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Corneal thickness and anterior chamber depth of the normal adult horse as measured by ultrasound biomicroscopy

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Abstract

Objective: To determine corneal thickness (CT) and axial anterior chamber depth (ACD) using ultrasound biomicroscopy (UBM) in normal adult horses. To compare corneal thickness measurements between UBM and ultrasonic pachymetry modalities.

Animals studied: Sixty eyes of 30 healthy adult horses aged 8-24 years.

Procedures: Ultrasonic pachymetry (velocity of 1640 m/s) was utilized to obtain measurements of the central, superior, temporal, inferior, and nasal cornea. Triplicate images of the same corneal locations were acquired using UBM (50 MHz). Images of the axial anterior chamber were used to measure ACD. Intraocular pressure (IOP) was estimated using rebound tonometry and axial globe length was measured using ultrasonographic biometry.

Results: CT (mean ± SD μm) measured by UBM was 854 ± 61 (central), 994 ± 58 (superior), 930 ± 57 (temporal), 979 ± 55 (inferior), and 898 ± 48 (nasal). CT measured by UBM was greater than that measured by ultrasonic pachymetry at all locations and was statistically significant at all locations except inferior (P = 0.0006-0.048). No sex nor age effect was detected for CT at any location. The repeatability of ultrasonic pachymetry was superior to that of UBM. Mean ± SD ACD was 5.74 ± 0.41 mm. A weak positive correlation was identified between central CT and IOP and between central CT and axial globe length.

Conclusions: Normal data for CT and ACD of the adult horse obtained using UBM are provided. CT determined by UBM was greater relative to pachymetry at all corneal locations.

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Keywords

UBM; equine; anterior segment; corneal thickness

Introduction

Ultrasound biomicroscopy (UBM) has proven to be a valuable diagnostic tool for evaluation of corneal and scleral disorders, anterior segment masses, the iridocorneal angle and ciliary cleft, as well as lens disorders in both human and animal patients.[1–7] Based on the ease of portability and increasing affordability, UBM has become a practical advanced imaging technique for use in equine ophthalmology. Clinical applications of UBM to investigate equine ocular pathology reported thus far include Descemet's membrane detachment, corneolimbal squamous cell carcinoma, stromal abscesses, and a corneal foreign body.[6–8]

Despite the utility of UBM, references with normal measurements of the adult horse cornea and anterior chamber are lacking. To date, a single *in vivo* study has investigated central corneal thickness in growing juvenile horses, and a single *ex vivo* study determined scleral thickness in enucleated globes, with no reports of normal *in vivo* parameters for adult horse eyes yet published.[9,10] The establishment of normative *in vivo* measurement values of the equine cornea and anterior chamber by UBM is necessary to aid in appropriate interpretation of this imaging modality applied to clinical cases.

Ultrasonic pachymetry is a commonly used modality for determining corneal thickness measurements in many species. Use of ultrasonic pachymetry to measure equine corneal thickness has been reported, including the determination of values for Miniature horses,[11] euthanized horses,[12] horses with ophthalmic abnormalities,[13,14] and to assess changes in corneal thickness following sedation and auriculopalpebral nerve block.[15] Ultrasonic pachymetry determines corneal thickness based on the time difference between ultrasound waves reflecting from the anterior and posterior surfaces of the cornea, and accurate results are dependent upon the pachymeter being set to the appropriate velocity of sound for the measured tissue. A limitation of ultrasonic pachymetry for measuring corneal thickness in horses is that the velocity of sound through the equine cornea has not been published. As such, use of the factory setting of 1640 m/s, appropriate for the human cornea, is typical though may not provide accurate corneal thickness measurements in horses. Despite this, the modality remains useful for serial monitoring of an individual horse or across horses so long as the velocity setting is known and consistent.

As UBM becomes more widely available it is likely that it will be used to assess many ocular parameters and disorders of the anterior segment. With increasing use of UBM, baseline values for the normal adult equine eye are needed. Additionally, determining how these values compare to those obtained with other methods is important. The objective of this study was to determine normal values for central and peripheral corneal thickness and anterior chamber depth (ACD) in the normal adult horse using UBM. A second objective was to compare corneal thickness values obtained with UBM to those obtained with ultrasonic pachymetry. It was hypothesized that the central cornea would measure

thinner than the peripheral cornea and that UBM and ultrasonic pachymetry would provide similar corneal thickness measurement values.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of the University of California-Davis and was performed in accordance with the Association for Research in Vision and Ophthalmology guidelines on the use of animals in research. All horses were research animals owned by the University of California-Davis.

Animals

Thirty healthy adult horses (15 mares, 15 geldings) with a mean age 14.4 ± 5.0 years were included. Breeds included the American Quarter Horse (n=12), Thoroughbred (n=12), Warmblood (n=3), American Paint Horse (n=2), and Lusitano (n=1). All horses underwent a complete ophthalmic examination including slit lamp biomicroscopy (SL-17 Portable Slit Lamp, Kowa Optimed Inc., Torrance, CA) pre- and post-pupillary dilation and indirect ophthalmoscopy following pupillary dilation with tropicamide 1% ophthalmic solution (Alcon Laboratories Inc., Fort Worth, TX). Intraocular pressures were obtained prior to pupillary dilation by rebound tonometry with a TonoVet Plus® (iCare Finland Oy, Helsinki, Finland) and fluorescein stain (BIO GLO™ sterile strips, 1 mg fluorescein sodium ophthalmic strip USP, Phoenix Pharmaceuticals, Belmont, CA) was used to determine if a corneal ulcer was present. Only horses with normal ophthalmic examination findings, normal intraocular pressures, and no retention of fluorescein stain were included. Included horses had no known history of ophthalmic disease. Tonometry, ultrasonic pachymetry, and ultrasound biomicroscopy were performed twice on both eyes of each included horse on two individual dates, approximately 40 days apart.

Ultrasonic pachymetry

Horses were sedated with intravenous detomidine hydrochloride (0.01 mg/kg; Dormosedan®, Zoetis Inc., Parsippany, NJ) and positioned with their head on a headstand with the head above the heart and in a normal head-carriage position. Auriculopalpebral perineural anesthesia was performed bilaterally with lidocaine hydrochloride 2% (VetOne, Boise, ID). Rebound tonometry was repeated with the TonoVet Plus in all eyes on the day of imaging prior to corneal anesthesia being provided with tetracaine hydrochloride 0.5% (Bausch & Lomb, Tampa, FL). An ultrasonic pachymeter with velocity setting of 1640 m/s (Pachette 3; DGH Technology, Inc., Exton, PA) was used to obtain corneal thickness measurements of the central, superior, temporal, inferior, and nasal cornea of both eyes of each horse. Peripheral corneal measurements were obtained 2 mm axial to the limbus. Measurements were obtained in triplicate, and to ensure accuracy of the data, only values with a standard deviation of < 20 μm were included. Rarely, horse movement resulted in a standard deviation > 20 μm and these readings were not recorded.

Ultrasound biomicroscopy

A commercially available ultrasound biomicroscope (Compact Touch STS UBM, Quantel Medical, France) with a 50-MHz linear transducer probe fitted with a ClearScan® probe

cover (ESI Inc., Plymouth, MN) filled with sterile water was used to acquire images of both eyes of each included horse. Optixcare® Eye Lube (CLC MEDICA, Ontario, Canada) was used as a coupling agent. The eye diagram and probe orientation guide within the imaging software were utilized for all image acquisitions. Transcorneal ultrasonography was performed with the probe positioned perpendicular to the globe such that the corneal epithelium, Descemet's membrane-corneal endothelial complex, and anterior lens capsule were of similar echogenicity, confirming perpendicular placement. Images of the central, superior, temporal, inferior, and nasal cornea were obtained in triplicate. Images of the axial anterior chamber, which included the cornea and anterior aspect of the lens, were obtained in triplicate for determination of ACD. All images utilized for data analysis included corneal epithelium, the Descemet's membrane and endothelium complex, and anterior lens capsule of similar echogenicity indicating that the probe was oriented perpendicular to the cornea at the time of image acquisition.

Corneal and anterior chamber depth measurements

Corneal thickness and ACD measurements were made using the Quantel Medical Compact Touch STS UBM software. Corneal thickness values were determined by measuring from the outermost aspect of the corneal epithelium to the innermost aspect of the Descemet's membrane and corneal endothelium complex. Central corneal thickness measurements were made at the maximum arch of the central cornea, and peripheral corneal measurements were made 2 mm axial to the external limbus (Fig 1). The axial ACD was measured as a line perpendicular to the maximum point of curvature of the anterior lens capsule extending to the corneal endothelium.

Ultrasonographic biometry

A-scan ultrasonographic biometry using a 10 MHz digital biometric ruler with a velocity setting of 1532 m/s (PacScan plus, SONOMED Model 300AP+, New Hyde Park, NY) was performed on both eyes of each horse to determine axial globe length. Transcorneal scans were performed with the probe positioned perpendicular to the axial cornea in line with the visual axis. Five measurements of each globe were obtained at each study time point.

Statistical analyses

Data normality was assessed by the Shapiro-Wilk test. Two-way analysis of variance (ANOVA) with Tukey's post-hoc testing was performed for comparison of corneal thickness measurements between modalities. Bland-Altman analyses were performed to assess agreement between modalities. To assess for repeatability of measurements within a modality between study timepoints, Pearson correlation coefficients were calculated. To assess for differences in corneal thickness and ACD based on sex and age, the population was compared as geldings vs. mares and horses < 13 years of age vs. horses 13 years of age. Unpaired t-tests were performed to evaluate differences in age and sex. Statistical analyses were performed using GraphPad Prism v. 9.1.2 (GraphPad software, Inc., San Diego, CA). For all analyses, significance was set at $P < 0.05$.

Results

Intraocular pressure and corneal thickness

Intraocular pressure (IOP) was normal in all horses with a mean ± SD of 18 ± 3 mmHg and no statistical differences between right and left eyes or between study timepoints. No statistical differences in mean corneal thickness measurements were identified at any individual locations between right and left eyes and so these data were averaged for each horse. When mean locational corneal thickness measurements between the two time points were averaged for each horse, the mean ± SD corneal thickness as measured by UBM was 854 ± 61 μm (central), 898 ± 48 μm (nasal), 930 ± 57 μm (temporal), 979 ± 55 μm (inferior), and 994 ± 58 μm (superior). Mean ± SD corneal thickness as measured by ultrasonic pachymetry was 825 ± 53 μm (central), 870 ± 48 μm (nasal), 879 ± 52 μm (temporal), 957 ± 48 (inferior), and 946 ± 56 μm (superior). Corneal thickness as measured via UBM was greater than corneal thickness measured via ultrasonic pachymetry at each location. This difference was statistically significant for all locations except the inferior location (Fig 2.).

The results of the Bland-Altman analyses are presented in Fig. 3. For the central cornea, the bias was 30 μm and the 95% limits of agreement (LoA) was −40 to 99 μm (Fig. 3A). The superior cornea had a bias of 48 μm and a 95% LoA of −29 to 125 μm (Fig. 3B). The temporal cornea had a bias of 51 μm and a 95% LoA of 4.2 to 98 μm (Fig. 3C). The inferior cornea had a bias of 19 μm and a 95% LoA of −60 to 99 μm (Fig. 3D), and the nasal cornea had a bias of 33 μm and a 95% LoA of −37 to 99 μm (Fig. 3E). Variability was largely consistent across the range of mean corneal thickness values.

A strong correlation in corneal thickness values between study timepoints when measured by ultrasonic pachymetry was found for the central (r = 0.92, 95% CI: 0.87-0.95; p <0.001), superior (r = 0.95, 95% CI: 0.92-0.97; p < 0.0001), temporal (r = 0.90, 95% CI: 0.83-0.94; p < 0.0001), and nasal (r = 0.94, 95% CI: 0.90-0.96; p < 0.0001) locations while the correlation for the inferior location was moderate (r = 0.61, 95% CI: 0.42-0.75; p < 0.001). For UBM, a moderate correlation between study timepoints was found for the central (r=0.57,95%CI: 0.35-0.72; p < 0.0001), superior (r = 0.63, 95% CI: 0.44-0.77; p < 0.0001), temporal (r = 0.69, 95% CI: 0.51-0.80; p < 0.0001), and inferior locations (r = 0.66, 95% CI: 0.47-0.80; p < 0.0001), while the nasal location had a weak correlation (r = 0.30, 95% CI: 0.04-0.53; p = 0.025). No statistical difference in corneal thickness at any location was detected between geldings and mares or between horses < 13 years of age and horses 13 years of age (Fig 4). A weak but significant positive correlation was identified between mean central corneal thickness measured by UBM and IOP (r = 0.38, 95% CI: 0.022-0.65; p = 0.039).

Mean ± SD axial globe length was 40.27 ± 1.63 mm, with no statistical differences between right and left eyes or between study timepoints. A weak but significant positive correlation was identified between mean central corneal thickness measured by UBM and axial globe length (r = 0.39, 95% CI: 0.038-0.66; p = 0.032).

Anterior chamber depth

No statistical differences in mean ACD values were detected between eyes or between study timepoints. When mean ACD values were averaged between eyes and across study time points for each horse, the mean ± SD ACD was 5.74 ± 0.41 mm (Fig 5). No statistical difference in anterior chamber depth was detected between geldings and mares or between horses < 13 years of age and horses 13 years of age. Anterior chamber depth and axial globe length were not correlated ($p = 0.17$).

Discussion

Quality images were obtained of the equine cornea and anterior chamber using *in vivo* UBM in standing sedated horses. Corneal thickness values obtained in this study were comparable to those obtained in other studies using high-resolution imaging techniques. For example, a study that used spectral-domain optical coherence tomography (SD-OCT) to assess corneal thickness in adult horses reported similar results in that the central cornea was thinnest and the nasal and temporal peripheral corneal locations were thinner than the superior and inferior locations.[16] Each of the corneal thickness values obtained by UBM in the current study were greater than those reported for SD-OCT. This is consistent with findings in dogs in which UBM also results in greater corneal thickness values relative to OCT.[17] A previous study identified that the mean central corneal thickness of 50 eyes of 50 warmblood horses aged 78 months or less as determined by UBM was 818 ± 41 μm.[9] As corneal thickness is known to increase with age in many species, this value is unsurprisingly lower than the mean central corneal thickness of 854 ± 61 μm obtained in the current study, which assessed mature adult horses.[18–20]

The current study identified that corneal thickness in horses determined by ultrasound biomicroscopy was greater relative to ultrasonic pachymetry at all corneal locations. This differs from a recent report in dogs in which the two techniques were found to produce similar central corneal thickness measurements with strong agreement between methods.[21] Another study that compared ultrasonic pachymetry to UBM in the assessment of corneal thickness of frozen canine corneas indicated that UBM consistently resulted in greater corneal thickness measurements.[22] As previously discussed, the accuracy of ultrasonic pachymetry in determination of corneal thickness is dependent upon the set velocity of the instrument being consistent with the speed of sound through the measured tissue.[23] The widely used velocity of 1640 m/s for ultrasonic pachymetry is appropriate for the human cornea, though the speed of sound in both the canine and bovine cornea has been demonstrated to be less than this, and it is likely that the same is true for the horse.[24–26] While the ultrasound velocities of several equine ocular tissues have been reported, to the author's knowledge, the speed of sound through the equine cornea has not been published to date.[27] As such, the corneal thickness measurements provided by ultrasonic pachymeters set to a velocity for the human cornea may not be accurate in horses. It is possible that this explains the difference in values obtained between the two modalities used in the current study. The current study found that the repeatability of ultrasonic pachymetry was superior to that of ultrasound biomicroscopy. This is suspected to be due to the greater chance of obtaining a slightly obliqued rather than perfectly perpendicular measurement of

corneal thickness with the larger UBM probe compared to the smaller ultrasonic pachymetry probe. Despite better repeatability, ultrasonic pachymetry has limitations relative to UBM in that it only provides numerical information on corneal thickness whereas UBM provides high-resolution images of the tissue of interest, and the accuracy of commercially available ultrasonic pachymeters for the equine cornea is questionable.

In the current study, a weak positive correlation between mean central corneal thickness measured by UBM and IOP was identified. A positive correlation between these values has also been reported in other species including dogs and humans.[28,29] For horses, this is unlikely to be clinically relevant for IOPs within the normal range. A weak but significant positive correlation was also identified between mean central corneal thickness measured by UBM and axial globe length. While a previous study did not identify a positive correlation between central corneal thickness and axial globe length in young horses, the study did identify a positive correlation between increasing age and both central corneal thickness and axial globe length.[9]

ACD measurements in the present study were made based on the true anterior chamber depth, defined as the posterior surface of the cornea (corneal endothelium) to the anterior surface of the lens (anterior lens capsule).[30,31] Previous studies that have reported on equine anterior chamber depth using biometry either defined ACD as the distance between the corneal epithelium and the lens or did not provide a definition for the measurement.[32,33] While the corneal epithelium to lens definition for measurement for ACD is commonly used for biometry for intraocular lens power calculations, the terminology can be misleading in that corneal thickness is also included in that measurement. A more recent study that utilized high-frequency ultrasound and UBM to assess various measurements of the growing equine eye used the same definition of ACD as was used in the present study.[9] The mean ± SD ACD in that study was 3.4 ± 0.4 mm, which is much lower than the value obtained in the present study (mean ± SD of 5.74 ± 0.41 mm), however that study examined horses ranging in age from young foals to 7 years, with a mean age of 23 months, while the mean age of the present study was 14 years. The major finding of that study was a positive correlation with increasing age for all evaluated ocular dimensions, and so this difference in ACD values between the two studies is likely explained by the difference in age.

There are few reports of the clinical use of UBM in horses; however, the utility of UBM as an important diagnostic for ocular surface and anterior segment disease has been made readily apparent in this limited body of literature. The normative data included in the present report provides an important reference that can be used as a comparison for clinical cases of both corneal and anterior segment disease. While UBM is inferior to SD-OCT in terms of image resolution, UBM has wider applicability for anterior segment imaging in that UBM can image nontransparent tissues including conjunctiva, sclera, and uvea as well as through dense corneal opacities that create significant artifact with OCT.[34] Limitations of UBM include the requirement of globe contact for image acquisition, which precludes use of UBM in fragile globes. Additionally, depth of penetration is limited to the anterior segment. Limitations of the present study include the inclusion of various horse breeds, which could impact globe size and thus corneal and ACD measurements.

The present study established normal values for corneal thickness and ACD of the adult horse measured by UBM. Relative to ultrasonic pachymetry, corneal thickness measurements were greater when assessed by UBM. While repeatability of ultrasonic pachymetry for measuring corneal thickness was superior to that of UBM, clinical applications of UBM are wider as it allows for high resolution imaging and measurements of the entire anterior segment in addition to determination of corneal thickness.

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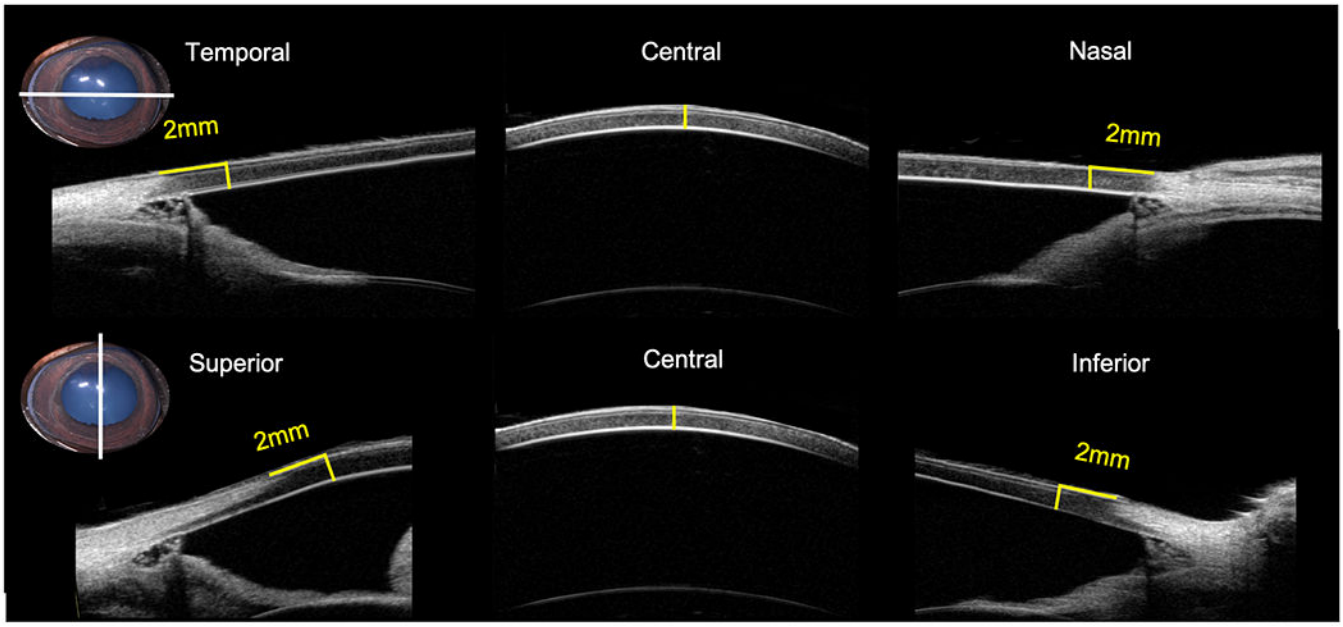

Figure 1.

Locations for determination of corneal thickness and anterior chamber depth using ultrasound biomicroscopy in the normal adult horse. Corneal thickness measurements were made at the location of the yellow lines. Peripheral corneal thickness was measured 2 mm axial to the external limbus.

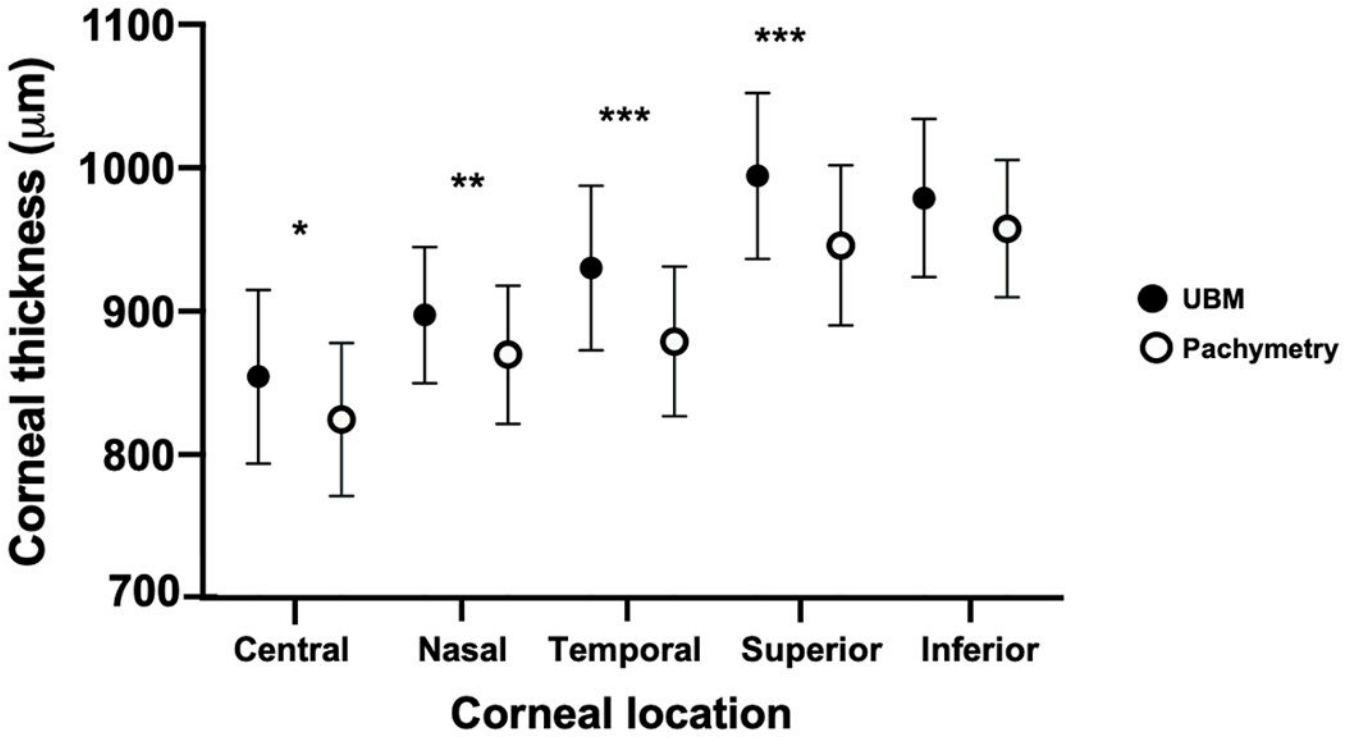

Figure 2.

Corneal thickness as determined by ultrasound biomicroscopy and ultrasonic pachymetry in normal adult horses. Data are presented as mean ± standard deviation P=0.048, *P=0.029, ***P<0.001

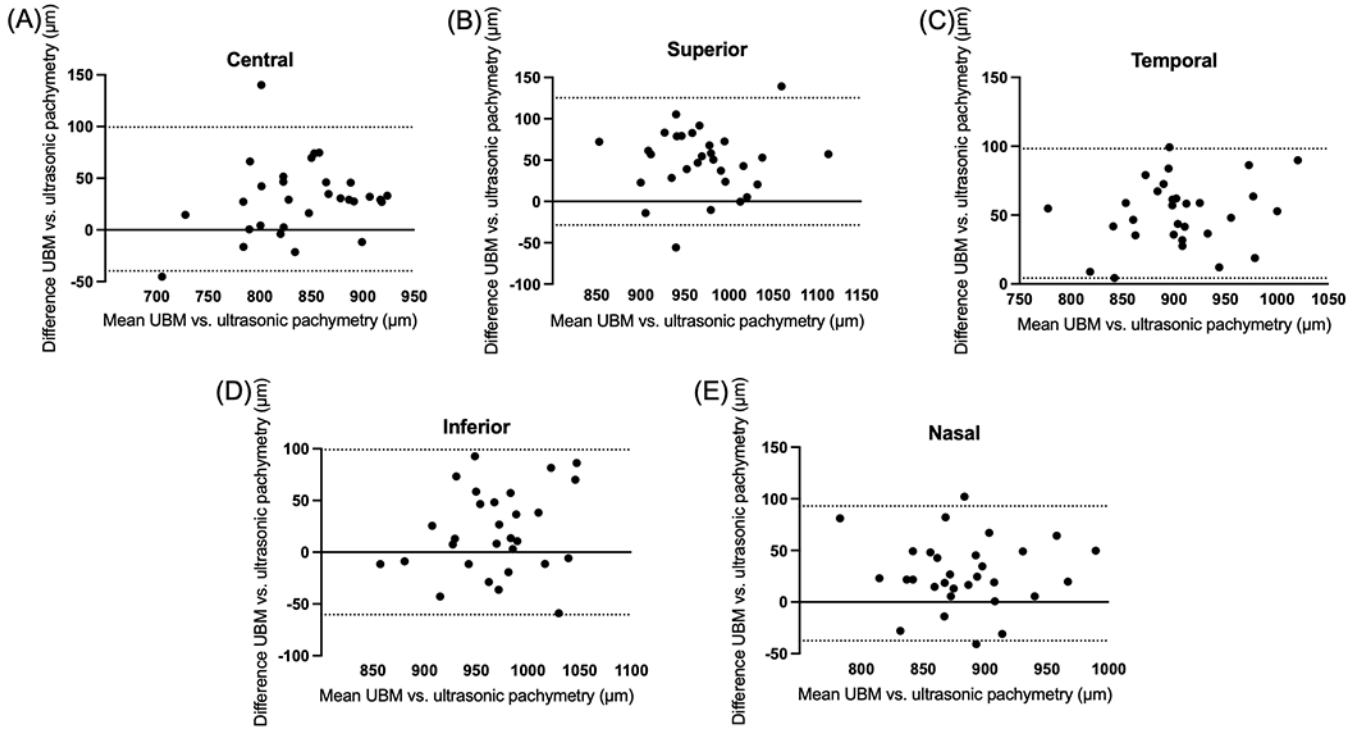

Figure 3.

Difference vs. mean Bland-Altman plots comparing ultrasound biomicroscopy and ultrasonic pachymetry for the measurement of central and peripheral corneal thickness in normal adult horses. Black dotted lines represent the 95% limits of agreement. (A) central (mean ± SD bias: 30 ± 35), (B) superior (mean ± SD bias: 48 ± 39), (C) temporal (mean ± SD bias: 51 ± 24), (D) inferior (mean ± SD bias: 19 ± 41), and (E) nasal (mean ± SD bias: 28 ± 33).

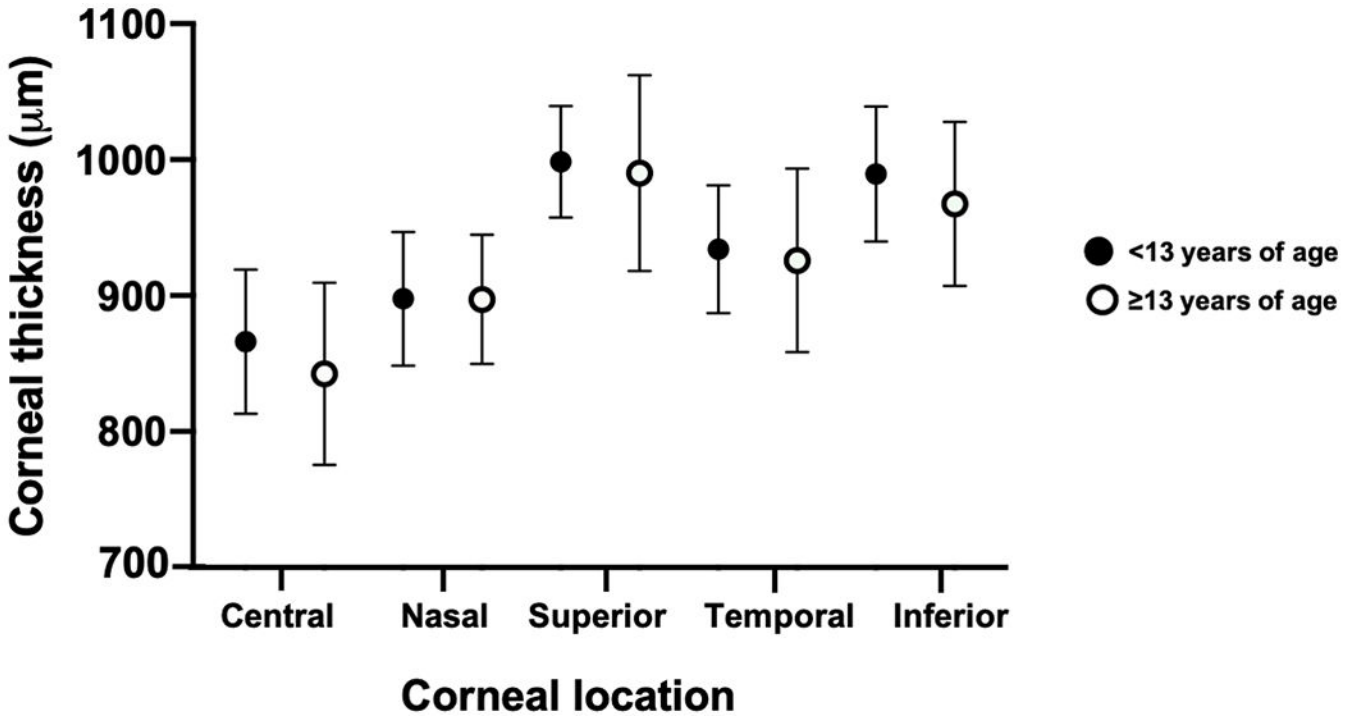

Figure 4.

Corneal thickness as determined by ultrasound biomicroscopy in normal adult horses grouped by age (<13 years or 13 years). Data are presented as mean ± standard deviation. No statistically significant differences were identified.

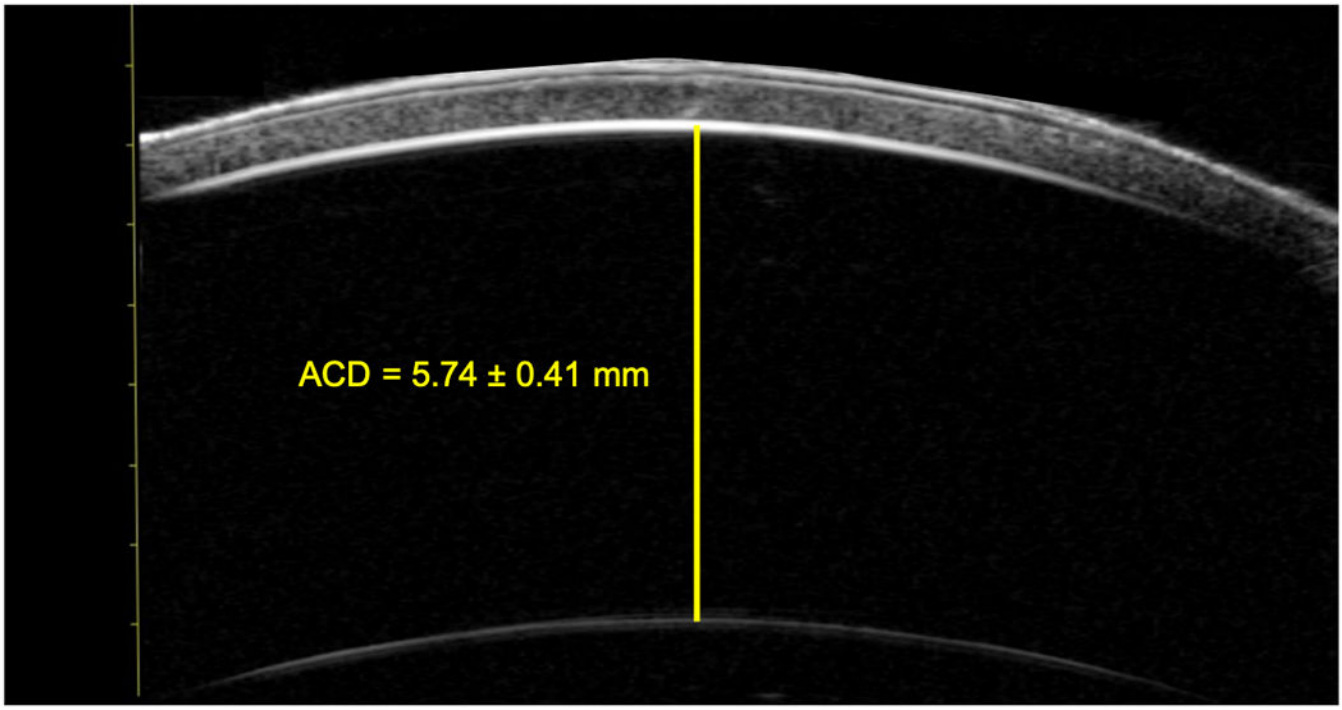

Figure 5.

Anterior chamber depth (ACD) of the normal adult horse eye as determined by ultrasound biomicroscopy. The yellow line represents where the measurement was obtained, beginning perpendicular to the maximum point of curvature of the anterior lens capsule extending to the corneal endothelium. Data are presented as mean ± standard deviation.