

Long-Term Cryoprotectant-Augmented Deep Freezing of the Animal Whole Globe for the Maintenance of Corneal Clarity

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Abstract

Purpose: Round-the-clock on-demand availability and corneal cloudiness are the two major problems with animal eyes for surgical practice. The purpose of this investigation is to assess the usability of long-term deep freezing for the preservation of enucleated sheep eyes and the adjunctive role of intracameral cryoprotective agent on corneal clarity.

Methods: Thirty-two enucleated fresh sheep eyes were obtained from the slaughterhouse. With an insulin syringe, 0.5 mL of aqueous was tapped from the 16 right globes; it was replaced by an equivalent intracameral cryoprotection solution. Treated eyes were kept in receptacles filled with 1 cc of the same cryoprotectant and stored in a deep freezer (-80°C). Left eyes were served as controls (untreated globes) and stored in the same condition. Six months later, globes were defrosted and corneal clarity was compared in a paired and masked fashion.

Results: All treated globes had clearer corneas as compared with untreated globes and they were all successfully applied for anterior segment surgical practice.

Conclusion: Long-term deep freezing with adjuvant intracameral cryoprotectant is effective for the preservation of corneal clarity of animal whole globe. This allows round-the-clock on-demand availability of animal eyes with clear corneas for surgical practice in ophthalmology skill labs.

Keywords: Ophthalmology Training, Animal Eye, Eye Model, Skill Lab, Corneal Clarity, Globe Preservation

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Introduction

Using practice animal eyes to simulate ophthalmic surgery on human eyes in ophthalmology skill labs (OSLs) is an established approach for improving residents' technical proficiency and confidence, and patient safety.^{1,2} However, postmortem ocular changes restrict round-the-clock availability of these eyes in OSLs and the ensuing corneal edema hampers visualization of the intraocular structures, which is a prerequisite for the practice of microsurgical intraocular techniques.^{3,4}

Cryopreservation (typically at around -196°C) is the only method that offers long-term storage of living cells and tissues, but the method is sophisticated and costly and has not been used for preservation of the whole globe. Further, it intends to maintain tissue viability for transplantation which differs from tissue maintenance for educational purposes. A variety of techniques have been applied to improve corneal clarity and/or intraocular visualization⁵⁻¹² but they are short-term, inadequate, or complicated.

Cryoprotective effect of dimethyl sulfoxide (DMSO) has clinically been shown.¹³ In fact, glycerin-cryopreserved grafts have been used for penetrating keratoplasty as a suitable alternative to fresh material; its use has been suggested for emergencies.¹⁴

The aim of this study is to investigate the adjunctive effect of cryoprotectants on the preservation of corneal clarity after long-term augmented deep freezing of animal whole globes for training purposes in OSLs.

Methods

Augmented deep-freezing storage

Thirty-two enucleated fresh sheep eyes were obtained from the abattoir. A 28-gauge needle was inserted into the anterior chamber of all right eyes through the corneoscleral limbus; 0.5 mL of aqueous was tapped, followed by replacement with 0.5 mL of cryoprotection solution containing glycerol as the cryoprotectant, Dulbecco's Minimum Essential Medium (DMEM) as the cell culture medium, and an antibiotic (Figure 1A). This is the conventionally available cryoprotection solution for the investigators; routinely used in cardiac valve cryopreservation. For corneal epithelial cell protection, sheep globes were positioned upside-down in plastic receptacles filled with 1 cc of the same solution (Figure 1B). For full anterior cornea exposure, the third lid was kept retracted. All left eyes were served as controls. The eyes were stored in a deep freezer at -80°C .

Six months later, globes were simply defrosted under lukewarm water flush for about one minute (Figure 1C) and their corneas' clarity were compared in a masked fashion by a single observer [SFM].

The study protocol complied with the Farabi Eye Hospital Research Council's requirements. The animals were sacrificed for purposes other than this experimentation and their remains were decomposed of in a standard fashion.

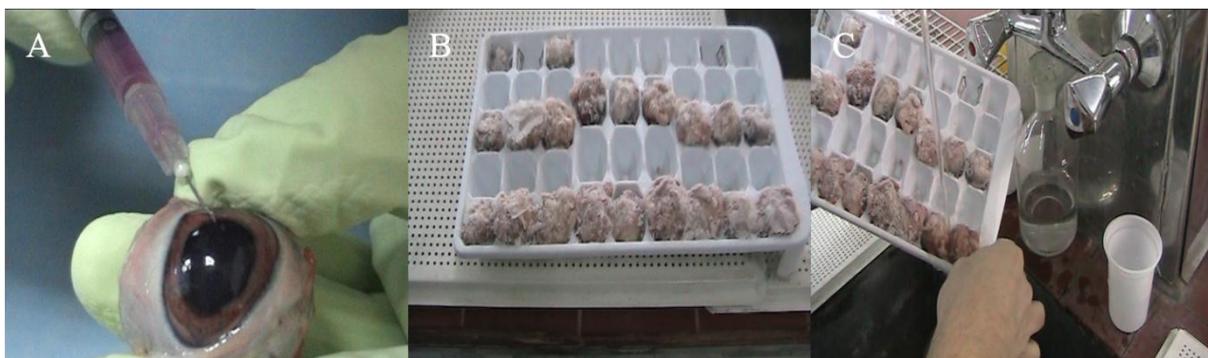


Figure 1. Augmented deep freezing technique of the animal whole globe: tap and injection of sheep eye anterior chamber; 0.5 cc of aspirated aqueous was replaced with 0.5 cc of cryoprotection solution (A). Sheep globes are kept cornea-side down in touch with cryoprotection solution (B). Globes are defrosted under lukewarm tap water flush (C).

Results

After six months, an ophthalmologist (SFM) compared corneas' transparency of treated and untreated sheep eyes in a paired fashion. All corneas of control 'uninjected' globes were hazier (Figures 2A and 2B). Suitability for performing anterior segment procedures such as capsulorhexis, phacoemulsification, and lamellar keratoplasties were evaluated too. All

globes had sufficient integrity to be mounted on the fixation system (Figures 2C, 2D). The stored eyes (of both groups) expressed troublesome iris pigment release during surgical practice (Figure 3). It should be noted that the 'uninjected' globes were also usable for procedures like trabeculectomy.

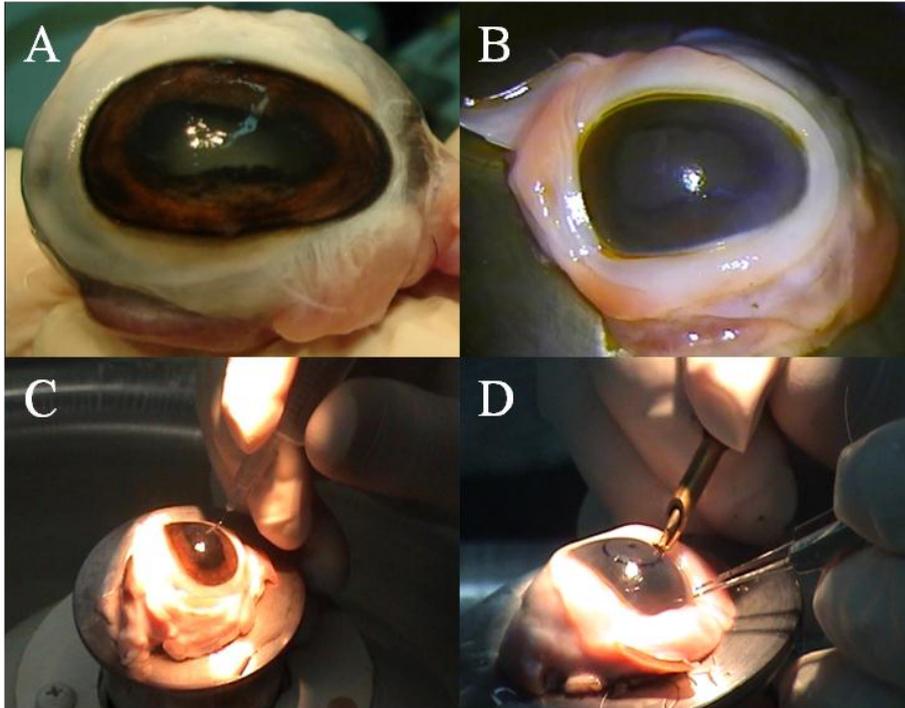


Figure 2. Following a 6-month augmented deep-freezing storage, treated eyes have clearer corneas (A) than their fellow frozen-only eyes (B). Sample anterior segment procedures (capsulorhexis and intrastromal corneal ring segment implantation) on globes with clear (C) and cloudy (D) corneas. Globe integrity was maintained in both groups following deep freezing, allowing their mounting on a globe fixation system.

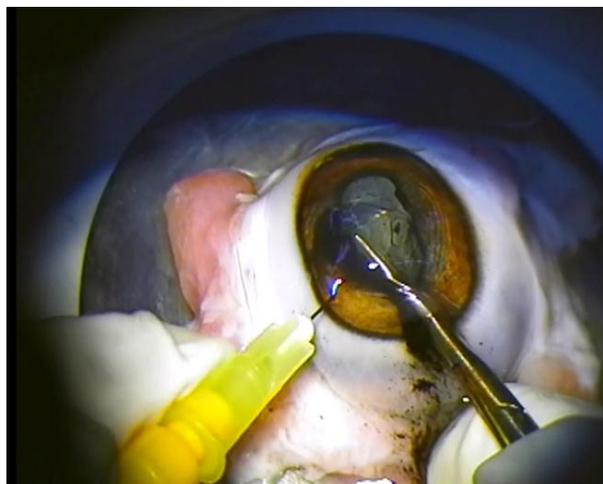


Figure 3. Iris pigments are heavily shed during surgical practice, attributable to postmortem changes.

Discussion

A variety of techniques have been advocated to improve intraocular visualization. These can be classified into two groups: corneal 'clarity enhancement' and corneal 'replacement' with artificial substances (Table 1). All of these methods are effective in short-term.⁵⁻¹¹

We have recently introduced sheep eyes as a viable ophthalmic surgery practice model¹⁵ and in fact tested its applicability on a self-designed eye fixation system.¹⁶ Our next challenge was to provide a reliable supply of practice eyes in terms of volume and round-the-clock availability for OSLs. To this end, we embarked on the current trial in which we could retain corneal transparency following a long-term preservation.

This method does not need sophisticated measures needed for conventional cryopreservation¹² in terms of controlled-rate freezers, liquid nitrogen storage systems, a 37° C water bath for thawing, tight standard cryoprotection solutions, and skilled staff.

During the freezing process, some changes, such as intracellular and extracellular ice crystal formation, accelerated dehydration, and solution effects, can potentially be harmful to the cornea.¹⁷ Vitrification is the process of modifying the freezing process into cryopreservation. It requires the addition of cryoprotectants like glycerol prior to cooling. We might have suppressed crystallization in the cornea (specifically corneal endothelium) through intracameral injection of cryoprotection solution and by exposure of the ocular surface

to the same solution from outside (corneal epithelium). Additionally, high viscosity of cryoprotectants may exert osmotic pull in the cornea. This could explain why this modified method resulted in clear corneas in the treated eyes six months following enucleation. This is an outstanding facility.

The relative difference in clarity could be verified in the sample images (Figures 2A vs. 2B).

The current study did not provide histopathological data. In fact, authors did pilot counting the corneal endothelial cells histopathologically but that was not feasible by our methods. Corneal confocal microscopy may provide such data in future studies; authors would like to emphasize that the primary purpose of this experiment was to assess the educational applicability of the method, and not its usability for formal tissue preservation.

The proposed intracameral injection needs a dedicated technician with delicate hands. Meticulous care should be taken during the puncture and access to the anterior chamber and the technician must avoid the lens anterior capsule and iris while protecting the globe from distortion. The alternative approach of submerging the whole globes in the cryoprotectant could be tested. However, this might have challenges during the process of defrosting. The troublesome iris pigment release during surgical practice is also most likely explained by the postmortem changes.

Table 1. Proposed methods for enhancing the corneal clarity and/or intraocular visualization in cadaver or animal eyes

Method	Mechanism
<i>Corneal clarity enhancements</i>	
AC injection of HMW substances: ^{5,6} OVD Glycerol-based lubricating jelly	Rapid dehydration of the cornea is achieved by increasing viscosity in AC and decreasing aqueous diffusion into the corneal stroma
Epithelial debridement or lamellar dissection	To remove the edematous epithelium and leaning the thick cornea
<i>Corneal replacement with artificial material</i>	
Contact lens ^{7,8} Temporary keratoprosthesis ⁹⁻¹¹	The opaque cornea of a cadaver eye is removed by trephination and glue is used to secure contact lens to the corneal rim. A silicone artificial cornea which is sutured to the trephined practice eye cornea; this provides an excellent view of all intraocular tissues.

AC: Anterior chamber, HMW: High molecular weight, OVD: Ophthalmic viscosurgical device

Virtual reality simulators have definite theoretical advantages over animal eye practice models; they are not dependent on animal sacrifice and are not affected by postmortem changes; they are available day and night and are unlimited in terms of surgical volume. Animal or cadaveric models on the other hand are still the closest approximation to real surgery,¹ are less costly, and can be used to simulate a broader range of ocular procedures.

Conclusion

The proposed 'augmented deep-freezing storage' of whole animal globes can potentially revive animal practice models as it realizes round-the-clock availability of such eyes for surgical practice with an easy, cost-effective, and time-efficient method.

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