Corneal Thickness in Pigs Measured by Ultrasound Pachymetry In Vivo

by ***Carsten Faber[1,*], Erik Scherfig[1,2], Jan Ulrik Prause[1,2] & Knud Erik Sørensen[2]***
[1]Eye Pathology Institute, University of Copenhagen, Denmark
[2]Department of Ophthalmology, Rigshospitalet, Copenhagen, Denmark

Summary
The objective was to evaluate the normal thickness and diameter of the pig cornea using, under general anesthesia, 13 female, young, domestic Danish Landrace pigs. The thickness was measured, for the first time in vivo, and were: 666 µm centrally, 657 µm nasally, 713 µm inferiorly, 669 µm temporally, and 714 µm superiorly (mean values). The cornea diameters were also measured, using calipers, and were: 14.9 mm horizontally and 12.4 mm vertically (mean values). Conclusion: The pig cornea is only slightly thicker than the human cornea. However, unlike the human cornea, no significant difference in thickness exists between the central and the tempero-nasal peripheral locations of the pig cornea. The pig could prove useful as source for corneas in human xenotransplantation.

Introduction
Corneal transplantation is the most frequently performed type of tissue grafting *(George & Larkin, 2004)*. It is a highly successful treatment of severe corneal disease. However, transplantation of tissue and organs in humans has a major setback: availability of donors. To solve this, there are currently many studies on the possible use of the pig as a source of hearts, kidneys, livers, Islets of Langerhans etc. for transplantation to humans *(Tai et al., 2007; Cox & Zhong, 2005; Zhong, 2007)*. Because of the unique immune privilege of the anterior chamber, corneal grafting may be the most likely xenotransplantation to succeed. This makes the pig eye a particularly interesting experimental model.
In the course of corneal transplantation, corneal thickness is a valuable indicator of graft failure and early rejection. Reliable reference material on the corneal thickness of the pig, however, does not exist. This study was conducted to investigate the normal thickness and diameter of the pig cornea.

**Correspondence:* Carsten Faber
Eye Pathology Institute, University of Copenhagen, Frederik den V's Vej 11, DK-2100 Copenhagen, Denmark
Tel: + 45 3532 6070
Fax: + 45 3532 6080
E-mail: carstenfaber@gmail.com

Materials & Methods
Measurements were performed on 13 female domestic Danish Landrace pigs (Lars Jonsson, Lynge, Denmark) weighing 43 kg, equivalent to an age of 104 days (median values). Their treatment was supervised by a veterinarian. The research protocol was approved by the Danish Animal Experiments Inspectorate, and was in accordance with the ARVO resolution for the use of animals in ophthalmic and vision research. Anesthesia was carried out as previously described *(Warfvinge et al., 2005)*.
The right eye of each pig was prepared with local administration of 0.4% benoxinate hydrochloride (Oxybuprocaine; SAD, Copenhagen, Denmark) and 5% povidone-iodine (SAD). To ensure ophthalmoscopy, the pupil was dilated using a combination of 10% phenylephrine hydrochloride (Metaoxedrin; SAD), 0.5% tropicamide (Mydriacyl; Alcon, Heinut, Belgium), and 1% atropine sulfate (SAD). The surface of the eye was protected from desicca-

Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

tion with a bandage keeping the eyelids closed before measurements and with isotonic saline solution during the measurements.

Ultrasound pachymetry (Pocket II, Quantel Medical, Bozeman, Montana, USA) was performed on the right eye of the pigs. Measurements were performed centrally and peripherally on the cornea next to the limbus, nasally, inferiorly, temporally, and superiorly. Each measurement was the mean of five readings. The horizontal and vertical diameters of the cornea were then measured with a pair of calipers at the widest points. Finally, the pigs were sacrificed by injection with 20 ml (200 mg/ml) pentobarbital sodium (KVL, Copenhagen, Denmark).

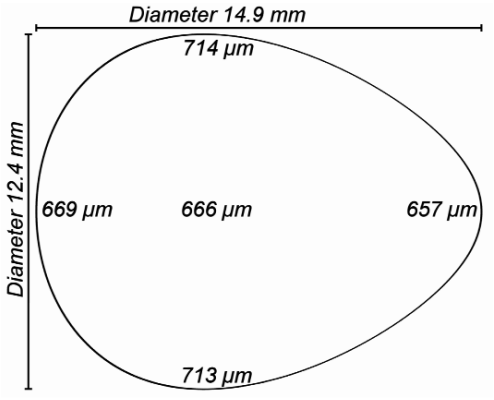

Figure 1. Sketch of the right cornea of the pig. Values inside the cornea designate the thickness.

Results

The corneal diameter and thickness are given in Table 1.

From Figure 1 it is seen that the cornea is thinnest along the longest diameter and has a nearly flat profile. The shortest diameter, however, is thicker in the periphery indicating a steep profile. As outlined in the figure, the shape of the cornea is not circular, but asymmetrically oval. Thus, the longest vertical meridian does not meet the longest horizontal meridian in the center of the cornea, but at a point temporally to the centre.

Discussion

Thickness

Using ultrasound the cornea of the pigs was measured at 666 µm thick centrally. A Medline search of the pig cornea only yielded data based on studies done ex vivo. One study, based on pig eyes from an abattoir using ultrasound, states a central corneal thickness of 980 µm (range 760 – 1,460 µm) *(Bartholomew et al., 1997)*. Another study found a thickness of 660 µm (range 507-829 µm). However, the authors found that the intraocular pressure in the enucleated pig eyes was too low and thus raised it with an injection of physiological salt solution

Table 1. Corneal diameter and thickness in pigs

Diameter of cornea (mm)	Mean (SD)	Median	Range
Horizontal	14.9 (0.5)	15.0	14.0-15.5
Vertical	12.4 (0.7)	12.5	11.0-13.0
Thickness of cornea (µm)			
Central	666 (68)	679	543-797
Nasal	657 (70)	665	497-748
Inferior	713 (41)	709	643-794
Temporal	669 (48)	677	574-755
Superior	714 (54)	714	632-832

(Wilmes et al., 1992). A third study reports a corneal thickness ranging between 600 and 700 mm on freshly enucleated pig eyes *(Bohnke et al., 1998)*. The lack of consistency in the measurements reported above clearly demonstrates the large margin of errors found in studies done on the cornea ex vivo.

Some of the reported variation in the pig cornea could also be attributed to differences in race, age, and sex of the pigs and difference in thickness between central and peripheral parts of the cornea.

There are no data on the effect of race on corneal thickness in pigs. Montiani-Ferreira *et al*. reported a difference between Labrador Retrievers and Beagle/Briards, but could not rule out weight as a confounding influence *(Montiani-Ferreira et al., 2003)*. Studies in humans indicate ethnic group-related differences in corneal thickness with a marginally thicker Caucasian cornea compared to Greenland Inuits, Blacks, Asians and Native Americans *(Doughty & Zaman, 2000)*.

There are no data on the effect of age on corneal thickness in pigs. The number of pigs in our study does not allow statistical analysis. Studies in cats, dogs, llamas, and horses indicate that the corneal thickness increases with age *(Gilger et al., 1991; Gilger et al. 1993; Gwin et al., 1982; Ramsey et al., 1999; Andrew et al., 2002)*. Beyond infant years there seems to be no dependence on age in humans *(Doughty et al., 2000)*.

There are no data on the effect of sex on corneal thickness in pigs. We only had female pigs at our disposal. In dogs males have thicker corneas than females *(Montiani-Ferreira et al., 2003; Gilger et al., 1991)*. In chicks, cats and horses gender has no effect *(Montiani-Ferreira et al., 2004; Andrew et al., 2001; Ramsey et al., 1999; Plummer et al, 2003; van der Woerdt et al., 1995; Gilger et al., 1993)*. In humans gender has apparently no influence on corneal thickness *(Doughty et al., 2000)*.

Evidently, the effect of race, age, and sex on corneal thickness in other animals, referred to above, is too small to explain the difference between our data and data previously reported on the pig.

With regards to central versus peripheral thickness, Bartholomew *et al.* (*1997*) states a porcine corneal thickness at the limbus of 1,190 µm (range 970 – 1,710 µm), which is thicker than values obtained from the central part. They, however, did not indicate in which peripheral part of the cornea measurements were done. Our study shows that the asymmetrically oval porcine cornea is of equal thickness at three points along the horizontal axis and that the superior and inferior peripheral parts are thicker than the central part. Similar differences exist for dogs and horses. In dogs the superior and temporal peripheral parts of the cornea are thicker than the central part (inferior and nasal locations were not measured) *(Gwin et al., 1982; Gilger et al., 1991)*. In horses the peripheral parts in all four quadrants are thicker than central part. Similar to the pig, the superior and inferior locations are thicker than the tempero-nasal locations *(van der Woerdt et al., 1995; Andrew et al., 2001; Ramsey et al., 1999)*. In cats and rabbits, no significant difference in thickness exits between central and peripheral parts *(Gilger et al., 1993; Chan et al., 1983)*. The human cornea is thicker in the peripheral tempero-nasal locations averaging at 672 µm 4 mm from the center *(Doughty et al., 2000)*.

Diameter

The group of pigs investigated in this study had an asymmetrically oval cornea with a horizontal (HCD) and vertical diameter (VCD) measuring 14.9 mm and 12.4 mm respectively (mean values). In the literature, the diameter of the pig cornea is reported to be 16.61 mm (range 15.50 – 17.79 mm) horizontally and 14.00 mm (range 12.80 – 14.99 mm) vertically *(Bartholomew et al., 1997)*. The ratio between the two parameters is similar to the one found in this study. It is likely that the differences in HCD and VCD are due to differences in size and race of the pigs studied.

The human cornea measured from the outside is also slightly oval, with a horizontal diameter of 11.7 mm and a vertical diameter of 10.6 mm. However, from the inside of the eye it is circular with a hori-

zontal and vertical diameter of 11.7 mm *(Doughty et al., 2000)*.

The pig cornea as a graft in human corneal xenotransplantation

In corneal transplantation, usually only the central 6-8 mm of the cornea is grafted. Thus the diameter of the porcine cornea is adequate. Our study implies a steep thickness profile in the superior-inferior direction. Probably, the difference in thickness between central and peripheral parts is smaller in a small diameter graft. This question needs to be addressed in further studies.

Conclusion

The pig cornea was measured in vivo using ultrasound pachymetry, and found to be only slightly thicker than the human cornea. However, unlike the human cornea there is no significant difference in thickness between the central and the peripheral locations along the greater diameter, and a steep thickness profile along the shorter diameter of the pig cornea. The pig could prove useful as source for corneas in human xenotransplantation.

References

Andrew S E, D T Ramsey, J G Hauptman & D E Brooks. Density of corneal endothelial cells and corneal thickness in eyes of euthanatized horses. Am J Vet Res. 2001, *62*, 479-482.

Andrew S E, A M Willis & D E Anderson. Density of corneal endothelial cells, corneal thickness, and corneal diameters in normal eyes of llamas and alpacas. Am J Vet Res. 2002, *63*, 326-329.

Bartholomew L R, D X Pang, D A Sam & J C Cavender. Ultrasound biomicroscopy of globes from young adult pigs. Am J Vet Res. 1997, *58*, 942-948.

Bohnke M, P Chavanne, R Gianotti & R P Salathe. Continuous non-contact corneal pachymetry with a high speed reflectometer. J Refract Surg. 1998, *14*, 140-146.

Chan T, S Payor & B A Holden. Corneal thickness profiles in rabbits using an ultrasonic pachometer. Invest Ophthalmol Vis Sci. 1983, *24*, 1408-1410.

Cox A & R Zhong. Current advances in xenotransplantation. Hepatobiliary Pancreat Dis Int. 2005, *4*, 490-494.

Doughty M J & M L Zaman. Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach. Surv Ophthalmol. 2000, *44*, 367-408.

George A J & D F Larkin. Corneal transplantation: the forgotten graft. Am J Transplant. 2004, *4*, 678-685.

Gilger B C, R D Whitley, S A McLaughlin, J C Wright & J W Drane. Canine corneal thickness measured by ultrasonic pachymetry. Am J Vet Res. 1991, *52*, 1570-1572.

Gilger B C, J C Wright, R D Whitley & S A McLaughlin. Corneal thickness measured by ultrasonic pachymetry in cats. Am J Vet Res. 1993, *54*, 228-230.

Gwin R M, I Lerner, J K Warren & G Gum. Decrease in canine corneal endothelial cell density and increase in corneal thickness as functions of age. Invest Ophthalmol Vis Sci. 1982, *22*, 267-271.

Montiani-Ferreira F, F Cardoso & S Petersen-Jones. Postnatal development of central corneal thickness in chicks of Gallus gallus domesticus. Vet Ophthalmol. 2004, *7*, 37-39.

Montiani-Ferreira F, S Petersen-Jones, N Cassotis, D T Ramsey, P Gearhart & F Cardoso: Early postnatal development of central corneal thickness in dogs. Vet Ophthalmol. 2003, *6*, 19-22.

Plummer C E, D T Ramsey & J G Hauptman. Assessment of corneal thickness, intraocular pressure, optical corneal diameter, and axial globe dimensions in Miniature Horses. Am J Vet Res. 2003, *64*, 661-665.

Ramsey D T, J G Hauptman & S M Petersen-Jones. Corneal thickness, intraocular pressure, and optical corneal diameter in Rocky Mountain Horses with cornea globosa or clinically normal corneas. Am J Vet Res. 1999, *60*, 1317-1321.

Tai H C, M Ezzelarab, H Hara, D Ayares & D K

Cooper. Progress in xenotransplantation following the introduction of gene-knockout technology. Transpl Int. 2007, *20*, 107-117.

van der Woerdt A, B C Gilger, D A Wilkie & S M Strauch. Effect of auriculopalpebral nerve block and intravenous administration of xylazine on intraocular pressure and corneal thickness in horses. Am J Vet Res. 1995, *56*, 155-158.

Warfvinge K, J F Kiilgaard, E B Lavik, E Scherfig, R Langer, H J Klassen, M J Young. Retinal progenitor cell xenografts to the pig retina: morphologic integration and cytochemical differentiation. Arch Ophthalmol. 2005, *123*, 1385-1393.

Wilmes C, J Draeger & M Kohlhaas. [Precision of lenticular thickness in relation to full stroma corneal thickness. Experimental results with the lamellar microkeratome]. Ophthalmologe. 1992, *89*, 147-150.

Zhong R. Gal knockout and beyond. Am J Transplant. 2007, *7*, 5-11.