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Experimental lamellar corneal graft in dogs using preserved equine pericardium







Ceratoplastia lamelar experimental em cães com pericárdio de equino conservado

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SUMMARY

The purpose of this paper was to study the preserved equine pericardium as a corneal lamellar graft of the dog. We performed in twelve adults, male and female mongrel dogs a superficial keratotomy 5 mm square and a fragment of equine pericardium of the same shape was sutured in place with multiple 7-0 nylon simple interrupted sutures. Each five animals were sacrificed in days 7, 15, 30 and 100 after surgery. The clinical and histological exams permitted us to assert that this membrane is a useful tissue to be used as a corneal lamellar graft of the dog.

UNITERMS: Cornea; Grafts; Pericardium.

INTRODUCTION

The superficial corneal lesions are a frequent problem presented to the veterinary ophthalmologists. One can have corneal tissue loss due to microorganisms or inflammatory cells destructive activities or by an iatrogenic way during neoplasia removals.

The cornea can be repaired by several means using synthetic or biological materials and his history comes from

the end of the eighteenth century.

In medicine the lamellar autogenous or homologous grafts experienced a spectacular increasing in the past 30 years due to the development of the microsurgery, and corneal preservation, although in veterinary ophthalmology its use are not yet well established.

In veterinary ophthalmology the repair of the non full-thickness lesions of the cornea can be done using the third eyelid flap, conjunctiva pedicle graft or conjunctival patch graft. Other procedures are widely used as tarsorrhaphy, corneoscleral transposition, tissue adhesives, contact lenses, and synthetic grafts.

The preserved biological membranes are used in medicine and in veterinary general surgery. Previous papers showed that homologous and xenologous pericardium, peritoneum, amniotic membrane, renal capsule preserved in glycerol are useful in the repair of superficial or full-thickness lesions of the cornea^{4,7,10}. Recently the amniotic membrane was used as an alternative substrate to treating persistent epithelial defects in human beings¹².

The purpose of this paper was to study the clinical and histological aspects of the experimental use of preserved equine pericardium in the repair of superficial defects of the cornea of the dog.

MATERIAL AND METHOD

The use of the animals was according to the ARVO Resolution concerning the use of animals in research. Twelve adult male and female mongrel dogs with normal eyes weighing about 10 kg were divided into 4 groups of 3 animals.

A lamellar, 5 mm square corneal defect was created 2 mm from the limbus at the 11 o'clock position using a #11 scalpel blade. A 5 mm square piece of equine pericardium, and stored in 98% glycerin, was dehydrated in saline solution and sutured in the corneal defect in a single interrupted pattern using 7-0 nylon sutures ([Fig. 1](#)).

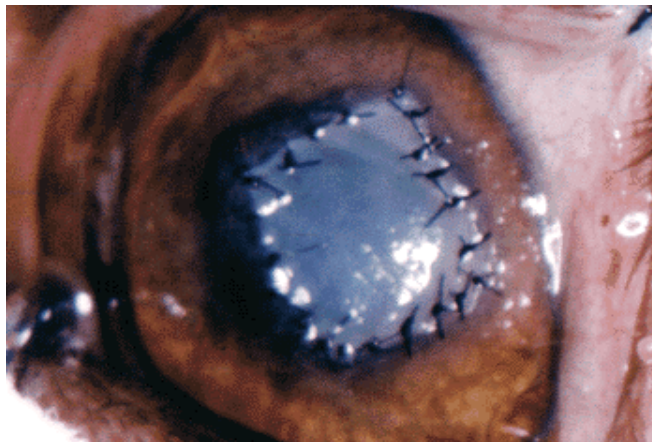


Figure 1

The eye of one dog just after the surgery showing the lamellar graft of equine pericardium.

Gentamicin eyedrops were used every 6 hours for 15 days after surgery. The eyes were examined daily by slit lamp biomicroscopy, tonometry, and direct ophthalmoscopy. Blepharospasm, corneal edema, corneal vascularization, and other signs of graft rejection were evaluated. The dogs from each group was sacrificed at days 7, 15, 30 and 100 after surgery, and the eyes enucleated, fixed in 10% buffered formalin, and embedded in paraffin. Sections were stained with hematoxylin-eosin, and examined by light microscopy.

RESULTS

Using an experimental lamellar graft of preserved equine pericardium to repair corneal defects of the dog we could see that the corneas were transparent during all experiment (100 days). The graft was totally opaque at the beginning, and at day 30 one had less opacification, and at day 100 the graft was reasonable transparent ([Fig. 2](#)). The animals presented a mild blepharospasm until 15 days after surgery and chemosis for a week. The conjunctival congestion was intense at the beginning, slight at the day 30 and a normal conjunctiva could be

observed at the end of the experiment.

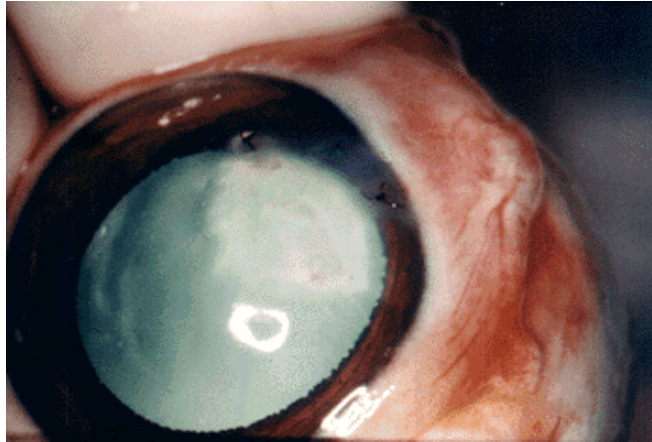


Figure 2

The same eye at day 100 showing the implant site with partial clearing of the implant.

New vessels were present at about day 10 and grew during the next 30 days towards the graft but at day 100 there were no more vessels. There were no significant signs in the anterior or posterior segments of the eye. Histologically we could see at the implant site a mild inflammatory cellular infiltrate at day 7. At day 100 one could see vessels, fibrosis, mild chronic inflammatory infiltrate, and a total epithelization of the cornea ([Fig. 3](#)) was observed. The anatomical and physiological aspects of the cornea were reestablished.

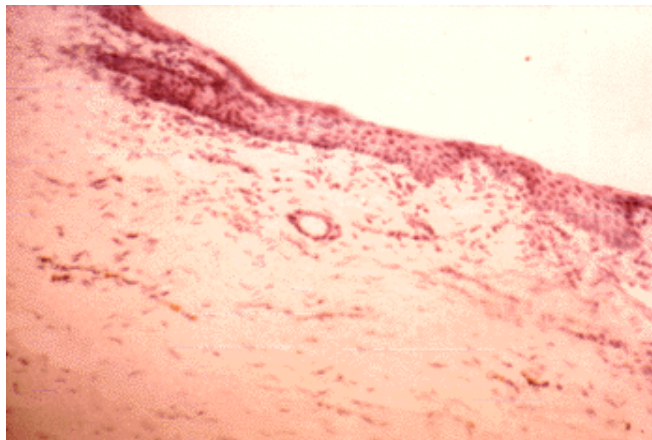


Figure 3

At day 100 are noted new vessels, fibrosis, mild chronic inflammatory process and epithelization at the implantation site (HE-100x).

DISCUSSION

The repair of partial thickness of the cornea has been made by several procedures with the purpose of restoring the architecture of the corneal tissues and preventing the leakage of the intraocular contents.

Blogg; Stanley⁵ had repaired defects of the cornea and sclera after limbal melanoma removal using the cartilage and conjunctiva of the third eyelid; although with good results this technique seems to be mutilating.

Conjunctival flaps have been widely used to cover completely or partially the cornea, they provide protection, direct blood supply which transport of antimicrobial and anticollagenolytic agents to the corneal

defects^{8,14,18,19}.

Tissue adhesives as methylmethacrylate have been used since 1968 in the treatment of corneal ulceration and perforation. The cyanoacrylate has a lower toxicity and is indicated to treat very small corneal punctures and with well-defined margins¹⁶. The Biofill had been used with good results¹⁷. Laus *et al.*¹¹ in an experimental study had used a gel-adhesive mixture (Colagel) in corneal lesions with poor results.

Hydrophilic soft contact lenses have been used in the treatment of superficial ulcers, deep ulcers, and refractory epithelial erosions⁹. The corneoscleral transposition covers the corneal defects by sliding corneal and scleral tissue adjacent to the defect¹⁵. This procedure has a disadvantage, as it causes an opacification at that point of the cornea.

The corneal grafts have not been used so frequently in veterinary ophthalmology although the autogenous techniques had an increase once these procedures are immunologically well accepted^{6,13}. The homologous graft frequently used in medicine has a restrict use in veterinary ophthalmology but the development of the methods of preservation of cornea for long periods²¹ and new microsurgical techniques will increase the use of the lamellar or penetrating keratoplasties.

The aim of these methods is to keep the integrity of the cornea by covering the cornea except the use of cartilage and conjunctiva of the third eyelid and the lamellar and penetrating keratoplasties that promote the substitution of the portion of the diseased cornea.

The opacification at the site of the graft is observed in almost methods of corneal lamellar repair but can be absent when treated by third eyelid flap, contact lens and temporary tarsorrhaphy. The lamellar graft of equine pericardium was opacified during almost all the experiment but at 100 day one could see a slight clear cornea and this is better when compared to the results of the treatment using the corneoscleral transposition¹⁵ and the cartilage and conjunctiva of the third eyelid⁵ where a dense scar is observed. This opacification is not of importance when it is located at the periphery and there is no visual deficit.

The vascularization of the cornea had begun at day 10, which is coincident with the observation of Wilkie; Wolf²⁰, who used a synthetic tissue in a penetrating keratoplasty. The same occurred when other biological membranes were used^{1,2,3,7}. At day 30 the fluorescein dye test was negative once the epithelium had been restored with total epithelization of the graft.

A granulation tissue appeared with the new vessels and at about the day 20 it was plentiful. When Wilkie; Wolf²⁰ used a synthetic tissue as a graft the granulation tissue elevated the graft and at the fifth week of implantation the graft was totally covered by the granulation, and the graft had been removed. When the pericardium was used one could not observe this elevation, and the graft was kept in site once it was integrated to the recipient cornea.

Miosis and low intraocular pressure were present in a few days after surgery probably due to a mild uveitis. The same occurred when other membranes were used in penetrating keratoplasties^{3,4}. It was not necessary any treatment to normalize this clinical signs.

At the histological exam one could see an inflammatory cellular infiltrate during all experiment, and at day 100 fibrosis and mild chronic inflammatory infiltrate could be seen. The epithelization began at day 7 and was complete at day 30.

These findings suggest that the anatomical and physiological aspects were restored. The equine pericardium preserved in glycerol would provide a helpful tissue to be used to repair superficial defects of the cornea of the dog and probably other species.

RESUMO

Com o objetivo de se estudar o comportamento, do ponto de vista clínico e histológico, do pericárdio de equino conservado em glicerina, nas reparações lamelares experimentais da córnea, foram constituídos 4 grupos com 5 cães cada. Após ceratectomia superficial de 5 mm x 5 mm um fragmento de igual tamanho de pericárdio de equino conservado em glicerina foi suturado usando-se fio monofilamentar de náilon nº 7-0 em pontos simples separados. Os animais foram sacrificados aos 7, 15, 30 e 100 dias de pós-operatório. Os resultados, tanto clínico quanto histopatológico obtidos, nos permitiram afirmar que o pericárdio de equino conservado em glicerina constituiu-se em boa alternativa de enxertia nos reparos lamelares da córnea de cães.

UNITERMOS: Córnea; Enxertos; Pericárdio.

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