

Density of corneal endothelial cells and corneal thickness in eyes of euthanatized horses

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Objective—To determine density of corneal endothelial cells and corneal thickness in eyes of euthanatized horses.

Sample Population—52 normal eyes from 26 horses.

Procedure—Eyes were enucleated after horses were euthanatized. Eyes were examined to determine that they did not have visible ocular defects. Noncontact specular microscopy was used to determine density of corneal endothelial cells. Corneal thickness was measured, using ultrasonic pachymetry or specular microscopy.

Results—Mean density of corneal endothelial cells was 3,155 cells/mm². Cell density decreased with age, but sex did not affect cell density. Values did not differ significantly between right and left eyes from the same horse. Cell density of the ventral quadrant was significantly less than cell density of the medial and temporal quadrants. Mean corneal thickness was 893 μm. Sex or age did not affect corneal thickness. Dorsal and ventral quadrants were significantly thicker than the medial and temporal quadrants and central portion of the cornea. We did not detect a correlation between corneal thickness and density of endothelial cells in normal eyes of horses.

Conclusions and Clinical Relevance—Density of corneal endothelial cells decreases with age, but corneal thickness is not affected by age or sex in normal eyes of horses. The technique described here may be useful for determining density of endothelial cells in the cornea of enucleated eyes. This is clinically relevant for analyzing corneal donor tissue prior to harvest and use for corneal transplantation. (*Am J Vet Res* 2001;62:(479–482)

The equine cornea comprises 5 layers: the outermost stratified squamous epithelium, epithelial basement membrane, stroma, Descemet's membrane, and innermost endothelium. Corneal endothelium constitutes a single layer of hexagonally shaped cells that interdigitate with each other¹ and create a barrier between the corneal stroma and aqueous humor. Endothelial cells limit imbibition of water and solutes from the anterior chamber into the corneal stroma.² A minimum critical density of endothelial cells is necessary to maintain corneal clarity and function in

humans.³ Disturbance of arrangement or density of corneal endothelial cells may have a profound effect on corneal transparency. Corneal endothelium may be compromised by a number of inherent disease processes or by surgical manipulations of the cornea or the anterior chamber of the eye. Increased understanding of pathophysiologic processes of diseases affecting the cornea of horses and the advent of novel surgical methods used to treat these common corneal diseases of horses necessitate further study of the corneal endothelium.

Innovative contact and noncontact specular microscopes used to measure density of corneal endothelial cells in humans rely on cooperation of the patient, who gazes at a specific target during acquisition of measurements. In other live animals, it may not be possible to obtain their cooperation and have them gaze at a specific target for a period sufficient for investigators to image endothelial cells of the peripheral corneal quadrants. Sudden and unpredictable head or eye movements, especially in large animals, may result in damage to the specular microscope. On the basis of pachymetry studies in live horses, the central portion of the cornea is the area most accessible for obtaining measurements.^{4,5} Therefore, it is necessary to determine whether the central area of the cornea can be used to predict density of the endothelial cells in alternate corneal locations in horses.

Density of corneal endothelial cells has been reported in normal eyes of humans,⁶ dogs,⁷ cats,^{8,a} and various laboratory animals.^{9–11} Age-related changes and pathologic conditions affecting cell density have been reported in humans^{12–14} and dogs.^{7,15,16} To the authors' knowledge, density of corneal endothelial cells has not been reported for normal or abnormal eyes of horses.

The purpose of the study reported here was to determine density of corneal endothelial cells in enucleated eyes of horses and discern whether cell density of the central portion of the cornea was representative of the entire cornea. We also wanted to determine whether there was a correlation between corneal thickness and density of corneal endothelial cells at corresponding measurement locations and whether these variables would be affected by specific factors (age, sex, right vs left eye).

Materials and Methods

Horses—Twenty-six horses (12 ponies, 7 Thoroughbreds, 4 Quarter Horses, and 3 mixed-breed horses) were used. Age, breed, and sex were determined from information provided by horse owners. Horses ranged from 4 to 288 months old (median, 66 months). Ten were females (range, 24 to 240 months; median, 132 months). Sixteen were males; 10 were castrated males (range, 36 to 288 months; median,

Received Mar 16, 2000.

Accepted Jun 15, 2000.

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Presented in part at the 30th Annual Meeting of the American College of Veterinary Ophthalmologists, Chicago, Ill, Nov 7, 1999.

108 months), and 6 were sexually intact males (range, 4 to 36 months, median, 9.5 months). None of the horses had a history or clinical signs consistent with ocular disease.

Horses were euthanatized for reasons unrelated to this study. Eyes were enucleated immediately after horses were euthanatized. The anterior segment of each eye was examined by use of a transilluminator with diffuse illumination and was considered normal. All measurements were made by a single observer (SEA).

Density of corneal endothelial cells—Density of corneal endothelial cells was calculated by use of a noncontact specular microscope^b in automatic or manual mode. This instrument uses differential focusing on the corneal epithelial and endothelial cell surfaces to image the endothelial cells in selected areas. A required working distance of 25 mm was used. Photographic areas measuring 0.2 × 0.5 mm were analyzed. In each location, 15 cells were selected for analysis of variables that included minimum, maximum, and mean cell size as well as SD. Density of endothelial cells was measured in the central and peripheral portions of 4 quadrants (dorsal, ventral, medial, temporal) of each cornea. Measurements for peripheral portions were made at a point approximately 5 mm from the limbus in the clear portion of the cornea.

Corneal pachymetry—Corneal thickness was measured by use of a specular microscope or by ultrasonic pachymetry in all eyes at 5 locations corresponding to those areas selected for measurement of cell density (central, dorsal, ventral, medial, and temporal portions of the cornea). The specular microscope was used to measure corneal thickness by differential focusing in most (137/260) measurement locations by use of the specular microscope in automatic mode. A 20-MHz ultrasonic pachymeter^c was used in 123 of 260 measurement locations when the specular microscope was used in manual mode. The pachymeter makes consecutive A-scan measurements of corneal thickness during recording and internally eliminates measurements that are > 5 μm from the mean before displaying a measurement. The transducer was apposed gently to the cornea in each of the 5 measurement locations without indenting the cornea. A value of 1,640 m/s was used for velocity of sound through the cornea.

Statistical analysis—Response variables in the study were density of corneal endothelial cells and corneal thickness. A split-plot ANOVA was used to evaluate the effect of grouping (sex) and repeated factors (side [right eye vs left eye] and measurement location [central and 4 peripheral areas]) on these variables. Post-hoc analyses were conducted, using the Tukey test. Data were expressed as mean ± SEM. Values of *P* < 0.05 were considered significant. The effect of age on response variables was evaluated graphically and by means of linear regression analysis. Means of the 26 data points were used to calculate *R*².

Results

Density of corneal endothelial cells—Mean ± SEM density of corneal endothelial cells for all eyes was 3,155 ± 765 cells/mm². Values did not differ significantly between the right and left eyes from the same horse. Cell density decreased significantly with age, as defined by the equation (cell density = 3,439 – [2.7 × age]) for the linear regression (*R*², 0.22; Fig 1). For all eyes, mean density of endothelial cells in the ventral portion of the cornea (2,968 cells/mm²) was significantly less than mean density of the medial (3,257 cells/mm²) and lateral (3,250 cells/mm²) portions of the cornea; however, it did not differ significantly from

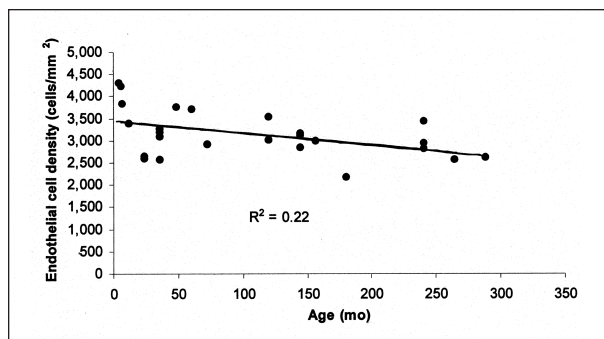


Figure 1—Correlation between age and density of corneal endothelial cells in normal eyes obtained from 26 euthanatized horses. The best-fit line for the linear regression is described by the following equation: density of corneal endothelial cells = 3,439 – (2.72 × age). Each symbol (•) represents the mean value for the eyes of 1 horse.

Table 1—Mean corneal thickness (mm) for 5 locations of each eye in normal eyes obtained from 26 euthanatized horses

Location	Right eye			Left eye		
	Lateral	Central	Medial	Medial	Central	Lateral
Dorsal	—	939*	—	—	926*	—
Central	861	858	882	866	859	898†
Ventral	—	914*	—	—	923*	—

Values were corrected for age. Pooled SEM for comparison of these means was 6.25.
*Value differs significantly (*P* < 0.05) from values for lateral, medial, and central locations in both eyes. †Value differs significantly (*P* < 0.05) from values for the central, lateral and dorsal locations in the right eye and central and medial locations in the left eye.
— = Location not examined.

values for the dorsal (3,086 cells/mm²) or central (3,216 cells/mm²) portions of the cornea. Pooled SEM for these mean values was 66. Side (right vs left) or other interaction terms did not significantly affect cell density. Mean endothelial cell density of male horses (3,351 cells/mm²) was found to be higher than the mean endothelial cell density of female horses (2,867 cells/mm²). This difference was not significant once age was removed as a confounding factor.

Corneal thickness—Mean corneal thickness for all eyes was 893 μm. There was a significant effect of location and a location-by-side interaction. Therefore, means of the 5 locations of each side were reported (Table 1). Post-hoc comparisons of these 10 means confirmed that the dorsal and ventral quadrants of the cornea were significantly thicker than the lateral and medial quadrants and central portion of the cornea. The exception was the left lateral quadrant, which was significantly thicker than the medial quadrant and central portion of the left eye and lateral quadrant and central portion of the right eye. Sex or other interaction terms did not significantly affect corneal thickness. Similarly, age did not affect corneal thickness (*R*², 0.07). We did not detect a correlation between density of endothelial cells and corneal thickness in normal enucleated eyes of horses (*R*², 0.0001).

Discussion

The corneal endothelium plays a critical role in maintenance of corneal clarity. Perilimbal mesenchy-

mal cells derived from the neural crest migrate from the embryonal lens to the acellular corneal stroma to form the corneal endothelium at 6 weeks of gestation in human fetuses.² A rapid decrease in density of corneal endothelial cells results from the period of endothelial cell genesis until 2 weeks after parturition in humans, followed by a loss of 0.56% cells annually. This monolayer population of endothelial cells does not undergo mitosis.² Mean density of corneal endothelial cells at birth in humans is 4,000 cells/mm² but decreases to 2,000 cells/mm² by the eighth decade of life.² In the study reported here, mean (\pm SEM) density of corneal endothelial cells in horses was $3,155 \pm 765$ cells/mm². Mean cell density decreased with advancing age in horses of this report. Correlation of decreasing density of corneal endothelial cells with advancing age has been reported in several other species.^{6,7,9,10} The finding of a higher endothelial cell density in males, compared with values in females, may have been attributable to a substantially lower median age of male horses (42 months) than female horses (132 months). Mean endothelial cell density was similar in all locations; however, it was significantly less in the ventral quadrant, compared with the medial and lateral quadrants. We are uncertain why the ventral location had a lower cell density than medial and lateral locations. Furthermore, we are uncertain of the clinical importance of this finding.

In humans, a minimal critical density of 400 to 700 cells/mm² is required to sustain corneal viability.¹⁷ Many ocular diseases may affect density of corneal endothelial cells in horses, but a minimum critical cell density necessary to maintain an optically clear cornea in horses has not been established. Ocular diseases that may affect density of endothelial cells and, subsequently, transparency of the cornea in horses include cornea globosa in Rocky Mountain Horses,^{4,18} recurrent uveitis, luxation of the anterior lens, and glaucoma.^{19,20} The effect of these conditions on density of corneal endothelial cells has not been reported. Ophthalmic surgical procedures that may compromise endothelial cells in horses include phacofragmentation^{21,22} and penetrating keratoplasty.²³ Density of corneal endothelial cells decreases following penetrating keratoplasty in humans,²⁴ and cell density is measured routinely as a screening procedure in human corneal tissue banks at the time of tissue harvest and as a means of evaluating viability of donor grafts prior to surgical placement. The effect of phacofragmentation or irrigating solutions designed for use in the anterior chamber during intraocular surgery on density of corneal endothelial cells has been reported in dogs^{22,25}; however, similar values have not been reported in horses.

Corneal thickness is considered a quantitative indirect measurement of endothelial cell density and function.³ In the study reported here, we did not detect an effect of age on corneal thickness in normal, enucleated eyes of horses. Ultrasonic pachymetry and specular microscope measurements of corneal thickness have been made in humans, and measurements made with the specular microscope were significantly less (mean, 32 μ m) than values obtained with the

ultrasonic pachymeter.²⁶ Comparisons were not made between equipment used in our study.

In 2 other reports,^{4,5} the thickest areas of the cornea were the dorsal and ventral quadrants; however, there were conflicting results concerning whether the dorsal or ventral quadrant was thickest in live horses. Distance from the limbus to the point at which corneal thickness measurements were obtained was 3 mm in 1 study⁴ but was not reported in the other study.⁵ In the study reported here, thickness of the peripheral portions of the cornea was measured 5 mm from the limbus and closely approximated results obtained in another study.³ It is possible that the location in the peripheral quadrant selected for measurements in our study may have corresponded with the location selected for measurement, which was not reported in that study.

Thickness of the central portion of the cornea increased with increasing age (up to 6 months old) in horses with normal corneas.⁴ In the study reported here, we did not detect an effect of age on corneal thickness. Discrepancy between results of our study and that other report may be attributable to a smaller number of horses and smaller age range in the study reported here, compared with those in the other study. In our study, only 1 horse was < 6 months old.

We did not detect a correlation between density of endothelial cells and corneal thickness in normal enucleated eyes of horses. Endothelial cells continuously secrete material for Descemet's membrane throughout life, resulting in increased composite thickness of the cornea. Use of corneal thickness as an indirect quantitative measurement of cell density and function does not account for the increase in thickness of Descemet's membrane with advancing age as a component of corneal thickness. In humans, density of corneal endothelial cells correlates poorly with thickness of the central portion of the cornea until a minimum critical density is reached.¹⁷

Use of a specular microscope to determine density of corneal endothelial cells is a practical way to evaluate corneal endothelium for eyes of horses that have been harvested for tissue banks or corneal transplantation. We believe that use of a specular microscope to determine endothelial cell density may have great potential for use in horses with conditions affecting the endothelium. A central location for measurement of density of corneal endothelial cells is sufficient to enable clinicians to generalize results for most corneal areas.

^aIgarashi R, Maeda Y, Igarashi O, et al. Examination of the normal feline corneal endothelium with a noncontact specular microscope (abstr), in *Proceedings*. 30th Annu Meet Am Coll Vet Ophthalmol 1999;79.

^bTopcon SP-2000P, Topcon America, Paramus, NJ.
^cDGH500, DGH Technology Inc, Exton, Pa.

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