

Original Research Article

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# Comparison of Tear Production and Corneal Integration after Corneal Reconstruction Using Autologous Corneo Conjunctival Graft and Decellularized Bovine Omental Graft in Cats

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## ABSTRACT

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The present study was based on 26 feline cases that were presented to Department of Veterinary Surgery and Radiology & Department of Veterinary Clinical Complex, College of Veterinary Science, Proddatur, Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram with various corneal affections. Clinical and detailed ophthalmic examination was carried out to diagnose various affections related to cornea which were later divided into two groups and treated using autologous corneoconjunctival graft and decellularized bovine omental graft. Tear production was measured pre and post operatively using STT strips and corneal integrity was evaluated using epithelial healing time. STT showed that the tear production was consistently high on the day of presentation and early post-operative days which was later resolved to normal. Epithelial Healing Time was 21 days in group 1 and more than 25 days in group 2 suggesting that epithelialization was faster in group treated with autologous graft than xenograft.

## Introduction

The cat eye has a relatively large cornea and lens, a rod dominated retina and tapetum cellulosum, which is well

adapted to vision in dim light. The cornea is a transparent, avascular tissue, unmyelinated rich nerve fibers, non- keratinized epithelium, well-organized lamellae in the corneal stroma, dehydrated state and

optimal spacing between collagen fibrils that is concave on the inside and smooth and convex on the outside through which light beams travel on the way to the retina, as well as a refracting and protecting membrane (Maggs, 2013). The normal physio- anatomical characteristics and functions are disrupted in corneal diseases leading to loss of corneal clarity is typically the first sign of corneal disease and resulting in vision loss in animals (Gelatt and Brooks, 2011 and Anoop *et al.*, 2016).

The corneal thickness in cat ranges from 0.4 to 0.6 mm and tends to be thickest in the periphery and thinnest in the center, which is an important principle to consider when performing corneal surgery. Corneal affections are treated medically, surgically or with both depending on the type, extent, duration and underlying cause. (Laguna *et al.*, 2015).

The aim of surgical treatment of corneal affections is to preserve structural integrity and maintain vision by conserving corneal transparency (Balland *et al.*, 2015). Murphy *et al.*, (2001) opined that the tear test was a primitive method of estimating the amount of tears produced and Schirmer tear test-I assesses both reflex and basal tear production whereas Schirmer tear test-II exclusively measures basal tear production (Morreale, 2003). Jegou and Tromeur (2014) reported that STT was less than 10 mm for the affected eyes in 9.7 per cent of the eyes in their retrospective study of corneal affections. The normal Schirmer's tear test in a cat >15 mm of wetting per minute (Morreale, 2003).

Thajunnisa *et al.*, (2020) reported that the tear production was consistently high on the day of presentation and early post-operative review days due to possible corneal irritation but returned to the normal range by day 60 with complete resolution of the condition. Chow and Westermeyer (2015) used fluorescein stain to determine epithelialization in his retrospective evaluation of corneal reconstruction using A Cell Vet alone in dogs and cats. EHT was determined by the time it took for the complete graft re-epithelization, assessed by means of a negative fluorescein uptake of the grafted cornea. (Laguna *et al.*, 2015)

## Materials and Methods

The present study was carried on clinical cases presented to the Department of Veterinary Surgery and Radiology, Department of Veterinary Clinical Complex, College of

Veterinary Science, Proddatur and Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram related to Corneal affections in felines over a period of 12 months. Cats were subjected to clinical and detailed ophthalmic examinations including STT, FDT to arrive at confirmatory diagnosis of corneal affections. The cats that were considered for surgical treatment were divided into two groups, with 6 animals in each group and in Group I, The corneal affections were surgically treated with autologous corneo conjunctival grafting where as in Group II, the corneal affections were surgically treated with decellularized bovine omental grafting that procured from the Department of Livestocks Products Technology, College of Veterinary and Animal science, Kerala Veterinary and Animal sciences university, Mannuthy, Kerala. Post operatively the quantitative production of the aqueous portion of the tear film was measured using commercially produced dye impregnated STT strips with a printed millimetre scale on them. It was done by inserting a test strip into the junction between the medial 2/3rd and lateral 1/3rd of the lower eyelid and leaving it there for one minute (Murphy *et al.*, 2001) (Fig. 1). Then the strip was removed, and the distance of wetness was immediately measured in millimetres. The presence and extent of the corneal ulcers were assessed using fluorescein dye in the form of sterile, impregnated strips". An additional seidel test was performed in suspected cases to conform corneal perforations and also to evaluate the corneal healing and integration. Sterile Normal Saline was used to wet the strip before applying it to the superior bulbar conjunctiva (Fig.2). The cat was allowed to blink multiple times to disperse the stain over the cornea and the excess stain was flushed out of the eye with sterile Normal Saline. In positive cases, the exposed corneal stroma was stained by the fluorescein dye (Miller, 2001). The presence of descemetocoele was identified by a clear, unstained area at the base of a deep corneal defect. The fluorescein uptake by the exposed stroma in corneal ulceration was made clearly visible with the application of blue light over the ulcerated area (Mitchell, 2011). These both tests were performed on the day of presentation and subsequently on 7<sup>th</sup>, 14<sup>th</sup>, 28<sup>th</sup>, 35<sup>th</sup> and 42<sup>nd</sup> post operative days. Epithelial Healing Time was determined by the time it took for the complete graft re-epithelization, assessed by a negative fluorescein uptake of the grafted cornea. If the donor graft was fresh, the donor epithelium was not removed; therefore, epithelial healing time was not applicable in these eyes (Laguna *et al.*, 2015).

## Results and Discussion

In the present study, the placement of dye-impregnated STT strips into the junction between the medial 2/3<sup>rd</sup> and lateral 1/3<sup>rd</sup> of the lower eyelid aided in the quantitative measurement of the aqueous portion of tear production in all the animals. No difficulties have been faced in recording the values. The total mean  $\pm$  SE values of STT in groups I and II were  $17.19 \pm 1.74$  and  $17.69 \pm 2.36$  respectively. Both groups showed a significant ( $<0.05$ ) decrease in values from the preoperative day to 42<sup>nd</sup> post-operative day. There was no significant difference in the values between the groups, and the tear production was consistently high on the day of presentation and 7<sup>th</sup> and 14<sup>th</sup> post operative days in both groups but returned to normal physiological range by 42<sup>nd</sup> post operative day (Tables 1 and Graph 1).

The fluorescein dye strips had satisfactorily stained the ulcerated areas on the corneal surface of the affected eyes. The uptake of the dye by the corneal stroma and stain was enhanced by cobalt blue light was used and to evaluate the corneal epithelial defects. In cases with descemetocoele, the Descemet's membrane did not take the dye, whereas the surrounding stroma had taken the dye. The presence of corneal ulcer was confirmed by fluorescein dye uptake in all cases during different periodic intervals.

On the 7<sup>th</sup> postoperative day, all the cases of group I and II were found positive for the fluorescein dye test. On the 14<sup>th</sup> postoperative day, cases 1, 2 and 6 of group I, 2 to 6 of group II were found positive. On the 28<sup>th</sup> postoperative day, all the cases of group I were found negative (Fig.3-8) and cases 2, 5 and 6 of group II were found positive. By the end of the present study, except case-2 of group II (Fig.10), all the animals were found negative for the fluorescein dye test (Fig.9, 11-14)

EHT was determined by the time it took for the complete graft re-epithelization, assessed by means of a negative fluorescein uptake of the grafted cornea. It was 14 days in cases 3 to 5 of group I and case 1 of group II, 28 days in case 2 of group I and cases 3 and 4 of group II, 35 days in cases 1 and 6 of group I and cases 5 and 6 of group II, and epithelial healing was not completed by the

end of our study in case 2 of group II.

STT showed that the tear production was consistently high on the day of presentation and early post-operative days which later returned to the normal range by end of study with complete resolution of the condition. These findings were supported by [Thajunnisa et al., \(2020\)](#). This may be due to constant irritation, pain and discomfort due to corneal affections on the day of presentation and due to graft on early preoperative days.

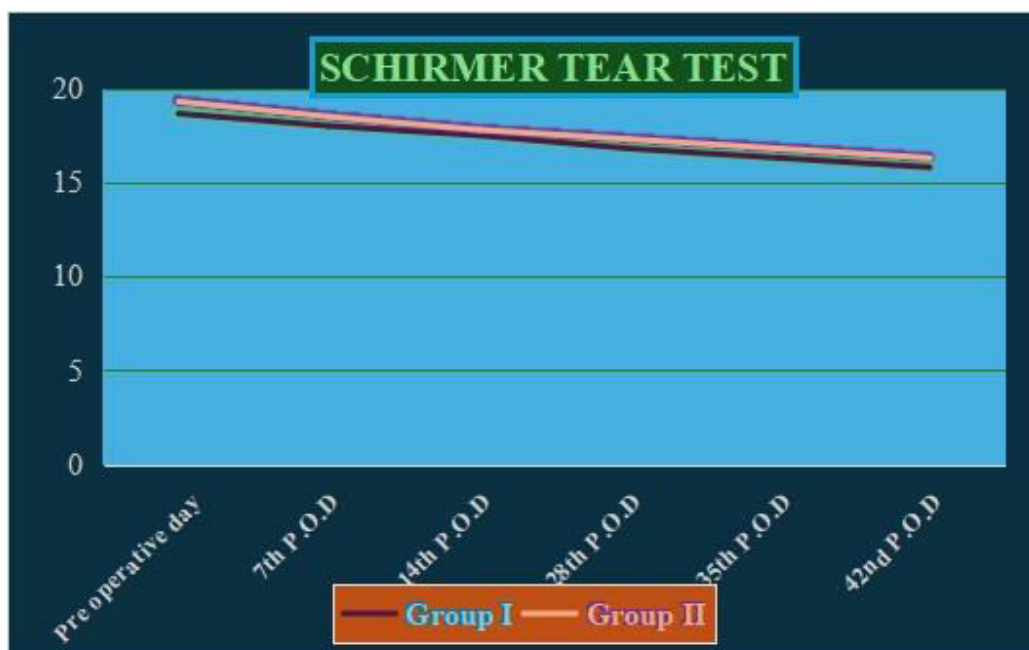
Fluorescein dye test was performed on post operative days to check the epithelialization of the graft by dropping normal saline over dye impregnated strip on superior bulbar conjunctiva. Similar procedure was performed by [Miller \(2001\)](#), [Laguna et al., \(2015\)](#) and [Chow and Westermayer \(2015\)](#). In case of descemetocoele, stain appeared as a doughnut-shaped ring in the walls of the ulcer but not at the centre, because descemet's membrane did not take up fluorescein stain, similar findings were reported by [Moore \(2005\)](#), [Mitchell \(2011\)](#) and [Maggs \(2013\)](#). The uptake of the dye by the corneal stroma was enhanced by the application of cobalt blue light over the corneal surface even in minor defects, similar finding was reported by [Moore \(2005\)](#). In group I treated with autologous graft were found negative for FDT by the end of 14<sup>th</sup> post operative day, whereas in group II treated with xenograft i.e., decellularized bovine omentum found negative for FDT by the end of 28<sup>th</sup> post operative day except in case 2. Similar results were obtained by [Vanore et al., \(2007\)](#) and [Balland et al., \(2015\)](#) who studied that graft integration was clinically evident by end of 35<sup>th</sup> day.

Epithelial Healing Time (EHT) was determined by the time it took for the complete graft re-epithelization, assessed by negative fluorescein uptake of the grafted cornea. Epithelial Healing Time (EHT) of cases treated with autologous graft i.e corneoconjunctival graft was 21 days. Similar study was recorded by [Laguna et al., \(2015\)](#) who reported the value of mean epithelial healing time as 19.2 days. In contrary to this, cases treated with xenograft i.e decellularized bovine omental graft showed mean epithelial healing time more than 25 days. The present study suggested that epithelialization was faster in group treated with autologous graft than xenograft.

**Table.1** The Mean  $\pm$  SE Values of Schirmer Tear Test (mm/min)

| Group   | Days of observation       |                             |                            |                            |                            |                           | Total Mean ± SE | p-value |
|---------|---------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|-----------------|---------|
|         | Pre-operative day         | Postoperative days          |                            |                            |                            |                           |                 |         |
|         |                           | 7 <sup>th</sup> day         | 14 <sup>th</sup> day       | 28 <sup>th</sup> day       | 35 <sup>th</sup> day       | 42 <sup>nd</sup> day      |                 |         |
| I       | 18.66 <sup>a</sup> ± 2.00 | 18.00 <sup>abe</sup> ± 2.19 | 17.50 <sup>cd</sup> ± 1.34 | 16.80 <sup>cd</sup> ± 1.80 | 16.30 <sup>dc</sup> ± 1.20 | 15.80 <sup>d</sup> ± 1.84 | 17.19 ± 1.74    | 0.005   |
| II      | 19.33 <sup>a</sup> ± 2.90 | 18.50 <sup>ab</sup> ± 2.50  | 17.80 <sup>b</sup> ± 1.80  | 17.30 <sup>bc</sup> ± 2.00 | 16.80 <sup>c</sup> ± 1.84  | 16.30 <sup>c</sup> ± 2.96 | 17.69 ± 2.36    | 0.009   |
| p-value | 0.289                     | 0.395                       | 0.401                      | 0.296                      | 0.209                      | 0.410                     |                 |         |

**Note:** Significant difference within the rows is shown in small letters (a, b, c, d, e, f)



**Graph:1** Mean  $\pm$  SE Values of Schirmer Tear Test (mm/min)



**Fig:1** Measuring Tear production using STT strip



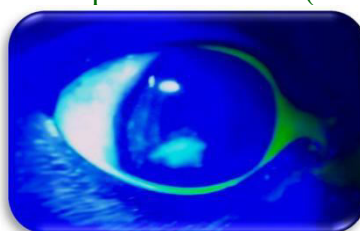
**Fig: 2** Performing Fluorescein dye test



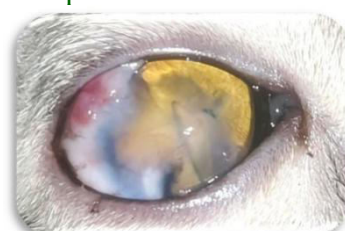
**Fig.3 Case 1: Deep Stromal Ulcer (OD) in Group I**



**Fig.3a** FDT on Pre operative day



**Fig.3b** FDT with cobalt blue light on pre operative day

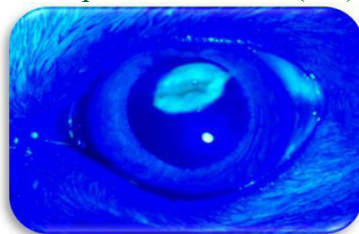


**Fig.3c** FDT negative on 28<sup>th</sup> day

**Fig.4 Case 2: Deep Stromal Ulcer (OS) in Group I**



**Fig.4a** FDT on pre operative day



**Fig.4b** FDT with cobalt blue light on pre operative day



**Fig.4c** FDT negative on 28<sup>th</sup> day

**Fig.5 Case 3: Deep Stromal Ulcer (OD) in Group I**



**Fig.5a** FDT on pre operative day

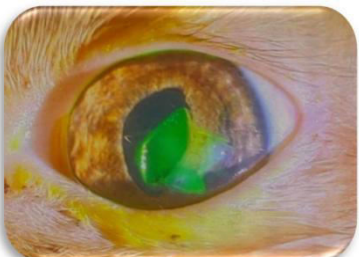


**Fig.5b** FDT with cobalt blue light on pre operative day

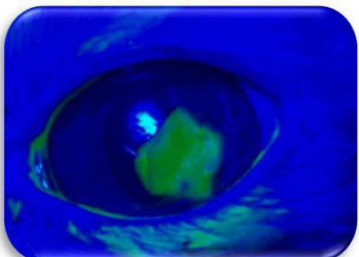


**Fig.5c** FDT negative on 14<sup>th</sup> day

**Fig.6 Case 4: Deep Stromal Ulcer (OS) in Group I**



**Fig.6a** FDT on pre operative day

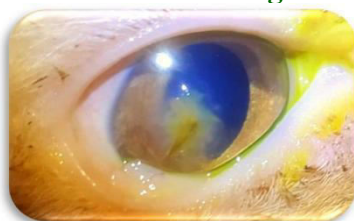


**Fig.6b** FDT with cobalt blue light on pre operative day

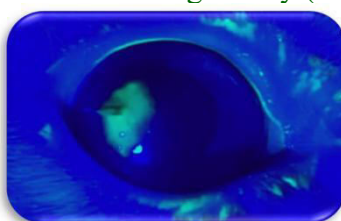


**Fig.6c** FDT negative on 14<sup>th</sup> day

**Fig.7 Case 5: Corneal Foreign Body (OD) in Group I**



**Fig.7a** FDT on pre operative day



**Fig.7b** FDT with cobalt blue light on pre operative day

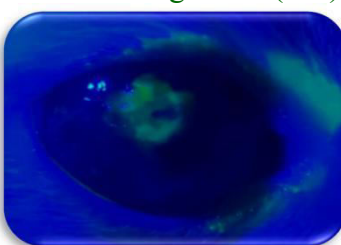


**Fig.7c** FDT negative on 14<sup>th</sup> day

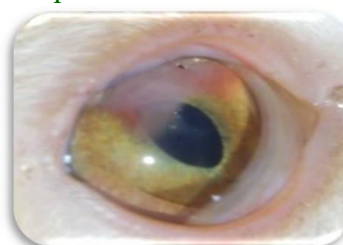
**Fig.8 Case 6: Perforating Ulcer (OD) in Group I**



**Fig.8a** FDT on pre operative day



**Fig.8b** FDT with cobalt blue light on pre operative day

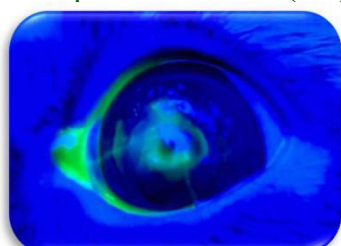


**Fig.8c** FDT negative on 28<sup>th</sup> day

**Fig.9 Case 1: Deep Stromal Ulcer (OS) in Group II**



**Fig.9a** FDT on pre operative day

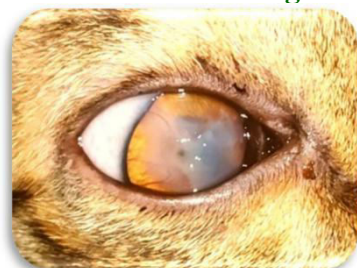


**Fig.9b** FDT with cobalt blue light on pre operative day

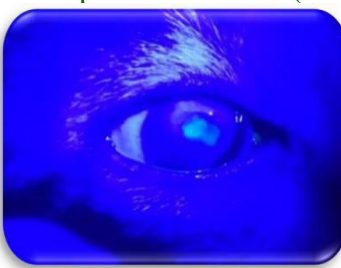


**Fig.9c** FDT negative on 14<sup>th</sup> day

**Fig.10 Case 2: Deep Stromal Ulcer (OD) in Group II**



**Fig.10a** FDT on pre operative day



**Fig.10b** FDT with cobalt blue light on preoperative day



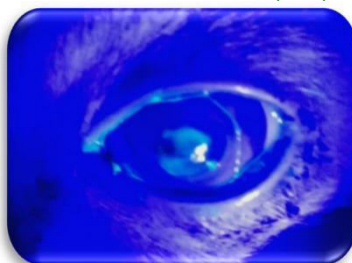
**Fig.10c** FDT was positive till end of study



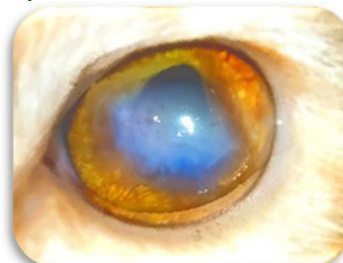
**Fig.11** Case 3: Descemetocoele (OS) in Group II



**Fig.11a** FDT on pre operative day



**Fig.11b** FDT with cobalt blue light on pre operative day

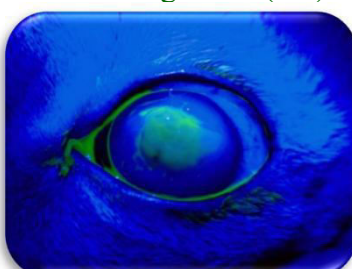


**Fig.11c** FDT negative on 28<sup>th</sup> day

**Fig.12** Case 4: Melting Ulcer (OS) in Group II



**Fig.12a** FDT on pre operative day



**Fig.12b** FDT with cobalt blue light on pre operative day

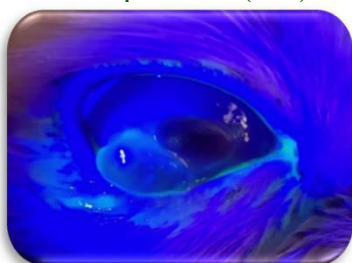


**Fig.12c** FDT negative on 28<sup>th</sup> day

**Fig.13** Case 5: Sequestrum (OD) in Group II



**Fig.13a** FDT on pre operative day



**Fig.13b** FDT with cobalt blue light on pre operative day

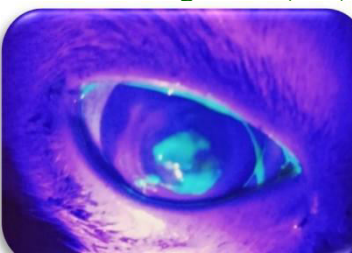


**Fig.13c** FDT negative on 35<sup>th</sup> day

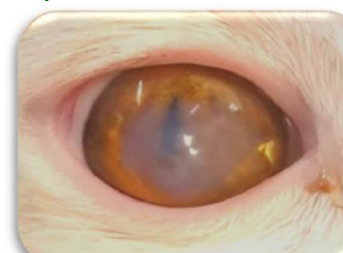
**Fig.14** Case 6: Perforating Ulcer (OD) in Group II



**Fig.14a** FDT on pre operative day



**Fig.14b** FDT with cobalt blue light on pre operative day



**Fig.14c** FDT negative on 35<sup>th</sup> day

This finding may be due to autologous graft that maintains continuous blood supply to the affected area and provides an immediate source of corneal stromal tissue to fill in the defect (Andrew *et al.*, 2001).

From this study, we conclude that there was no significant difference in the values between the groups, and the tear production was consistently high on the day of presentation and 7<sup>th</sup> and 14<sup>th</sup> post operative days in both groups but returned to normal physiological range by 42<sup>nd</sup> post operative day whereas EHT suggested that corneal integration and epithelialization was faster in group treated with autologous graft than xenograft which may be due to autologous graft that maintains continuous blood supply to the affected area and provides an immediate source of corneal stromal tissue to fill in the defect.

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### Author Contributions

David Jeevanraj Giddalur: Investigation, formal analysis, writing—original draft. Rambabu Kalaka: Validation, methodology, writing—reviewing. P. Vidya Sagar:—Formal analysis, writing—review and editing. A. Prasanth Babu: Investigation, writing—reviewing. K. Anjali: Resources, investigation writing—reviewing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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