

# Endothelial density analysis by age group of corneal donors in an eye bank in Paraná State, Brazil

## *Análise da densidade endotelial por faixa etária dos doadores de córnea em um banco de olhos no Estado do Paraná, Brasil*

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### ABSTRACT

**Objective:** To analyze the endothelial density of corneas donated for transplant in different age groups. **Methods:** The study evaluated 244 corneas from 122 male and female donors in Cascavel Eye Bank - PR from the analysis of medical records containing specular microscopy of the donors. **Results:** Most corneal donors were male (67.2%). The average donors age was 53.8 years, ranging from 5 to 70 years. The age group of 61 - 70 years with the largest number of donations, corresponding to almost 41% and the average endothelial density was 2645 cells / mm<sup>2</sup> (ranging from 1897 to 4201 cells / mm<sup>2</sup>). In the first decade of life, mean endothelial density was 2923 cells / mm<sup>2</sup>; in the second decade 2757 cells / mm<sup>2</sup>; in the third 2846 cells / mm<sup>2</sup>; in the fourth 2627 cells / mm<sup>2</sup>; in the fifth decade, 2830 cells / mm<sup>2</sup>; on the sixth 2605 cells / mm<sup>2</sup> and on the seventh decade of life 2570 cells / mm<sup>2</sup>. **Conclusion:** This study demonstrated that the mean endothelial density of corneas donated for transplant of the first decade of life (2923 cells / mm<sup>2</sup>) when compared to the seventh decade alone (2570 cells / mm<sup>2</sup>) was reduced. In the evaluation from the second to the sixth decade, there was no pattern, sometimes there was an increase, sometimes a decrease in the average endothelial density.

**Keywords:** Endothelial density; Cornea; Tissue donors; Age groups

### RESUMO

**Objetivo:** Analisar a densidade endotelial das córneas doadas para transplante nas diferentes faixas etárias. **Métodos:** O estudo avaliou 244 córneas de 122 doadores de ambos os sexos no Banco de Olhos de Cascavel- PR a partir da análise de prontuários médicos contendo a microscopia especular dos doadores. **Resultados:** A maioria dos doadores de córnea era do sexo masculino (67,2%). A idade média dos doadores foi 53,8 anos com variações entre 5 e 70 anos. A faixa etária dos 61-70 anos com o maior número de doações, correspondendo a quase 41% e a densidade média endotelial foi de 2645 células/mm<sup>2</sup> (variando de 1897 a 4201 células/mm<sup>2</sup>). Na primeira década de vida, a densidade endotelial média foi 2923 células/mm<sup>2</sup>; na segunda década 2757 células/mm<sup>2</sup>; na terceira 2846 células/mm<sup>2</sup>; na quarta 2627 células/mm<sup>2</sup>; na quinta década, 2830 células/mm<sup>2</sup>; na sexta 2605 células/mm<sup>2</sup> e na sétima década de vida 2570 células/mm<sup>2</sup>. **Conclusão:** Este estudo demonstrou que a densidade endotelial média das córneas doadas para transplante na primeira década de vida (2923 células/mm<sup>2</sup>) quando comparada isoladamente à sétima década (2570 células/mm<sup>2</sup>) teve redução. Já na avaliação da segunda até a sexta década não houve um padrão, ora havendo acréscimo, ora decréscimo da densidade endotelial média.

**Descritores:** Densidade endotelial; Córnea; Doadores de tecidos; Grupos etários

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## INTRODUCTION

The corneal endothelium is assessed and investigated by the Ophthalmology field, mainly through specular microscopy. <sup>(1)</sup> This tissue is formed by the simple squamous epithelium and covers the inner surface of the cornea. <sup>(2)</sup> The endothelium has a single layer of interdigitated cells, which are arranged in mosaic pattern and present hexagonal and regular shapes. <sup>(3)</sup> Endothelial cells fix through occlusion zones and have several mitochondria. They use ATP-powered pumps to withdraw water towards the aqueous humor in order to maintain constant hydration in the corneal stroma <sup>(2)</sup>. Corneal transparency is maintained by the ion pump in the endothelium, which accounts for stromal deturgescence and dehydration. <sup>(4)</sup> The hypothetical lack of this transport mechanism could lead to stromal edema and result in low vision acuity. <sup>(5)</sup>

The endothelium is formed by a monolayer comprising 400,000 cells, which rest on the posterior limiting lamina of the cornea. <sup>(6)</sup> Individuals' endothelial density ranges from 3,500 mm<sup>2</sup> to 4,000 mm<sup>2</sup> cells at birth; it decreases after adolescence and presents standard density ranging from 1,400 mm<sup>2</sup> to 2,500 cells mm<sup>2</sup> in adulthood. <sup>(7)</sup>

Thus, the aim of the current study was to assess the endothelial density of corneas removed from corpses belonging to different age groups - from the first to the seventh decade of life - that were donated for transplantation.

## METHODS

Observational, retrospective and descriptive study carried out in the Eye Bank of Cascavel County, Paraná State, based on the analysis of medical records reporting the specular microscopy of donated corneas.

The Eye Bank of Cascavel County accepts corneas from donors in the age group 4-70 years. The current study adopted the manual cell labeling technique and used Kodo equipment to analyze the corneal tissue.

Corneas collected for donation at the Eye Bank of Cascavel County from April to July 2018 were included in the study; no exclusion criteria were applied to the investigated sample.

The current research involved human beings; thus, it followed Resolution 466/12 set by the National Health Council. The study was approved by the Research Ethics Committee of the University Center of Assis Gurgacz Foundation, under Opinion n. 15792319.0.0000.5219. Researchers requested exemption from the Informed Consent Form (ICF).

## RESULT

Optisol was the preservation medium used in all corneas and the specular microscopy evaluation was carried out after

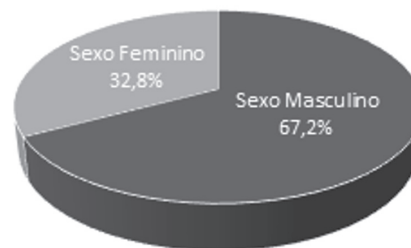


Figure 1. Corneal donors per sex

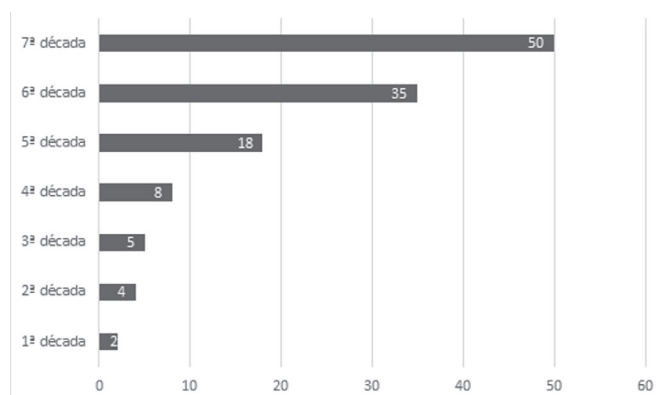


Figure 2. Number of donors per age group

twelve hours of tissue preservation; the mean time between corneal enucleation and preservation was 5.15 hours (standard deviation = 3.48).

The total number of donors recorded from April to July 2018 was 122; thus, totaling 244 donated corneas. Eighty-two (82) donors were men (67.2% of total donations) and forty (40) were women (32.8% of total donations), as shown in Figure 1.

Donors' age ranged from 5 to 70 years; their mean age was 53.8 years. Mean endothelial density was 2,645 cells/mm<sup>2</sup>, its lower limit was 1,897 cells/mm<sup>2</sup> and the upper limit was 4,201 cells/mm<sup>2</sup>.

Table 1 shows endothelial density values based on donors' decade of life - information comprise mean endothelial density, its upper and lower limits, as well as the standard deviation of each decade of life. Mean endothelial density recorded for donors in the first age group (up to 10 years old) was 2,923 cells/mm<sup>2</sup>, its upper limit was 3,267 cells/mm<sup>2</sup> and the lower limit was 2,551 cells/mm<sup>2</sup>; Mean endothelial density recorded for the second age group (from 11 to 20 years old) was 2,757 cells/mm<sup>2</sup>, its upper limit was 3,184 cells/mm<sup>2</sup> and the lower limit was cells/mm<sup>2</sup>; the third age group (from 21 to 30 years old) recorded mean endothelial density equal to 2,846 cells/mm<sup>2</sup>, its upper limit was 3,636 cells/mm<sup>2</sup> and the lower limit was 1,901 cells/mm<sup>2</sup>. The fourth age group (from 31 to 40 years old) recorded mean endothelial density equal to 2,627 cells/mm<sup>2</sup>, its upper limit was 3,202 cells/mm<sup>2</sup> and the lower

Table 1  
Endothelial density - in mm<sup>2</sup> - per decade of life

	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>
Mean	2,923 (±481.53)	2,757 (±652.65)	2,846 (±219.91)	2,627 (±122.32)	2,830 (±909.33)	2,605 (±465.27)	2,570 (±176.06)
Upper limit	3,267	3,184	3,636	3,202	4,201	3,333	4,102
Lower limit	2,551	2,192	1,901	2,004	2,257	1,897	1,897

limit was 2,004 cells/mm<sup>2</sup>. Mean endothelial density recorded for the fifth age group (from 41 to 50 years old) was 2,830 cells/mm<sup>2</sup>, its upper limit was 4,201 cells/mm<sup>2</sup> and the lower limit was 2,257 cells/mm<sup>2</sup>; the sixth age group (from 51 to 60 years old) recorded mean endothelial density equal to 2,605 cells/mm<sup>2</sup>, its upper limit was 3,333 cells/mm<sup>2</sup> and the lower limit was 1,897 cells/mm<sup>2</sup>, and the seventh age group (from 61 to 70 years old) recorded mean endothelial density equal to 2,570 cells/mm<sup>2</sup>, its upper limit was 4,102 cells/mm<sup>2</sup> and the lower limit was 1,897 cells/mm<sup>2</sup>.

## DISCUSSION

Most donors (67.2%) in the current study were men; this finding was in compliance with the literature;<sup>(8-10)</sup> moreover, this percentage can change depending on the study.

The mean age of donors at Hospital de Olhos de Cascavel (Cascavel Eye Hospital) was 53.8 years; it was close to the one observed in a study carried out at Santa Casa Hospital of São Paulo, wherein donors' mean age was 55.57 years<sup>(8)</sup>, as well as to the mean age observed in a University Eye Bank (52.85 years).<sup>(9)</sup>

The upper age limit in the current study was 70 years - younger than the that observed in other studies whose upper age limits were 97<sup>(8)</sup> and 80 years.<sup>(10)</sup> The lower age limit observed in the survey was 5 years, although the literature also reports 9-month-old donors.<sup>(8)</sup> It is worth emphasizing that the standard for corneal donations in Cascavel Eye Bank comprises donors in the age group 4-to-70 years.

The number of donors has gradually increased as their age advanced. The seventh age group (from 61 to 70 years old) accounted for most donations (40.98%), whereas the first age group (up to 10 years old) accounted for only 1.63% of donations - this age group recorded the smallest number of donations.

Endothelial density, which refers to the number of endothelial cells per millimeter, was also analyzed in the current study; this parameter plays an essential role in the clinical evaluation of corneas because it determines corneal function and ability to respond to stress.<sup>(5)</sup> The mean donor endothelial density observed in Cascavel Eye Bank was 2,645 cells/mm<sup>2</sup>. This number was close to the one recorded for living Egyptian<sup>(11)</sup> and Indian<sup>(12)</sup> patients, who presented endothelial density equal to 2,647 cells/mm<sup>2</sup> and 2,525 cells/mm<sup>2</sup>, respectively. Other studies have also recorded similar mean donor endothelial density for a healthy Caucasian population (2,732 cells/mm<sup>2</sup>)<sup>(13)</sup> and for Filipino patients (2,798 cells/mm<sup>2</sup>).<sup>(14)</sup>

According to some authors, the endothelium does not have the ability to regenerate<sup>(2)</sup>. Thus, as the cornea cannot undergo mitosis, the number of cells in it decreases due to several factors, such as aging.<sup>(5)</sup> However, new research<sup>(15)</sup> has observed increased endothelial density in living patients 24 weeks after the injection of corneal endothelial cells supplemented with ROCK inhibitor (rho-associated protein kinase); this outcome may indicate the regeneration ability of the endothelium.

According to the literature, endothelial density gradually decreases from individuals' birth to death, and it leads to the emergence of areas lacking cells in the central corneal region.<sup>(16,17)</sup> The first age group (up to 10 years old) analyzed in the current study recorded the highest endothelial density (2,923 cells/mm<sup>2</sup>); however, this number should be interpreted with caution, since this age group only comprised two donors. The second age group (from 11 to 20 years old) recorded endothelial density decrease by 166 cells/mm<sup>2</sup> in comparison to the first age group; however,

the number of donors in this age group was larger (four donors). The third age group (from 21 to 30 years old) recorded smaller endothelial density increase (89 cells/mm<sup>2</sup>) than the previous age group. In addition, it presented five donors, one more than the second age group. The fourth age group (from 31 to 40 years old) comprised eight donors and recorded endothelial density equal to 2,627 cells/mm<sup>2</sup>; the number of endothelial cells in this age group decreased by more than 200 cells/mm<sup>2</sup> in comparison to the previous age group.

Endothelial density has increased from the fourth (from 31 to 40 years old) to the fifth (from 41 to 50 years old) age group. This outcome may be associated with donors' cause of death, which was not evaluated in the current study, as well as with the difference in the number of donors between these age groups; the fourth age group recorded eight donors, whereas the fifth age group recorded eighteen donors - this number was more reliable than the one recorded for the fourth age group.

In addition, there was significant decrease in endothelial cell density from the fifth age group (from 41 to 50 years old) onwards. This age group recorded endothelial cell density equal to 2,830 cells/mm<sup>2</sup>, the sixth age group (from 51 to 60 years old) recorded 2,605 cells/mm<sup>2</sup> and the seventh age group (from 61 to 70 years old), 2,570 cells/mm<sup>2</sup>. This outcome can be explained by the fact that, besides age, trauma, inflammation and other diseases can decrease the number of endothelial cells<sup>(18)</sup>, as well as that some pathologies are more prevalent from this age group onwards.

A Brazilian study<sup>(19)</sup> has conducted specular microscopy in vivo in 784 corneas in order to analyze the endothelial density of patients throughout life. Results have shown decreased endothelial density over the years. Numbers observed from the first to the seventh 10-year-based age group were 3,156 cells/mm<sup>2</sup>, 3,027 cells/mm<sup>2</sup>, 2,834 cells/mm<sup>2</sup>, 2,737 cells/mm<sup>2</sup>, 2,576 cells/mm<sup>2</sup>, 2,344 cells/mm<sup>2</sup> and 2,280 cells/mm<sup>2</sup>, respectively. Based on the comparison of the Brazilian study<sup>(19)</sup> to the current one - which evaluated the endothelial density in dead donors - the mean endothelial density in living patients was higher than that of dead donors in the first (up to 10 years old), second (from 11 to 20 years old) and fourth (from 31 to 40 years old) age groups. Endothelial density values recorded for donors in the third (from 21 to 30 years old), fifth (from 41 to 50 years old), sixth (from 51 to 60 years old) and seventh (from 61 to 70 years old) age groups stood out.

The literature lacks studies focused on investigating the endothelial density of corneal donors. The studies available were conducted with living patients, a fact that hinders the discussion about and comparison of data to other scientific studies.

It is necessary giving greater relevance to research about endothelial density, since it is a valuable parameter used to assess the long-term survival of corneal grafts.<sup>(20)</sup>

## CONCLUSION

There was significant decrease in endothelial density from the first (up to 10 years old) to the seventh (from 61 to 70 years old) age group, when they were compared in separate. However, endothelial density values recorded from the second (from 11 to 20 years old) to the sixth (from 51 to 60 years old) age group were not linear; sometimes they decreased in comparison to the previous age group, sometimes they increased in comparison to it. It is important emphasizing that the lack of difference in endothelial density between the second (from 11 to 20 years old) and the sixth (from 51 to 60 years old) age group may be

explained by sample size, since some age groups presented very small number of donors.

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