach to the cornea through tear film instability or corneal injury, they may be able to invade and colonize the corneal epithelium and stroma.

The healthy corneal epithelium and tear film of the horse represent formidable physical barriers to epithelial and stromal invasion of bacteria or fungi, but injuries to the ocular surface of horses are unfortunately common. Bacteria such as Pseudomonas sp. readily adhere to injured or diseased epithelium at the edge of an epithelial defect but do not adhere well to intact corneal epithelium or to stroma. Fungi in horses interestingly seem to be able to adhere to healthy corneal epithelial cells in the absence of a normal PTF layer.
Once microbial organisms reach the stroma, they spread radially by migrating between the stromal collagen lamellae (Fig. 9). A vicious cycle may be initiated after relatively minor injury to the cornea or tear film, with the “second injury” occurring because of the release and action of massive amounts of powerful inflammatory cytokines. These cytokines trigger a rapid and extensive infiltration of the corneal stroma by PMNs and a few T cells that are chemotactically attracted from the limbal circulation and the tear film. The destruction of corneal stroma is thus caused by the liberation of proteolytic enzymes by both the microbial organisms and the PMNs. Excavations in the corneal epithelial cells form around adhered Pseudomonas sp. bacteria. These bacteria disappear into the substance of the corneal epithelial cell at these sites such that the microbes are no longer on the epithelial surface and are not susceptible to antibiotics. The Pseudomonas sp. organisms can be seen replicating within the epithelial cells or migrating through the epithelial cells and into the anterior stroma. The invasion of epithelial cells by Pseudomonas sp. renders the bacteria transiently resistant to host defenses and the effects of topical antibiotics. Entry of Pseudomonas sp. into the corneal epithelial cell involves active metabolic processes. The entire process of microbial adherence and early stromal penetration in the experimental rabbit eye occurs in only 1 hour.

Once Pseudomonas sp. organisms reach the stroma, they replicate and spread both radially and deeply, migrating between the stromal collagen lamellae. The intrastromal spread of the bacteria is facilitated by bacterial production of serine proteases, MMP-2, and MMP-9. All of these proteases are increased in the tears of horses with ulcers. These enzymes are potent proteoglycanases that rapidly destroy stromal ground substance. Corneal microbial invasion is immediately followed by infiltration of the corneal stroma by PMNs chemotactically attracted to the horse cornea from the limbal blood vessels and the PTF. After the PMNs enter the cornea, a destructive process involving necrosis of stromal kerocytes and phagocytosis of these degenerating stromal cells by PMNs occurs. This process does not seem to be related to increased bacterial MMP production. As PMNs migrate peripherally in the anterior stroma, the overlying EBM is destroyed, thus leading to sloughing of the overlying epithelium (Fig. 10). The enlarged epithelial defect further enhances the migration of PMNs into the corneal stroma. A loss of collagen fibrils accompanies degradation of the collagen framework of the corneal stroma. Most evidence indicates that true corneal collagenolysis results from the proteases produced by the PMNs. If the replication and spread of microbes is not halted by the host response or instillation of antibiotics, and the PMN-derived protease-induced stromal necrosis cannot be ameliorated, the process of stromal degradation ultimately leads to total loss of stromal tissue and corneal perforation.

Pathogenic fungi are better able to adhere to the cornea and PTF, chemotactically attract more leukocytes, are associated with higher levels of MMP-9, and penetrate deeper vertically into the cornea than non-pathogenic fungi. Pathogenic fungal organisms have a unique propensity to move or “tunnel” deep in the stroma toward the Descemet’s membrane of horses. Total corneal ulceration ultimately requires the degradation of the collagen framework of the corneal stroma. Most evidence indicates that true corneal collagenolysis results from the proteases produced by the PMNs. Bacterially derived proteases probably cannot degrade intact collagen fibrils, but they can contribute to stromal collagenolysis once they reach the stroma. Pathogenic fungi are better able to adhere to the cornea and PTF, chemotactically attract more leukocytes, are associated with higher levels of MMP-9, and penetrate deeper vertically into the cornea than non-pathogenic fungi. Pathogenic fungal organisms have a unique propensity to move or “tunnel” deep in the stroma toward the Descemet’s membrane of horses. Total corneal ulceration ultimately requires the degradation of the collagen framework of the corneal stroma. Most evidence indicates that true corneal collagenolysis results from the proteases produced by the PMNs. Bacterially derived proteases probably cannot degrade intact collagen fibrils, but they can contribute to stromal collagenolysis once they reach the stroma.
the initial collagen breakdown is initiated by a true collagenase.\textsuperscript{48} Thus, the destruction of corneal tissue results from the liberation of proteolytic enzymes by both the microbial organisms and the PMNs.\textsuperscript{48} An organism’s virulence (i.e., ability to rapidly cause serious disease) relates to its ability to adhere and to induce inflammation and activation of destructive enzymes. If the infection is halted by antibacterial therapy or the host inflammatory response, the ocular surface undergoes a process of repair, which includes vascularization, glycosaminoglycans synthesis, collagen resynthesis (i.e., scar formation), and re-epithelialization.\textsuperscript{48}

Clinical History and Appearance of Equine Ulcerative Keratitis

Many early cases of equine ulcerative keratitis initially present as minor corneal epithelial abrasions or ulcers with slight pain, blepharospasm, epiphora, and photophobia.\textsuperscript{2,10} Small corneal abrasions and ulcers are detected through oblique transillumination and fluorescein dye retention. At first, anterior uveitis and corneal vascularization may not be clinically pronounced; a slowly progressive, indolent course often belies the seriousness of the condition. Both superficial and deep corneal vascularization as well as painful uveitis may occur. Extensive in-
trastromal lesions, vascularization, conjunctival injection, and corneal edema may then become evident. Corneal collagen breakdown or “melting” appears as a gelatinous, gray opacity to the margins or central regions (or both) of an ulcer. Deep penetration of the stroma to the Descemet’s membrane with corneal perforation is a possible sequela to all cases of equine corneal ulcers.10,21

Infection and hyperprotease activity should be considered likely in every equine corneal ulcer. *Staphylococcus* sp., *Streptococcus* sp., and *Pseudomonas* sp. are frequent bacterial corneal pathogens of the horse. Fungal involvement should be suspected if a corneal ulcer has received prolonged antibiotic or corticosteroid therapy (or both) with either slight or no improvement, if there is a history of corneal injury with vegetative material, or if an ulcer fails to vascularize. *Fusarium* and *Aspergillus* sp. are frequent fungal pathogens of the equine cornea.21,35 The tear film of all eyes with corneal ulcers has significantly elevated levels of proteases.8 Tear film neutrophils are associated with release of highly destructive protease and collagenase enzymes, which in the horse can result in rapid corneal stromal degeneration or melting and perforation.8,21–24

Diagnostic Techniques for Ulcerative Keratitis

It is important to approach each case of ulcerative corneal disease in the horse in a systematic manner. Most cases can be diagnosed on the basis of standard laboratory techniques. Fluorescein and rose bengal stains must be used (Table 1). Cyto logic examination and microbiologic cultures of deep or rapidly progressing ulcers are very beneficial at determining the proper therapy.10,21 Corneal cultures should be obtained first, and then followed by corneal scrapings for cytology.10,21 Vigorous corneal scrapings at the edge and base of the lesion to detect bacteria and deep hyphal elements can be obtained with the handle end of a sterile scalpel blade and topical anesthesia. Superficial swabbing cannot be expected to yield the organism (or organisms) in a high percentage of cases, because the organisms are deep

Fig. 16. (A) A large diameter melting ulcer is thin in the center. (B) The ulcer is covered with a double amnion transplant. (C) Two months later central granulation tissue remains.

Fig. 17. (A) A fluorescein positive melting ulcer is treated with a double amnion transplant. (B) Two weeks later, a large amount of granulation tissue is present. (B) Five weeks later, the corneal granulation tissue is reducing in size. (D) Nine weeks postoperatively, the cornea continues to remodel.
within the ulcer. The highest yield of data from corneal scrapings can be obtained by examination of material deep in the lesion.\textsuperscript{10,21}

Staining with fluorescein dye for detection is critical in the horse.\textsuperscript{10,21} The hydrophilic fluorescein dye moves into the regions between damaged epithelial cells and into the stroma when the corneal epithelium is absent. Corneal abrasions stain only faintly with fluorescein, ulcers stain very brightly, and a descemetocele can be recognized as a dark, non-fluorescein staining clearing at the bottom of a deep ulcer. Descemetoceles do not retain fluorescein dye, whereas deep ulcers with some stromal present will stain with fluorescein. Rose bengal retention indicates tear film instability and occurs in early stages of fungal keratitis,\textsuperscript{50} keratoconjunctivitis sicca (KCS), corneal scars, and presumed viral keratitis in the horse.\textsuperscript{10,21,24}

The Seidel’s test is used in horses for detecting corneal rupture and aqueous humor leakage. The affected cornea is anesthetized with a topical anesthetic. A large pool of fluorescein is obtained from a moistened fluorescein strip applied to the region of the cornea where a leak is suspected to be located. A full-thickness corneal defect leaking aqueous humor will result in a color change in the pool of fluorescein dye as the aqueous humor dilutes the fluorescein. The orange color will turn from orange to green (Fig. 11D) if leakage is slight or become clear if aqueous leakage is prominent.

Tear film instability leads to poor healing of corneal ulcers in the horse. The normal tear film is continuous over the cornea with blinking maintaining this continuity. The tear film becomes

![Image](https://example.com/image1.png)

**Fig. 18.** (A) The entire cornea is melting in this eye. (B) A double amnion transplant is partially coming off 2 wk later. (C) At 8 wk postoperatively, the cornea is clear where the amnion came off and slightly opaque where the amnion adhered.

![Image](https://example.com/image2.png)

**Fig. 19.** (A) An iris prolapse from a bacterial fungal ulcer was treated with a penetrating keratoplasty. (B) An infectious endophthalmitis resulted in enucleation.
unstable and breaks up to result in ulcers if blinking does not occur often enough. The tear film breakup time (TFBUT) test is another use of fluorescein dye. Fluorescein dye is placed on the cornea and not flushed off. The lid is manually blinked three times and held open to expose the tear film to evaporation. Dark dry spots will appear under cobalt blue filtered light as part of normal evaporation of tears. The time required for a dry spot to appear on the corneal surface after blinking in the TFBUT in a normal healthy horse eye is \( \approx 20 \) s. A TFBUT of \(<10–15\) s is abnormal and associated with instability of the mucin layer of the tear film in horses.

**Therapeutic Methods for Ulcerative Keratitis**

Horses with corneal ulcers are often in pain, and topical treatment is usually difficult. In a fractious horse or one with a painful eye needing frequent therapy, subpalpebral lavage treatment systems are used. Various techniques have been described, but we prefer to use a length of silicone tubing with a single hole and footplate positioned in the superior palpebral fornix. The ventral palpebral fornix can also be used for lavaged tubing placement, but increased absorption of medications applied topically to the dorsal cornea achieved higher concentrations of the medications into the cornea and anterior chamber compared with administration ventrally. A standard needle holder, 12-gauge needle with the hub removed, 1–2 m of silicone tubing (0.065-in OD), and
standard dermal suture material are needed. Sedation, eyelid akinesia, sensory eyelid blocks, and topical anesthesia are generally sufficient to place this tubing system.21

Medical Therapy
Medical therapy always comprises a major thrust of ulcer control, albeit tempered by judicious use of adjunctive surgical procedures.21 This intensive pharmacologic attack should satisfy the therapeutic objectives of ulcer sterilization, reduction in tear film protease activity, and decrease in uveitis and be modified according to its efficacy. Treatment frequently needs to be sustained for weeks. Repopulation of bacteria and fungi can occur during and after the administration of topical antibiotics in the horse.41 This is especially true for gram-positive bacteria such as *Streptococcus* sp.41

Once the cause of a corneal ulcer is presumptively diagnosed on the basis of cytology or is conclusively proven on the basis of culture, the objectives of medical therapy must be carefully considered to ensure comprehensive treatment. First, bacterial and fungal growth must be halted, and the microbes rendered non-viable. Second, tear film protease activity must be inhibited. Third, anterior uveitis must be controlled to prevent blinding sequelae.21 Visual outcomes of several potentially catastrophic equine keratopathies are listed in Table 2.

**Ulcer Sterilization**

The ulcer must be sterilized, although sterility does not necessarily equate with rapid or complete healing.10,14 Infection initiates and complicates ulcerative disease processes, but the hyperproteinase
activity in the PTF and stroma perpetuate it. The killing of microbes is not that difficult in most cases. Appropriate antibiotics, such as gentamicin, ciprofloxacin, or tobramycin ophthalmic solutions or ointments, may be used topically to treat gram-negative bacterial ulcers.\textsuperscript{10,21,35} Amikacin (10 mg/ml) may also be used topically for gram-negative bacteria. Chloramphenicol may penetrate the cornea but is a bacteriostatic drug that should be used carefully. Cefazolin (55 mg/ml) is best for treatment of equine ulcers with gram-positive bacterial infections and can be proportionally combined in a therapeutic cocktail with ophthalmic tobramycin, serum, and ophthalmic natamycin.\textsuperscript{10} An increasing resistance to tobramycin and gentamicin by \textit{Pseudomonas} and \textit{Streptococcus} sp. bacteria of horses has been noticed.
in Florida. These tobramycin- and gentamicin-resistant Pseudomonas sp. bacteria remain sensitive to ciprofloxacin, such that ciprofloxacin is the present standard of care for Pseudomonas sp. in Florida.

Miconazole, natamycin, fluconazole, voriconazole, clotrimazole, and itraconazole have been used successfully for topical treatment of fungal ulcers in the horse.10,21,42,70–74 (Table 3). Anti-fungals may need to be used for several weeks. Systemic antifungals may be beneficial, but their efficacy is not proven. Intravenous miconazole is associated with severe anaphylactic reactions in the horse, and it should be used with caution. Oral itraconazole does not reach therapeutic levels, whereas oral fluconazole and voriconazole do reach effective antifungal levels in the horse eye.70,75

### Control of Uveitis

In the horse, as in other species, iridocyclitis to some degree is the usual and expected sequela to ulcerative keratitis. Uveal inflammation is incited through an axon reflex mediated by the ophthalmic branch of the trigeminal nerve, which is sensory for the cornea, conjunctiva, and uvea.10,21 The sensory nerve endings release substances that induce the uveitic response. The horse is particularly sensitive to this corneal ulcer-associated axon reflex uveitis. Miosis, aqueous flare, hypopyon, and hypotony are present to some degree in eyes with ulcers. Controlling the uveitis may be as difficult as healing the cornea.

Anterior uveitis in horses with corneal ulcers should be treated by both topical and systemic routes.10,21 The systemically administered non-steroidal anti-inflammatory drugs (NSAIDS) phenylbutazone (2 mg/kg, PO, q 12 h) and flunixin meglumine (1 mg/kg, PO, IV, IM, q 12 h) can be used orally or parenterally with both effective in reducing uveal exudation and relieving ocular discomfort in horses. Flunixin does seem to be slightly more effective than phenylbutazone. Topically administered NSAIDS such as diclofenac, flurbiprofen, suprofen, and bromfenac can be used in conjunction with systemic NSAIDs to further suppress signs of anterior uveitis in horses.

Topically applied anti-cholinergics (e.g., 1% atropine) are effective in causing pupillary dilatation and stabilizing the blood–aqueous barrier to reduce aqueous flare. Pupillary dilatation protects the visual axis from occlusion, and it may minimize development of synechiae.10,21 Synechiae are adhesions between the iris and lens and can occur very quickly. Synechiae formation can prevent dilatation of a miotic pupil to result in blindness. Topical atropine (1%) and phenylephrine (2.5%) in combination can dilate some pupils when synechiae are present. Tissue plasminogen activator (TPA) can also aid synechia breakdown in some cases.10,21 Relaxation of the ciliary muscles also eliminates ciliary spasm, which is a factor in ocular discomfort.

### Table 1. Ulcer Stain Order

<table>
<thead>
<tr>
<th>Stain</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescein (FL)</td>
<td>Stain first. Identifies ulcers if positive.</td>
</tr>
<tr>
<td>Rose bengal (RB)</td>
<td>Stain immediately after fluorescein staining.</td>
</tr>
<tr>
<td>RB retention</td>
<td>Indicates tear film instability.</td>
</tr>
<tr>
<td>Mucin tear film layers</td>
<td>Blocks RB staining.</td>
</tr>
<tr>
<td>RB stains</td>
<td>Exposed epithelial cells, mucous, and stroma (slow absorption).</td>
</tr>
<tr>
<td>Poor prognosis</td>
<td>for rapid healing if an ulcer is FL positive and RB positive</td>
</tr>
</tbody>
</table>

### Table 2. Visual Outcome and Ocular Survival of Ulcers, Iris Prolapse, and Stromal Abscesses

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Visual Outcome</th>
<th>Globe Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcers</td>
<td>&gt;90%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Iris prolapse</td>
<td>~40%</td>
<td>67% (ulcers) to 80% (lacerations)</td>
</tr>
<tr>
<td>Stromal abscesses</td>
<td>&gt;80–92% depending on the depth of the abscess</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Antifungal Drugs

<table>
<thead>
<tr>
<th>Antifungal Drugs</th>
<th>Topical Formulation and Frequency</th>
<th>Systemic Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natamycin</td>
<td>5%; q 6 h</td>
<td>Not available</td>
</tr>
<tr>
<td>Miconazole</td>
<td>1%; q 6 h</td>
<td>Anaphylactic reactions</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>1% with 30% DMSO; q 6 h</td>
<td>Itra: 5 mg/kg, PO, q 24 h</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.2%; q 6 h</td>
<td>14 mg/kg, PO loading dose</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>—</td>
<td>30 mg/kg, PO, q 24 h</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.15%; q 6 h</td>
<td>—</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>1% q 6 h</td>
<td>4 mg/kg, PO, q 24 h</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>0.1% solution; q 6 h</td>
<td>—</td>
</tr>
<tr>
<td>Povidone iodine solution</td>
<td>2%; q 12 h</td>
<td>—</td>
</tr>
<tr>
<td>Silver sulfadiazine</td>
<td>Dermatologic cream 1%; q 12 h</td>
<td>—</td>
</tr>
</tbody>
</table>

*Dose: 5 mg/kg PO q 12 h × 7 days and then 5 mg/kg PO q 24 h.*
topical atropine more than four times per day should be watched closely for symptoms of reduced gut motility and, in rare cases, colic.10,21,76

Collagenolysis Prevention
Severe corneal inflammation secondary to bacterial (especially Streptococcus and Pseudomonas spp.) or, less commonly, fungal infection may result in sudden, rapid corneal liquefaction and perforation.8,10,2124,28,43,77–80 Activation and/or production of proteolytic MMP or other enzymes by corneal epithelial cells, leucocytes, and microbial organisms are responsible for stromal collagenolysis in the horse.22 An undetermined protein, enzyme, cytokine, or other component of the horse tear film attracts PMNs to the ulcer.22–24

MMPs and serine proteases such as neutrophil elastase are elevated in the tears of both eyes of horses with an ulcer in only one eye.8,21,22 The ulcerated eye has higher tear protease levels than the non-ulcerated eye. MMP-2 increased 83%, MMP-9 increased 232%, and neutrophil elastase (NE) increased 172% in the PTF of equine eyes with ulcers.21,22 MMP tear levels decrease as an ulcer resolves/heals.22,23 It is more difficult to therapeutically reduce the tear film MMP levels in infected ulcers.22

MMPs are inhibited by tissue inhibitors of metalloproteinases (TIMPS), serum, ethylenediamine tetra-acetic acid (EDTA), tetracyclines, doxycycline, ilomostat, and acetylcysteine. Serine proteases (such as NE) are inhibited by serum and α-1-proteinase inhibitor (AIP; pre-albumin inhibitor in the horse). Although tear film NE does not seem to have a direct collagenolytic effect,22–24 NE is still a problem because it inhibits the inhibitory activity of TIMPS against MMPs. MMP inhibits the equine NE inhibitor pre-albumin inhibitor, which makes the NE more active (Table 4). The large amount of NE may thus cause indirect breakdown of equine corneal stromal collagen by increasing the MMP activity.21-23

Serum and other antiproteases have been successfully used in the treatment of severe dry eye, persistent corneal epithelial defects, and other severe ocular surface disorders in horses and other animals (Table 5).1,22–24,80–84 Serum contains α-2-macroglobulins, fibronectin, vitamin A, and platelet-derived growth factors (PDGFs) that induce the proliferation, migration, and differentiation of corneal epithelial cells.84 Serum rather than fresh frozen plasma (FFP) was significantly superior in stimulating these corneal epithelial cell changes. The superior epitheliotrophic capacity of serum might be caused by the higher concentration of proliferation mediators such as EGF and PDGF and its higher content of vitamin A.84 A long clotting time (≥120 min), a sharp centrifugation (3000 g for 15 min), and dilution with balanced salt solution (BSS) improve the ability of serum eye drops to support the metabolism of corneal epithelial cells.82 For many years, we have routinely and successfully used topically administered, unpreserved autogenous serum in the corneal ulcers of horses with evidence of collagenolysis, infection, and/or chronicity.21,23,24,79,80 This serum can be administered topically as often as possible, and it is replaced by new serum every 8 days. It can be stored frozen, at room temperature, or in a refrigerator.2,21–24,80 Serum is by nature non-allergenic and its biomechanical properties are similar to normal tears.83 Contamination of the serum is unlikely, but the serum should be closely monitored for changes in turbidity that could be evidence of contamination. Ten percent

<table>
<thead>
<tr>
<th>Table 4. Antiproteases for Topical Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antiprotease Drugs</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Serum (α-2-macroglobulin, α-1-proteinase inhibitor, and platelet-derived growth factors)</td>
</tr>
<tr>
<td>Plasma (α-2-macroglobulin and α-1-proteinase inhibitor)</td>
</tr>
<tr>
<td>EDTA 0.2%</td>
</tr>
<tr>
<td>Ilomostat 0.1%</td>
</tr>
<tr>
<td>Acetylcysteine 5–10%</td>
</tr>
<tr>
<td>Doxycycline 0.1%</td>
</tr>
<tr>
<td>α-1-Proteinase inhibitor 0.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5. Melting Ulcer Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drugs</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Topical antibiotics: gentamicin; tobramycin; ciprofloxacin; 1% amikacin; 1% doxycycline, or cefazolin (55mg/ml)</td>
</tr>
<tr>
<td>Topical antifungal if cytology is positive for hyphae: voriconazole (1%)</td>
</tr>
<tr>
<td>Topical autogenous serum</td>
</tr>
<tr>
<td>Topical EDTA (0.2%)</td>
</tr>
<tr>
<td>Topical atropine (1%): reduce dose when pupil is dilated</td>
</tr>
<tr>
<td>Systemic flunixin meglumine</td>
</tr>
<tr>
<td>Systemic doxycycline</td>
</tr>
</tbody>
</table>
acetylcysteine or 0.17% dipotassium EDTA can also be administered in superficial and/or severe corneal ulcers until stromal liquefaction diminishes and healing occurs.\textsuperscript{9,21–24,78,80} In some eyes, both EDTA and serum may be needed to arrest corneal stromal melting.\textsuperscript{9,21–24,78,80} I tend to minimize the use of acetylcysteine in my horse cases because it can disturb the stability of the tear film and make the horses rose bengal positive. Topical and/or systemic doxycycline can also reduce tear film protease activity in equine corneal ulcers.\textsuperscript{90,85}

6. Profiles of Matrix Metalloproteinase Activity in the Equine Tear Fluid During Corneal Healing in Horses With Ulcerative Ocular Surface Disease

Tissue breakdown occurs with the normal metabolic activity of the cornea.\textsuperscript{10,86} Proteolytic enzymes are important in the slow turnover and remodeling of the normal healthy corneal stroma. Inherent tissue protease inhibitors normally balance the activities of these proteolytic enzymes to prevent excessive degradation of the normal healthy tissue. An imbalance in homeostasis between proteases and protease inhibitor levels caused by excessive levels of tear film and corneal proteases can cause pathological degradation of corneal stromal collagen and proteoglycans.\textsuperscript{10,86–88a} This may be similar to acute laminitis in horses where excessive proteases induce pathology at the hoof lamellar dermal–epidermal interface.\textsuperscript{88b}

The tear film proteases have been previously evaluated in both normal and diseased eyes of animals and humans.\textsuperscript{23,79,89,90} In ulcerated horse corneas, tear film levels of MMP-2, MMP-9 (also called gelatinase A and B, respectively), and NE are significantly elevated compared with age-matched normal controls\textsuperscript{8} and are hypothesized to contribute to the breakdown of stromal collagen.\textsuperscript{8} Ulcerative keratitis with extensive stromal involvement displays rapid progression that can lead to corneal perforation in many horse eyes, probably because of this upregulated proteolytic activity (Figs. 7, 9, 14–17, 21, and 26).\textsuperscript{10}

Total tear film MMP activity decreased as corneal healing progressed compared with levels measured on day 1 in horses with ulcers.\textsuperscript{23} The general trend was a decrease, but different patterns of tear film MMP activity were observed. There was an increase in proteolytic activity after keratectomy and penetrating keratoplasty but not after amniotic membrane transplant or conjunctival graft surgery.\textsuperscript{23}

The mean of the total MMP activity (±SD) measured in the tear fluid of the ulcerated eye (2.44 ± 1.44) of the 10 horses studied was significantly higher than the one in the contralateral eye (0.81 ± 0.68; \textit{p} = 0.006) on the first day of admission.\textsuperscript{23} The mean MMP activity in these ulcerated eyes significantly decreased (−82.4%) between the first of admission and the day when the ulcer was completely healed (\textit{p} = 0.0002).\textsuperscript{23}

The MMP-9 and MMP-2 enzymes are present at a significantly higher level in the tear film of horses with ulcerative keratitis.\textsuperscript{8,23} The majority of host-derived and microbial tear film enzymes are MMPs. Enzymes of bacterial or fungal origin (i.e. exogenous proteases) can contribute to ulcerative keratitis, either directly or indirectly, through the activation of corneal proteases (i.e., endogenous proteases or proteinases).\textsuperscript{23} Seven of 10 horses had negative bacterial and fungal cultures, possibly caused by the anti-

Fig. 26. Early fungal plaque formation in the horse. (A) A cellular infiltrate surrounds the fluorescein staining region. (B) Tear film instability is generalized over the lesion. (C) The condition has resolved with granulation tissue formation 3 wk later.
microbial therapy started before the visit at the Veterinary Medical Teaching Hospital (VMTH). Bacteria were isolated from two cases and fungi were isolated from two cases.23

Multiple studies have reported elevated levels of proteases in tears from animals and humans with ulcerative keratitis25 and changes in protease activity during the healing process of the ulcerated cornea, but precise MMP activity profiles based on the collection and the analysis of many serial tear fluid samples have not been reported in either animals or humans. We documented for the first time that total MMP activity decreases in equine tears as the corneal epithelial and stromal healing occur.23

These results of corneal wound healing in the horse are in accordance with those previously reported of skin wound healing.23 Total MMP activity was highest in the early phases and decreased as the skin wound healing proceeded.23 The ratio of MMP to tissue inhibitor of MMP (TIMP) in skin wound fluids is inversely correlated with the skin healing process.23 Therefore, a combination of increased concentrations of MMPs with decreased concentrations of TIMPs is responsible for an increased proteolytic environment that contributes to the failure of skin wounds to heal and may also be true for the horse cornea.23 The TIMPs in the tear film of horses have not yet been studied. Other studies suggest also that persistence of high levels of proteolytic activity in corneal wounds is responsible for the failure to heal.23,91

Our data support the use of aggressive therapy to rapidly reduce the activity of tear proteases in case of ulcerative keratitis. Equine serum, which contains strong and broad protease inhibitors such as α-2-macroglobulin and α-1-proteinase inhibitor, was used topically in all the cases presented here. Various other antiprotease compounds are also available (N-acetylcysteine or NAC and EDTA) that use different mechanisms to inhibit different families of proteases present in equine tears.23,24,80 A combination of inhibitors may be indicated for severe corneal ulceration in horses.10,21,80 Treatment strategies for healing corneal ulcers in horses should be directed toward reducing microbial activity, decreasing uveitis, and reducing tear film concentrations of MMPs, because reduced MMP activity is associated with improvement of the clinical signs of ulcerative keratitis in horses.

7. Inappropriate Therapy for Ocular Surface Failure Associated With Infection

Corticosteroid therapy by all routes is contraindicated in treatment of equine corneal infections. Even topical corticosteroid instillation to reduce the size of a post-mycotic corneal scar or blood vessels may be disastrous if bacteria or fungi remain indolent in the corneal stroma and/or the tear film remains unstable on the scarred corneal surface.10,21

8. Adjunctive Corneal Ulcer Surgical Therapy

Frequently during clinical treatment of corneal ulcers in the horse, surgery becomes necessary to augment the medical treatments.10,21,22,43

Keratectomy

Keratectomy can be very useful in treating superficial ulcers and melting ulcerative keratitis of horses. Removing necrotic infected tissue by keratectomy speeds healing, minimizes scarring, and decreases the stimulus for iridocyclitis.10,21

Chemical Cautery

There is anecdotal evidence that chemical cautery of superficial ulcers with small amounts of topically applied phenol or trichloroacetic acid is effective in treating superficial ulcers among yearlings and adult horses. It should not, however, be performed on descemetoceles or rapidly progressive ulcers displaying evidence of collagenolysis, because the chemical agent will cause corneal rupture.10,21

Conjunctival Grafts

Conjunctival grafts or flaps (CFs) are frequently used in equine ophthalmology for clinical treatment of deep, melting, and large corneal ulcers, descemetoceles, and perforated corneal ulcers both with and without iris prolapse (Figs. 6, 11, 12, 18, and 20).10,21,22,92 CFs are best mobilized from the bulbar conjunctiva.10,21 I do not recommend using the conjunctiva immediately adjacent to the nictitans, however, because postoperative nictitans movement can put tension on the sutures holding the conjunctival graft to the cornea and thereby result in premature CF release. CFs can be transposed and sutured onto the cornea to provide sufficient tissue to strengthen most weakened corneas, but they are not as strong as corneal grafts. Conjunctival autografts contain limbal stem cells, blood vessels, and lymphatics, thus offering significant antibacterial, antifungal, antiviral, antiprotease, and anticollagenase effects. With conjunctival grafts, PMNs, antibodies, plasma, and α-2-macroglobulins are immediately placed in the corneal ulcer bed.10,21,22 Systemic antibiotics can enter the ulcer site at higher levels through leakage from the conjunctival graft vasculature. The fibrovascular, or deeper, layer of the conjunctival transplant offers immediate fibroblasts and collagen with which to begin rebuilding the corneal stroma. Conjunctival grafts will result in various sizes and degrees of corneal scars.10,21 Scarring can be minimized, however, by removal of necrotic cornea with keratectomy before graft placement. Postoperative topical corticosteroids can reduce this postoperative scar tissue formation to a minimum, but corneal scarring after conjunctival grafts should be anticipated.

Conjunctival autografts are more difficult to perform than nictitating membrane flaps, but they are simpler than corneocconjunctival and corneoscleral transpositions and penetrating keratoplasty surger-
ies. They are also easier to perform in the horse than in other species, because the horse has a great deal of very mobile bulbar conjunctiva. Conjunctival autografts from either bulbar or palpebral conjunctiva should be thin, and they should not include Tenon’s capsule or the bulbar fascia. Tenon’s capsule should be stripped or cut from the graft so that the graft lies over the corneal defect before suture placement. The inclusion of Tenon’s capsule may contribute to surgical failure by increasing traction on the transplanted conjunctival graft. Conjunctival flaps should have tension-relieving sutures placed at the limbus to prevent the graft pulling away from the ulcer bed prematurely. Conjunctival pedicle grafts using bulbar conjunctiva from the dorsal or temporal quadrants are my preference, because the conjunctiva in those areas is surgically available and the pedicle flaps cover only the ulcer surface, thereby allowing postoperative observation of the pupil and anterior chamber as the graft does not cover the entire cornea. Melting ulcers should always be stabilized with medical therapy, if possible, before graft placement to prevent protease digestion of any absorbable sutures holding the conjunctival graft in place and premature graft release. Temporary tarsorrhaphies are performed concurrently with conjunctival grafts to minimize blinking movements to the corneal sutures and to allow quick graft adherence to the stroma.

Conjunctival Pedicile Flaps

A conjunctival pedicle flap is made by incising the conjunctiva (excluding Tenon’s capsule) with tenotomy scissors 1 mm posterior to and parallel to the limbus. The distance of the limbus to the corneal lesion determines the length of this incision. The flap is undermined posteriorly toward the fornix. A perpendicular incision is made at the distal end of the flap, and an incision parallel to the first incision and limbus is made as wide as is necessary to cover the corneal lesion. The flap is rotated over the corneal defect and sutured in place with absorbable 5-0 to 7-0 suture in a simple interrupted pattern. The degree of rotation should be <45°.

When performing a CF or graft, remove as much necrotic cornea as possible before placing the corneal graft. Make the CF thin but not so thin it is white. Tears in the flap should be sutured. CF fibrosis and failure may be associated with iris prolapse under the flap. Perform a Seidel’s test on every ulcer before CF placement. Aqueous humor leakage induces fibroplasia of the corneal side of the flap such that this surface becomes thickened and fibrotic and does not completely adhere to the wound. The flap may still partially adhere but vascularization of the cornea may fail if there is a corneal leak. Flap bruising manifesting as purple areas of the CF may indicate flap ischemia. A white CF has become avascular and may prematurely retract. CF bulging may indicate iris prolapse under the flap and can be monitored by assessing intraocular pressure (IOP) and positive Seidel’s test. Anchor the CF with sutures at the limbus to reduce flap tension to prevent premature flap retraction. Continue topical antiproteinases after flap placement or the absorbable sutures may be dissolves prematurely and result in CF retraction. Infection of a CF with Streptococcus sp. bacteria or Aspergillus sp. fungi is often associated with excessive levels of proteases in the tear film and can literally melt a CF in hours. Antimicrobial therapy should still be aggressive after CF placement.

9. Amniotic Membrane Grafts for Ocular Surface Reconstruction

Ophthalmic AMT or grafting is indicated for surface reconstruction of both the conjunctiva and cornea as a patch for either surface or as a carrier for the growth or expansion of epithelial cells for transplantation. The innermost layer of the placenta, the amniotic membrane or amnion consists of a basement membrane and a thick avascular stroma. This relatively acellular material is very strong and has become the most widely used tissue for ocular surface reconstruction in human ophthalmology. Its uses and indications are constantly evolving and expanding in both human and veterinary ophthalmology. In severe melting disease of the ocular surface in horses, where traditional surgical placement of a conjunctival graft would render a sighted or a potentially sighted globe blind because of extensive fibrosis, the AMT should be considered as the primary surgical treatment option for dogs and horses.

Scientific Basis of AMT Clinical Application

AMT is an effective clinical therapy for ocular surface pathology because it has the ability to facilitate the migration and differentiation of epithelial cells and the reinforcement of cellular adhesion of those same cells, the ability to modulate stromal scarring, and the ability to decrease ocular surface inflammation. It has also been shown to have antiangiogenic and antimicrobial effects. The amniotic membrane consists of three basic layers: an epithelial monolayer, a basement membrane, and an avascular, hypocellular stromal matrix. The epithelium is usually damaged and non-viable, if not completely denuded by the preparation, preservation, and storage procedures for amnion, so is not usually a consideration for therapeutic procedures. Amniotic basement membrane compositionally resembles that of the conjunctival basement membrane, making it a nearly ideal material for ocular reconstruction. It has a network of compacted reticular fibers that give it considerable tensile strength. The fact that this basement membrane provides support to the fetus for the length of gestation stands as testimony to its strength. It has very good structural integrity,
transparency, and elasticity and is known to promote epithelial cell migration, adhesion, and differentiation. Amnion, like the basement membranes of normal cornea and conjunctiva, consists of types IV, V, and VII collagen and fibronectin. The laminin 1 and 5 of amnion facilitate corneal epithelial cell adhesion and anchorage to the stroma.\textsuperscript{1,93,94,96} It is a superior substrate for the growth and proliferation of epithelial cells and their progenitors because it allows them to maintain their normal morphology and differentiation, prevents epithelial cell apoptosis, prolongs their lifespan, and in the case of progenitor cells, maintains their clonogenicity.\textsuperscript{1,93} These properties of amnion allow the direct transplantation of it onto recipient corneas to replace limbal stem cell deficiencies, facilitate epithelialization of longstanding ulcerations, and repair corneas damaged from a variety of insults.\textsuperscript{93}

The stromal side of the membrane consists of a loose matrix of fibroblasts, proteoglycans, glycoproteins, and collagen and is rich in fetal hyaluronic acid.\textsuperscript{1,93} Several growth factors have been identified in the stromal matrix of amnion including hepatocyte growth factor, TGF, EGF, and keratoctye growth factor, which may contribute to this scar reduction action. Amnion has been shown to suppress TGFβ signaling and the proliferation and myofibroblastic differentiation of normal corneal, limbal, and conjunctival fibroblasts.\textsuperscript{1,93} This action explains why AMT can reduce scar formation after ocular surface reconstruction and injury. Several amnion stromal growth factors may also support re-epithelialization after AMT.\textsuperscript{1,93} The amnion stromal matrix also has the ability to suppress the expression and activity of certain inflammatory cytokines that originate from the ocular surface epithelia and are present in abundance on the diseased ocular surface. These cytokines include IL-1α, IL-2, IL-8, interferon λ, tumor necrosis factor (TNF)β, TNFα, basic fibroblast growth factor, and PDGF.\textsuperscript{1,93} This cytokine modulation explains some of the anti-inflammatory properties of AMT. The stromal matrix also decreases ocular inflammation by attracting and sequestering inflammatory cells that infiltrate the diseased ocular surface. These inflammatory cells are inactivated by rapid apoptosis in the amnion stroma. The decrease in the presence and activity of inflammatory cells may also be caused by reduced levels of proinflammatory cytokines and chemokines that further decrease their recruitment. Additionally, natural inhibitors of MMP-2 and MMP-9, and nitric oxide synthase, and potent anti-inflammatory proteins including IL-10 and IL-1 receptor antagonists have been found in amniotic stroma.\textsuperscript{1,93} Various antiangiogenic substances have been recognized in amnion as well.\textsuperscript{93}

Corneal neovascularization is a non-specific response of the cornea to inflammation, and may be initiated during infectious and sterile corneal wounds and immune-mediated reactions.\textsuperscript{93} The exact mechanisms of corneal neovascularization have not been fully determined, but the migration, proliferation, and differentiation of endothelial cells are upregulated by various growth factors that are liberated during inflammation.\textsuperscript{93} These same proinflammatory chemicals are suppressed in the presence of AMT. This previously described anti-inflammatory action is a way in which amnion decreases corneal neovascularization. Another is through the release of soluble antiangiogenic factors from the epithelial and mesenchymal cells of amniotic membrane including the IL-1 receptor antagonist, all four types of TIMP, collagen 18, IL-10, thrombospondin-1, and pigment epithelium-derived factor (PEDF).\textsuperscript{1,93} These factors decrease vascular endothelial cell proliferation. AMT has been noted to prevent and induce regression of corneal neovascularization in clinical cases.\textsuperscript{1,93,99}

There is evidence that the amniotic membrane has diverse properties against bacterial and viral infections.\textsuperscript{1,93,96} The fact that the chorionamnion prevents the spread of bacteria from the mare to the fetus suggests that it may function as a physical barrier against infection.\textsuperscript{1,93} Additionally, various compounds that promote antimicrobial immunity can be induced in amnion, including various ILs, interferons, TNFα, activin A, inhibin A, pre-B cell colony-enhancing factor, and leukemia inhibitory factor.\textsuperscript{1,93} Freshly isolated amnion displays constitutive immunity against many viral infections and it contains its own interferon, which differs from interferon-α, β, and λ or TNF.\textsuperscript{1,93} Amnion is also able to store antibiotics and release them over several days.\textsuperscript{1,93}

**Indications for AMT**

The anti-inflammatory and antiangiogenic properties of amnion make it an ideal substance to mitigate the destructive forces at work in several types of equine keratopathy.\textsuperscript{1,93} By decreasing the concentration of inflammatory cytokines, AMT can reduce the severity of corneal melting and corneal neovascularization and improve the likelihood of organic reparation of both epithelial and stromal defects. AMTs perform protective functions in corneas that have been structurally compromised by disease or keratectomy. By modulating corneal fibroblasts, it can decrease scarring and improve corneal clarity.\textsuperscript{1,93,99} We are beginning to use AMT with attached equine limbal stem cells as a treatment for corneal ulcerations of many types including those occurring as the result of limbal stem cell deficiencies because amnionic membrane (AM)\textsuperscript{8,14} can accommodate proliferating and differentiating epithelial cells.\textsuperscript{7,8,13,22} AMT therapy in human ophthalmology is considered superior to the placement of conjunctival grafts and tarsorrhaphy because it provides a more optically and cosmetically acceptable outcome. In veterinary ophthalmology, AMT has resulted not only in superior cosmetic outcomes but has also permitted greater potential for
vision compared with the more traditional therapy of conjunctival grafting.\textsuperscript{1,93}

In horses, AMT has been used with great success in clinical cases of severe ocular surface diseases such as ulcerative keratitis and keratomalacia to preserve globe integrity and a clear visual axis and as an adjunct in the reconstruction of the cornea and conjunctiva after excision of surface neoplasms.\textsuperscript{85,100,104} It has also been used successfully in clinical cases of keratomalacia, ankyloblepharon, and after resection of corneal neoplasia in dogs and cats.\textsuperscript{102–104} AMT is a versatile surgical technique in both human and veterinary ophthalmology with a constantly expanding list of potential indications.\textsuperscript{93}

**AM Harvesting and Storage**

We have had success using the guidelines concerning procurement and processing of tissue reported by the Food and Drug Administration (FDA) for our equine placentas.\textsuperscript{1,93} Although we do not screen donor mares for specific diseases or infections, we take care to use amnion harvested aseptically from healthy placentas from healthy mares with no known history of transmissible disease.

The fetal component of the placenta that consists of the inner amnion and the outer chorion forms the limits of the sac that encloses the fetus.\textsuperscript{1,93} The amniotic epithelial monolayer is adjacent to the fetus, whereas the spongy stromal matrix sits adjacent to the chorion. The amnion is relatively easily separated from the chorion by blunt dissection because the amniotic stroma is only loosely connected to the chorion.\textsuperscript{1,93} The amnion is recognized as the avascular portion. The amniochorion is placed in 2 l of phosphate-buffered saline (PBS)\textsuperscript{a} with 5 mg EDTA for 30 min and washed completely three times in PBS. It is cut to a manageable size (5 × 5-cm squares) and placed epithelial side up on nitrocellulose laboratory paper.\textsuperscript{93} The amnion is then placed in a 1:1 mixture of glycerol and Hanks balanced salt solution\textsuperscript{a} and frozen at −80°C until further use. Amnion may also be freeze-dried or lyophilized for storage.\textsuperscript{1,93}

**AM Application Principles**

The most frequent use of AMT in veterinary ophthalmology to date has been in the equine eye because of the relative ease of procuring and handling equine amnion compared with that of small animals and the catastrophic severity of corneal diseases experienced by horses.\textsuperscript{93,100,101} However, equine amnion has also been used successfully in corneal and conjunctival reconstruction in small animal patients.\textsuperscript{101–105} There are three basic principles for the application of AMT on which the surgical technique chosen for an individual case is based.\textsuperscript{93}

**Inlay or Graft Technique**

In this procedure, the amnion is tailored to the size of the defect and is meant to act as a scaffold for epithelial cell migration. This graft becomes integrated into the host tissue. The amnion is secured with its basement membrane up facing away from the stroma to allow migration of surrounding migrating epithelial cells onto and across the membrane.\textsuperscript{93}

**Overlay or Patch Technique**

The patch technique uses amnion as a sort of biological contact lens to protect the healing surface of the defect below it. The patch may also reduce inflammation by its barrier effect against the chemical mediators in the tear fluid. When used as a patch, the amnion is secured to the cornea with its basement membrane side down that prevents adhesion and incorporation of the patch such that the amnion is not incorporated into the cornea and sloughs. I now believe that amnion sloughing 7–10 days postoperatively yields the least scarring and provides the most therapeutic benefit in horses with severe ocular surface disease.\textsuperscript{93}

**Filling-in or Layering Technique**

Multiple pieces of amnion trimmed to the size of the defect are placed within the depth of the ulcer crater. A larger graft is secured to the edges of the defect in an inlay fashion. An additional larger patch may be placed superficial to the AMT to help protect the deeper layers.\textsuperscript{93} Double layer amnion grafting is also very beneficial in horses with severe ocular surface disease.

In preparation for surgical placement of AM, the eye should be prepped routinely with an antimicrobial wash, usually dilute povodine-iodine, and a topical anesthetic agent applied. Loose epithelium surrounding an ulcer, or over an area of bullous keratopathy should be debrided with forceps, microsurgical sponges, and a microsurgical blade.\textsuperscript{93} Any diseased or fibrotic conjunctiva or necrotic melting cornea should be completely resected before placement of the graft or patch. Once the wound or defect is adequately prepared, the amnion should be trimmed to the desired size on the paper and oriented atop the lesion. It can be difficult to orient the amnion if it comes off of its nitrocellulose membrane.\textsuperscript{93} The epithelial side is shiny, and the stromal surface can be identified by the presence of vitreous-like strands.\textsuperscript{93}

Typically, simple interrupted or continuous sutures will be used on any graft-type applications.\textsuperscript{93} Most grafts are fashioned so that the graft is 1 mm larger than the defect to which it is to be applied.\textsuperscript{93} Patch applications usually involve even larger pieces of amnion and are usually sutured to the limbus, again with 7-0 or 8-0 vicryl or its equivalent. In most of our equine cases, the AM was affixed to the corneal or conjunctiva with either 7-0 or 8-0 vicryl in a simple interrupted or continuous pattern.\textsuperscript{93}

Sutureless techniques are in development in an attempt to improve the ease of application, decrease the time needed for application and anesthesia if indi-
Medical therapy for the primary disease process continues several weeks postoperatively for horses with AMT. The most common and serious complications of AMT are the spread of an underlying infection to adjacent cornea or the anterior chamber in cases of infectious keratitis and the failure of the AMT to be retained.23 In cases of extensive ocular disease, the membrane may either dissolve or be sloughed necessitating its replacement.2,4,5,7,22

Nictitating Membrane Flaps
Nictitating membrane flaps are recommended for superficial corneal diseases including corneal erosions and ulcers, eyes with facial nerve paralysis, temporary prevention of exposure keratitis, and to reinforce a bulbar conjunctival graft (Fig. 8).10,21,22

Enucleation
Panophthalmitis after perforation through a stromal ulcer has a grave prognosis.21 Whether or not treatment is applied, phthisis bulbi likely will result after a chronically painful course; therefore, to spare the unfortunate horse this chronic discomfort, enucleation is the humane alternative.10,21,22 Histopathologic examination of the globe is recommended.

10. Disruption of the Equine Ocular Surface by Fungi: The Biological Continuum of Equine Ulcerative Keratomycosis

Fungal organisms are characterized by the absence of chlorophyll and include mushrooms, molds, yeast, and fungi.10,106 Fungi are heterotrophic organisms that are usually filamentous and multicellular. The filaments are also called hyphae, which can be interrupted or divided by cross-walls or septa.106 Septate filamentous fungi include several species common to the equine eye (e.g., Fusarium, Aspergillus, Penicillium).10,37,43 Each hypha has a surrounding, definitive cell wall made up of chitins, glucans, and mannans.106 Chitin, which is a structural polysaccharide, is a cell wall component of fungal hyphae that is absent in vertebrate systems. The plasma membrane of fungal hyphae, interior to the cell wall, contains ergosterol, which is a cell membrane sterol frequently targeted by antifungal agents.106

Fungal organisms are ubiquitous in the equine environment and are considered normal inhabitants of the equine conjunctival microflora that have a powerful pathogenic potential.10,37,39,43,72,107,108 Geographic environmental differences undoubtedly exist to account for variation among particular fungal species in specific regions. Previous reports have indicated a propensity for fungal keratitis in the summer and fall months.10,73 but other reports do not find this.40 This may result from temperature and humidity factors favoring fungal replication, or it may result from an increase in the amount of time that horses spend in their stalls.109 The seasonality of infection in the southern United States shows that ulcerative keratomycosis occurs throughout the year, with most cases occurring in...
the late fall to winter months (i.e., October through January).72

In the semi-tropical environment of Florida, the conjunctiva in 95% of normal horses was found to have a fungal flora that included Aspergillus, Penicilium, Alternaria, Cladosporium spp., or some combination of the four.37 The equine globe is large and prominently placed, and the equine environment is full of plant material that often harbor fungal elements. The normal equine eye also has a conjunctival bacterial flora that consists primarily of Gram-positive, non-pathogenic bacteria.41,107,109 The presence of a few Gram-negative rods are also normal. Bacteria suppress the fungal populations of the cornea and conjunctiva by competing for nutrients.10

The pathogenesis of ulcerative fungal keratitis commonly begins with superficial corneal disease that results in an epithelial defect and stromal invasion by the commensal fungal organism or seeding of fungi from a foreign body of plant origin.42,43 Abnormal tear film stability may also predispose to fungal keratitis.50 Stromal destruction results from the release of proteases and other enzymes from the fungi, chemoattracted leucocytes, and keratocytes.8,21,22,43

Use of topical corticosteroids has been implicated in enhancing fungal replication and in predisposing the cornea to fungal infection and decreasing the efficacy of antifungal agents.110,111 A previous report stated that 10 (62.5%) of 16 horses with ulcerative keratitis resulting from fungi had been treated with topical or subconjunctival corticosteroids.35 In another study, only 6 (15.4%) of 39 horses had been treated with topical corticosteroids,72 but topical antibiotics had been used in 32 (82.1%) of 39 of these horses before referral and diagnosis.72 Topical antibiotic therapy may suppress susceptible bacterial strains while allowing multiplication of resistant organisms,10 thus making conditions more favorable for fungal replication. Previous treatment with topical antibiotics may have as great an influence as previous topical corticosteroid treatment on the occurrence of equine ulcerative keratomycosis.21,72

Veterinary clinicians have hypothesized for many years that the large size of the horse eye, some inherent ocular surface weakness, and corneal trauma were necessary for fungal invasion and infection of the horse cornea.1 I no longer believe that corneal trauma is necessary for most cases of fungal invasion of the horse cornea but believe that some aggressive fungi can induce primary disease of the tear film and cornea in horses. The reason why keratomycosis is so common in the horse—and so rare in animals such as the dog, cat, and cow—may be that the horse exhibits immunoprotective deficiencies of the tear film or cornea (or both) that predispose to fungal infection.

Diagnosis of Equine Keratomycosis

Equine keratomycosis is represented clinically and pathologically as a range or continuum of lesions. One type of lesion may proceed to the next or even revert to the previous clinical form. There seem to be several clinical variations of keratomycosis in the horse.

Clinical suspicion for fungal involvement and response to antifungal therapy can also aid in making the diagnosis (Table 6). In one report, cytology (86.1% positive) and culture (84.6% positive) were effective means of making the diagnosis of keratomycosis.72 Because of the propensity for deep invasion by fungal organisms, however, negative cytologic or culture results may be obtained from superficial cytology specimens.21 Histopathologic examination of keratectomy specimens can also be highly effective for obtaining evidence of fungi, but these specimens must be obtained after keratectomy and thus are less frequently available for diagnostic purposes.72

Forms of Equine Keratomycosis

Fungi with high pathologic potential have the ability to adhere strongly to the epithelial or stromal tissue and also attract large numbers of PMNs that release excessive amounts of MMPs into the stroma that allow vertical or deep movement of the fungi into the cornea. Fungi of low pathogenicity adhere poorly, do not attract PMNs, are associated with small quantities of MMPs, and move laterally or horizontally in the corneal stroma.59 Fungi infecting the horse cornea appear to have some affinity for Descemet’s membrane. Fungal hyphae and varying degrees of PMN infiltration are often found deep in the horse cornea.21,22,42,43 This deep corneal fungal invasion to the level of Descemet’s membrane can progress further in some eyes and lead to liberation of fungi into the anterior chamber, iris, and lens to cause horrific endophthalmitis.21,22,42,43,108

Fungal-Induced PTF Instability

This may be the earliest form of equine ocular surface pathology in the horse. Fungal “induced pre-corneal tear film instability” is either induced directly by fungal organisms and proteases or is a

Table 6. Suspect Fungal Involvement of the Ulcer

<table>
<thead>
<tr>
<th>Suspect Fungal Involvement of the Ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does not heal quickly</td>
</tr>
<tr>
<td>Does not vascularize</td>
</tr>
<tr>
<td>Is associated with plant material</td>
</tr>
<tr>
<td>Has been treated with corticosteroids</td>
</tr>
<tr>
<td>Follows prolonged topical antibiotics</td>
</tr>
<tr>
<td>Has rose bengal stain retention</td>
</tr>
<tr>
<td>Has positive cultures</td>
</tr>
<tr>
<td>Looks dry</td>
</tr>
<tr>
<td>Has positive cytology</td>
</tr>
<tr>
<td>Has positive PCR</td>
</tr>
<tr>
<td>Has a thick brown/yellow corneal plaque</td>
</tr>
</tbody>
</table>

AAEP PROCEEDINGS / Vol. 56 / 2010 91
reaction to fungi in the tear film (Figs. 26 and 27). Environmental irritants may play a role in the pathogenesis and persistence of this problem. It appears to represent a qualitative form of KCS. These eyes are mildly uncomfortable, rose bengal positive, and fluorescein negative. The cornea appears dry and roughened, but the tear levels are normal. Treatment is topical serum and antifungals and fly masks. Eyes with this problem can develop ulceration.

Epithelial Non-Ulcerative Keratomycosis

Epithelial non-ulcerative keratomycosis is the least recognized form of equine keratomycosis. The fungi are diffusely located over the entire cornea in the superficial layers of the corneal epithelium. There is mild ocular pain, the cornea is gritty to the touch, and there is very faint to no fluorescein retention (Fig. 28) and negative rose bengal staining. Observation with a slit-lamp biomicroscope shows epithelial opacities. Hyphae are found with cytology and the condition responds to topical miconazole.

Subepithelial Keratomycosis

Subepithelial keratomycosis represents another type of “early equine keratomycosis” and is also difficult to recognize without the slit lamp. The corneal lesions may be either punctate (Figs. 29 and 30) or diffuse (Fig. 31) and are located beneath the epithelium. The opacities are fluorescein positive in some cases but not all. A few eyes are rose bengal positive. The subepithelial form of keratomycosis can progress to the non-ulcerative epithelial form of keratomycosis. Non-ulcerative epithelial and subepithelial keratomycosis in the horse may represent a preliminary phase of a continuum of clinical signs of equine keratomycosis.

Other differential diagnoses for subepithelial keratomycosis are immune-mediated keratitis, viral keratitis, KCS, and corneal scarring.

Epithelial Ulcerative Keratomycosis

Does loss of epithelium after subepithelial invasion lead to the more classic forms of keratomycosis? The more fungal and neutrophil proteases are involved, the deeper is the fungal problem. Stromal keratomycosis and deep melting ulcers are the more classic situations. Therefore, it is not a good idea to “grid” fungal ulcers!

Ulcerative keratitis refers to a disruption of the corneal epithelium, with varying amounts of stromal loss that may have concurrent bacterial or fungal infection (or both) (Figs. 12, 18, 24, and 32). Ulcerative keratomycosis is a serious, sight-threatening disease in the horse, and the veterinary liter-
ature is replete with cases of poor visual outcome in the various types of equine keratomycosis. Vision after keratomycosis in the horse may be retained in as few as 50% of eyes, with nearly one half the eyes with ulcerative keratomycosis reported to become blind, require enucleation, or both. In our opinion, this high failure rate is caused by a lack of aggressive surgical therapy early in the disease, when ulcerative keratomycosis is most effectively treated.

Clinical signs associated with equine ulcerative keratomycosis include miosis, blepharospasm, epiphora, and photophobia. The diagnosis of keratomycosis is made on the basis of finding fungal hyphae, mold, or yeast during cytologic or polymerase chain reaction (PCR) examination of a corneal scraping, culture of the corneal lesion, and/or histologic examination of a surgical keratectomy specimen.

Combined medical and surgical therapy is indicated if ulcers are extremely deep, are not responding to medical treatment, or worsen despite medical treatment. Surgeries for keratomycosis include conjunctival pedicle grafts, bridge grafts, hood...
grafts, island grafts, and full-thickness penetrating keratoplasty.\textsuperscript{10,21,22,43} Surgical treatments may leave the horse with a larger scar, but bulbar conjunctival grafts usually prevent corneal rupture and allow for physical support, a regional blood supply, and a supply of endogenous antiproteases to the ulcer site.\textsuperscript{10,21,22,42,43}

\textit{Aspergillus} sp. is the most common cause of equine ulcerative keratomycosis among cases presented to the University of Florida and in previous reports.\textsuperscript{10,35,112} Corneal aspergillosis seems to be a very destructive fungal infection in the horse. Aggressive medical and surgical therapy for ulcerative keratomycosis in horses should result in a positive visual outcome and ocular survival in \textgreater 90\% of eyes.\textsuperscript{72} Despite this success, however, therapy is quite prolonged, and scarring of the cornea may be prominent. New antifungal and antifibrotic medications are needed.

\section*{Thick Pigmented Fungal Plaques}

Some horse eyes with keratomycosis develop large-diameter pigmented plaques on the corneal surface that can be \textgreater 1 mm thick (Figs. 26, 32, and 33). Stromal infiltration of PMNs is pronounced beneath the plaques. The plaques are generally surrounded by intense corneal vascularization. Surgical removal of the plaque is necessary to speed healing. The tear film is generally unstable near plaques.\textsuperscript{21}

\section*{Superficial Stromal Abscesses}

Superficial stromal abscesses may be caused initially by superficial corneal ulcers (Fig. 34). The ulcers rapidly heal, and the epithelium is intact with superficial stromal abscesses. Bacteria and fungi may be involved. Most of these respond to aggressive topical medical therapy with antifungal medications such as voriconazole.\textsuperscript{21}

\section*{Full-Thickness Fungal Stromal Abscesses}

Fungal stromal abscesses may be superficial initially and then move vertically (Figs 35 and 36). All layers of the cornea can be affected. Surgery with penetrating keratoplasty is necessary in many of these eyes.\textsuperscript{21}

\section*{Deep Fungal Stromal Abscesses}

A stromal abscess can result from stromal inoculation with bacteria or fungi through a small or large corneal epithelial defect (Figs. 37–44).\textsuperscript{21} They may also arise from the iris.\textsuperscript{113} The organisms become encapsulated in the corneal stroma after re-epithelialization of the corneal ulcer over the infection site. Yellow-white opacities at the level of the posterior stroma and endothelium are present with the possibility of anterior chamber involvement. There is corneal edema and vascularization anterior to the abscess. The vascularization may be asymmetrical. The vast majority of deep stromal abscesses are caused by fungi.\textsuperscript{21} Lamellar keratoplasty is the preferred surgical treatment method.

\section*{Iris Prolapse}

Iris prolapse is defined as corneal, limbal, or scleral perforation (or some combination of the three), with iris protrusion through the wound after trauma or deep ulceration. These three conditions all may be associated with fungal infection (Figs. 6, 19, 20, 23, 43, and 44). Iris prolapse is an example of the eye
trying to heal itself. It is not hopeless, but only ~47% are visual after therapy.114

Lens and Fungi
Descemet's membrane and the lens capsule are both type IV collagen. Fungi can be associated with lens capsule invasion in the horse (Fig. 21). A persistent uveitis is present, but the condition has not been successfully treated.113 Fusarium spp. and Cladosporium spp. have been cultured from eyes with lens capsule invasion.113

11. Therapy for Equine Keratomycosis
Treatment must be directed against the fungi and against the corneal and intraocular inflammatory responses that occur after fungal replication and hyphal death. I would argue that it is not that hard to kill fungi but that the dead hyphae are difficult to remove by the ocular defense mechanisms that result in long-term inflammation and slow healing.10,21,42,43 Dead fungi attract leucocytes. The primary leukocyte attracted is the PMN, which is too small to phagocytize fungal hyphae. The PMNs release large amounts of proteases that can be detrimental to the horse cornea. Macrophages are large enough to remove hyphae but are present in insufficient numbers to remove the hyphae rapidly. The dead hyphae are thus also inflammatory.

The levels of ophthalmic antifungal drugs in the horse cornea needed to achieve fungicidal effects are so difficult to obtain that many such agents, including miconazole, are generally considered to exhibit fungistatic activity only. Loss of corneal epithelium results in increased corneal and aqueous drug concentrations.10,21,42,43 Long duration of antifungal drug exposure has been recommended for complete fungal destruction and resolution of clinical signs, but this may not be completely true.10,21,42,43 Natamycin is often used topically in the horse.10,21,42,43 It is a polyene antifungal agent that is the only commercially available topical ophthalmic antifungal medication in the United States. It is very effective against Fusarium sp., less so against Aspergillus sp., and least effective against yeasts.10,21,42,43 Natamycin is produced by the bacteria Streptomyces natalensis. This bacteria is a part of the normal corneal microflora of the horse and may produce endogenous tear film levels of natamycin that normally suppress the fungal populations of the horse cornea. A lack of efficacy of natamycin against Aspergillus spp has been noticed.
in Florida. Silver sulfadiazine is a topical antimicrobial agent with both antifungal and antibacterial activity that is believed to be fungicidal and is used in equine eyes.\textsuperscript{10,21,42,43} Dilute (1:50) povidone-iodine is effective against bacteria, fungi, viruses, and protozoa, and it has also been used therapeutically for corneal ulcers.\textsuperscript{10,21,42,43} It may have particular efficacy against \textit{Fusarium} isolates.\textsuperscript{10,21,42,43} Itraconazole and dimethyl sulfoxide (DMSO) are being used successfully for equine keratomycosis in the northern parts of the United States but do not work well for me in Florida.\textsuperscript{116}

Miconazole (1%) has been used successfully and frequently as a topical antifungal agent.\textsuperscript{10,21,42,43} It is most effective against \textit{Aspergillus} spp. It is an imidazole antifungal drug with excellent corneal penetration, especially in those corneas with denuded epithelium.\textsuperscript{10,21,42,43} The 1% solution also does not retard corneal healing or cause pathologic changes during corneal epithelial regeneration.\textsuperscript{10,21,42,43} In Florida, microbial resistance to miconazole has been noted with a reduction in efficacy against \textit{Fusarium} spp. but is still effective against \textit{Aspergillus} spp.\textsuperscript{74} Antifungal susceptibility testing on fungal isolates from horses is recommended, but it can take several weeks to obtain results, and the results are not always consistent with clinical outcomes. The gynecologic version of miconazole is no longer recommended for topical use because it can be very irritating to the eye.

Voriconazole (1%) is a triazole antifungal drug that topically penetrates the intact horse cornea at therapeutic levels and is effective against both \textit{Fusarium} spp. and \textit{Aspergillus} spp.\textsuperscript{70} I have found it to be very effective in fungi infecting the eyes of horses in Florida.

Frequency of treatment is another important aspect to consider with antifungal agents. We currently initiate topically administered antifungal therapy for equine keratomycosis with the combination of natamycin and miconazole, or vori-
conazole alone, applied three to four times per day over the first few days. This treatment frequency was determined empirically because the intensity of iridocyclitis was often noted to be magnified dramatically on the day after topical antifungal administration at treatment frequencies higher than this. Sudden death of stromal fungi because of initiation of antifungal drug therapy can result in acute iridocyclitis in some eyes. Topical antifungal medications can be increased to six times per day on subsequent days if necessary.

Iridocyclitis is a frequent finding in horses with ulcerative keratomycosis, and it must be aggressively treated and controlled. This iridocyclitis can temporarily escalate in intensity after hyphal death once antifungal therapy is initiated. Flunixin meglumine, which is a prostaglandin synthetase inhibitor, is the most frequently used and efficacious NSAID for systemic treatment among horses we see for iridocyclitis. It unfortunately also reduces the speed of vascularization of the corneal ulcers. Phenylbutazone does not seem to have an effect on corneal vascularization but is also not as effective as flunixin at reducing ocular pain. One percent atropine sulfate, which is a parasympatholytic agent, is used in all cases for its mydriatic and cycloplegic effects (i.e., to dilate the pupil, stabilize the iris blood vessels, and diminish ciliary body muscle spasms associated with the axon reflex uveitis occurring with equine corneal ulceration). Topical 2.5% phenylephrine may be useful in conjunction with atropine (q 8 h) to achieve mydriasis in uveitic eyes, although phenylephrine by itself is a poor mydriatic for the horse eye.

12. Perforation of the Ocular Surface: Iris Prolapse in the Horse

Corneal perforation with iris prolapse may be a sequela of traumatic insult to the globe or orbit and of infectious and non-infectious ulcerative keratitis. Iris prolapse in the horse most frequently occurs after acute ocular trauma, particularly sharp and perforating corneal injuries causing rupture of the cornea, limbus, or sclera. Corneal perforation also unfortunately occurs secondary to enzymatic degradation of stromal collagen and ground substance caused by infectious and non-infectious ulcerative keratitis.

Fig. 38. (A) Asymmetrical vascularization is a cardinal sign of a stromal abscess. Note the intense corneal vascularization dorsally that is partially covering the abscess. Hypopyon is also present in this eye. (B) A fan like area of corneal vascularization at the six o’clock position obscures a deep stromal abscess.

Fig. 39. (A) A deep stromal abscess is removed by deep lamellar endothelial keratoplasty. (B) An endothelial scar is present 5 mo postoperatively.
The prognosis for horses with perforating corneal lacerations is generally considered to be guarded depending on the size of the corneal lesion, location of the corneal perforation, the degree of iridocyclitis before perforation, the length of time iris prolapse is present, and the mechanism for the iris prolapse (Figs. 6, 19, 20, 23, and 45). Perforating lacerations caused by sharp injuries are generally associated with a better prognosis than those caused by blunt or missile injuries. High-energy, blunt ocular trauma may result in hyphema or globe rupture (or both) at the limbus or equator where the sclera is thinnest. The prognosis of iris prolapse with corneal lacerations and mild hyphema is slightly more favorable, albeit still guarded, compared with that for horses having corneal lacerations and total hyphema. In one study in horses, iris prolapse and hyphema comprising greater than an estimated 10% of the anterior chamber resulted in blindness, phthisis bulbi, or enucleation. Both clinical and surgical guidelines for treatment of iris prolapse in the horse, which are based on the possibility of useful vision and ocular survival, will assist in choosing a therapeutic plan of action. Perforating lacerations confined to the cornea and measuring 15 mm or less in length tend to have a favorable visual outcome after surgical repair. Iridectomy does not generally exacerbate postoperative uveitis or adversely affect visual outcome, and it may facilitate postoperative mydriasis and prevent septic endophthalmitis. Conversely, perforating corneal lacerations measuring 15 mm or more in length and extending to, along, or beyond the limbus are inclined to a poor visual outcome and enucleation. Chances of retaining vision may also be substantially reduced in perforating corneal wounds accompanied by hyphema (even when comprising only 10–50% of the anterior chamber). If there is only a small amount of hyphema, endog-
enous tissue plasminogen activator (TPA) on the equine iris surface can digest the fibrin and blood clots. Large amounts of anterior chamber fibrin and blood clots will disappear faster after the injection into the anterior chamber of TPA (50–150 μg in 0.1 ml via intracameral injection). Eyes with perforating corneal ulcers of >2-wk duration, as well as eyes with melting ulcers, and ulcers with concomitant fungal and bacterial infections (or both) tend to have a poor visual outcome or result in enucleation because of endophthalmitis.21,114 Eyes with bacterial or fungal corneal ulcers of long duration may have substantially more preoperative iridocyclitis and be at a greater risk of developing aggressive endophthalmitis postoperatively than ulcers of short duration, thus predisposing to more extensive development of anterior and posterior synechiae, cataract, glaucoma, and retinal detachment.114 If uncontrolled, iridocyclitis may predispose to fibropu-
pillary membrane formation with subsequent poste-
rior synechiae and cataract.21,120

Surgical Repair of Iris Prolapse
Several concepts are important in the microsurgery of iris prolapse repair.10,21 The corneal endothelium is very sensitive to mechanical trauma such that, when manipulating or holding corneal tissue, only the stroma and epithelium should be held with the corneal forceps. The edges of traumatic corneal perforation are not generally debrided, but the edge of a perforation caused by an ulcer may need debride-
ment. If an iris prolapse is fresh, an attempt is made to replace the protruding iris. If the iris tissue is damaged or necrotic, the protruding iris is excised with electrocautery.10,21 Hemorrhage is always a risk if the iris is transected. Partial-thick-
ess sutures (1/2 to 3/4 depth) are used in the cornea; full-thickness penetrating sutures are never used. The cornea is sutured with a simple interrupted pattern (1 mm apart) using 5-0 to 7-0 vicryl or nylon suture. After partial closure of the wound, blood and fibrin clots in the anterior chamber are carefully removed by gentle anterior chamber irriga-
tion with lactated Ringer’s solution (LRS), or more appropriately digested with TPA (50–150 μg in 0.1 ml via intra-cameral injection). TPA can successfully digest blood clots in the anterior chamber of horses that have been present for 2 wk.10,21 Wound closure is completed, and the anterior chamber is reformed with hyaluronic acid, LRS, or an air bub-

Fig. 42. (A) A deep stromal abscess is treated with a deep lamellar endothelial keratoplasty. (B) An endothelial scar is present 3 yr postoperatively.

Fig. 43. (A) A deep stromal abscess with hypopyon is treated with a deep lamellar endothelial keratoplasty. (B) The graft is slightly wrinkled 1 yr postoperatively.
ble. A Seidel’s test is used to test for suture line integrity.

13. Chemoattraction of Neutrophils to the Equine Ocular Surface: Corneal Stromal Abscesses

The complete etiology of stromal abscessation is not totally known in the horse but may have several causes. Corneal stromal abscess in the horse can be a vision-threatening sequela to apparently minor superficial corneal ulceration. A corneal stromal abscess may develop after epithelial cells adjacent to a small epithelial defect or a micropuncture divide and migrate over the puncture wound to seal infectious agents or foreign bodies in the stroma (Figs. 28, 38–44, and 46–50). This re-epithelialization forms a barrier that protects the bacteria or fungi from topically administered antimicrobial medications. The avascularity of the cornea and absence of corneal lymphatics slow the recognition of such infectious agents by the ocular immune system, but soluble microbial antigens, cytokines, and collagen fragments may diffuse to the limbus for exposure to the lymphatic system, enter the bloodstream through perilimbal vessels, or diffuse into the aqueous humor to attract rapid migration of neutrophils into the cornea. These neutrophils release degradative enzymes into the stroma to cause further collagen degeneration and exacerbate the initial chemoattraction for more neutrophil and leucocyte migration.

The micropuncture hypothesis does not, however, explain clustering of cases of stromal abscesses to a specific farm or location or groups of stromal abscess cases at certain times of the year. It does not
explain the cases of stromal abscesses in related animals. Some stromal abscesses seem to be a result of a systemic disease or are caused by microendothelial puncture from the iris and anterior chamber. A familial or genetic predisposition in the horse for stromal abscessation may exist. Superficial stromal abscesses tend to be associated with bacteria and deep stromal abscesses caused by fungi. The pathogenicity of fungi may also vary in stromal abscesses with fungi of low pathology staying relatively superficial and moving horizontally in the stroma and more pathologic fungi moving deeper vertically in the stroma.

Bacteria or fungi can infect corneal stromal abscesses, or they can be sterile. Many cytologic, microbiologic, and histopathologic specimens obtained pre- and postoperatively from stromal abscesses fail to yield diagnostic results, although infection with bacteria or fungi may have triggered abscess formation. Subsequent medical therapy may sterilize the abscess, but the degenerating PMNs release proteases that cause the stromal keratitis, pain, and uveitis. Horses that undergo early surgery in the course of this disease tend to have a more rapid recovery than those in which surgery is delayed. Re-epithelialization of stromal abscesses interferes with both routine diagnosis and treatment. A stimulus for epithelialization or absence of an inhibitor of epithelialization, which is not found in equine ulcerative keratitis, must be present in cases of stromal abscess for them to form. Re-epithelialization after corneal scrapings in medically treated horses is rapid and quite dramatic. Representative preoperative cultures are difficult, if not impossible, to obtain because of both the intact, epithelialized surface of the abscess and the deep location of the lesions in the cornea. The epithelium and stroma over deep stromal abscesses generally are relatively healthy, and they are difficult to remove.

The diagnosis of stromal abscessation is made on the basis of a focal, yellow-white, stromal infiltrate with associated corneal edema. Recurrence of stromal abscesses is possible. The recurring lesions tend to be orange in color. Some eyes with stromal abscesses have the initial clinical signs suggestive of minor corneal trauma and slight anterior uveitis. Fluorescein dye retention is either negative or positive over an area much smaller...
than the diameter of the corneal lesion. Single or multiple abscesses may be present. A mild-to-fulminating iridocyclitis occurs secondary to what initially appears to be a relatively benign corneal disease, thus suggesting to some clinicians that the condition is not likely to be painful or result in blindness.\textsuperscript{10,21,24,108,121--123} Corneal vascularization is variable but generally superficial at presentation such that the vascularization may obscure observation of the precise location of the abscess. Asymmetrical patterns of corneal vascularization are a classic sign of a stromal abscess (Figs. 38, A and B, 40, and 43).\textsuperscript{10,21,24,108,121--123}

A chronic stromal abscess with secondary uveitis may be difficult to differentiate from eyes with equine recurrent uveitis. Melting and infected ulcers of horses are also associated with severe anterior uveitis, but each is distinct in general appearance from a stromal abscess, with ulcers invariably retaining fluorescein stain over most of the lesion and stromal abscesses only retaining stain over a small area of the lesion during the acute stages (if at all).\textsuperscript{10,21,24,108,121--123}

**Stromal Abscess Therapy**

Some investigators have suggested that medical therapy for stromal abscesses may be more appropriate than surgical therapy.\textsuperscript{122} Vascularization will heal stromal abscesses if the vessels grow to the proper depth, but the healing process may take several months. Many superficial stromal abscesses will initially respond positively with less uveitis to topical mydriatic/cycloplegics and to topical and systemic antibiotic and NSAID therapy, but many gradually worsen clinically, thus requiring surgical intervention. Repeated scraping to remove the corneal epithelium may allow better drug penetration for superficial stromal abscesses but does little to heal deep stromal abscesses.

**Stromal Abscesses**

Stromal abscesses do not completely heal until they become vascularized, either directly from a conjunctival graft or indirectly from corneal vascular in-growth.\textsuperscript{10,21,24,108,121--123} Deep and superficial corneal vascularization must occur in deep stromal abscesses for the lesion to resolve. Abscess material projecting into the anterior chamber will not vascularize and will continue to stimulate iridocyclitis. Some degree of corneal vascularization is generally present, but it seems to grow much more slowly than the 1–2 mm/day
reported in the horse. Vascularization of deep stromal abscesses may only occur superficial to the abscess, or to the abscess margin, but not vascularize the deeper lesion itself. The ability of the horse eye to vascularize the stroma near Descemet’s membrane is very poor. Superficial vessels will not make a 90° turn to grow into the deeper cornea. This suggests that factors are being released from the abscess that influence the vascular response. The cyclooxygenase and lipoxygenase inflammatory pathways are instrumental in corneal angiogenesis, and use of flunixin meglumine, which is an inhibitor of the cyclooxygenase pathway, for anterior uveitis will, at higher doses, decrease the speed of corneal vascularization. We have noticed that vascular perfusion and formation of corneal vessels increases when we change from flunixin meglumine to phenylbutazone, when a treatment of systemically administered flunixin meglumine is inadvertently missed, or when the dose of flunixin meglumine is reduced. There is an important balance in the use of flunixin meglumine between reducing the iridocyclitis, which can potentially blind the horse whether the cornea heals or not, and resolving the stromal keratitis.

Most deep stromal abscesses are of a solid, inspissated, rather than liquefied, nature such that they cannot be “drained.” A very few stromal abscesses are liquefied in the early stages of abscess formation and may be removed by drainage by cannulation. Abscesses projecting into the anterior chamber require surgical removal by lamellar keratoplasty in most situations.

If significant improvement in the signs associated with a deep stromal abscess does not occur within the first 48–72 h of intense and appropriate medical therapy (Table 7), surgery can improve results and reduce the duration of medical therapy. If medical therapy alone is used, systemic NSAID therapy should be carefully adjusted to allow control of the anterior uveitis without significantly inhibiting the corneal vascularization necessary to heal the corneal stromal abscess. Corneal abscesses vascularize at a slow rate, however, and waiting for the cornea to heal may result in loss of vision from complications of the severe endophthalmitis that can be found with this disease.

Deep stromal abscesses generally have a poor therapeutic outcome with medical therapy alone because they vascularize poorly or not at all, and most stromal abscesses involving Descemet’s membrane are, in my experience, fungal infections. Deep lamellar and penetrating keratoplasties are used in eyes with abscesses near Descemet’s membrane and in eyes with rupture of the abscess into the anterior chamber. This aggressive surgical therapy can be very successful and is performed both to eliminate antigenic stimulation from the sequestered organisms and to remove the necrotic debris, metabolites, and toxins from the degenerating leucocytes and microbes in the abscess. Keratectomy specimens may also be the only way to obtain a definitive etiologic diagnosis via culture, histology, or PCR to institute proper antimicrobial therapy. Placing a conjunctival pedicle graft over a deep lamellar keratectomy site rapidly restores the physical integrity of the cornea by supplying fibrovascular tissue to fill in the stromal defect, and it delivers a focal, direct blood supply such that the need for vascularization is met, and angiogenic factors are no longer required to stimulate the ingrowth of limbal blood vessels. In addition, conjunctival grafts allow plasma, leucocytes, antibodies, and systemically administered antibiotics to reach the diseased cornea.

14. Ocular Surface Disruption

Recurrence Erosion (Chronic Superficial Ulceration)

Indolent or non-healing superficial erosions are superficial ulcers characterized by decreased adhesion of healing corneal epithelial cells to the epithelial basement membrane. They are relatively common in the horse. Foals and adult horses may be affected. Typically indolent ulcers are

<table>
<thead>
<tr>
<th>Table 7. Medical Therapy for Stromal Abscesses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug</strong></td>
</tr>
<tr>
<td>Topical natamycin (5%)</td>
</tr>
<tr>
<td>Topical antibiotic: bacitracin, neomycin, polymyxin B</td>
</tr>
<tr>
<td>Topical atropine (1%)</td>
</tr>
<tr>
<td>Systemic flunixin meglumine</td>
</tr>
<tr>
<td>Systemic omeprazole</td>
</tr>
<tr>
<td>Systemic antibiotics: doxycycline (D);</td>
</tr>
<tr>
<td>trimethoprim sulfa (TMS)</td>
</tr>
<tr>
<td>Itra: 5 mg/kg, PO, q 12 h</td>
</tr>
<tr>
<td>Fluc: 14 mg/kg, PO loading dose, and then 5 mg/kg, PO, q 12 h × 7 days, and then 5 mg/ kg, PO, q 24 h</td>
</tr>
</tbody>
</table>

AAEP PROCEEDINGS / Vol. 56 / 2010 103
sterile with no detectable abnormality of the eyelids, tear film, or palpebral conjunctiva that would prevent healing of the ulcer. The etiology is poorly understood in all species, but is likely to involve a complex process of failure of normal basement membrane regeneration and absent or delayed hemidesmosome attachment after minor superficial insult. Overexpression of MMPs by abnormal basal epithelial cells in combination with anomalous expression of adhesion macromolecules such as fibronectin and laminin may be contributing factors. A hyaline membrane covering the ulcer site may hinder communication between epithelial and stromal cells to prevent healing. In some cases, the disease may represent a primary EBM disease. Rarely, a focal endothelial abnormality and corneal edema, for example, in association with anterior synechia, may cause a non-healing epithelial erosion. Affected eyes show only moderate discomfort and lacrimation, and there is an area of discrete, static shallow ulceration with typically redundant non-adherent epithelial margins and a narrow zone of corneal edema. Fluorescein uptake may be faint because of partial reformation of the corneal epithelium. Some degree of vascularization occurs in only around one third of indolent ulcers. All eyes with indolent ulcers should be carefully examined for underlying mechanical causes of superficial ulcers such as ectopic cilia, and cytology and bacterial and fungal cultures should be carried out to identify an infectious etiology. Qualitative KCS may result in superficial erosions in horses, indicating a need for rose bengal staining and tear evaluation with the Schirmer tear test in horses with superficial ulcers.

Debridement of the redundant epithelial margins and base is the initial treatment of choice. Generally debridement is done only once and not multiple times. This may be used in conjunction with a therapeutic soft contact lens to physically protect regenerating basal epithelium. Where initial debridement fails, grid keratotomy, superficial lamellar keratectomy, and conjunctival grafting are further treatment options. Thermal cautery has been used successfully in two horses with non-healing erosions where epithelial debridement had failed. Topical polysulfated glycosaminoglycan (50 mg/ml in artificial tears) instilled into the eye three times daily or by continuous infusion is reported anecdotally to be successful in effecting re-epithelialization caused by modulation of serine protease expression in dogs but does not seem to help the horse. The prognosis in all cases is guarded because non-healing erosions are potentially recurrent and continued dehiscence of the poorly adherent repair epithelium by the mechanical action of the eyelids and/or poor tear film stability may be difficult to overcome.

15. Therapeutic Replacement of the Equine Ocular Surface

Targeted Lamellar Keroplasty and Full-Thickness Corneal Transplantation for Inflammatory Keratopathies in the Horse: A Paradigm Shift in Equine Corneal Transplantation

General Concepts of Corneal Transplantation

Corneal transplantation can be performed for optical, therapeutic, tectonic, and cosmetic reasons in horses. Penetrating keratoplasty (PK) is full-thickness microsurgical transplantation of the corneal epithelium, stroma, and Descemet’s membrane/endothelium. Corneal transplantation by PK is the most widely practiced form of organ transplantation in humans, with nearly 60,000 surgeries performed worldwide every year. Because of the immunologic privilege of the cornea, PK in humans with noninflammatory dystrophic keratopaties has a success rate approaching 75% 5 yr postoperatively. Keratitis, infection, corneal vascularization, and/or the need for re-operations dramatically reduce the success rate of PK in human patients.

PK: Humans and Horses

PK has long been the standard procedure for treating irreversible opacification of the human cornea from various infectious and noninfectious keratopaties. The terms penetrating and perforating can be a little confusing. The term PK is defined by its reference point of the incision in the globe; therefore, when a full-thickness PK graft is performed, the incision penetrates or enters the globe but perforates or enters and exits the cornea. The success rate of PK in noninflamed human corneas is high compared with those in other human organ transplants because of the low rate of immunologic rejection of corneal tissue. Graft rejection does, however, occur in human patients with vascularized, infected corneas, and subsequent repeat PK procedures in these eyes are inevitably less successful. Severe keratitis causing total destruction of limbal stem tissue is also considered a risk factor for PK failure in humans. Performing PK in the horse is always done under the extreme risk factors associated with corneal graft rejection.

Corneal transplantation for treatment of severe inflammatory nonulcerative and ulcerative keratopaties with corneal vascularization, infection, and...
Keratomalacia (melting) has been performed successfully in horses at the University of Florida since 1993 (Figs. 6, 18–20, 22, 23, and 36). Full-thickness PK may be performed in horses for therapeutic and tectonic reasons for melting ulcers with extensive stromal loss, iris prolapse/descemetocoeles, and full-thickness stromal abscesses. PK has become a viable, routine, and successful surgical technique in horses with severe keratitis and is associated with a very good visual outcome in most cases (80%). This is despite the fact that nearly all the corneal transplant donor tissue grafts in horses having PK vascularize postoperatively and exhibit troubling degrees of full-thickness corneal opacification at the surgical site.

**PK in the Horse: Surgical Procedure**

Several surgical procedures for corneal transplantation have been described for the horse. PK for full-thickness stromal abscesses or ulcers/iris prolapses with missing corneal tissue was used first. PK has become a viable, routine, and successful surgical technique in horses with severe keratitis and is associated with a very good visual outcome in most cases (~80%). This is despite the fact that nearly all the corneal transplant donor tissue grafts in horses having PK vascularize postoperatively and exhibit troubling degrees of full-thickness corneal opacification at the surgical site.

**PK: Donor Cornea Preservation**

Donor cornea is harvested from fresh equine globes within 24 h of death. Globes are obtained from horses euthanized for noninfectious diseases such as colic or laminitis. The corneas are hemisected and stored frozen at −20°C in a broad-spectrum antibiotic solution or frozen in corneal storage media. Topical natamycin (5%) and miconazole (1%); or 1% voriconazole. Topical antibiotic: gramicidin, neomycin, polymyxin B. Topical atropine (1%). Topical autogenous serum. Topical cyclosporine A (begun in 2003). Topical flurbiprofen. Systemic fluinixin meglumine. Systemic omeprazole. Systemic antibiotics: doxycycline (D) or trimethoprim sulfa (TMS). Systemic antifungals: itraconazole (Itra) or fluconazole (Flu).

**PK: General Surgical Considerations**

PK involves full-thickness removal and replacement of a diseased portion of the cornea. Corneal sutures induce refractive instability by causing disruption of the corneal surface topography but are necessarily used to heal the vertical stromal PK incision. Various sizes, types, and particular suture patterns have been used in PK surgeries.

Preoperative medications for PK, PLK, DALK, and DLEK are listed in Table 8. Topical serum and 2% cyclosporine A are added postoperatively. All PK, PLK, DALK, and DLEK procedures are performed under general anesthesia and muscular paralysis. An operating microscope is used for each procedure.

Hemorrhage from blood vessels of corneal vascularization is controlled with topical phenylephrine (2.5%), pressure, and/or electrocautery. The anterior chamber is reformed in each procedure by injecting viscoelastic solution (hyaluronate sodium, 10 mg/ml) into the anterior chamber. The viscoelastic solution also moves the iris posteriorly and breaks down any adhesions between the cornea and the iris. Direct contact with the lens capsule is to be avoided by reforming the anterior chamber with the viscoelastic solution that is left in the anterior chamber. The viscoelastic solution left in the anterior chamber disappears in 24 h.

**PK: Surgical Technique**

The surgical approach for PK in horses is as follows: The size of the corneal lesion is determined with calipers. The recipient globe is stabilized with scleral fixation sutures of 5-0 nylon. A corneal trephine of appropriate size is centered over the diseased area and rotated with minimal downward pressure to obtain a clear-cut, round incision with vertical sides. The incision with the trephine approaches to just near, but should not penetrate, Descemet’s membrane. The remaining intact deep stromal tissue is vertically incised with a #65 Beaver blade to enter the anterior chamber.

---

Table 8. Medications Used Preoperatively and Postoperatively for PK, posterior lamellar keratoplasty (PLK), deep anterior lamellar keratoplasty (DALK), and deep lamellar endothelial keratoplasty (DLEK)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose and Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical natamycin (5%) and miconazole (1%); or 1% voriconazole</td>
<td>q 6 h</td>
</tr>
<tr>
<td>Topical antibiotic: gramicidin, neomycin, polymyxin B</td>
<td>q 6 h</td>
</tr>
<tr>
<td>Topical atropine (1%)</td>
<td>q 6 h</td>
</tr>
<tr>
<td>Topical autogenous serum*</td>
<td>q 6 h</td>
</tr>
<tr>
<td>Topical cyclosporine A* (begun in 2003)</td>
<td>q 8 h</td>
</tr>
<tr>
<td>Topical flurbiprofen*</td>
<td>q 6 h</td>
</tr>
<tr>
<td>Systemic fluinixin meglumine</td>
<td>1 mg/kg, IV, IM, PO, q12 h</td>
</tr>
<tr>
<td>Systemic omeprazole</td>
<td>1 mg/kg, PO, q 24 h</td>
</tr>
<tr>
<td>Systemic antibiotics: doxycycline (D) or trimethoprim sulfa (TMS)</td>
<td>D: 10 mg/kg, PO, q 12 h</td>
</tr>
<tr>
<td></td>
<td>TMS: 30 mg/kg, PO, q 12 h</td>
</tr>
<tr>
<td></td>
<td>Itra: 5 mg/kg, PO, q 12 h</td>
</tr>
<tr>
<td></td>
<td>Fluc: 14 mg/kg, PO loading dose, and then 5 mg/kg, PO, q 12 h × 7 days, and then 5 mg/kg, PO, q 24 h</td>
</tr>
</tbody>
</table>

*Postoperative only.
being careful to avoid the iris, corpora nigra, and lens. The button of diseased host tissue is removed with corneal section scissors. The keratectomy buttons from the deep stromal abscess cases are processed for aerobic and fungal culture and sensitivity, cytology, and histopathology.

The anterior chamber is reformed by injection of viscoelastic solution before placing the graft.\textsuperscript{21,34,123} A full-thickness button of cornea that was 1 mm diameter larger than the recipient bed is trephined from the endothelial to the epithelial side of the donor cornea. The frozen cornea is most easily handled slightly, but not completely, thawed. The donor button is grasped with fine-toothed forceps while paying particular attention to the orientation of the epithelium/endothelium, placed on a gauze swab, and kept moistened with lactated Ringer's solution. The epithelium is not removed from the corneal donor button.

The donor cornea is placed in the recipient bed, and four cardinal sutures of 8-0 polyglactin 910\textsuperscript{o} or 9-0 nylon are placed at the 12-, 6-, 9-, and 3-o'clock positions.\textsuperscript{21,34,123} Simple interrupted sutures are placed to fill in the remaining sectors in each quadrant, or alternatively, a simple continuous suture pattern is placed to hold the graft. Once the donor cornea is sutured into place, viscoelastic solution is again injected at the limbus with a 20- to 22-gauge needle to completely reform the anterior chamber. The viscoelastic solution is not removed from the anterior chamber.

A conjunctival pedicle or amniotic membrane graft can then be sutured over the keratectomy/graft site in those eyes with evidence of infection, melting, or vascularization to achieve more rapid assimilation of the graft into the cornea.\textsuperscript{21,34,123} A temporary lateral tarsorrhaphy is performed to minimize eyelid trauma to the PK. Autogenous serum to decrease tear film proteinases attacking the graft and eyelid trauma to the PK. Autogenous serum to de-...
stromal abscesses.10,21 Deep stromal abscesses are some degree but is otherwise normal in many deep artificial cornea may be vascularized and edematous to host tissues.150,154,155

Targeted Anterior Stromal Lamellar Transplantation
Anterior lamellar keratoplasty (ALK) replaces the stroma, stromal keratocytes, and the epithelial layer with its transient amplifying cells.149 The healthy endothelium and Descemet’s membrane are retained to reduce the risk of endothelial allograft reaction.150,152,153 Preservation of Descemet’s membrane and endothelium slows corneal graft rejection and speeds corneal healing.152,153 Indications for ALK include anterior stromal scars, corneal melting disorders, descemetocoeles, small corneal perforations, corneal tumors, corneal infection, and keratoconus.152–154

The traditional technique of ALK is to manually dissect the stromal host tissue layer by layer with the use of corneal lamellar dissectors until Descemet’s membrane is exposed. There is reported to be a weak adhesion between the deep stroma and adjacent Descemet’s membrane in humans, and the goal of ALK is to use this transition zone to more easily manually dissect the stroma from Descemet’s membrane.155 It is still difficult to obtain a precise and perfectly smooth lamellar dissection manually and to venture deep enough to remove the majority of deep diseased stroma without causing perforation. Thus, the visual outcomes in humans after manual ALK are generally less than perfect because of collagen disruption at the interface between donor and host tissues.150,154,155

Deep ALK (DALK) is a new surgical technique to safely remove the entire corneal stroma down to bare Descemet’s membrane. It has the ability to remove all diseased stroma to lead to better visual outcomes156 and lowers the risk of endothelial cell allograft rejection that would occur with PK.152 Indications for DALK include keratoconus, chemical and thermal corneal burns, patients with vascularized corneas who are at high risk of endothelial rejection, and corneal perforations.145,150,154,155

Targeted Posterior Stroma and Descemet’s Membrane/Endothelium Lamellar Transplantation
The inherent philosophy of split-thickness or lamellar corneal surgery is to replace only the diseased portion of the cornea, leaving the normal tissue intact. In other words, to do the least amount of resection for the greatest amount of benefit.34,138,139,145,150 The superficial cornea may be vascularized and edematous to some degree but is otherwise normal in many deep stromal abscesses.10,21 Deep stromal abscesses are rarely liquefied abscesses but represent regions of corneal degeneration and focal neutrophilic invasion of the posterior stroma, Descemet’s membrane, and endothelium.10,108 Many deep stromal abscesses have extension into the anterior chamber and are believed to be a result of fungal infection.10,108,123 Surgical removal of the diseased posterior stroma and endothelium by lamellar corneal transplantation is warranted for horses with deep stromal abscesses confined to the posterior stroma and horse eyes with deep stromal abscesses with anterior chamber invasion that have a persistence or progression of severe pain and vision-threatening uveitis in the face of aggressive medical therapy.10,54,150,158

Posterior LK (PLK) involves replacement of diseased posterior corneal stroma, and Descemet’s membrane and endothelium with donor tissue while retaining the epithelium and anterior corneal stroma.150–152,157,158 The purpose of PLK is to target and replace the diseased Descemet’s membrane and endothelium and retain the overlying epithelium and stroma to reduce disturbing the topography of the ocular surface. This procedure is also termed DLEK.34,138,139 The PLK and DLEK techniques are recommended for therapy of Fuchs’ endothelial dystrophy, pseudophakic bullous keratopathy, iridocorneal endothelial syndrome, and failed penetrating grafts.138,139,150

Several variants of the PLK and DLEK procedures have been developed in humans. In general, a lamellar disc of endothelium and Descemet’s membrane is selectively excised with microscissors and a special trephine from the recipient eye through a small scleral incision or tunnel. The donor Descemet’s membrane and endothelium is inserted into the defect.139,150–159

The PLK and DLEK procedures have been improved with the use of automated microkeratomes.150 The Descemet’s membrane stripping endothelial keratoplasty (DSEK) procedure is a modification of the PLK. Descemet’s stripping endothelial keratoplasty and Descemet’s stripping automated endothelial keratoplasty (DSAEK) use a mechanical microkeratome to harvest the donor corneal graft and mechanical stripping of the diseased host endothelium and Descemet’s membrane.159 The visual results with DSEK and DSAEK are comparable in humans to vision after DLEK and seem to offer significant advantages over standard penetrating keratoplasty for human patients with persistent corneal edema.160

Targeted Epithelial Layer Lamellar Transplantation
Severe ocular surface diseases caused by total limbal stem cell deficiency can be treated by epithelial layer lamellar transplantation.1,150,161 The transplantation of epithelial cells restores epithelial stratification and barrier function and stabilizes and reduces the existing keratitis. Targeted replacement of corneal epithelium can be accomplished by limbal transplantation of intact limbal epithelial stem cells attached to AMTs1,145,150,154 or transplantation of sheets of cultivated epithelial stem cells.1,149,150,161
16. Equine LKs

DALK

**DALK: Surgical Technique**

DALK is indicated in horses for rapidly progressive, large diameter, catastrophic, melting corneal ulcers with and without exposing of Descemet’s membrane. Achieving a “true” DALK procedure down to bare Descemet’s membrane is difficult in the horse because there does not seem to be a “space” or “loose adhesion” between the equine Descemet’s membrane and posterior stroma. Hydrodissection, viscodissection, and corneal dissectors are used to bluntly dissect most of the diseased stroma from the equine Descemet’s membrane. Pressure by injecting air or a viscoelastic through a 30-gauge needle into the remaining deep stroma can be used to further dissect and detach the remaining diseased stroma from Descemet’s membrane. This pressure associated microdissection produces a dome-shaped detachment of the stroma from Descemet’s membrane that allows the remaining diseased stroma to be removed to expose the smooth and refractile Descemet’s membrane. Cases of microperforation of Descemet’s membrane during this part of the surgery can be resolved by injecting air into the anterior chamber.

This procedure replaces all diseased corneal tissues anterior to Descemet’s membrane and has been done successfully in horses with melting ulcers (n = 2), descemetoceles (n = 2), and corneal perforations with iris prolapse (n = 2). All horses did well except one with a melting fungal ulcer that was enucleated (Figs. 15, 22, 24, and 25).

**PLK**

The equine epithelium is nonulcerated and edematous, and the anterior stroma is vascularized but otherwise normal in most equine deep stromal abscesses. Despite the name, the inflammation of deep stromal abscesses is localized to Descemet’s membrane and the endothelium. For horses with deep stromal abscesses and anterior chamber fungal invasion through the endothelium that persist, or even progress, with severe pain and vision-threatening uveitis in the face of aggressive medical therapy, surgical removal of Descemet’s membrane and the endothelium containing the abscess by lamellar corneal transplantation is necessary to save the globe and preserve vision. PLK in horses is recommended for deep stromal abscesses in the axial cornea that are 10 mm or less in diameter. The overlying epithelium may be edematous but is intact, and the anterior stroma may be vascularized but should not have a cellular infiltrate (Figs. 40 and 44).

**PLK: Surgical Technique**

The surgical approach for PLK (Figs. 10–17, 27, and 28) in horses is as follows: a three-sided, rectangular, anterior lamellar corneal flap, hinged on one side, is constructed by hand dissection to two-thirds stromal thickness over the stromal abscess. The superficial flap of epithelium and anterior stroma is made at least 1 mm larger on each side than the diameter of the deep stromal abscess. A Martinez corneal dissector is used to undermine and elevate the superficial corneal layers to expose the abscess. The flap is gently raised, the abscess is measured with callipers, and a trephine, #65 Beaver blade, and corneal transplant scissors are used to remove the posterior stromal abscess, Descemet’s membrane, and endothelium. Hemorrhage is controlled as previously described. The deep keratectomy button is processed for aerobic bacterial and fungal culture and sensitivity, cytology, and histopathology.

The anterior chamber is reformed by injection of viscoelastic solution. A circular donor graft of posterior stroma, Descemet’s membrane, and endothelium 1 mm larger than the defect is cut from donor tissue using a trephine. The graft is placed in the corneal defect and sutured every 2 mm using 8-0 absorbable suture material in a simple interrupted pattern. The three-sided superficial flap is then sutured in place using 8-0 absorbable suture material. The viscoelastic is not removed from the anterior chamber. Partial temporary tarsorrhaphies are placed in all eyes to protect the graft during recovery.

**PLK: Results**

The medical records of 54 horses receiving PLK were reviewed. The cases consisted of 20 Thoroughbreds, 12 American Quarter Horses, 7 Warmbloods, 3 Paint Horses, and 12 horses of seven other breeds. PLK was performed in 11 stallions, 19 geldings, and 24 mares. The ages of horses receiving PLK ranged from 7 wk to 25 yr.

The PLK procedure was performed for single (n = 49) or multiple (n = 5) medically non-responsive deep stromal abscesses of the axial cornea. Graft size was most commonly 6–8 mm in diameter but ranged from 4 to 10 mm in diameter. A conjunctival flap or AMT was used in conjunction with PLK in one case each.

Complications of PLK included flap ulcers (some infected; n = 20), aqueous leakage from wound dehiscence or suture microleaks (n = 16), graft failure requiring surgical repair or replacement (n = 4), suture abscesses (n = 4), flap vascularization (all to some degree), flap edema (all), hyphema (n = 1), persistent edema (n = 1), and Descemet’s membrane retraction from the graft (n = 1). The superficial PLK flap is delicate and commonly partially ulcerates but rarely gets infected to seriously delay healing. The superficial PLK flap vascularizes in most cases. Epithelial bullae of the superficial flap occurred in one case. Gift protrusion occurred in one case.
The donor graft remained transparent for up to 7 days and then opacified. Subepithelial opacification and graft opacification can be seen with a slit lamp. Suture marks will be present in the cornea. 136 The visual outcome was positive in 53/54 eyes (98.1%) that had PLK. 136 The average time for complete healing when defined as being off all medications in PLK cases with a successful visual outcome was 30.8 ± 9.5 days. PLK is associated with a shorter surgery and treatment time than PK and DLEK. 34, 136, 147

DLEK: Surgical Procedure

DLEK is recommended for medically nonresponsive deep stromal abscesses in the peripheral cornea that are 10 mm or less in diameter and have moderate vascularization and edema of the overlying epithelium and anterior stroma with no stromal cellular infiltrate. The DLEK transfers healthy Descemet’s membrane and endothelium while preserving the corneal surface and topography. A fully intact epithelium with no corneal sutures is present postoperatively in the axial cornea. 34, 136, 145

A limbal incision up to 25 mm in length is made to two-thirds stromal depth. 34, 136, 148 A stromal pocket is formed manually over the deep stromal abscesses with a Martinez corneal dissector. The superficial flap of normal epithelium and vascularized stroma is gently retracted, and Descemet’s membrane and endothelium with the abscess is removed with a trephine and corneal scissors. The anterior two thirds of the donor corneal tissue is removed by manual dissection and discarded, and a trephine 1 mm larger in diameter than the recipient site used to obtain the circular lamellar donor graft of normal Descemet’s membrane and endothelium. The superficial corneal flap is partially sutured at the limbus, the endothelial donor graft gently inserted into place with Utrata forceps, and the limbal incision is then sutured closed. The graft self-adheres to the recipient stroma by action of the endothelial pump but may need to be repositioned by a needle inserted at the limbus. The graft is supported during the immediate postoperative period by viscoelastic injected into the anterior chamber. 34, 136, 148

DLEK replaces the diseased posterior stroma and endothelium and has proven successful for surgical therapy of deep stromal abscesses in the horse (Figs. 37, 42, 43, and 48–50). 34, 136, 148 Complications of DLEK in the horse include suture abscesses, suture incision microleaks, incision edema, and graft misalignment. The donor graft remains transparent for up to 7 days and vascularizes and opacifies. The superficial epithelium and stroma remain transparent in DLEK. The endothelial scar is typically vascularized, eventually becomes opaque, and is similar between PLK and DLEK but much better than with PK. The visual outcome approaches 90% for the horse eyes that had the DLEK procedure. 34, 136, 148

DLEK does not disrupt the stroma in removing the diseased endothelium and Descemet’s membrane and may eventually prove useful for cases of persistent corneal edema and deep stromal abscesses in the horse once technical issues are resolved. 150, 159, 161

DLEK: Results

The medical records of 66 horses receiving DLEK were reviewed. 136 The cases consisted of 19 Thoroughbreds, 22 American Quarter Horses, 6 Tennessee Walking Horses, and 19 horses of five other breeds. DLEK was performed in 6 stallions, 25 geldings, and 35 mares. The ages of horses receiving DLEK ranged from 4 mo to 20 yr. 136

The DLEK procedure was performed for medically nonresponsive deep stromal abscesses of the peripheral cornea (n = 66). 136 Graft size was most commonly 6–8 mm in diameter but ranged from 4.5 to 11 mm in diameter. A CF was placed over the limbal incision in one case. The donor graft remained transparent for up to 7 days and then vascularizes and opacifies. 136

Complications included ulcers near the incision (n = 4), aqueous leakage from wound dehiscence or suture microleaks (n = 3), graft failure requiring surgical repair (n = 3), hyphema (n = 2), focal nonprogressive cataract formation (n = 2), graft movement or slippage postoperatively (n = 2), and graft protrusion (n = 1). 136 Corneal edema associated with the extensive corneal dissection was quite pronounced immediately postoperatively and then nearly completely resolved over several weeks time in DLEK cases. 136

Subepithelial opacification anterior to the graft and graft opacification caused by presumed graft rejection could be seen with a slit lamp in all cases. 136 The intervening cornea again became transparent. Enucleation (n = 4) was necessary because of satellite abscesses and recurrent deep stromal abscesses from presumed fungal regrowth. 136 The recurrent deep stromal abscesses were a particularly aggressive type of deep stromal abscess that were strikingly orange colored, often found in multiple sites in the same eye, and associated with aggressive iridocyclitis. They were associated histologically with microfragmentation of Descemet’s membrane and have also been noted at the University of Florida in eyes that received medical therapy only and not the DLEK procedure. 136

The visual outcome was positive in 59/66 eyes (89.4%) that had DLEK. The average time for complete healing when defined as being off all medications in DLEK cases with a successful visual outcome was 35.8 ± 14.7 days. The DLEK is associated with a shorter surgery and treatment time than the PK. 136
Epithelial Layer Lamellar Transplantation

The next target for component surgery of the cornea is replacement of corneal epithelium. Regeneration of corneal epithelium can be accomplished by limbal transplantation of intact limbal epithelial stem cells attached to AMT or transplantation of sheets of cultivated epithelial stem cells. The transplantation of epithelial cells using either method restores epithelial stratification, barrier function, and stabilizes keratitis.

Scientists at the University of Florida have successfully attached equine limbal stem cells to equine AM for targeted epithelial layer transplantation. This technique is available for use in the horse.

Corneal Transplantation in Horses: Clinical and Technical Observations

Seventeen PKs were performed in horses at the University of Florida from 1993 to 2000, 88 transplants of all three types were done between 2000 and 2004, and the remaining 101 cases done in 2005–mid-2007. At the time of the submission of this manuscript in May 2010, we have done >283 corneal transplants in horses.

Frozen corneas were used in 95% of the procedures with satisfactory results. It is easiest to handle the frozen cornea when it is partially, rather than completely, thawed. Corneas could be stored at −20°C for 6 mo and still be used satisfactorily. Despite the damage freezing does to the endothelium, many equine donor grafts that were initially opaque while frozen became transparent in the cornea after several days. The vast majority eventually developed some degree of opacity, but a few long-term PLK and DLEK grafts again regained their transparency, suggesting that the horse endothelial cells are capable of expansion and migration to cover damaged endothelial cells.

Bulging of the iris and the corpora nigra into the incision site intraoperatively did occur occasionally. Adhesions or synechia between the abscess and iris were often present. Thick fibrin membranes were often noted intraoperatively on the posterior cornea, anterior iris face, and lens. The pupil was often partially occluded by these membranes. Hypopyon was allowed to drain from the anterior chamber. Iris membranes should be removed with caution or not removed at all because of the risk of hemorrhage. Retrocorneal membranes may be gently dissected from the cornea with viscodissection, but vigorous traction can cause serious hyphema. It is safe to leave the viscoelastic inside the horse anterior chamber. Most of it was gone in 24 h, and no complications were associated with its use.

Sixty-five of the 151 (43.1%) deep stromal abscesses and full-thickness stromal abscesses in this series were positive for fungi histologically. Bacteria were cultured in three stromal abscesses and fungi in only two stromal abscess cases.

Abscesses projecting into the anterior chamber are avascular and can be associated with 1- to 2-mm-thick retrocorneal membranes. These avascular retrocorneal membranes rarely, if ever, heal by vascularization and must be surgically removed by PK, PLK, or DLEK or persistent uveitis and blindness results. It is not necessary to remove the entire deep stromal abscesses to achieve success surgically but as much of the central portion of the deep stromal abscesses as possible should be excised.

The superficial cornea retains transparency in mist cases of PLK and DLEK. The grafts initially remain clear in PLK and DLEK despite being frozen. Rejection manifested by graft swelling begins 5–7 days postoperatively in all types of transplants. The incisions should also be carefully examined for microleaks using Seidel’s test at this time. Uveitis accompanies the rejection and graft swelling and is most pronounced in PK. The rejection is manifested by loss of transparency and occurs in all cases in the short term. The least graft opacification is apparent in the DLEKs and the most with PK. Suture tracks in the recipient cornea were often present at PLK and PK surgical sites.

PK cases were more severe in nature with more preoperative corneal destruction, worse uveitis, and absence of corneal stroma. The deep stromal abscesses generally had fairly normal stroma contiguous to the lesions. Simply removing the deep stromal abscesses and not replacing the tissue with a transplant results in generalized corneal edema that takes months to resolve. Descemet’s membrane was multifocally fragmented in the failed DLEK eyes, suggesting an aggressive infectious regrowth or immunologic response.

Corneal graft rejection comprises a series of complex immune responses that involve the recognition of the foreign donor graft antigens by the host’s immune system, leading to the initiation of an effector immune response by the host immune system against foreign antigens culminating in graft rejection and graft decompensation. Corneal graft rejection is primarily a cell-mediated immune response controlled by CD4+ T lymphocytes. The cornea’s normal immune privilege is hindered by keratitis and neovascularization.

Rejection of a corneal transplant graft is a process in which a corneal graft that has been clear for several days in horses or 2 wk in humans suddenly succumbs to graft edema in conjunction with anterior segment inflammatory signs. Rejection of the graft epithelium, stroma, and endothelium can occur in isolation or in combination.

Corneal grafts done for noninflammatory dystrophic conditions are the most successful of clinical transplants in humans, with those done for acquired corneal degenerative diseases having a high risk of rejection. Corneal inflammation, infection, and/or vascularization contribute significantly to the failure or rejection of corneal grafts. All eyes in horses that have corneal transplants...
would be considered high risk for failure or rejection based on the human criteria as the grafts are placed in inflamed, infected, and vascularized corneas. Graft rejection is manifested by a loss of transparency. Vascularization abandons the immune privilege of the cornea by allowing antigens to leave the graft through blood vessels and lymphatics and also by allowing host antigen-presenting cells into the graft. 165 Smaller grafts in humans are associated with lower rejection rates, and high-volume surgeons have better success presumably because of better postoperative management decisions. 10,142–144,166,167

In one study of corneal transplantation in people, the 1-yr graft survival rate was 88%; the 5-yr survival rate was 74%, and at 10 yr, the survival rate was 62%. 140 Another human PK study indicated the probability of developing graft rejection or graft failure 15 yr postoperatively was 23% and 28%, respectively. 168 Thirty percent of PK eyes in humans have at least one episode of rejection, and 5–7% eventually lead to graft failure. 141 The rejection rate in heavily vascularized human eyes after PK is 65%, with 50–66% of these capable of being controlled medically. 141 Vascularization of the host cornea is the most important risk factor for graft rejection. 141 The endothelial rejection rate directly correlates with the degree of vascularization. 141 An avascular cornea is at low risk for rejection. 141 Vascularization at least 2 mm into the stroma in three or more quadrants places the graft in a high-risk category for rejection. 141 Most of the horses operated on at the University of Florida were thus in the high-risk category because of corneal vascularization and the endothelial location of so many of the lesions.

Repeat graft surgery, infection, synechia, and keratitis also increase the likelihood for PK graft rejection in humans. 141 Most PK graft infections in humans occur 1 yr postoperatively because of ulcers, KCS, and viral infections. 109 Placing a CF as we did in many of our horse PK patients probably guaranteed graft rejection but was deemed necessary to support the weakened tissue surrounding the lesion in specific horses. 136

Prevention of corneal graft rejection lies with the reduction of the donor antigen tissue load, tissue matching, and suppressing the host immune response. 141 Topical and systemic corticosteroids pre- and postoperatively are the mainstay of high-risk corneal transplant management in humans. 141 Topical and systemic cyclosporine A may also be used to prevent or reverse graft rejection in humans after corneal transplantation. 141 We feel the use of topical corticosteroids is very risky in these horse cases because excessive proteinase activity and infection are often present. 70 We also feel, however, that this type of clinical dogmatism deserves further study. It may be that some steroids could be used in the horse and others could not. There is a gap in our knowledge, and further controlled studies are warranted.

Partial graft rejection and scar formation have been unavoidable with PK, PLK, and DLEK procedures in our high-risk horse patients despite the use of topical 2% cyclosporine A. 136 There did not seem to be any harm and little benefit to using topical cyclosporine A in our high-risk horse patients, although topical 2% cyclosporine A treatment is effective in reducing the risk of allograft rejection in high-risk human patients. 141

PK has the inherent problem of creating an inherently weak vertical stromal wound that requires surface corneal sutures. 138,139 The incised stroma never regains the structural integrity and strength of normal stroma. Topographic distortion of the cornea caused by corneal sutures results in refractive instability in humans after PK. 138,139 The PK graft epithelium often sloughs leaving the graft open to infection and excessive tear film proteinase activity. PK incisions and suture tracts can often dehisce and leak aqueous humor and was common in cases at the University of Florida. Corneal sutures must provide a great compressive force to create a watertight seal of the junction between the donor and recipient tissue. 170 The sutures loosen as the wound heals, the stromal edema resolves, and the tissue contracts to allow tissue separation and aqueous leakage in some cases. 138,139 Vertical incisions are more prone to leak at low IOP levels. 170 Sutures are a leading cause of graft infection and vascularization. 139 Use of nylon sutures could be beneficial to the horse patients because nonreactive nylon is less likely than vicryl to attract inflammatory cells. 140 We used vicryl because it was absorbable and we did not wish to have to remove nylon sutures. Loose sutures cause graft vascularization, infection, and rejection. 141 Corneal sensory nerve denervation is also a problem after PK in humans and may take 1 yr postoperatively to resolve. 171 We did not recognize a loss of corneal sensation to be a clinical problem in our horses but intend to study this in future horse surgical corneal transplant cases. Contaminated donor tissue, incomplete excision of infected recipient tissue, and environmental microbial infection can be causes of microbial keratitis after PK. 169 Loose sutures and exposed knots allow for bacterial and fungal invasion. Mucous on a suture is also a nidus for infection. 169

The location and size of the transplant sites in the cornea may influence corneal rejection. Graft sites closer to the limbus, presumably because of their proximity to limbal blood vessels, and keratoplasties >8.0 mm in diameter are associated with increased rejection rates in humans. 142–144,166,167 Large grafts in the horse also seemed to have a more dramatic scarring effect, but these were also the most severe cases. Paradoxically, vascularization provides tectonic and nutritional support to the horse corneal transplant graft, and a donor graft is not considered healed until it vascularizes. 10,108,123,136,147 Grafts closer to the limbus are considered more desirable for transplant in horses with deep stromal abscesses because of
their proximity to limbal blood vessels, yet this proximity to the limbus undoubtedly increases the rejection rate. The existence of a maximal size for donor cornea in the horse should also be appreciated (i.e., use the minimal graft size necessary to achieve the therapeutic goal desired).

Corneal transplants have been performed in horses using both fresh and frozen grafts. Fresh grafts maximize endothelial cell preservation, thereby minimizing postoperative corneal opacity. The endothelium of frozen cornea is damaged by changes in pH, osmolality, solute concentration, and ice crystal formation. Because fresh cornea is not often available, most corneal transplants in horses are still done quite satisfactorily with frozen grafts. The frozen grafts in horses display transparency initially but begin to become opaque within 5–7 days after surgery.

Among equine PK cases in which conjunctival or amniotic grafts are used, the function of the corneal graft is tectonic (i.e., it maintains corneal integrity). Fresh, clear corneal grafts are undoubtedly preferable, but such fresh grafts are not always available. In these cases, frozen tissue with presumed dead endothelium was used successfully. Donor corneal grafts in most equine cases perform their tectonic role superbly, but we have seen a number of frozen horse corneal grafts again become completely clear several months postoperatively. This may be caused by migration of recipient endothelial cells into the donor graft. Vascularization of the grafts, indicating rejection, begins 5–7 days postoperatively in the horse. The resulting scar, which is typically vascularized and eventually opaque, is similar between PLK and PK but smaller with DLEK.

These surgical procedures are still evolving in the horse, but corneal transplantation is a tectonically viable surgery with an overall vision retention success rate of 88.5% in treating vascularized and infected equine corneal disease. It is not possible to achieve corneal clarity for purely optical reasons after corneal transplantation in the horse, but the surgery is feasible. Excessive deposition of fibrotic repair tissue in the cornea leads to special challenges because this repair tissue is opaque, and its contraction alters the corneal shape undoubtedly interfering with the function of focusing light on the retina.

Each of our severe keratopathy horse cases had a major disturbance of the ocular immune system that strongly favored graft rejection. Nevertheless, we saved many eyes with medically nonresponsive conditions that would have been lost. Corneal transplantation in horses can have good visual outcomes, although graft rejection, graft failure, and scar formation still occur too frequently with all three of these procedures. Automated microkeratomes, artificial anterior chambers, specialized corneal forceps and scissors, and new pharmacologic approaches to infection and graft rejection could technically improve the transplant surgical procedures.

17. Equine Corneal Transplantation: Discussion

Corneal transplantation for treatment of severe inflammatory keratopathies with corneal vascularization, infection, and keratomalacia (melting) has been performed successfully for therapeutic and tectonic reasons in horses at the University of Florida since 1993. PK for full-thickness stromal abscesses or ulcers/iris prolapses and PLK and DLEK for deep stromal corneal abscesses with an intact epithelium and vascularization, but no cellular infiltrate of the anterior stroma in the axial cornea, are used for deep stromal abscesses in the horse.

A positive visual outcome was achieved for PK, PLK, and DLEK in 77.9%, 98.1%, and 89.4%, respectively. DALK has been recently used for large diameter melting ulcers and descemetoceles. These surgical procedures represent the current medical standard of care for medically nonresponsive catastrophic cornea disease in the horse. We have proven we can do corneal transplant surgery successfully for therapeutic and tectonic reasons in horses, but we must now take it to the next level. Corneal transplantation in horses results in good visual outcomes, although graft rejection, graft failure, and graft opacification still occur too frequently with the PK, DLEK, PLK, and DALK procedures. Corneal inflammation, infection, and vascularization contribute significantly to the failure or rejection of corneal grafts. Vascularization of the host cornea is the most important risk factor for graft rejection because it abolishes the immune privilege of the cornea by allowing antigens to leave the graft through blood vessels and lymphatics and also by allowing host antigen-presenting cells into the graft. All eyes in horses that have received corneal transplants would be considered high risk for failure or rejection based on the human criteria because the grafts are placed in inflamed, infected, and vascularized corneas. Graft rejection is manifested by a loss of donor tissue transparency.

The donor graft rejection process in the horse is persistent, overwhelming, and cannot be stopped at the present time. This is undoubtedly because of a lack of understanding of this powerful rejection process. Topical corticosteroids and cyclosporine A have been used in limited cases at the University of Florida in attempting to prevent and/or reverse the rejection but had no effect.

Better techniques of donor graft preservation and an understanding of both the triggers for rejection and the rejection process itself in corneal transplantation in the horse are critical to overcoming the rapid donor graft rejection process noted in the horse. It is unlikely that we will ever achieve the high level of transparency in the corneal transplants in our horse patients that physicians do in human patients receiving corneal transplants. A small corneal scar is considered a satisfactory outcome in
the horse. Corneal transplants in humans with corneal vascularization and/or infection are rejected and scar similar to our horse patients and are considered failures needing re-operation. Some clinicians may prefer to use the term “corneal grafting” rather than “corneal transplantation” for our horse patients because the use of frozen corneal tissue without tissue matching is more of a grafting material and substitute for missing or damaged corneas.

18. Equine Corneal Transplantation: Conclusions

Conventional corneal transplantation, in the form of PK, involves full-thickness replacement of the cornea and is a highly successful procedure in humans and horses. Despite the incredibly positive experience with PK in the horse, I now suggest that this procedure should be rarely used in the treatment of equine keratitis. I believe that we should abandon PK in most equine corneal cases because of the postoperative complications, the intense postoperative care needed, and the fact that all donor grafts rapidly become opaque because of rejection. The cornea is anatomically a multilayered structure, and many equine keratopathies primarily affect individual layers of the cornea. Targeted LK seems more appropriate to the microsurgical therapy of the most common equine keratopathies because only the graft becomes opaque and not the entire thickness of the cornea. LKs thus provide quicker visual rehabilitation and an improved safety profile compared with standard PK in the horse. Selective lamellar surgical replacement of only the diseased corneal layers while retaining unaffected normal corneal layers represents a new paradigm shift in the field of corneal transplantation in humans and the horse. Continued evolution of these relatively new techniques in veterinary ophthalmology is helping to reduce complications and further improve outcomes in horses with serious corneal disease.

19. Future of Equine Ocular Surface Disease

Will it ever be possible to do a true corneal transplantation for optical reasons in the horse? It is really not possible at this time but as we develop a better understanding of the immunology involved in equine corneal graft rejection and determine how best to modulate stromal proteases and infectious organisms of the horse cornea, it may some day be possible.

I thank the membership of the AAEP for this honor. I am shocked and deeply moved by the special recognition. I was just trying to help the horses. I was truly blessed by my parents, Betty Jane and Henry Eugene Brooks. They instilled in me the lifelong desire to achieve and learn, and gave me the freedom to excel. They are the best parents a boy could have. I love you very much and wish you had heard me lecture. This is dedicated to the following: Dr. Edward L. Mockford, Drs. Julian and Fern Krakowski, Ms. Madeline Stewart, Ms. Florine Laws, Ms. Mae Ehrhardt, Omer and Doris Walton, Dick and Eleanor Seiger, Bob and Wilma Finley, Mr. Robert E. Price, and Ms. Marietta Baumgartner for putting up with me talking in class, educating me, and having faith in me from first grade and higher; the horses—I wish I had been faster to learn; the very special Dr. Bo Reich; all my ophthalmology residents, faculty, and graduate students at the Universities of Tennessee and Florida—I learned so much from all of you, you are amazing people, and I am forever in your debt; Dr. Andy “Braveheart” Matthews, the best equine corneal specialist in the world—long live Scotland; Drs. Ann Dwyer, Carol Clark, and Derek Knottenden, who are non-opthalmologists setting new standards of care in equine ophthalmology; and Dr. Brian Gilger and the faculty of North Carolina State University—Brian and I and many others are doing our best to improve the science of equine ophthalmology.

References and Footnotes

17. Brooks DE, CK Clark, GD Lester. Cochent-bonnet aesthe-
19. Gan L, Fagerholm P, Kim H. Effect of leukocytes on cor-
20. neal cellular proliferation and wound healing. Invest Oph-
22. Pian T, Foster C, Wasson P, et al. Role of fibronectin and fibri-
23. nogen in healing of corneal epithelial scrape wounds. Invest Ophthal-
25. JD. Surgical and nonsurgical trauma. In: Tasman W,
26. Ollivier FJ. Medical and surgical management of melting
27. corneal ulcers exhibiting hyperproteinase activity in the
30. matrix metalloproteinase activity in equine tear fluid during
31. corneal healing in 10 horses with ulcerative keratitis. Vet
34. various compounds to inhibit activity of matrix metallopro-
35. teinases in the tear film of horses with ulcerative keratitis. Am
37. Neanderland MH. Healing of experimentally induced cor-
40. administration of epidermal growth factor on healing of
41. 1130–1135.
42. Miyahara S, Kiryu J, Miyamoto K, et al. In vivo three-
43. dimensional evaluation of leukocyte behavior in retinal mi-
crocirculation of mice. Invest Ophthalmol Vis Sci 2004;45:
44. 4197–4201.
45. Brooks DE, Andrew SE, Biros DJ, et al. Ulcerative ker-
atitis caused by beta-hemolytic Streptococcus equi in 11
47. Swank A, Hosgood G. Corneal wound healing and the role of
49. 1007–1017.
51. factors (EGF, PDGF-BB and TGF-beta 1) on cultured equine
52. epithelial cells and keratocytes: implications for wound
54. Robertson SA, Andrew SE. Presence of opioid growth fac-
55. tor and its receptor in the normal dog, cat and horse cornea.
58. normal horses following experimental application of topical
59. antimicrobial or antimicrobial-corticosteroid ophthalmic
61. Scotty NC. Equine keratomeysis. Curr Tech Equine
62. Andrew SE, Willis AM. Diseases of the cornea and sclera.
63. In: Gilger BC, ed. Equine ophthalmology. St. Louis,
64. MO: Elsevier; 2005;157–252.
65. Curiel JM, Murphy CJ, Jang SS, Bellhorn RW. Nutrition-
69. Fleisch SMJ, Fletcher EL, Pier GB. Evidence that precel-
70. lular factors may be involved in the non-specific inhibition of
bacterial adherence to intact cornea. Invest Ophthalmol
72. Zaidi TS, Fleisch SMJ, Pier GB. Ocular mucus modulates
Pseudomonas aeruginosa adherence to both intact and in-
73. 847.
74. Stern GA, Schultz GS. The pathogenesis of bacterial infec-
tions of the cornea. In: Tasman W, Jaeger EA, eds. Du-
ance’s foundations of clinical ophthalmology. Philadelphia,
75. PA: Lippincott; 1996;1–11.
76. Kloetz SA, Au YK, Misra RP. A partial thickness epithelial
defect increases the adherence of Pseudomonas aeruginosa
1074.
77. Brooks DE, SE Andrew, HM Denis, et al. Rose bengal
positive epithelial microerosions as a manifestation of
78. corneal keratomeysis. Trans Am Ophthalmol Soc
80. Gray LD, Kreger AS. Rabbit corneal damage produced by
Pseudomonas aeruginosa infection. Infect Immun 1975;12:
419–422.
81. Huang X, Hazlett L. Analysis of Pseudomonas aeruginosa
corneal infection using an oligonucleotide microarray.
83. Kreger AS, Gray LD. Purification of Pseudomonas aerugi-
 nosa proteases and microsopic characterization of pseudo-
monal protease-induced corneal damage. Infect Immun
84. Brown SI, Bloomfield SE, Tam WI. The cornea-destroying
zyme of Pseudomonas aeruginosa. Invest Ophthalmol Vis
caused by protease and elastase from Pseudomonas aerugi-
86. Monod M, Capoccia S, Lechenne B, et al. Secreted pro-
292:405–419.
87. Iglewski BH, Burns RP, Gipson IK. Pathogenesis of cor-
nal damage from Pseudomonas exotoxin A. Invest Ophthal-
88. Dong X, Shi W, Zeng Q, Xie L. Roles of adherence and
type IV collagen substrates in the growth patterns of fungal
89. Reichert R, Stern G. Quantitation of adherence to and
the interaction between Pseudomonas aeruginosa and the corneal epithelium: an electron microscopic study. Arch Ophthal-
 mol 1985;103:1221–1225.


*Fischer Scientific, Hampton, NH 03842.

*Biorad Laboratories, Richmond, CA 94547.

*cTissuemend II, Stryker Biotech, Hopkinton, MA 01748.

*dVet BioSIST®, Smiths Medical, Waukesha, WI 53186.

*eAcrivet, Salt Lake City, UT 84088.

*fAcrivet, Salt Lake City, UT 84088.

*gBausch & Lomb (Vision Care), Rochester, NY 14609.

*hHylartin V, Pfizer Animal Health, New York, NY 10017.

*iEthicon, Somerville, NJ 08876.