

Experience of using autologous cornea for deep and penetrating keratoplasty in dogs and cats

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Abstract. This paper presents a generalized experience in the treatment of deep corneal ulcers, descemetoceles and perforated corneal ulcers in which keratoplasty was used using autologous cornea. Deep corneal ulcers are characterized by an acute inflammatory process and the risk of developing many complications leading to loss of vision. Conservative treatment of such pathologies takes a long time and is not always successful. Keratoplasty for corneal transplantation is considered an effective method. The outcome of the operation depends on the transplanted material and postoperative treatment tactics. The work conducted a study on the effectiveness of using autologous cornea in 32 animals with subsequent inclusion of a regenerative drug in the treatment regimen. The authors describe the clinical picture, the surgical technique, and provide the results of dynamic observation of animals. Based on the results of clinical observations, it was established that the use of autologous cornea for deep corneal ulcers can be an effective treatment method. In none of the 32 cases examined, rejection was recorded, while the morphofunctional characteristics of the cornea were preserved. Drug maintenance therapy, including a regenerative drug, showed good results in the postoperative period. The use of a regenerative drug accelerated the healing process and made it possible to maintain the transparency of the cornea and its curvature.

1 Introduction

Stromal corneal ulcers, descemetoceles and perforated corneal ulcers are a fairly common pathology in veterinary ophthalmology and often cause decreased or loss of vision, and in more severe cases, the eye as an organ [1,2,3,4]. In this regard, the treatment of these ophthalmopathies is a pressing problem in the practice of a veterinary specialist. There are a large number of methods for treating deep and perforated corneal ulcers, both medical and surgical [5,6,7]. As for conservative treatment, it is not effective for perforated corneal ulcers, and for deep defects it often does not solve the main task of the veterinary ophthalmologist - to make the cornea as transparent as possible while maintaining its curvature [6]. Therefore, at the moment, the only effective way to treat animals with such

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pathologies is surgery, which includes the use of various materials for corneal transplantation in case of perforation or closure and filling of very deep defects [1,6,8].

All materials and methods of transplantation have their own advantages and disadvantages. In particular, various types of artificial cornea (Alloplant, amniotic membrane, porcine intestinal submucosal layer, etc.) are well suited for lamellar keratoplasty, but do not solve the problem of perforated ulcers. For example, Vanore M. et al identified the treatment of deep ulcers using porcine small intestinal submucosal (SIS) graft as a possible effective alternative to traditional implants. Derek W.Y Chow et al concluded that the porcine bladder submucosal corneal reconstruction technique (ACell Vet) can be used in practice, but in their study the results were better in cats than in dogs.

Donor cornea (lyophilized or frozen) is equally suitable for lamellar and penetrating keratoplasty, but there is a risk of rejection when used. It is difficult to use fresh donor corneas, since veterinary banks of donor corneas are not common, and this material is stored for a limited amount of time [7]. Methods of surgical treatment of deep and perforated corneal ulcers, such as covering with a conjunctival flap, are also often used, the method itself is simple to perform, there is practically no rejection, the flap takes root well, but a significant disadvantage of this method is gross scarring and, as a result, lack of transparency in the area corneal lesions.

Autologous corneal transplantation has previously been used to treat deep stromal corneal ulcers, descemetoceles, and perforated corneal ulcers [9,10,11]. The essence of the method is to move your own cornea to the site of the defect and fix it with sutures. According to various authors, the use of autologous cornea is preferable, since graft rejection is practically excluded and such a cornea does not need to be prepared and stored in advance [12, 13, 15]. A number of sources contain data on autologous corneal transplantation in dogs, but there is no data on autologous corneal transplantation in cats after removal of the corneal sequestrum [12, 13, 14, 15]. I would also like to note that the sources do not contain a detailed and substantiated description of postoperative treatment, which would include regenerative therapy to improve the quality of healing and reduce recovery time.

2 Material and methods of research

The material for the study was 32 animals (14 cats and 18 dogs) of various breeds and age and sex groups diagnosed with corneal ulcers of varying severity and corneal sequestration. All animals had one eye injured. The studies were carried out on the basis of the Department of Biology and Pathology of Small Domestic, Laboratory and Exotic Animals of the Federal State Budgetary Educational Institution of Higher Education "MSAVM and B - MVA named after K.I. Scriabin" and the Center for Emergency Veterinary Ophthalmology and Eye Microsurgery.

As shown in Table 1, autologous corneal transplantation was used in 18 dogs and 14 cats for the following ophthalmopathies: perforated corneal ulcer (4 dogs), descemetocoele (12 dogs), and deep stromal corneal ulcer (2 dogs). In cats – corneal sequestration (8 cats), descemetocoele (3 cats) and perforated corneal ulcer (3 cats).

Table 1. Types of ophthalmopathies in dogs and cats for which keratoplasty was performed using autologous cornea.

Diagnosis	Kind of animal	
	Dogs	Cats
Corneal sequestration	–	8

Perforated corneal ulcer	4	3
Descemetocele	12	3
Deep stromal corneal ulcer	2	–
Total	18	14

Clinical cases of corneal sequestration in cats were characterized by the following picture: when examining the cornea using slit biomicroscopy, it was revealed that the depth of the sequestration was 2/3 of the thickness of the cornea, neovascularization of the cornea and pronounced perifocal edema were noted. During a general ophthalmic examination, blepharospasm and photophobia and slight purulent discharge were noted.

In animals with perforated corneal ulcers, pathological changes were as follows. Two dogs had a similar clinical picture - severe blepharospasm and photophobia, profuse purulent discharge. Biomicroscopy revealed a perforated corneal ulcer with a diameter of about 2 mm with pronounced perifocal edema and the presence of neovascularization. The Seidel test gave a positive result. Intraocular pressure is reduced due to perforation, the eyeball is collapsed, and anterior serous exogenous uveitis is also observed.

In a cat with a perforated corneal ulcer, clinical changes were characterized by mild blepharospasm and photophobia, minor serous exudate, and no corneal edema or neovascularization. Biomicroscopy of the cornea revealed a defect with a diameter of about 2 mm, the Seidel test was positive. Hypotonia of the eyeball and miosis were also noted.

The clinical picture of descemetocele and deep stromal ulcers of the cornea was similar, the difference was in the depth of the defect - with descemetocele the depth respectively reached Descemet's membrane, and with a stromal ulcer the depth reached 2/3 of the thickness of the cornea. Otherwise, during a general ophthalmic examination, we observed photophobia and blepharospasm, purulent exudate. Slit biomicroscopy revealed a corneal defect with a diameter of about 3-4 mm with pronounced perifocal edema and symptoms of keratomalacia. The fluorescein test is positive, the Seidel test is negative.

In the process of our research, as well as according to data from foreign authors, we identified the following indications for autologous cornea transplantation: 1. Corneal sequestration of cats with a diameter of no more than 5-6 mm, not of a through nature, and also in the absence of pronounced total corneal edema and keratomalacia of most of the corneal area; 2.Deep stromal ulcers of the cornea and descemetocele with a diameter of no more than 5-6 mm, also in the absence of keratomalacia, subtotal or total corneal edema; 3. Perforated corneal ulcers with a diameter of the perforated area of no more than 3 mm, and also in the absence of extensive areas of keratomalacia, subtotal or total corneal edema.

All these conditions are necessary in order to be able to select a donor graft from a normal area of the cornea, which should be 1 mm larger than the diameter of the defect in penetrating keratoplasty, and in layer-by-layer keratoplasty, correspond to the diameter of the defect after primary surgical treatment. That is, it is necessary to assess in advance the possibility of such a transplant, based on the size of the defect after surgical treatment, and how much normal cornea will remain for autotransplantation.

Surgical treatment of corneal ulcers and corneal sequestration was performed under general anesthesia using intravenous propofol (6 ml/kg) as induction and then maintaining anesthesia using isoflurane and oxygen. Then the surgical field was prepared according to generally accepted methods, the eyeball was removed and fixed, and local anesthesia was carried out by instillation of eye drops containing proximetacaine hydrochloride. The operation was performed under an operating microscope, magnification – 10.

In the case of descemetocele (Fig. 1) and deep stromal corneal ulcers (Fig. 2), primary surgical treatment of the defect was performed, which consisted of removing non-viable

and necrotic tissue, areas of keratomalacia, and purulent infiltrates. Then, using a corneal trephine suitable for the diameter of the ulcer, a bed was prepared for further transplantation and fixation of the graft. After preparing the bed, an area of normal cornea was determined (preferably paralimbal) and a non-through trepanation was performed using a corneal trephine with a diameter identical to the prepared bed. Next, a keratectomy was performed to a depth of $\frac{1}{2}$ of the corneal thickness using a corneal delaminator and corneal scissors. The autologous cornea was placed in a prepared bed and fixed first with simple interrupted sutures at 12, 6, 3 and 9 o'clock, and then with a simple continuous suture (8-0 nylon thread, atraumatic or 8-0 vicryl, also atraumatic) or further with interrupted sutures. After this, tarsorrhaphy was performed for a period of 30 days.

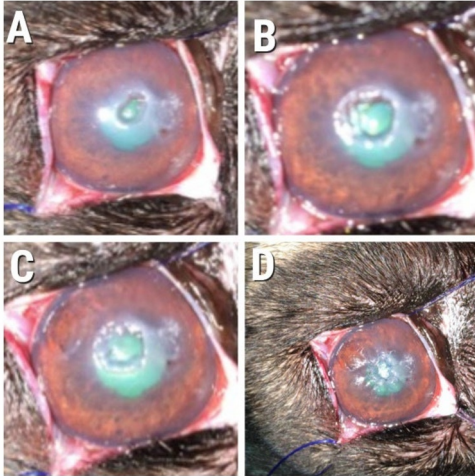


Fig. 1. Technique for deep lamellar keratoplasty using autologous grafting in a dog with descemetocoele. A – descemetocoele. B – primary surgical treatment of the ulcer and preparation of the bed for transplantation. C – preparation of the autograft and placing it in the prepared bed. D – fixation of autologous cornea with simple interrupted sutures.

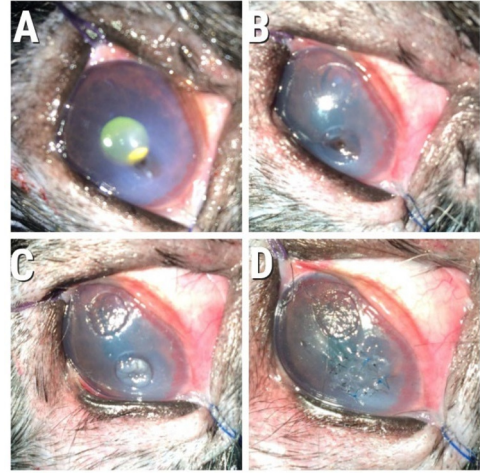


Fig. 2. Technique for deep lamellar keratoplasty using autologous transplantation in a dog with a deep stromal corneal ulcer. A – deep stromal corneal ulcer. B – primary surgical treatment of the ulcer and preparation of the bed for transplantation. C – preparation of the autograft and placing it in the prepared bed. D – fixation of autologous cornea with simple interrupted sutures.

For corneal sequestration of cats (Fig. 3), the sequence of actions during surgery was as follows. We first determine the diameter of the sequester using a corneal compass, select a corneal trephine of the appropriate diameter, and use the latter to perform a superficial trepanation of the cornea. Next, we perform a layer-by-layer keratectomy using a delaminator and scissors, that is, we remove the body of the sequester to healthy, unchanged corneal tissue, while simultaneously preparing the bed for the future transplant. After these manipulations, the procedure is the same as for autologous corneal transplantation for deep stromal corneal ulcers and descemetocoele.

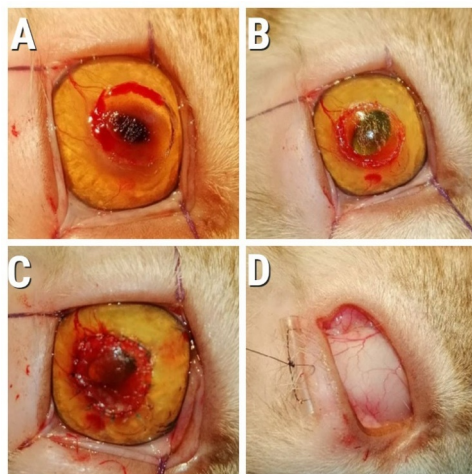


Fig. 3. Technique for deep lamellar keratoplasty using autologous cornea in a cat with corneal sequestration. A – layer-by-layer removal of the body of the corneal sequestrum. B – prepared bed after removal of the corneal sequestrum. C – fixation of the autologous cornea with simple interrupted sutures. D – tarsorrhaphy.

Perforated corneal ulcers (Fig. 4 and Fig. 5). The complexity of surgery for perforated ulcers lies in a number of nuances. Firstly, the eyeball lacks tone due to perforation, and this significantly complicates lamellar keratectomy to prepare an autologous cornea. Secondly, one must always take into account the fact that a perforated cornea is a gateway to intraocular infection, which can significantly complicate the postoperative period. Therefore, when performing primary surgical treatment of the wound, it is necessary to thoroughly rinse the anterior chamber of the eyeball using antiseptic solutions. And thirdly, before sealing the defect, it is necessary to ensure that the intraocular structures of the eye, in particular the lens, iris and retina, are not damaged.

If the above conditions are met, the algorithm of actions is as follows. Primary surgical treatment of the ulcer is also carried out, the anterior chamber of the eyeball is washed from fibrin, pus or blood. The bed for the transplant is prepared. Further, for the convenience of layer-by-layer keratectomy on a collapsed eyeball, you can fill the eyeball with a viscoelastic preparation based on sodium hyalurate, which will maintain eye turgor and the possibility of corneal trepanation. If the diameter of the perforated ulcer is no more than 2 mm, then provisional sutures can be placed on the edges of the wound and carefully juxtaposed, thereby ensuring the tightness of the eyeball, and then also injecting viscoelastic. After determining the location of the normal cornea for transplantation, layer-by-layer trephination and keratectomy are performed. The prepared autologous cornea is placed in the bed and fixed. As the final stage of the operation, tarsorrhaphy is performed for a period of 30 days.

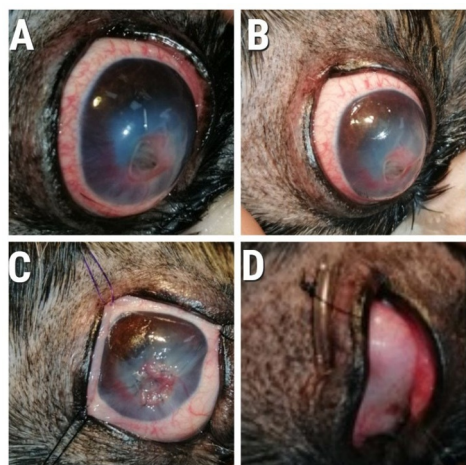


Fig. 4. Technique for penetrating keratoplasty using autologous cornea in a dog with a perforated corneal ulcer. A – perforated corneal ulcer. B – preparation of an autologous graft and primary surgical treatment of the ulcer. C – fixation of the autologous cornea with simple interrupted sutures. D – tarsorrhaphy.

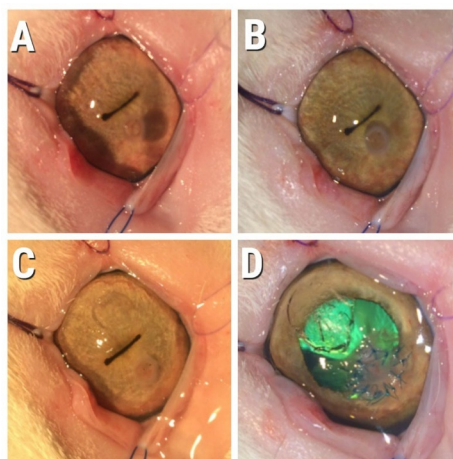


Fig. 5. Technique for performing penetrating keratoplasty using autologous cornea in a cat with a perforated corneal ulcer. A – perforated corneal ulcer. B – primary surgical treatment of the ulcer. C – preparation of an autologous transplant. D – fixation of autologous cornea with simple interrupted sutures.

As postoperative therapy, we used an antibiotic in the form of eye drops from the group of 4th generation fluoroquinolones, 1 drop 4 times a day, and a regenerative drug in the form of Reparin-Helper® eye drops, 1 drop 4 times a day. The interval between drops is 15 minutes, the duration of therapy is 30 days. When performing penetrating keratoplasty, mydriatic was added to these drugs in the form of eye drops, 1 drop 2 times a day for 14 days, in order to prevent the formation of anterior and posterior synechiae.

3 Results

Postoperative examination was carried out on the 7th and 30th days. Since the cornea was covered by the third eyelid, on the 7th day the patient's condition was assessed using indirect signs. In particular, the general condition of the animal was assessed (appetite, thirst, mobility), and the area of the eyeball was also assessed. When assessing the latter, palpation, through the upper eyelid, the following criteria were noted: pain, increased local temperature, tone of the eyeball and its size. During a visual examination, the presence and nature of exudate, the degree of hyperemia of the conjunctiva of the eyelids, the integrity of the sutures holding the third eyelid, and the presence of leakage of intraocular fluid were assessed.

As a result, when examining all operated animals on the 7th day, we did not find any pathological changes in the animal's organ of vision or behavior. The animal's appetite, thirst and mobility are preserved. On palpation of the eyeball there was no pain, no purulent discharge, no increase in local temperature, the tone of the eyeball and its size were preserved. Hyperemia of the conjunctiva of the eyelids and a moderate amount of catarrhal exudate were observed; the integrity of the sutures was preserved. An important indicator of a high-quality operation in animals that underwent penetrating keratoplasty was the absence of leakage of intraocular fluid, which indicated the tightness of the structure and good engraftment of the graft.

On the 30th day after the operation, the sutures from the third eyelid were removed, that is, the eye was opened and the sutures from the cornea were removed under local

anesthesia. In all operated animals, we noted good engraftment of the autologous cornea. The fluorescein test and Seidel test showed a negative result. Biomicroscopy of the cornea and anterior segment of the eyeball revealed moderate perifocal edema and varying degrees of fibrosis; there were no pathological changes in the anterior chamber of the eyeball. There was no fibrosis in the area where the autologous cornea was selected; the integrity and curvature of the cornea was preserved. Vision was preserved in all clinical cases. In animals that underwent end-to-end autologous corneal transplantation, neovascularization was observed in the transplant area. After removal of the sutures, all animals were prescribed an eye ointment based on cyclosporine A 2 times a day, the duration of therapy was 30 days, in order to minimize scarring and completely eliminate inflammatory phenomena on the cornea.

4 Discussion

As can be seen from Table 2, we obtained the highest quality results after transplantation of an autologous cornea in the surgical treatment of corneal sequestration in cats. In five cases out of eight, there was an almost completely transparent cornea (an example of a nubecula), both in the transplant area and in the transplant selection area, and in one case each in dogs diagnosed with descemetocoele and deep stromal ulcer of the cornea. The macula was observed in 13 animals (examples of the macula), including a cat that received a end-to-end graft. We also consider this a good result, since with the macula the clouding of the cornea is insignificant and does not affect the quality of the animal’s vision. In six dogs with a perforated corneal ulcer and in two dogs with descemetocellus, the outcome of treatment was leukoma, this is due to the specific characteristics of healing. As a rule, in dogs, unlike cats, the healing process is more intense with greater formation of connective tissue, newly formed vessels and granulate. Which ultimately leads to more severe scarring of the cornea. In all cases, the clouding of the cornea in the area of material selection is minimal and almost imperceptible, which indicates high-quality healing of the cornea and engraftment of the graft. The result of surgical treatment fully justified the use of autologous cornea - there was no rejection in any case, and the degree of corneal opacification in almost all cases was insignificant, which made it possible to preserve the morphofunctional characteristics of the cornea and, as a result, vision.

Table 2. The degree of corneal scarring after autologous corneal transplantation for various types of corneal ulcers in dogs and cats, 60 days after surgery.

Kind of animal	Diagnosis	Nubecula	Macula	Leukoma
Dogs (18 heads)	Perforated corneal ulcer (4 cases)	–	2	2
	Descemetocoele (12 cases)	1	5	6
	Deep stromal corneal ulcer (2 cases)	1	1	–
Cats (14 heads)	Corneal sequestration (8 cases)	5	3	–
	Perforated corneal ulcer (3 cases)	1	2	–
	Descemetocelle (3 cases)	3	-	-
Total: 32 animals	32 clinical cases	11	13	8

5 Conclusions

Based on the studies conducted, we can conclude that in certain cases, the use of autologous cornea for lamellar and penetrating keratoplasty for small-area corneal defects (no more than 5 mm for non-penetrating and no more than 3 mm for penetrating) is absolutely justified and can serve as an excellent alternative for classical methods such as conjunctivoplasty, donor cornea transplantation and the use of biomaterials. The method of using autologous cornea for keratoplasty is relatively simple to perform and does not require storage or preparation of material for transplantation. The only limitation to consider is the size of the corneal ulcer. The result of surgical treatment also fully justified the use of autologous cornea - there was no rejection in any case, and the degree of corneal opacification in almost all cases was insignificant, which made it possible to preserve the morphofunctional characteristics of the cornea and, as a result, vision. It should also be noted that the area on the cornea where the graft was taken was almost completely restored - transparency and curvature were preserved.

Postoperative treatment, which, in addition to antibiotics, included the use of the regenerative drug Reparin-Helper®, also played an important role in graft engraftment and corneal healing. The regenerative drug stimulated healing and had an anti-inflammatory effect, due to which fibrosis in the defect area was significantly less. This gave us the opportunity to abandon the use of keratoprotectors and non-steroidal anti-inflammatory drugs. It should also be noted that replacing keratoprotectors and NSAIDs with the regenerative drug Reparin-Helper® was not only more effective, but allowed us not to overload the regimen for the animal owner (which increases the chance of completing the prescribed therapeutic course).

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