

Preliminary study of rabbits as an animal model of mammalian eye transplantation and literature review

Estudo preliminar de coelhos como modelo animal para transplante de olho em mamíferos e revisão da literatura

Emmerson Badaro¹ , Paula Cassini¹ , Gabriel Costa de Andrade¹ , Gustavo Büchele Rodrigues¹ , Eduardo Amorim Novais¹ , Eduardo Büchele Rodrigues¹ 

¹ Universidade Federal de São Paulo, São Paulo, SP, Brazil.

How to cite:

Badaro E, Cassini P, Andrade GC, Rodrigues GB, Novais EA, Rodrigues EB. Preliminary study of rabbits as an animal model of mammalian eye transplantation and literature review. Rev Bras Oftalmol. 2022;81:e0026.

doi:

<https://doi.org/10.37039/1982.8551.20220026>

Keywords:

Eye/transplantation;
Electroretinography; Optic
nerve; Retina; Blindness; Rabbit;
Models, animal; Anastomosis,
surgical

Descritores:

Olho/transplante;
Eletrorretinografia; Nervo
óptico; Retina; Cegueira;
Coelho; Modelos animais;
Anastomose cirúrgica

Received on:
Nov 12, 2021

Accepted on:
Jan 14, 2022

Corresponding author:

Emmerson Badaró
Rua Botucatu, 821 – Vila Clementino
Zip code: 04023-062 – São Paulo, SP, Brasil
E-mail: ebadaro@me.com

Institution:

Universidade Federal de São Paulo, São
Paulo, SP, Brazil.

Conflict of interest:
no conflict of interest.

Financial support:

this work was supported, in part, by São
Paulo Research Foundation (Fapesp), #
2011/17363-3.



Copyright ©2022

ABSTRACT

Purpose: To describe an innovative animal model of eye transplantation used in rabbits.

Methods: six Dutch-belted male rabbits were submitted to lateral orbitotomy in the right eye, wide retrobulbar anatomy exposure, dissection of the structures, identification and distal section of the optic nerve followed by anastomosis either by vicryl (group 1) or fibrin glue (group 2). Electroretinography recording was performed before the section of the optic nerve and every 30 seconds after, to monitor the function of retina. Left eye was used as control group.

Results: After optic nerve resection and anastomosis, stable ERG amplitude of the right eye was lost after 302 seconds in group 1 and after 296 seconds on group 2. Left eye kept longer stable ERG amplitude curves.

Conclusions: The animal model of whole eye transplantation was effective in describing a novel technique to be used in rabbits, with success of the anatomic procedure. Further studies will clarify the best anastomosis methods and maintenance of function of the receptor organ.

Translational relevance: this animal model of whole eye transplantation provides a novel perspective for blind patients and the research models, since we describe a novel mammal animal model. This model can be used as basis of a human model of whole eye transplantation in future studies.

RESUMO

Objetivo: Descrever uma técnica cirúrgica inovadora para transplante de olho em um modelo animal em coelhos.

Métodos: Seis coelhos machos com Dutch Belted foram submetidos à orbitotomia lateral do olho direito, com ampla exposição da anatomia retrobulbar, dissecação do cone muscular, exposição e secção distal do nervo óptico seguida de anastomose por vicryl (Grupo 1) ou cola de fibrina (Grupo 2). O registro da eletrorretinografia foi realizado antes da secção do nervo óptico e a cada 30 segundos após, para monitorar a função da retina. O olho esquerdo foi usado como grupo controle.

Resultados: Após a ressecção do nervo óptico, a estabilidade da amplitude da eletrorretinografia foi perdida no olho direito após 302 segundos no Grupo 1 e após 296 segundos no Grupo 2. O olho esquerdo manteve eletrorretinografia estável por períodos mais longos.

Conclusão: O modelo animal de transplante total de olho foi eficaz em descrever uma nova técnica cirúrgica para ser utilizada em laboratório com coelhos, com sucesso do procedimento anatômico. Novos estudos esclarecerão os melhores métodos de anastomose e manutenção da função do órgão receptor.

INTRODUCTION

Approximately 36 million people worldwide suffer from blindness, with up to 20% or 7.4 million, with a vision of light perception or no perception of light.^(1,2) The most common causes for blindness and vision impairment worldwide were cataract and under correction of refractive error, which are reversible with treatment, whereas glaucoma, macular degeneration, and diabetic retinopathy are the most prevalent causes of irreversible vision loss and blindness.⁽³⁾ The main factor of irreversible blindness is related to severe retinal ganglion cell (RGC) axon damage, since after these cells are damaged, they do not regain their function.⁽⁴⁾ A potential alternative to these patients is whole eye transplantation, which may provide vision to a blind patient with viable RGCs as long as the optic system is preserved.

The first attempt of whole eye transplantation was in 1885, when a rabbit eye was transplanted to a human orbit.⁽⁴⁾ Over the next decades, several articles were published on the field, however little progress was obtained and most studies were performed in cold blood animals.^(4,5) Due to its highly specialized function with complex and unique anatomy, whole eye transplantation with restoration of visual function has always been a challenge for researchers. After years of continued experiments and literature search on the field, in 1977, a group of specialists considered total eye transplantation was “doomed to failure by the ganglion cell axon’s inability to withstand cutting, by the difficulty of insuring adequate circulation of blood to the transplanted eye during or shortly after operation, and lastly by immune rejection of foreign tissue”.⁽⁵⁾

In 2009, Ellenberg et al. defined three main impediments for human whole eye transplantation: maintenance of donor eye viability, optic nerve regeneration with restoration of topographic organization, and avoidance of immunological rejection.⁽⁴⁾ These three factors have conducted most research groups aimed at achieving the success of whole eye transplantation.

The lack of literature on reproducible animal models can be used as start point to a research team.⁽⁴⁾ Also, most models are in cold vertebrates and some in small mammals. An ideal animal model should allow access to the optic nerve with enough safety to all intra orbital structures, especially vascular and neural. It is still not clear in the literature which is the best method for optic nerve neurotaphy, whether it is using biological fibrin glue or suture neurotaphy.

For many years, rabbits have been widely used in ophthalmic animal research, and knowledge of

anatomy, physiology and research surgical techniques of the eye and orbit anatomy have allowed the development of the research and the application in other areas. Wide access to middle-sized research laboratories, the possibility of reproducibility and unique anatomical characteristics are some of the factors that make rabbits an excellent subject to be used as a model in ophthalmic research.

Rabbit orbits are situated in the side of the cranial skull, and their openings are almost at right angle to the transverse plane of the head. The orbital walls are well developed in both height and length, but the depth is shallow. Orbit and globe are large when compared to animal size.

The orbit is roughly circular, it has about 25mm diameter, with the skull being about 108mm long and 50mm wide. The rabbit’s globe measures 16mm to 19mm anteroposteriorly, 17mm vertically, and 18mm to 20mm horizontally. A large Harderian gland (19mm long, 12mm to 15mm wide, and 4mm to 6mm thick at its largest point) occupies the lower anterior part of the orbit. It is medial to the lacrimal gland and almost completely surrounded by a large venous sinus. A very small intraorbital gland is beneath the zygomatic arch.⁽⁶⁾

The optic nerve approaches the globe from below, ascends over the posterior surface in virtual contact with the sclera, penetrating the latter at a very acute angle. These characteristics, together with the animal’s middle size, allow easy and fast access to the eye and orbit structures. Similarities to human anatomy and physiology, as well as extensive knowledge of rabbit research make this animal very important for ophthalmology research.

Therefore, our purpose was to describe an innovative animal model of eye transplantation used in rabbits.

METHODS

Animals

A total of six male Dutch Belted rabbits, weighing from 1.8kg to 2.5kg were used in the experiments. All experimental procedures were performed in accordance with the Association for Research in Vision Ophthalmology® (ARVO) statement for use of laboratory animals, with Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985), with the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (revised 1986) and the US Animal Welfare Act, as well as university and

national guidelines for research on animals. Animals were anaesthetized by intramuscular injection of a solution containing 1mL of ketamine (50mg/mL) and 0.4mL of xylazina (10mg/mL). Topical tetracaine (Bausch & Lomb, Inc., Tampa, Florida, United States) was used for additional anesthesia. Pupils were dilated with 2.5% phenylephrine hydrochloride (Akorn, Inc., Buffalo Grove, Illinois, United States) and 1% tropicamide (Akorn, Inc.) eye drops.

Before the surgical procedure

After the placement of a lid speculum, all animals were submitted to ophthalmological examination in both eyes, including anterior biomicroscopy with portable slit lamp and fundoscopy (Keeler Instruments, Broomall, Pennsylvania, United States), to exclude any previous pathology and anatomical diseases.

Animals were divided into two groups regarding the optic nerve anastomosis method: Group 1 (three animals) had the anastomosis performed by termino-terminal vicryl suture; and Group 2 (three animals) had the termino-terminal anastomosis performed by fibrin glue.

Surgical procedure

The right eye was used as the eye under investigation, while the left eye was used as control.

Topical 5% povidone-iodine was used followed by saline solution washout for site preparation. The rabbit's fur was cut close to the skin with a razor blade.

A lateral cantal skin incision with 3.0cm was performed and exposed the lateral orbital wall. Lateral orbitotomy with removal of the lateral orbital wall allowed access to the retrobulbar space, and dissection of the muscular cone exposed the optic nerve (Figure 1). The optic nerve was isolated taking extreme care not to injure it, not to touch by hand or by any forceps at this time. Care was also taken not to injure orbital and ocular structures, including veins, arteries and extraocular muscles, aiming at maintaining the best anatomy and vascularization.

Two guidance sutures using 7.0 curved needle vicryl were performed before sectioning the optic nerve. With the optic nerve clearly exposed and wide access allowed (Figure 2), fine scissors were used to section it most distal to the globe possible. The termino-terminal anastomosis of the optic nerve was immediately performed in loco using guidance sutures of vicryl (Group 1) or adhesive fibrin glue (Group 2). Four sutures were used in vicryl group as superficial as possible.

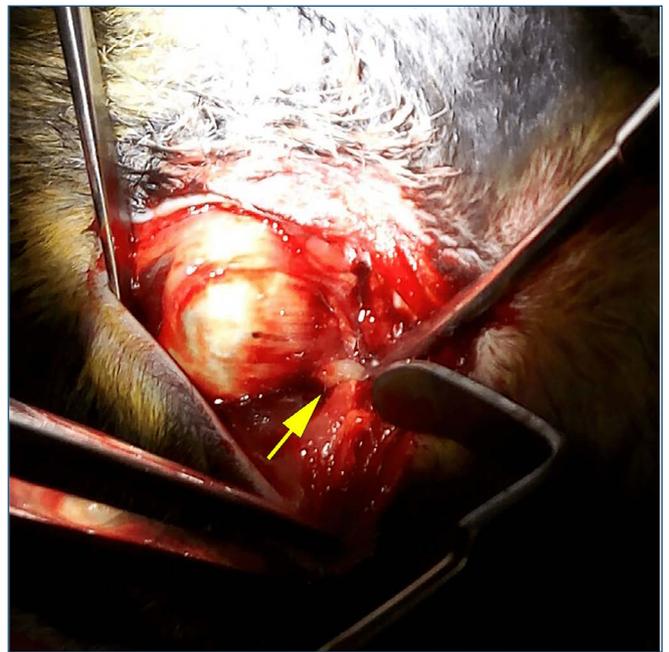


Figure 1. After lateral orbitotomy, wide exposure of the anatomy is obtained and optic nerve (yellow arrow) is clearly exposed.

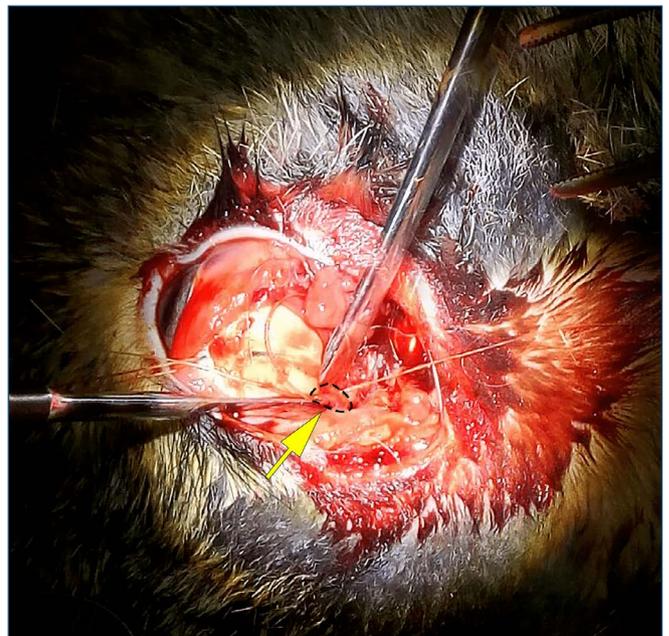


Figure 2. Wide exposed of the orbit anatomy. Optic nerve (yellow arrow) is seen isolated by two guidance sutures (black line) before sectioning most distal to the globe possible.

Retina function analysis by electroretinography and electroretinography acquisition protocol

The pupils were maximally dilated with 1% tropicamide eye drops. The cornea was anesthetized with topical tetracaine drops, and unipolar contact lenses with electroretinography (ERG) jet electrodes (Universe SA, La Chaux-de-Fons, Switzerland) were placed on cornea with 2% methylcellulose (Ophthalmos, São Paulo, Brazil). A

1.5mm gold reference electrode filled with electrolytic gel was placed at the temporal-superior canthus, while the ground electrode was also filled with gel and placed on the earlobe and fixed using a conductive paste after shaving the areas. Animals were then presented in an Ephios portable ERG system (Ephios AB, Rejmyre, Sweden).

The room where the experiment was conducted was kept dark during the ERG recording. The fixed Ganzfeld working distance to the eye was 2.5cm. Full field ERG with responses were obtained every 30 seconds until 5 minutes of null responses were found. Stable ERG was considered as measurement of eye viability. Electroretinography b-wave amplitudes were measured from the trough of the "a" wave to the peak of the "b" wave. Peak-to-peak amplitudes were used to measure the optic nerve responses. After 5 minutes of null responses, the damage on retina cells was considered irreversible, and the experiments were discontinued.

After the surgical procedures, animals were re-examined by anterior biomicroscopy and indirect ophthalmoscopy to exclude iatrogenic lesions and validate ERG measurements. In the end of the experiments, rabbits were euthanized with an intravenous injection of 120-mg/kg sodium pentobarbital.

Research ethics committee approval was obtained by CEUA 1342111214 – *Universidade Federal de São Paulo*.

RESULTS

At baseline and after the procedures, findings in anterior biomicroscopy and funduscopy of all eyes were negative for hemorrhage, retinal detachment, intraocular opacities and other signs of previous diseases.

Lateral orbitotomy allowed wide access to the retrobulbar space. Safe and easy identification of orbit structures was obtained with this technique. Dissection of the optic nerve and other orbit structures without injuring them was performed safely. The reproducibility of the surgical steps was considered satisfactory.

Electroretinography responses

Baseline curves were present in all animals. Electrodes placed on the cornea were used for ERG recordings, which served as an index for neuronal activity in the outer retina ("a" wave for photoreceptors and "b" wave for bipolar cells).

After optic nerve resection, stable ERG amplitude was lost after 302 seconds on Group 1 and after 296 seconds on Group 2. This was considered the end of the experiments and the animals were then euthanized.

DISCUSSION

The attempts of visual recovery after severe visual impairment are challenging and demand long-term research. Many techniques have been studied in different animal models, such as in cold blood vertebrates, but only few and small studies in mammals. The differences in anatomy including vasculature and neurological system of the eye of each species limit the knowledge on the subject. Besides great efforts in this subject, surgical solutions to complete blindness are lacking in the literature and clinical practice.

Ellenberg et al.⁽⁴⁾ reviewed 17 articles regarding total eye transplantation, with a total of seven articles describing 173 mammalian eye transplantations. Of the seven studies, only two have demonstrated partial recovery of visual function, both in rats and none in rabbits.⁽⁷⁻⁹⁾ Therefore, achieving success in eye transplantation in rabbits would be a milestone in ophthalmology, and this knowledge may contribute to future studies in humans.

Neurological regeneration and functional recovery are challenges on a sensorial system such as visual. Although central nervous systems are considered capable of intrinsic regeneration after axons are damaged,⁽¹⁰⁾ cranial nerves have very limited recovery. Over the last decades, biomaterials have been studied to induce regeneration of peripheral axons, and have a potential application on total eye transplantation. Growth factors such as neurotrophin, nerve growth factor and brain derived neurotrophic factor, used in combination with biomaterials, may be useful.⁽⁸⁾ The ideal technique to deliver these materials to the eye and to the optic nerve may be through intra vitreous injection in combination with biological suture. The incorporation of stem cells and stem-like cells that secrete growth – promoting cocktail of factors on the site of injury, are under study.^(10,11) The use of biomaterials may provide a positive environment to improve functional regeneration of vision.

Shi et al. demonstrated ocular viability with perfusion of retina and ERG responses after enucleation with anastomosis between carotid and ophthalmic arteries with at least partial maintenance of donor ocular viability in swine eyes.⁽¹²⁾

Lima et al.⁽¹³⁾ demonstrated function regeneration of optic nerve in small animal model following a crush injury to the optic nerve in mice. With adequate stimulation, using a co-injection of an inflammatory stimulator, cyclic adenosine monophosphate analogue and targeted gene deletion, RGCs are able to regenerate axons on the full length of the visual pathway. They were also able to

demonstrate partial restoration of the optomotor response, depth perception and circadian entrainment, and partial restoration of pupillary light reflex.

Different optic nerve suture techniques may be suggested after resection, such as the microsurgical suture neuroorrhaphy, currently the gold standard for peripheral nerve suture, and the use of biological and fibrin sealants.⁽¹⁴⁾ The rationale of avoiding neuroorrhaphy is the trauma promoted to the nerve end, the time taken and the limited anatomical space on the orbit. Repairing nerves without or at least with fewer sutures should be easier, faster, less traumatizing and, therefore, should promote better visual recovery. Although it was not the main purpose of the study, no difference was found regarding ERG amplitude curves and time of responses when comparing fibrin glue and vicryl suture. This may be due to the relative short period of retina viability, and our group believes that biological fibrin sealant has a better rationale and may present better functional outcomes.

Eye transplantation demands revascularization of the ocular and orbital structures, supplied through their respective branches of the ophthalmic artery. Without immediate reperfusion, retinal function of an enucleated eye demonstrated by ERG amplitudes is greatly decreased or absent within 5 minutes.⁽¹⁵⁾ Maintenance and restoration of retinal function, as measured by ERG, has been demonstrated ex vivo in isolated perfused eyes for up to 10 hours.⁽¹⁵⁾ In these experiments, eyes were enucleated, and perfusion was initiated immediately. The eyes were perfused under positive pressure with various buffered perfusates.

In order to achieve success in eye transplantation, vascular anastomosis plays an important role. This step wasn't the objective of this study and so it was not evaluated, but to achieve optic nerve viability, we believe vascular anastomosis should be considered as an important step of the surgery.

Using fluorescein angiography, Sher et al. demonstrated revascularization of the retina after microsurgical anastomosis of autotransplanted ovine eyes and in canine eyes following anastomosis of the ciliary artery of dogs to the femoral artery in rats.⁽¹⁶⁾

Vascular anastomosis may also present better results when traditional hand-sewn technique is avoided. The penetrating needle can induce vascular wall damage, which affects the healing response and increases the risk of subendothelial matrix exposure and thrombus formation.⁽¹⁷⁾ In our study, this was not evaluated.

With the advances in microsurgery and immunomodulation, together with new therapies in neurodegeneration,

strategies for overcoming the challenges previously thought to undermine the possibility of eye transplantation are emerging.

The present lack of visual prognosis after complete blindness and the consequent individual, family and society costs are imperative motivations towards the research to promote visual recovery. In this study, we promoted a novel surgical technique and animal model for total eye transplantation. Considerable work is still needed to confirm the retina and vascular maintenance of the donor eye and to overcome the challenges of whole-eye transplantation.

CONCLUSION

In conclusion, this study of an animal model for preliminary total eye transplantation was successful in presenting a reproducible rabbit model. Lateral orbitotomy allowed wide access to the retrobulbar space. Electroretinography measurements evaluate maintenance and restoration of the retinal function. Future studies should be performed regarding increase of ocular viability using microsurgery techniques, immunomodulation, vascular anastomosis and neurodegeneration therapies.

ACKNOWLEDGMENTS

São Paulo Research Foundation (Fapesp), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and CAPES-rede nBioNet. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) This work was supported, in part, by Fapesp (# 2011/17363-3).

REFERENCES

- Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, Mariotti SP. Global data on visual impairment in the year 2002. *Bull World Health Organ.* 2004;82(11):844-51.
- Flaxman SR, Bourne RR, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, et al.; Vision Loss Expert Group of the Global Burden of Disease Study. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. *Lancet Glob Health.* 2017;5(12):e1221-e1234.
- Bourne RR, Jonas JB, Flaxman SR, Keeffe J, Leasher J, Naidoo K, et al.; Vision Loss Expert Group of the Global Burden of Disease Study. Prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe: 1990-2010. *Br J Ophthalmol.* 2014;98(5):629-38.
- Ellenberg D, Shi J, Jain S, Chang JH, Ripps H, Brady S, et al. Impediments to eye transplantation: ocular viability following optic-nerve transection or enucleation. *Br J Ophthalmol.* 2009;93(9):1134-40.
- Scientists urged to hold firm to eye transplant goal. *JAMA.* 1978;240(12):1227.
- Peiffer RL, Jr, Pohm-Thorsen L, Corcoran K. Models in ophthalmology and vision research. In: *The Biology of the Laboratory Rabbit*. 2nd ed. Academic Press; 1994. Chap. 19, p. 409-33. doi: 10.1016/B978-0-12-469235-0.50025-7.
- Freed WJ, Wyatt RJ. Transplantation of eyes to the adult rat brain: histological findings and light-evoked potential response. *Life Sci.* 1980 Aug 11;27(6):503-10.

8. Koppányi T, Baker C. Further studies on eye transplantation in the spotted rat. *Am J Physiol* 1925;71:344-8.
9. Zor F, Polat M, Kulahci Y, Sahin H, Aral AM, Erbas VE, Karagoz H, et al. Demonstration of technical feasibility and viability of whole eye transplantation in a rodent model. *J Plast Reconstr Aesthet Surg*. 2019;72(10):1640-1650.
10. Haggerty AE, Oudega M. Biomaterials for spinal cord repair. *Neurosci Bull*. 2013;29(4):445-59.
11. Ritfeld GJ, Roos RA, Oudega M. Stem cells for central nervous system repair and rehabilitation. *PM R*. 2011;3(6 Suppl 1):S117-22.
12. Shi J, Ellenberg D, Kim JY, Qian H, Ripps H, Jain S, Chang JH, Azar DT. Restoration of electroretinogram activity in exenterated swine eyes following ophthalmic artery anastomosis. *Restor Neurol Neurosci*. 2009;27(4):351-7.
13. Lima S, Koriyama Y, Kurimoto T, Oliveira JT, Yin Y, Li Y, et al. Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. *Proc Natl Acad Sci U S A*. 2012;109(23):9149-54
14. Isaacs J, Klumb I, McDaniel C. Preliminary investigation of a polyethylene glycol hydrogel "nerve glue". *J Brachial Plex Peripher Nerve Inj*. 2009;4:16.
15. Gouras P, Hoff M. Retinal function in an isolated, perfused mammalian eye. *Invest Ophthalmol*. 1970;9(5):388-99.
16. Sher H, Cohen RJ. Revascularization of isolated extracorporeal canine eyes by direct microsurgical anastomosis. *J Microsurg*. 1980;1(5):399-402.
17. Liang X, Sun G, Hu B, Li J. Microvascular sutureless adhesive bonding anastomosis with a new soluble hollow stent. *J Craniofac Surg*. 2013;24(5):1823-7.