INTRODUCTION

The corneal endothelium is a single layer interlocking multi-sided cells making up the most posterior layer of cornea. Endothelial layer is of fundamental importance in maintaining the transparency of the cornea and changes in endothelial cells can occur due to aging, eye diseases, drugs or intraocular surgery. Specular microscopy is among the most reliable techniques for analysing the corneal endothelium (ABIB & BARRETO, 2001; ANDREW, S. et al. 2001; PIGATTO et al., 2006; PIGATTO et al., 2008; FRANZEN et al., 2010; BERCHT et al., 2015; COYO et al., 2016; TERZARIOL et al., 2016). Furthermore, specular microscopy is beginning to be used as part of the ophthalmic examination before and after cataract removal procedures in dogs (NAGATSUYU et al., 2014).

Due to the existence of interspecies variations in endothelial parameters, knowledge of normal data on the endothelium in each species is necessary. Previous studies have shown that rabbit corneal endothelium repairs by cell division and...
migration (MORITA, 1995). Rabbits have been widely employed in the evaluation of the effects of surgical procedures and drugs on the corneal endothelium (ATILLA et al., 2003; MENCUCCI et al., 2005; ARI et al., 2015). However, there are few published references regarding endothelial density in rabbits. SAILSTAD & PEIFFER, (1981) evaluated corneas from healthy rabbits using specular microscopy and reported that average number of cells for rabbits was 2998 cells per mm². MORITA (1995) reported that endothelial density decreases in rabbits over 12 months old. Moreover, there are no data regarding the effect of aging on corneal endothelial hexagonality in rabbits. The knowledge of the normal endothelial parameters is important not only for clinical evaluation but also for assisting in the planning of future studies related to corneal transplantation in rabbits. This study was carried out with the objective of evaluating the cell density and cell morphology of the corneal endothelium of rabbits of different ages using contact specular microscopy. In addition, it aimed to evaluate if there is a difference in the endothelial parameters in relation to the age of the animals.

MATERIALS AND METHODS

Thirty-six healthy eyes from 18 New Zealand white rabbits, males and females, obtained from the São Nunca rabbitry (Araricá, RS, Brazil) were used in this study. Rabbits died of death from natural causes for reasons unrelated to this study and died of disease that did not directly affect the eye. All stages of the study were performed according with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in studies related to ophthalmology. Enucleation was performed within 4 hours of death. Ophthalmic examination was performed immediately after enucleation. The examination consisted of biomicroscopy with slit lamp (Portable Slit Lamp SL 15, Kowa, Japan) and fluorescein test (Fluorescein strips, Ophthalmos, SP, Brazil). Each group consisted of 12 eyes. Group I comprised rabbits aged 6 months, Group II comprised rabbits aged 12 months, and Group G III comprised rabbits aged 4 years. Eyes were studied immediately after enucleation and kept in a wet chamber. The eyes were taken to the specular microscope and examined using a contact specular microscope (Celmax, Medical Service, Brazil) with software available for endothelium analysis (Specular Corneal Microscopy, Celmax). All eyes were submitted to specular microscopy and those who had some alteration were not included in the study. From each sample, three clear images were captured. In each image at least 60 endothelial cells were analysed. All evaluations were performed by the same investigator. Parameters studied included endothelial cell density and hexagonality. The data obtained were expressed as mean ± SD. and by using one-way analysis of variance to compare the data among age groups. Differences were considered statistically significant if the P value was less than 0.05.

RESULTS

No changes were noted in the slit lamp examination. With the specular microscope it was possible to analyse, capture images, and quantify the cell density and hexagonality of the corneal endothelium. In all the analysed images a regular pattern of size and shape of the endothelial cells was observed throughout the entire cornea (Figure. 1). The mean cell density for GI was 2307 cells per mm², for GII was 1895 cells per mm², and for GIII was 1818 cells per mm² (Table. 1). For GI, the mean cell density was 2336±367.99 cells per mm² for the right eye and 2278.33±294.15 cells per mm² for the left eye. For

Figure 1 - Specular micrographs of the normal corneal endothelium of a rabbit. A. Normal corneal endothelium of a rabbit from G III. B. Normal corneal endothelium of a rabbit from G I.
Corneal endothelial cell density and morphology in rabbits’ eyes using contact specular microscopy.

Table 1 - Mean endothelial cell density (cell mm$^{-2}$) and pleomorphism in rabbits evaluated through specular microscopy.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
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</thead>
<tbody>
<tr>
<td>Mean endothelial density</td>
<td>2307</td>
<td>1895</td>
<td>1818</td>
</tr>
<tr>
<td>Pleomorphism</td>
<td>74.33</td>
<td>71.83</td>
<td>64.02</td>
</tr>
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GI, the mean cell density was 1875.17±164.26 cells per mm$^2$ for the right eye and 1914.33±190.50 cells per mm$^2$ for the left eye. For GII, the mean cell density was 1865.83±283.29 cells per mm$^2$ for the right eye and 1771.50±215.84 cells per mm$^2$ for the left eye. Cell density decreased significantly with age (P<0.001) among the three groups.

Pleomorphism in the rabbits of GI was 74.33±10.08. In the rabbits of GII it was 71.83±11.38. In the rabbits of GIII it was 64.02±28.80. Significant differences (P<0.001) were evident between GI and GIII and GII and GIII but not between Groups I and II. The data obtained did not differ significantly between the right and the left eye from the same rabbit.

DISCUSSION

Specular microscopy, optical microscopy, and scanning electron microscopy (SEM) are among the most widely used methods for corneal endothelium analysis (MORITA et al., 1994; PIGATTO et al., 2009; ALBUQUERQUE et al., 2015; COYO et al., 2016; FAGANELLO et al., 2016). There is consensus that the preparation of corneas for studies using SEM causes cell retraction by reducing the original cell area and increasing the endothelial cell density (VIRTANEN et al., 1984; FAGANELLO et al., 2016).

Nevertheless, SEM has been used to study the ultrastructure of endothelial cells of different species of animals (PIGATTO et al., 2004; PIGATTO et al., 2005a; PIGATTO et al., 2005b; PIGATTO et al., 2009; TAMAYO-ARANGO et al., 2009). Due to the fact that there is no cellular retraction and it can be used in animals and living humans, specular microscopy is considered the gold standard technique for assessing the corneal endothelium in people and animals (MORITA, 1995; ABIB & BARRETO, 2001; PIGATTO et al., 2006; PIGATTO et al., 2008; FRANZEN et al., 2010; BERCHT et al., 2015; ALBUQUERQUE et al., 2015; COYO et al., 2016). Specular microscopy has also been employed in the evaluation of dogs undergoing cataract removal surgery (NAGATSUYU et al., 2014). Specular microscopy is a widely used tool in many researches, and been demonstrated to be extremely reliable, and reproducible. In healthy rabbits observations of the corneal endothelium employing a specular microscope have already been performed (SAILSTAD & PEIFFER, 1981; MORITA, 1995).

In previous studies, rabbits underwent general anaesthesia prior to specular microscopy examination (SAILSTAD & PEIFFER, 1981; MORITA, 1995). However, in the present study we made the choice to use the eyes of animals. This methodology using the eyes of animals that have been sacrificed has been used successfully in previous studies (PIGATTO et al., 2006; PIGATTO et al., 2008; FRANZEN et al., 2010; ALBUQUERQUE et al., 2015; COYO et al., 2016). It is well established that the endothelial structure is preserved for up to 6 hours after death (FRANZEN et al., 2010; ALBUQUERQUE et al., 2015). In the current study, the maintenance of the eyes in a humid chamber allowed the examinations without interfering with the transparency of the cornea. The use of eyes from abattoirs or breeders avoids animals being anaesthetized only to perform specular microscopy. Moreover, results obtained with this methodology can be extrapolated and compared to values obtained from living animals.

Rabbits have been widely used as an experimental model for ophthalmic research (ATILLA et al., 2003; MENCUCCI et al., 2005; ARI et al., 2015). The goal of the present study was to evaluate the cell density and percentage of hexagonal cells because these are the most reliable parameters for evaluating the integrity of the corneal endothelium (ABIB & BARRETO, 2001; PIGATTO et al., 2006; NAGATSUYU et al., 2014). SAILSTAD and PEIFFER (1981) observed the corneal endothelium of 14 young adult rabbits using a specular microscope. The authors reported an average density of 2998 cells per square millimeter. MORITA (1995), studying corneal endothelial cell of rabbit eyes by specular microscopy reported an endothelial cell density between 2180 and 3460 cells per mm$^2$. In the present study, the endothelial cell density varied between 2336 and 1771.50 cells per mm$^2$. In other studies, scanning electron microscopy was used to quantify the endothelial density in rabbits (DOUHTY, 1998; PIGATTO et al., 2005a).

However, due to the cellular retraction caused by the preparation for scanning electron microscopy and the increase in the number of cells per square millimeter, they cannot be compared with the values obtained with a specular microscope. It is very well established
in many species that a decrease in endothelial density occurs with aging (MORITA et al., 1994; FRANZEN et al., 2010; ALBUQUERQUE et al., 2015; BERCHT et al., 2015; COYO et al., 2016). Results of this study regarding the mean cell density in different age groups were similar to the results obtained for other species of animals with a mean decrease in endothelial cell density with increasing age.

Regarding the endothelial morphology in rabbits, there are no studies evaluating the effect of aging on corneal endothelial hexagonality in rabbits. MORITA (1995) examined rabbits aged between 6 months and 15 months with a specular microscope and found cells with five and six sides. PIGATTO et al. (2005a) evaluated the morphology of 3-month-old rabbits using SEM with a focus on polygonality of the endothelium. Most cells were hexagonal (75%) in shape, with pentagonal (14%) and heptagonal (11%) cells constituting the greater portion of the remaining corneal endothelium. Some studies evaluating animals of a unique age group have already been conducted and concluded that most cells have six sides (PIGATTO et al., 2004; PIGATTO et al., 2005; PIGATTO et al., 2006; PIGATTO et al., 2008; IGATTO et al., 2009; TAMAYO-ARANGO et al., 2009; FRANZEN et al., 2010; ALBUQUERQUE et al., 2015; COYO et al., 2016). In humans with a healthy cornea, more than 60% of the corneal endothelium cells are six-sided (McCAREY et al., 2008). A decrease in the number of endothelial cells with six sides commonly occurs with aging (ALBUQUERQUE et al., 2015; COYO et al., 2016). In the present study, it was observed that with the aging of the rabbits there was a decrease in the number of hexagonal-shaped cells. With regard to the parameters evaluated, no statistical differences were observed between both eyes of the same rabbit. Previous studies reported the absence of differences regarding the endothelial parameters obtained between healthy eyes (MORITA et al., 1994; PIGATTO et al., 2006; PIGATTO et al., 2008; FRANZEN et al., 2010; ALBUQUERQUE et al., 2015; BERCHT et al., 2015). These results obtained in the present study were consistent with those reported in rabbits were the density of corneal endothelial cells is similar in the left and the right eye and in females and males.

CONCLUSION

Corneal endothelium of rabbits suffers changes due to advancing age both in terms of cell density and morphology. The data obtained in this study may serve as a reference for further studies.

ACKNOWLEDGEMENTS

We are grateful to the ‘São Nunca’ rabbitry (Araricá, RS, Brazil) for providing the physical space and for giving us the eyes used in this research. We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing a scholarship to Gustavo Brambatti.

REFERENCES


