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Evaluation of vacuum trephined corneal allograft transplantation for corneal ulcers and opacities in dogs

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Abstract
The study on evaluation of vacuum trephined corneal allograft transplantation for corneal ulcers and opacities was carried out in the Department of Surgery and Radiology, Veterinary College, Bangalore with the patients which had refractory corneal ulcers which were not responding to the medical treatment. The fluorescein dye test was used to confirm the corneal ulcer. The ulcers that were refractory to any of the medications were considered for corneal transplantation using vacuum trephined corneal allograft. Healthy donor eyes were harvested from the cadaver within one hour after death, the cornea along with sclera was carefully dissected and was stored in the corneal storage medium for transplantation. On the day of surgery, the recipient cornea was surgically removed and bed was prepared for allograft transplantation. The corneal button was trephined to match the diameter of the defect and was secured to the recipient bed using cardinal suture pattern under magnifying loupe magnification with polyglactin 910 6-0 size suture material. Temporary tarsorrhaphy was performed to prevent self-mutilation and to protect the eye from the environment. Post-operatively ceftriaxone @ 25 mg/kg body weight and prednisolone 1 mg/kg body weight twice daily were administered intramuscularly for 10 days. The moxifloxacin with dexamethasone eye drops were instilled into the eye after removal of tarsorrhaphy sutures. The corneal graft was successfully accepted by the host and the cornea became nearly transparent and the near satisfactory vision was retained by 60 days post-operatively.

Keywords: Vacuum trephine, allograft transplantation, corneal ulcer

1. Introduction
The canine eye is an active organ that constantly adjusts the amount of light it lets in and focuses on objects near and far. The cornea is the outermost portion of the fibrous tunic, which is a transparent, avascular structure that serves to transmit and refract light and has a protective function for the intraocular contents. The cornea relies on the precorneal tear film and aqueous humor for nutrition and waste. Corneal ulceration occurs commonly in canine patients, and its treatment depends mainly on the type of ulcer/defect. The most common causes for corneal ulcers in dogs include trauma, foreign bodies, infection, inadequate lacrimal secretion, and corneal endothelial dysfunction, spontaneous, toxic, and anatomic/functional abnormalities in adnexa. The corneal defects are being treated with topical anti-inflammatories, antibiotics, autologous serum, third eyelid flap, conjunctival flaps etc. The corneal ulcers which do not show the tendency to heal with the medical management and any of the surgical procedures require the corneal allograft transplantation. A new technique of equipping Vacuum trephine for corneal allograft transplantation is gaining popularity and thus improves the quality of life of the dogs, which would otherwise go blind.

2. Materials and Methods
In the present study, vacuum trephined corneal allograft was transplanted for non-healing corneal ulcers and opacities in six clinical cases of dogs presented to the department of veterinary surgery and radiology, veterinary college hospital, Bengaluru. The instruments required for vacuum trephined corneal graft transplantation were: General ophthalmic surgical set (Plate 1) and Vacuum trephine (Plate 2) used to trephine the precise diameter of the corneal button from the donor eye.

2.1 Harvesting of the donor cornea
The healthy donor eyes were collected from the dogs immediately after euthanasia or death by enucleation of the globe. After enucleation, a sterile gauze sponge was placed around the sclera to secure the eye and the cornea was excised along with part of the sclera by dissecting off from the other tunics of the eye using corneal scissors.

The donor cornea thus obtained was cleane.d with the normal saline and 1:1000 diluted povidone iodine solution. The sclera along with cornea thus collected was suspended with concave side upwards into the corneal preservation media (Cornisol). This cornea was stored by refrigeration at 4 °C till its use at the time of transplantation to the recipient corneal bed.

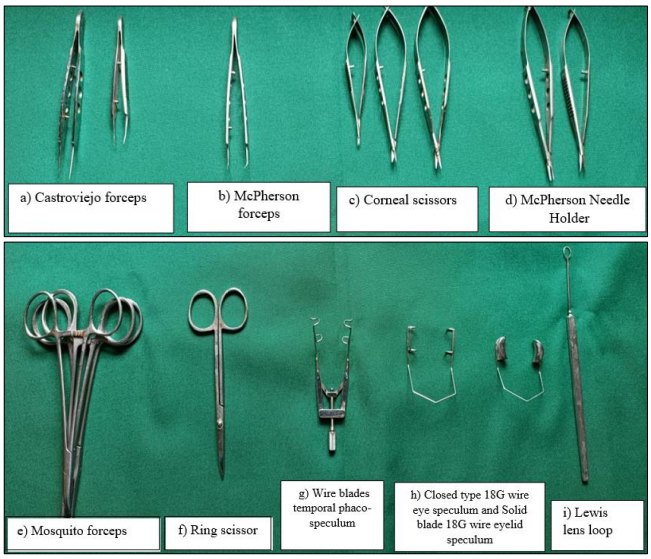

Plate 1: Ophthalmic surgical set

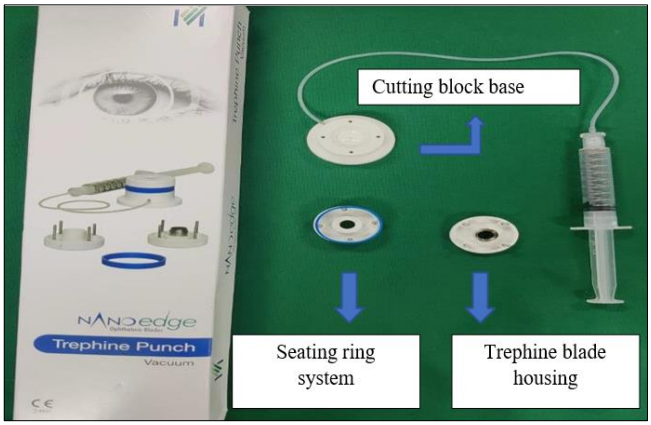

Plate 2: Vacuum trephine

2.2 Positioning of the patient

The dogs were positioned on lateral recumbency, with the affected eye facing the ophthalmic surgical microscope. The sterile drape was applied to cover the whole body by keeping only the operating eye open. The eye lid speculum was applied to keep the eye open throughout the surgery. The temporary sutures were applied to the bulbar conjunctiva to secure the globe to avoid movement during the surgical procedure.

2.3 Preparation of the recipient bed

The area of the cornea which was ulcerated or opacified was marked using trephine blade by partial incision into the thickness of the cornea. Further the diseased cornea was dissected full thickness along the line of partial thickness incision using corneal scissors (Plate 9). The anterior chamber was irrigated with Ringer's lactate solution to clear the collagenous or fibrin material suspended in the anterior chamber fluid. Following irrigation of anterior chamber, the recipient bed was made ready for transplantation of the corneal button by applying hydroxypropyl methyl cellulose solution (Oxana PFS 2% ®) to maintain the contour of the recipient bed.

Plate 3: Donor cornea with sclera dissected out

Plate 4: Cornisol medium

Plate 5: Positioning of the eye to be operated

Plate 6: Stay sutures applied to secure the globe**.**

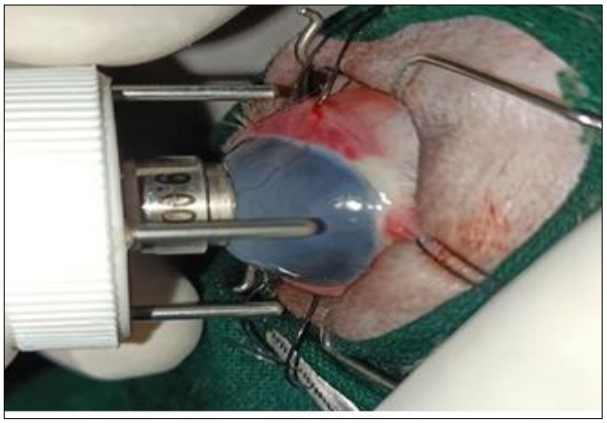

Plate 7: Marking diameter of the diseased cornea with trephine blade

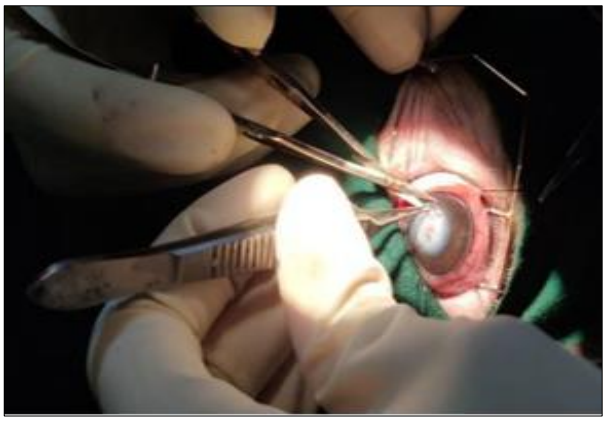

Plate 8: Dissecting the diseased cornea with Corneal scissors

Plate 9: Cardinal sutures at each quadrant

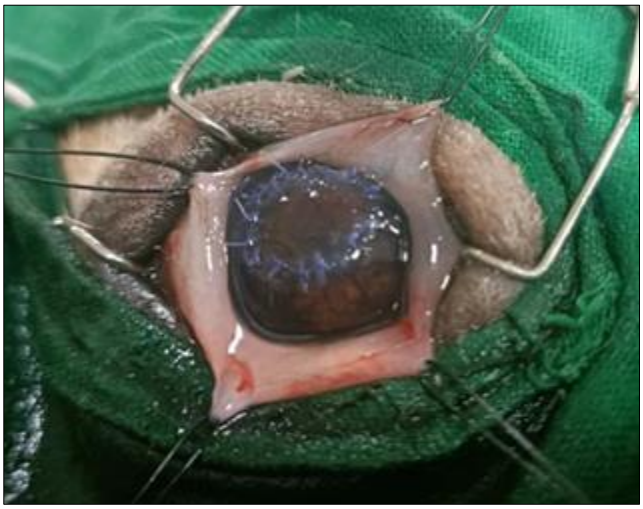

Plate 10: Additional simple interrupted sutures

Suturing the donor corneal button to the recipient bed

The vacuum trephined corneal button (allograft) was carefully placed over the recipient bed. The corneal button was sutured to the recipient bed with four cardinal sutures in the four quadrants with Polyglactin 910 no 6-0. Once the allograft was stabilised, an additional eight to sixteen simple interrupted sutures were applied.

3. Results and Discussion

The physiological parameters like rectal temperature, heart rate, respiratory rate were in the normal range throughout the period of study and were not affected by the corneal ulcers and opacities. These observations were similar to the findings of Plummer (2009) [16], Goulle (2012) [10] and Anwar *et al.* (2014) [2].

The hematological parameters like total erythrocyte count, Haemoglobin, Total Leukocyte count, Differential leukocyte counts e carried out in the study were in the normal range throughout the period of study and were not affected by the corneal ulcers and opacities. This was in accoradance with Nagashree (2019) [15], Mahapatra (2020) [14], Wichayacoop *et al.* (2009) [19], Costa *et al.* (2019) [5] and Gogova *et al.* (2020) [9].

Plate 11: Day 0 image immediately after stabilizing the corneal allograft to the recipient bed

Plate 12: Day 14 image showing vascularization from all the quadrants towards the allograft

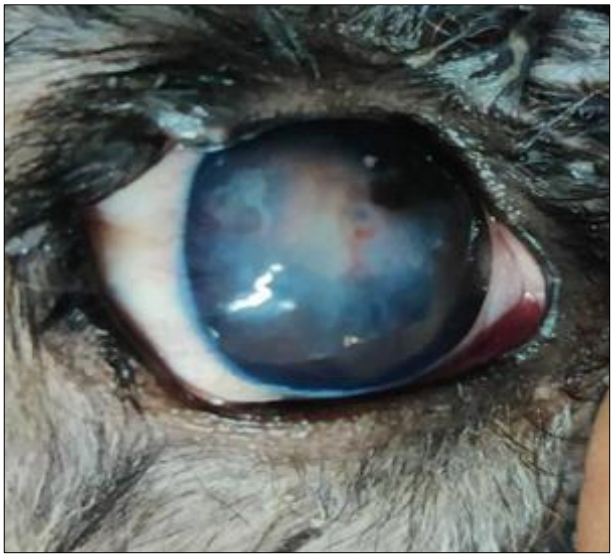

Plate 13: Day 28 image depicting the irregular granulation tissue

Plate 14: Day 45 image showing acceptance of the corneal allograft without any complications.

Plate 15: Day 60 image showing focal spot of haziness and few superficial visible vessels from a part of the limbus (2 mm)

The biochemical parameters like serum Alanine aminotransferase and serum creatinine carried out in the study were well within the normal range and were not affected by the technique used to carry out the procedure. This was in accordance Bagley and Lavach (1994) [3], Prasanna (2022) [17].

The clinical evaluation of the operated eye was carried out with the parameters like Schirmer tear test, Fluoroscein dye test were normal and remained non-significant throughout the period of study. This is in accordance with Williams and Burg (2017) [20].

All the dogs showed the reflexes of vision function tests *viz*., Menace reflex, Palpebral reflex, Pupillary light reflex and Cotton ball test by the end of last day of the observation. This is similar to the findings of Felchle and Urbanz (2001) [6].

The assessment of healing cornea was achieved with parameters like Corneal clarity, Corneal edema, and Corneal vascularization. The corneal clarity was assessed with following grades: 0: No haziness in cornea, 1: Focal spot of haziness, 2: Iris details visible, 3: Iris details not visible, pupillary details visible and 4: Totally opaque cornea (Lacerda *et al.,* 2017) [13]. All the cases before the surgery were graded 3 and 4. All the cases attained grade 2 by the end of 45 days. This was in corroboration with the observations were in accordance with Lacerda *et al.* (2017) [13], Hansen and Guandalini (1999) [12] and Gisha (2017) [8].

The corneal edema was graded while assessing the corneal healing as grade 0: No Signs of edema, grade 1: Mild edema, grade 2: Moderate edema and grade 3: Severe edema (Fenzl *et al*., 2004 and Antonia *et al*., 2014) [7, 1]. Corneal oedema was absent (grade 0) in all the dogs on day zero. There was moderate corneal haze in four dogs and severe corneal haze by the 3rd post-operative day. All the cases attained grade o and grade 1 by 45th post-operative day. All the cases attained grade o and grade 1 by 45th post-operative day. This could be due to the anti- inflammatory and antibiotic medications properties could have assisted in the reduction of oedema and enhancement of the healing. This was in accordance with Gisha (2017) [8] and Grixti and Malhotra (2018) [11].

The scoring of the corneal vascularization was followed as according to Boisjoly *et al.*,1993; Nagashree (2019) [4, 15] score 0 – No visible vessels, score 1 – Mild superficial vascularisation, thin vessels visible with magnification, score

2 – Profuse superficial vascularisation visible to naked eye and score 3 – Extensive vascularisation with thick vessels originating from all quadrants. The corneal vascularization was graded as one in four cases and zero in two cases. This tributed to the healing of the donor cornea in all dogs due to combined effects of anti-inflammatory, antibacterial medicines and acceptance of the donor cornea by the recipient corneal bed. This was in corroboration with Boisjoly *et al.* 1993; Gisha (2017) [8, 4], Grixti and Malhotra (2018) [11] and Nagashree (2019) [15].

4. Conclusion

The corneal transplantation is a feasible and less invasive substitute than enucleation in which the vision of the dog can be restored to enhance the quality of life. Precise trephining of the size of the donor and recipient cornea which helps to minimize corneal scar and to facilitate primary healing of the wound for better vision. However, Graft rejection, Corneal opacity, post-operative suture dehiscence are the most encountered complications.

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