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Consideration of growth (age)-related effects on globe size and corneal thickness in ovine eyes for use in laboratory studies

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Summary 250

Purpose: To assess differences in eyeball mass, corneal diameter and central corneal thickness (CCT) in slaughterhouse-procured ovine eyes.

Methods: Over a 12 year period, measurements of eye globe mass, horizontal cornea diameter (HCD), and CCT were routinely undertaken within 2 h post-mortem. Only eyes free of obvious mechanical damage or disease were used.

Results: From measurements on 736 quality-selected and trimmed eyes, globe wet mass ranged from 10.4 to 25.2 gm, HCD from 19.0 to 26.5 mm, and CCT measured by ultrasound pachymetry from 0.543 to 0.836 mm (with an overall average of 0.690 mm \pm 0.056 mm). The eye globe mass was strongly correlated to HCD ($r^2 = 0.829$). CCT correlated with globe mass ($r = 0.543$) and to HCD ($r = 0.402$). Based on the different anatomical measurements, a lamb's eye would be expected to have a thinner cornea (average 0.640 mm) than that of an adult outbred ewe (average 0.730 mm). In freshly-procured eyes showing signs of slight corneal oedema, CCT was greater (average 0.856 \pm 0.052 mm), and up to 24 h cold storage resulted in predictable increases in CCT from 6 to 24 %, especially in eyes showing signs of corneal oedema before storage.

Conclusions: Based on the correlations obtained, differences in ovine eyes can be attributed to growth-related differences in the animals and thus, indirectly to their expected age. A simple measure of the HCD in ovine eyes used for laboratory studies would be a useful indicator to provide in reporting these studies.

Key words: ovine, sheep, lamb, eyeball mass, corneal diameter, corneal thickness, pachymetry

[INTRODUCTION]

One aspect of developing our understanding refractive status and its relation to vision is to use animals eyes, both for general comparisons,¹⁻³ and to develop or evaluate procedures (e.g. for refractive surgery) that would not be easy to undertake on human eyes *in vivo*.⁴⁻⁹ Such *ex vivo* studies to assess the structural effects of incisions or trephine application or laser pulses to the cornea have been carried out on ovine eyes, as have some studies of the optical properties of the cornea.¹⁰⁻¹² Corneal thickness must be considered as an important factor in such assessments, but only one of these studies provides this information.⁵ The average value given (of 0.590 mm) for 'fresh lamb eyes' is consistent with one text source that states that the central corneal thickness in 'sheep' is 0.5 mm,¹ but remarkably different from another text where values of between 0.8 and 2 mm are given,¹³ again for 'sheep' eyes. Another more recent anatomical study reported corneal thickness of 0.689 mm for a single sheep eye.¹⁴ It can also be noted that while one text states that the cornea diameter in 'sheep' eyes is 27 mm,¹ the lamb eyes were noted to have an average corneal diameter of just 16.4 mm.⁵ Other studies have simply indicated that 'sheep' eyes were used,^{4,6-9,12} with one of these reports indicating that the horizontal corneal diameter in 1 year old sheep was 24 mm.⁹ With a general expectation that corneal oedema will likely develop post-mortem, it is also relevant to note that the published reports indicate that while some of these eyes were used 'fresh' (i.e. within 3 h post-mortem, or what can be considered as recent post-mortem material),^{5,8,11,12} other assessments could have been made on corneas that were at least 6 h post-mortem and even up to 24 h.^{4,7,10,15}

Overall, with such a wide range of values reported for corneal thickness and corneal diameter for ovine eyes, a systematic study was initiated to assess not only corneal thickness and diameter and also globe size (mass) which is considered to be a general indicator of the animal size.¹³ The present report is an analysis of ovine eyes periodically received from a slaughterhouse over a period 12 years usually designated as being from lambs or from outbred adult ewes slaughtered for meat production. With this accepted difference, the inter-relationships between globe mass, corneal diameter and corneal thickness could be investigated. In addition, the opportunity was taken to further investigate how often corneal oedema might be encountered in these types of eyes and the extent to which further changes in corneal thickness might occur over 24 h.

MATERIALS AND METHODS

The material for the present studies was obtained from a local slaughterhouse who provided recent post-mortem ovine eyes, mainly from the Scottish Blackface breed. Based on conversations with the slaughterhouse personnel, some of the eyes were expected to be from lambs (likely aged from between 4 months and 12 months) while others were from older outbred older ewes (that would more likely be aged between 2 and 5 years). However, specific information on the age of individual slaughtered animals was not available.

Following an initial written request to the Meat Hygiene Service (MHS) UK, small batches of eyes (usually 10 to 20) were made available on verbal request without any restrictions. No specific approval documentation was required by either the slaughterhouse or university on the use of the material. All animal husbandry and slaughter was undertaken by trained personnel under the MHS UK regulations, with the animals being humanely killed by electrobolt and the whole eyes being available as soon as possible after slaughter and decapitation, a process that can take 1 to 2 h in total for a batch of eyes to be obtained. Specific instructions were given to remove the eyeball from the orbital cavity and to trim away the eyelids and some of the extraocular muscle tissue. The eyeballs were then rinsed with cold tap water, placed in closed plastic bags (then usually placed in a plastic container) and taken to the laboratory usually between 10.00 and 13.00 h; the transport time (usually at ambient

temperature) was routinely less than 30 min. Once at the laboratory, the bag of eyes was always placed on ice and measurements taken as soon as possible from some eyes, with all assessments and measurements being made by the author. Some eyes were kept, in closed plastic bags on ice for either 6 to 7 h (over the working day) or the bags placed in a 4°C refrigerator for 22 to 25 h (i.e. overnight), and then corneal thickness measurements made.

All eyes were first inspected for overall quality and several in any particular batch were routinely rejected as showing signs of abnormality (where the cornea was not obviously transparent) or gross damage to the globe and cornea (such as perforation or lacerations). The eyes that appeared to be in reasonable condition were further checked to see that there were no gross signs of corneal or conjunctival disease, substantial vascularization at the limbus or across the conjunctiva, or any growths or nodules. A few eyes did show small localized areas of conjunctival haemorrhage, but this was considered to be induced during the eye procurement.

If the overall appearance of an eye was acceptable, the next step was to trim off the remainder of the fat, connective and muscle tissue from the eyeball using fine dissecting scissors. The optic nerve was also carefully severed just outside the eyeball, which was occasionally wetted with physiologic saline (at room temperature, RT) to reduce the chance of desiccation-related changes in the eyes during these preparation steps that usually took several minutes for each eye.

As soon as the trimming was completed, the globe mass of such a quality-selected eyeball (nominally free of extraneous fat and extraocular muscle tissue) was then measured to within 0.1 g on a pan balance. Both the horizontal corneal diameter (HCD) and vertical corneal diameter (VCD) (see Figure 1) were then measured to within 0.5 mm with a calliper-based rule. The corneal surface was routinely re-wetted with saline, and a single measurement of central corneal thickness (CCT) made with an ultrasound pachymeter (Corneoscan Model 8500, Allergan Humphrey, Toronto, Canada) with the ultrasound velocity setting at 1580 m/s. The globe was simply held manually and the probe applied as perpendicular as possible to the central region of the corneal surface. Only light contact was needed and, most commonly, an audible beep immediately confirmed a successful CCT reading being made. For a few eyes (25, taken from 5 different batches), three repeat measures were made at the same location over a period of 10 to 15 s. A single measurement of central corneal thickness (CCT) was considered acceptable since such repeat measures were all within a 0.01 mm range indicating repeatability of the CCT measures to within 1 % of the average values.

All data were entered into spread sheets in Systat v. 11.0 (Systat, Evanston, IL) to generate global statistics and graphical output. All data sets, or sub-sets, were checked for having a normal distribution using the default Shapiro-Wilk test and a cluster analysis (based on K-means hierarchical sequencing) being undertaken, both as included in Systat. Variability across data sets was also assessed by calculation of a 95 % confidence interval (95 % CI) as based on ± 1.96 SD. As appropriate, sets of data were compared using Friedman rank order tests, with the level of statistical significance set at $p < 0.05$. Unweighted linear regression analyses were also carried out to assess inter-relationships between measurements, generating assessment of significance ($p < 0.05$) and strength of the correlations (Pearson r or r^2 values as appropriate).

RESULTS

Overall features and anatomical characteristics of the quality-selected eyes

Figure 1 shows a typical ovine eyeball, as received, with a small amount of extraocular fatty and muscle tissue still attached. For some eyes, this excess tissue was substantial. The cornea of the ovine eye is distinctly oval in external profile, aligned with the horizontal pupil. The main part of the bulbar conjunctiva generally appeared to be non-pigmented, but the narrow limbus

region surrounding the cornea quite often showed some pigmentation (which is presumably a characteristic of the breed). Some eyes in a batch were distinctly smaller and others larger. At some periods of the year, all the eyes were small and from lambs. If specific requests to the slaughterhouse were made for 'sheep' (rather than lamb) eyes, then the latter were usually obtained and were routinely distinctly larger in size.

Overall, from all batches received, the wet mass values of the eyeballs were found to range from 10.4 to 25.2 g, with a mean (\pm SD) of 17.8 ± 3.6 g. Figure 2A shows the distribution of eyeball mass values to be distinctly bimodal ($p < 0.001$). The two groupings identified (F ratio = 2211) were from 10.4 to 16.7 g (sub-group mean 13.5 g) and 16.8 to 25.2 g (sub-group mean of 20.0 g). For these eyes, the HCD values ranged from 19.0 to 26.5 mm (mean 23.4 ± 2.0 mm) (Figure 2B), while the VCD values were between 13.5 and 22.5 mm (mean 18.3 ± 1.8 mm). The HCD and VCD values were inter-related in a predictable manner (graph not shown, correlation $r^2 = 0.753$). An average corneal diameter [calculated from $(\text{HCD} + \text{VCD})/2$] was 20.9 ± 1.8 mm. The distribution of HCD values was distinctly negatively skewed ($p < 0.001$) and while not as notable as the eyeball mass values, a cluster analysis on HCD values indicated two very distinct sub-groups (F ratio = 2264, $p < 0.001$) which had HCD values between 19 and 22.5 mm (sub-group mean of 21.1 mm) and 23.0 to 26.5 mm (sub-group mean of 24.7 mm). The CCT values of these eyes ranged from 0.543 to 0.836 mm, with a group mean of 0.690 mm and an SD of 0.056 mm. A histogram (Figure 2C) shows that most CCT values fell within the range between 0.650 and 0.750 mm, but there were a few eyes with either much lower (i.e. < 0.5 mm) or rather higher (i.e. > 0.8 mm) values. The distribution of the CCT data (Figure 2C) appears almost unimodal, but did not fit a normal distribution ($p = 0.001$) and application of a cluster analysis again indicated two groups albeit at a slightly lower confidence level (F ratio = 1546); the two groups were between 0.540 to 0.690 mm (sub-group mean of 0.640 mm) and also 0.690 to 0.840 mm (sub-group mean of 0.730 mm).

Statistical correlations

Overall, the HCD was highly correlated to eyeball mass (Figure 3A) with a similarly strong association for VCD (not shown). For the entire set of 736 eyes, the eyeball mass predicted HCD ($r^2 = 0.829$), with a 0.5 mm increase in HCD for each g increase in the eyeball. For HVD this was 0.43 mm / g ($r^2 = 0.768$). A comparison between the eyeball wet mass values and CCT is shown in Figure 3B. This reveals a modest ($r = 0.423$) but statistically-significant ($p < 0.001$) positive correlation. For each g increase in eyeball mass, the CCT was found to be 0.007 mm thicker, or could be different by 0.07 mm if the eyeball mass differed by 10 g (as it easily can be).

CCT changed in a predictable fashion in relation to HCD. This is shown in Figure 4 in two different ways. Firstly (Figure 4A), all data points are used to assess the apparent dependence of CCT on the HCD. This, as indicated by the regression line, reveals a modest positive correlation ($r = 0.402$, $p < 0.001$) but there is clearly a wide spread to the CCT data. If the CCT data is grouped according to HCD, in 0.5 mm increments, the apparent inter-relationship indicates slight non-continuity (Figure 4B) rather than CCT necessarily being a continuous (linear) variable related to HCD. Notwithstanding, there is clearly some inter-dependency of CCT on cornea size. The slightly smaller CCT values were quite often associated with the sub-group having the smaller HCD values.

Post-mortem changes in corneal thickness

A small number of other eyes were considered to have corneas that showed slight signs of oedema rather than being obviously transparent based on simple visual inspection, i.e. the cornea appeared slightly hazy and the visible iris detail was not quite as sharp. These were only

occasionally encountered, usually with just a single example in a particular batch when found. This oedema, when it was present, appeared to be predominantly in the outmost epithelial cell layer of the cornea, with only slight involvement of the stroma. This conclusion was made because removal of the epithelium (by light scraping with a single-edged razor) substantially reduced the oedematous appearance. While rejected for further use in specific experiments, measurements of CCT were made on some of these eyes as well as some assessments of the changing oedema with further post-mortem storage.

The CCT measures, taken on the eyes as received, clearly indicated that when some visible oedema signs were present there was a noticeable increase in the measured corneal thickness. From 33 such eyes, the CCT values ranged from 0.802 to 0.934 mm (mean 0.856 ± 0.052 mm), with most values being well outside the distribution of CCT values given in Figure 2C. These CCT measures were not included in the main analyses.

For 22 normal appearing (corneal oedema-free) sheep eyeballs (from 4 different batches) left on ice for 6 to 7 hours in small closed plastic bags (having been previously washed with tap water), the mean corneal thickness was found to increase from 0.707 ± 0.062 mm to 0.750 ± 0.072 mm; this represents 6.1 % increase in corneal thickness ($p = 0.017$). The corneas of these eyes, at the re-measurement did not show obvious signs of oedema.

If eyes were left overnight in a 4°C refrigerator in the same closed plastic bags, then the corneas of all the eyes showed mild but distinct signs of oedema, and the mean CCT was found to have increased to 0.841 ± 0.059 mm, which represents an 18.9 % increase in thickness ($p < 0.001$). This change is still however less than that which can occur if the corneas already show slight signs of oedema when received. For a set of such eyes that showed visible signs of corneal oedema even prior to overnight storage, the CCT values changed from 0.846 ± 0.069 mm to 1.047 ± 0.089 mm (average 23.7 % increase, 18 eyes from 15 different batches). The net increase, of 0.201 mm, was not only statistically significant to that before cold storage ($p < 0.001$), but also statistically greater than that seen with eyes that did not have obvious signs of corneal (epithelial) oedema.

DISCUSSION

The outcome of the present systematic study indicates that there should be predictable differences in the thickness of ovine corneas based on what would logically be expected to be growth-related changes in the globe size, i.e. if an eyeball is heavier or has a larger diameter cornea (with the eyes generally being from a specific breed) then this is also surely an age-related effect. Overall, ovine eyes (i.e. from *Ovis aries* genus, with numerous different breeds) might be considered to be from younger animals (i.e. lambs, with the same term being applied to the meat product) or older animals (with the term mutton perhaps applied to the meat product). There do not appear to be any specific breed-independent criteria for defining the exact difference, i.e. the age at which a lamb is sent for slaughter will be dependent on the adequacy of its growth to generate a suitable carcass for meat production, for example.

The sets of measurements of corneal thickness in this study were undertaken over an extended time period and were, in part, prompted by the paucity of data available on this aspect of ovine corneas in the literature at the time these studies were started.^{1,5,13,14} These previous reports indicated that central corneal thickness values could be between 0.500 and 2 mm, albeit with the use of different measuring techniques. The present studies were undertaken as soon as possible, from some eyes in all batches received, as part of routine quality control measures, i.e. to assess how many eyes were suitable for various laboratory-based studies. Overall, in the analyses of the data obtained, it was assumed that the eyeballs were not only from healthy animals but that they were essentially free of any substantial post-mortem artefacts. The measurements were made as soon as practically possible after the slaughter of the animals,

while the emphasis was on preparing just a few samples for specific experiments especially since the additional step of trimming off all residual fat and muscle tissue takes time and is unlikely to be done in routine use of corneas in laboratory-based studies. The overall outcome is consistent with other recent studies comparing corneas from lambs and adult sheep, allowing for the possibility that corneal thickness values may be slightly different between different breeds of sheep.¹⁵

The analyses are presented to show that ovine eye eyes obtained via routine slaughterhouse procedures can be substantially different, but that a simple measure of horizontal corneal diameter (HCD) should provide a useful indicator for size of the eyes. This size is very obviously dependent on eyeball mass, and is predictably different in lambs versus outbred ewes. Stated another way, while actual animal age was not specifically available, the size differences reflect the predicted age of the animals. The dynamic changes and differences are surely important and for, example, to state that the globe mass of a 'sheep' eye is 15 g,¹³ or that the a corneal diameter value of 27 mm would be that expected for a 'sheep' eye,¹ is clearly not consistent with the data obtained in the present study. In developing model eye constructs for 'sheep',² such differences need to be considered. As with HCD, CCT appears to be a continuous variable that changes with eye growth (Figure 4B) with thicker corneas being more likely to be found for larger eyes, providing no substantial post-mortem oedema has developed.

The last aspect of the present study, on the post-mortem changes in CCT that can readily occur, hopefully serves to emphasise the importance of obtaining and using the eyes as soon as possible after slaughter. Even within a few hours, small post-mortem-related changes in CCT can occur and even though this time period is not always specified, a few hours has been that for use of corneas in some laboratory-based assessments.^{4,5,8,11,12} With the use of slaughterhouse material, it is reasonable that there will be some delay (up to a few hours) between death and access to the eyes. It has been noted that an ovine cornea 'keeps its properties for a few hours post-mortem if regularly hydrated',¹¹ and the storage prior to use in closed plastic bags (as used in this study) should facilitate this. With corneal thickness logically being very important in any assessment of the optical properties of the cornea or the effects of surgical incisions, post-mortem changes in the corneas of slaughterhouse-procured ovine eyes need to be given more consideration; even a few hours delay between death and use of the eyes can result in noticeable increases in corneal thickness. While this may be evident as oedematous changes, actual measurements of corneal thickness would obviously be useful to underpin the normality of the corneas.

For all of the present studies, information on the exact age of the animals being slaughtered was not available. While all such livestock routinely had individual logs of source and age details generated, it was not possible to follow the slaughter of individual animals. Lambs could be slaughtered for meat over a period of several months since birth, and perhaps to 1 year or even slightly longer than this depending on their growth and their designated use. No specific time period is apparently imposed for designation of an outbred ewe. Based on conversations with the slaughterhouse personnel, smaller eyes were from lambs, but when specific requests were made for adult sheep eyes, the batches contained mainly the larger eyes. Other sources of inter-eye variability could arise because of the gender of the animals (which could be male lambs versus slightly older females, with castrated wether lambs perhaps being slaughtered at a slightly younger age) and / or unavoidable differences in growth of the eyes versus the individual body weight gain; the latter parameter is that which will usually determine when lambs will be sent for slaughter rather than simply their age. Overall, eyes from lambs can be expected to have slightly thinner corneas (by approximately 0.1 mm) than adult sheep, with the respective CCT values being around 0.640 mm and 0.730 mm respectively. The data presented in Figure 4 emphasises the possible distinction in that the expected CCT values

change as HCD values shift from smaller to larger eyes at about 21.5 to 22 mm. With the projected differences in CCT based on differences in either HCD or globe mass, it should be noted that the corneas from sheep versus lamb eyes could differ by more than the expected inter-eye variability (as SD). Notwithstanding, these results are presented to indicate that ‘ovine’ (‘sheep’) eyes can be remarkably different in size and that it would therefore be useful to provide an indication of such size differences for material being used in laboratory-based research.

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Figure captions

Figure 1. An as-received ovine eye from the slaughterhouse, with some adherent fatty and muscle tissue still attached, to illustrate horizontally aligned pupil and the associated longer horizontal corneal diameter (HCD) compared to the vertical corneal diameter (VCD). A mm rule measure is below the eye image.

Figure 2. Histogram to show distribution of (A) eyeball wet mass values, (B) horizontal corneal diameter, and (C) central corneal thickness values for quality-selected ovine eyes obtained over a 12 year period.

Figure 3. Scatterplots to (A) illustrate dependence of horizontal corneal diameter on eye globe wet mass (with the line being that from an unweighted linear regression, $r^2 = 0.829$) and (B) the relationship between central corneal thickness and wet mass of trimmed eye globes (with the line being that from an unweighted linear regression, $r = 0.423$).

Figure 4. Plots to illustrate central corneal thickness values in relation to the measured horizontal corneal diameter (HCD) for quality-selected ovine eyes obtained over a 12 year period (with the line being that from an unweighted linear regression, $r = 0.402$) or (B) with the corneal thickness data is presented as mean \pm 95 % confidence interval (based on 1.96 SD) for each 0.5 mm interval of HCD.

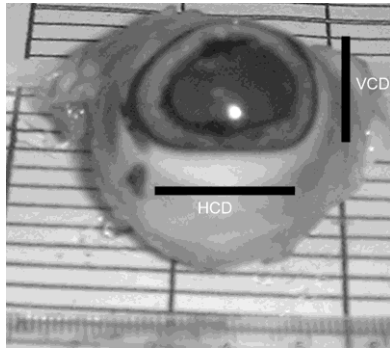


FIGURE 1

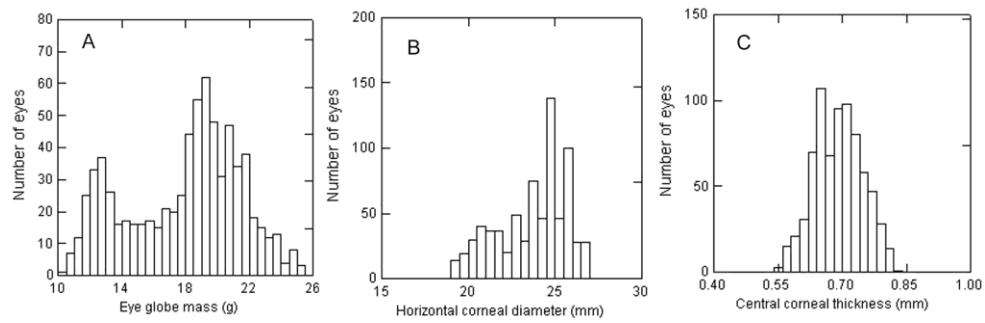


FIGURE 2

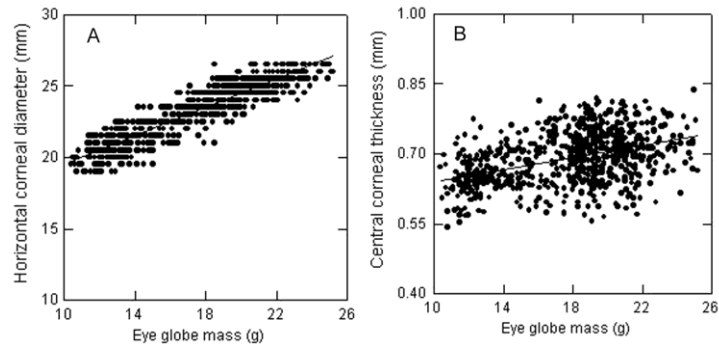


FIGURE 3

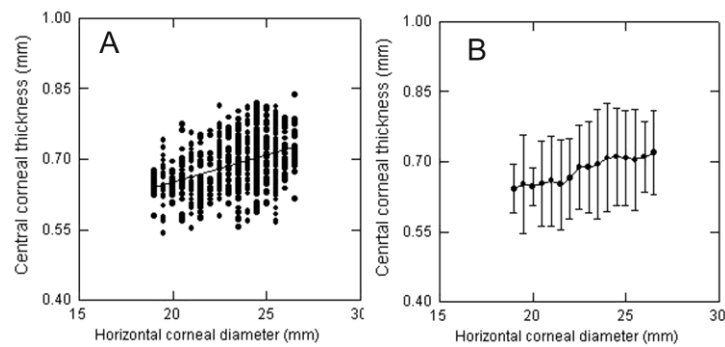


FIGURE 4