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Studies on evaluation of autologous concentrated growth factor membrane for the treatment of corneal ulcer in dogs

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Abstract

The efficacy of Concentrated Growth Factor Membrane (CGFM) in the healing of deep corneal ulcers was studied in clinical cases in dogs presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru. Six dogs which were having deep corneal ulcer, melting stromal ulcer, descemetocoele and staphyloma were selected irrespective of age, breed and sex. All of the six dogs selected were Shih Tzu. The age of affected animals ranged from four months to 24 months with four males and two females. In all the six cases under the study, autologous concentrated growth factor membrane of adequate size to cover the lesion was obtained by following Sacco's protocol (2006). Scoring for corneal opacity (area and severity), neovascularisation and fluorescent staining (area and severity) were recorded at appropriate intervals and significant changes were noticed before and after treatment with CGFM. All the animals which were visually impaired on day zero with respect to the affected eye improved gradually and had complete restoration of vision by the end of study period. By promoting early epithelialisation and healing with minimal scarring, the autologous CGFM prepared as per Sacco's protocol resulted in restoration of complete vision function.

Keywords: Dogs, eye, corneal ulcer, concentrated growth factor membrane, CGFM

Introduction

Cornea acts as a barrier to shield the eye from the outside world (Kaushal *et al.*, 2024) ^[1]. Numerous factors such as trauma, precorneal tear film shortages or defects, irritants, abnormalities of the eyelids or lashes, immune-mediated or allergic inflammation, foreign bodies or the inability to blink, could cause corneal ulcers. Breed-specific predispositions based on eyelid and craniofacial conformation has also been linked to an increased incidence of corneal ulcers (Packer *et al.*, 2015) ^[2]. There are four types of corneal ulcers: superficial keratitis, deep corneal keratitis, descemetocoele keratitis and perforation keratitis (Kim *et al.*, 2009) ^[3]. Appropriate treatment of corneal ulcer is required for preventing the development of more complicated ulcer forms, which could result in increased ocular pain, corneal perforation and blindness (James- Jenks *et al.*, 2023) ^[4]. Many treatments have been tried in corneal lesions. Some of these methods included corneal tissue sealants, conjunctival grafts, third eyelid flap, tarsorrhaphy and keratotomy, applications of soft contact lens, amniotic membrane application, small intestinal submucosa application, renal capsule application, pericardial application, stem cell applications and platelet-rich plasma application (Melek *et al.*, 2022) ^[6]. Concentrated Growth Factor (CGF) is a new generation of platelet concentrate products, which exhibits an interesting clinical and biotechnological application potential (Borsani *et al.*, 2015) ^[5]. This study was undertaken to evaluate the efficacy of concentrated growth factor membrane for the treatment of corneal ulcer in dogs.

Materials and Methods

The study on evaluation of autologous concentrated growth factor membrane for healing of corneal ulcer was conducted in clinical cases of dogs presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru. Six dogs with

Corneal affections like deep corneal ulcer, melting stromal ulcer, descemetocoele and staphyloma which were found fit for surgery were chosen for the study irrespective of age, breed and sex. A detailed and thorough clinical and ophthalmic examination was carried out in all the six cases under present study. Evaluation of haematological parameters (total erythrocyte count (millions/cmm), haemoglobin (g/dL), total leukocyte count (thousands/cmm), differential leukocyte count (%) and platelet count (thousands/ μ L)) and biochemical parameters (serum creatinine (mg/dL), blood urea nitrogen (mg/dL) and serum alanine aminotransferase (U/L)) were done before and after treatment with CGFM. Under general anaesthesia, following aseptic precautions and sufficient

wound debridement, corneal grafting was done with CGFM. All the corneas were protected using tarsorrhaphy which was retained up to seven days.

Ophthalmic examination

A detailed ophthalmic examination was carried out to assess the condition of the eye. The tear production was assessed using STT-1 (figure 1) and the extent of ulceration was assessed by means of fluorescein dye test (figure 2). The Semi quantitative preclinical ocular toxicity scoring (SPOTS) system of grading for monitoring changes in corneal opacity and vascularisation as described by Eaton *et al.* (2017)^[8] was also done before and after CGFM graft.



Fig 1: Schirmer Tear Test with the test strip in-situ



Fig 2: Positive fluorescein test indicated by dye retention at the ulcerated site

Pre-operative preparation

Solid food was withheld in all dogs for 12 hours and water for 6 hours prior to surgery. The affected eye was thoroughly lavaged with normal saline and peri ocular area was cleaned with sterile cotton. The eyelashes were clipped close to the palpebral border and eye was irrigated with sterile normal saline solution and 1:40 dilution povidone iodine solution.

Anaesthetic protocol

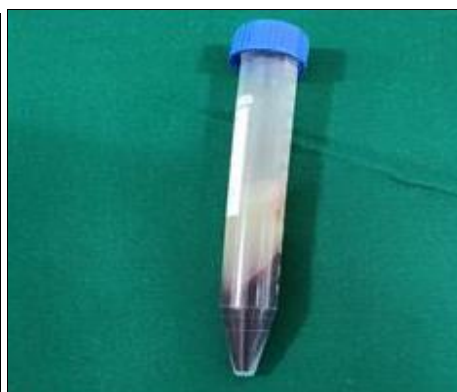
All the dogs were premedicated with atropine sulphate (Atropine Sulphate® injection IP, (0.6 mg/ml), Harson Laboratories, Akota, Baroda - 20) at the dose rate of 0.045 mg/kg body weight intramuscularly. Sedation was achieved with administration of xylazine hydrochloride (Xylaxin® injection USP, (20 mg/ml), Indian Immunologicals Ltd., Hyderabad - 33) at the dose rate of 1.0 mg/kg body weight intramuscularly. General anaesthesia was induced and maintained with thiopentone sodium (Thiosol® injection (500 mg), Neon laboratories Ltd, Mumbai) at the dose rate of 12.5mg/kg intravenously given to effect.

Preparation of autologous concentrated growth factor membrane (CGFM)

According to the Sacco's (2006) protocol for preparation of the autologous Concentrated Growth Factor (CGF) membrane, a 7ml of fresh blood was collected in 15mL anticoagulant-free tube with a scalp vein (21-23G), under sedation prior to surgery. The tubes were immediately centrifuged in a centrifuge (Eppendorf Centrifuge 5804/5804 R, Eppendorf SE, Hamburg, Germany) with a special program of centrifugation; acceleration x 30", 2,700 rpm x 2', 2,400 rpm x 4', 2,700 rpm x 4', 3,000 rpm x 3' and deceleration x 36" and stop. Following centrifugation red blood cells aggregated at the tube's bottom, concentrated growth factor gel in the center, and platelet-poor plasma (PPP) at the tube's top. The gel in the middle part of the sample and the red blood cells underneath were gently removed from the tube and the red blood cells below were cut out with sterile scissors. The clots were gently pressed between sterile gauze to obtain the CGF membrane (Figure 3).



A



B

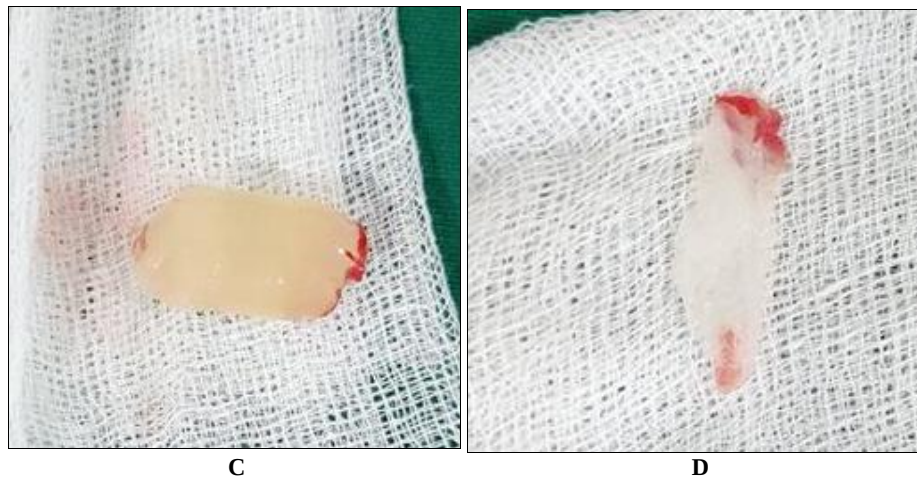


Fig 3: Preparation of autologous Concentrated Growth Factor Membrane (CGFM), A: The high- speed Eppendorf Centrifuge, B: Tube after centrifugation with separated CGF gel, C: Concentrated growth factor gel, D: Concentrated growth factor membrane obtained after pressing with sterile gauze

Surgical technique

Animal was positioned in lateral recumbency with affected eye facing upwards and draped. The superficial cornea was debrided to remove loose epithelium and debris along the margin of the ulcer. Corneal scissor was used to excise the non-viable epithelial tissue. The eye was flushed with 1:40 povidone iodine solution. The CGFM graft was applied over the lesion and secured using polyglactin 910(VICRYL[®]™, Johnson and Johnson Pvt.Ltd, Maharashtra-36) size 6-0. Simple interrupted sutures were placed to secure the graft to bulbar conjunctiva. Peri operatively, continuous irrigation with sterile isotonic normal saline was done to rehydrate the graft and to prevent desiccation of cornea. A temporary tarsorrhaphy was performed in all the 6 cases using 3-0 nylon (TRULON®, Healthium Medtech Ltd, Bangalore-58) to protect the cornea and the graft in all the dogs (Figure 4).

Post-operative care

Post-operatively an Elizabethan collar was advised in all cases till complete healing to prevent self-mutilation and injury to the eye. Ceftriaxone (Intacef, Intas Pharmaceuticals Ltd., Ahmedabad-54) was given parenterally at the dose rate of 20 mg/kg body weight, in all the cases on the day of surgery. Use of moxifloxacin eye drop (Ophthocare-M, Corise Healthcare Pvt Ltd., Thane-01) at the dose rate of one drop twice a day topically was advised in cases which retained fluorescein stain after the seventh post-operative day. Cephalexin (Cephavet®, Sava Healthcare Ltd., Gujarat-35) at the dose rate of 20 mg/kg body weight was given orally twice a day for five days and meloxicam at the dose rate of 0.2 mg/kg body weight was given for three days (Melonex, Intas Pharmaceuticals Ltd., Ahmedabad). The tarsorrhaphy sutures were removed on seventh day post-grafting.



Fig 4: Surgical procedure: A: Positioning of the animal for surgery, B: Sterile draping for surgery, C: CGFM graft secured to bulbar conjunctiva using polyglactin 910 size 6-0 in simple interrupted pattern, D: Temporary tarsorrhaphy done using 3-0 nylon

Results and Discussion

In almost all the cases presence of corneal edema and scar tissue negatively affected the visual function scoring. Those animals which had an extensive corneal injury took longer time to regain visual functions. It was assessed by menace response, PLR and cotton ball test. By day 21st post grafting, complete vision function was noticed in all the six cases under present study. This was in agreement with the findings of Melek *et al.* (2022) ^[6] who used CGFM graft for treating corneal alkaline burns in Rabbits.

The reflex and basal aqueous tear production was measured by STT-1in the present study. The higher readings in schirmer tear test pre-operatively as compared to post-operative readings could be due to the excessive lacrimation due to pain and ocular irritation which gradually reduced as the ulcer healed. This was in accordance with the findings of Williams and Burg (2017) ^[7]. The Semi quantitative preclinical ocular toxicity scoring (SPOTS) system of grading for monitoring changes in corneal opacity and vascularisation was done before the surgery, on the day of surgery (0th day) and post-surgery on 7th, 14th, 21st and 28th days as described by Eaton *et al.* (2017) ^[8]. A minimal corneal opacity (severity and area) with score one was present in all the dogs except in one dog at the end of the study period. This was attributed to the findings of Riestra *et al.* (2016) ^[9] who reported in her study that blood products can modulate TGF- β activity thereby reducing tissue fibrosis and promoting cell regeneration.

In the present study all the six dogs showed positive fluorescein dye test on affected eye on the day of presentation which could be due to the fluorescein sodium stain taken up by exposed corneal stroma (Singh *et al.*, 2004) ^[10]. A minimal fluorescein staining (area and severity) with score one was present in three dogs by 7th post-operative day. By 14th post-operative day all the six cases showed negative fluorescein staining. This was similar to the findings of Ajin (2023) ^[11]

Who did autologous PRFM graft for deep corneal ulcer in dogs and was attributed to the epithelialisation of the cornea due to the grafted membrane. In the present study corneal neovascularisation increased during the healing period up to 14th day post grafting. By 21st day post grafting, corneal vascularisation was absent in all the cases except two. By day 28 it was almost nil in all the six cases. This finding was similar to the observations of Mishra *et al.* (2021) ^[12] who reported that anti-inflammatory nature of platelets and its growth factors promoted rapid healing with earlier and complete withdrawal of neovascularization.

Various haematological parameters estimated under the present study were within the normal range. The values of serum creatinine, BUN and alanine transaminase were also within the normal range and there were no significant difference observed between the pre and post- operative values. This was in accordance with the findings of Kim *et al.* (2009) ^[3], Vidhyasree (2022) ^[13]. In all the six cases under the study, a membrane of adequate size to cover the lesion was obtained by following Sacco's. The only complication observed after treatment with CGFM graft was corneal opacity. A minimal corneal opacity was present in all cases except one at the end of observation period. This was similar to the findings of Melek *et al.* (2022) ^[6] and Ajin (2023) ^[11] who used autologous grafts for corneal ulcer treatment. Bernardi *et al.* (2017) ^[14] stated that tissue regeneration was one of the current therapies proposed. Due to the autologous nature of these concentrates, all biocompatibility concerns were avoided. High quantity of growth factors, such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-B1 (TGF), TGF-B2, Fibroblast Growth Factor (FGF), Vascular Endothelial Growth Factor (VEGF) and Insulin like Growth Factor (IGF) were derived from platelets. They were reported to promote cellular proliferation, matrix remodeling and neo-angiogenesis (Figure 5, 6, 7, 8, 9 and 10).

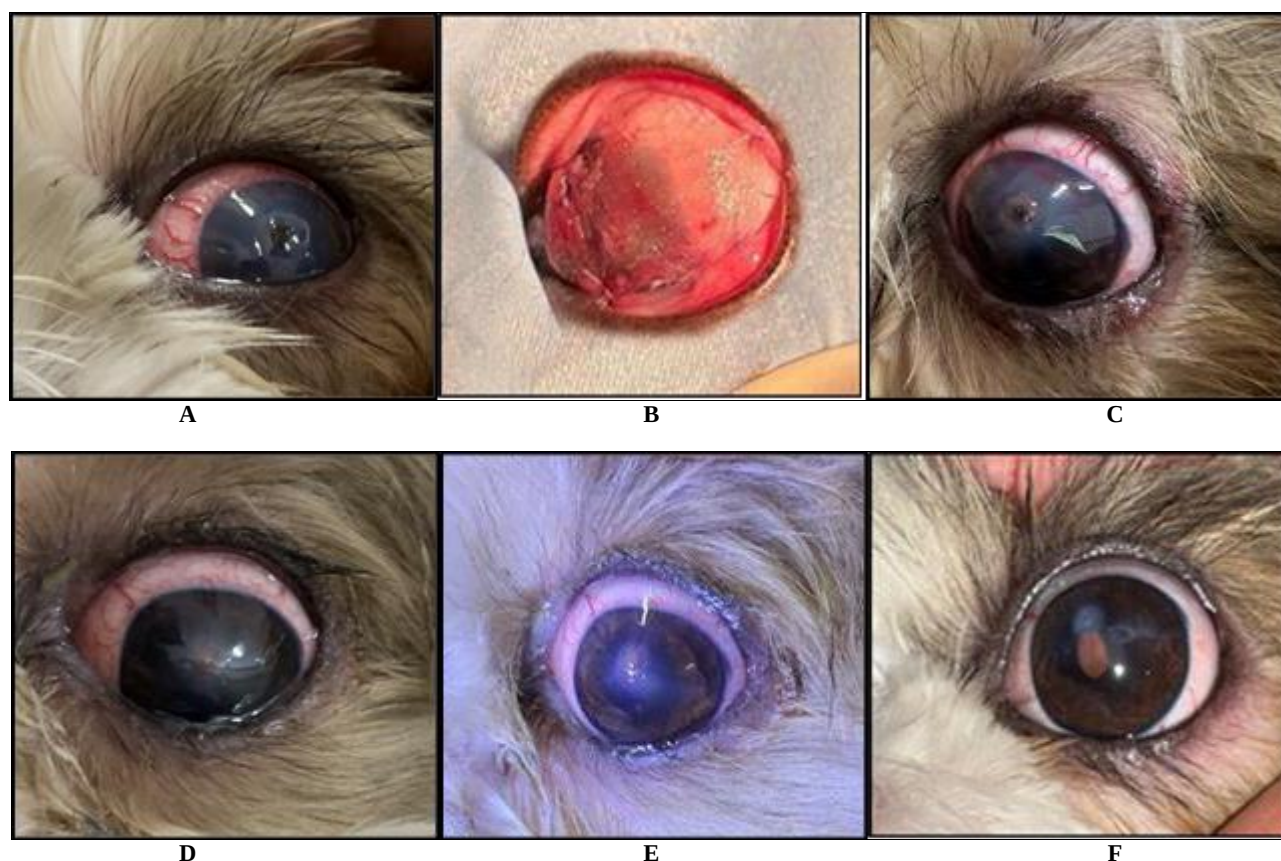


Fig 5: Pre and post-operative images of case 1, A: Descemetocoele, B: On the day of surgery, C: Neovascularisation and granulation, D and E Scar tissue formation, F: Minimal corneal opacity

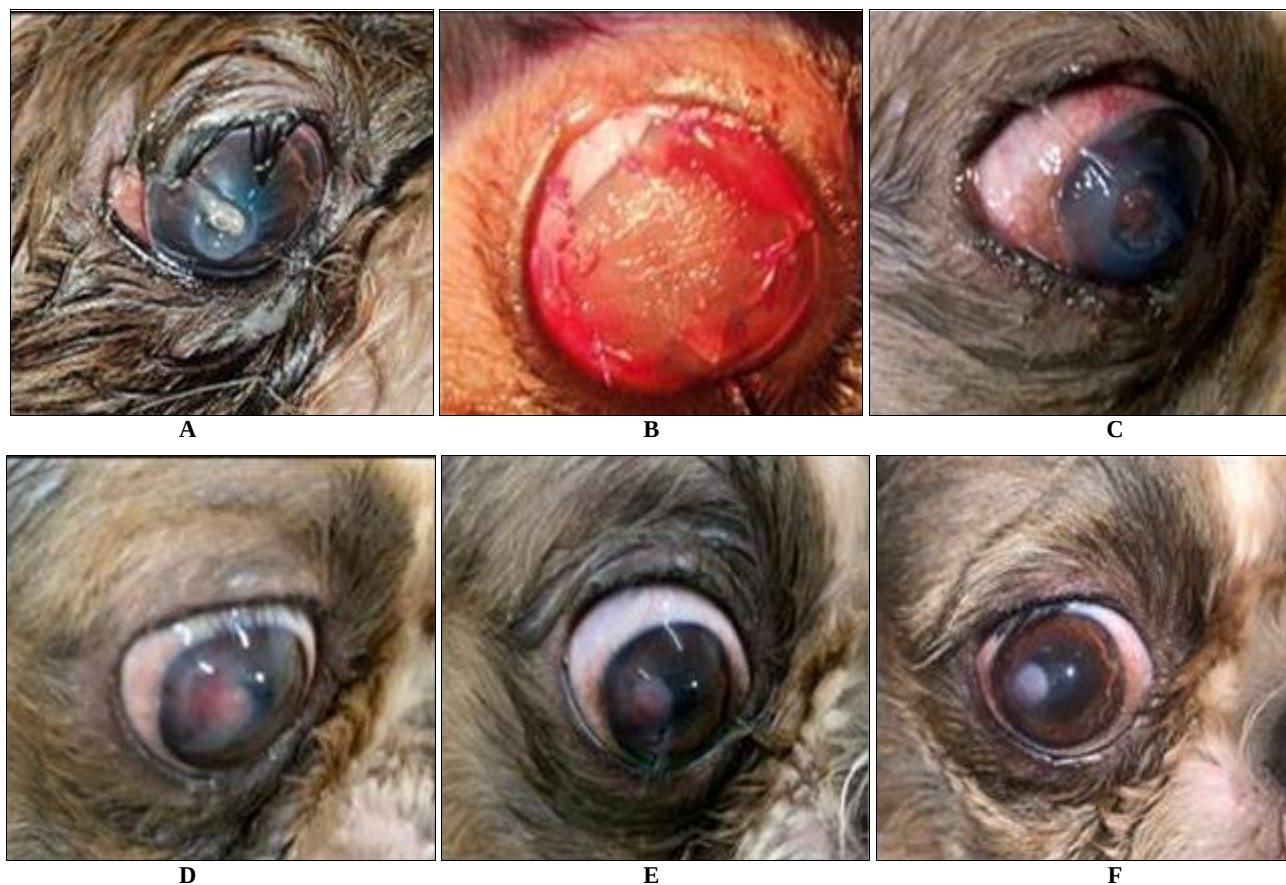


Fig 6: Pre and post-operative images of Case 2, A: Melting stromal ulcer, B: On the day of surgery, C: Granulation and edema, D and E Scar tissue formation, F: Minimal corneal opacity

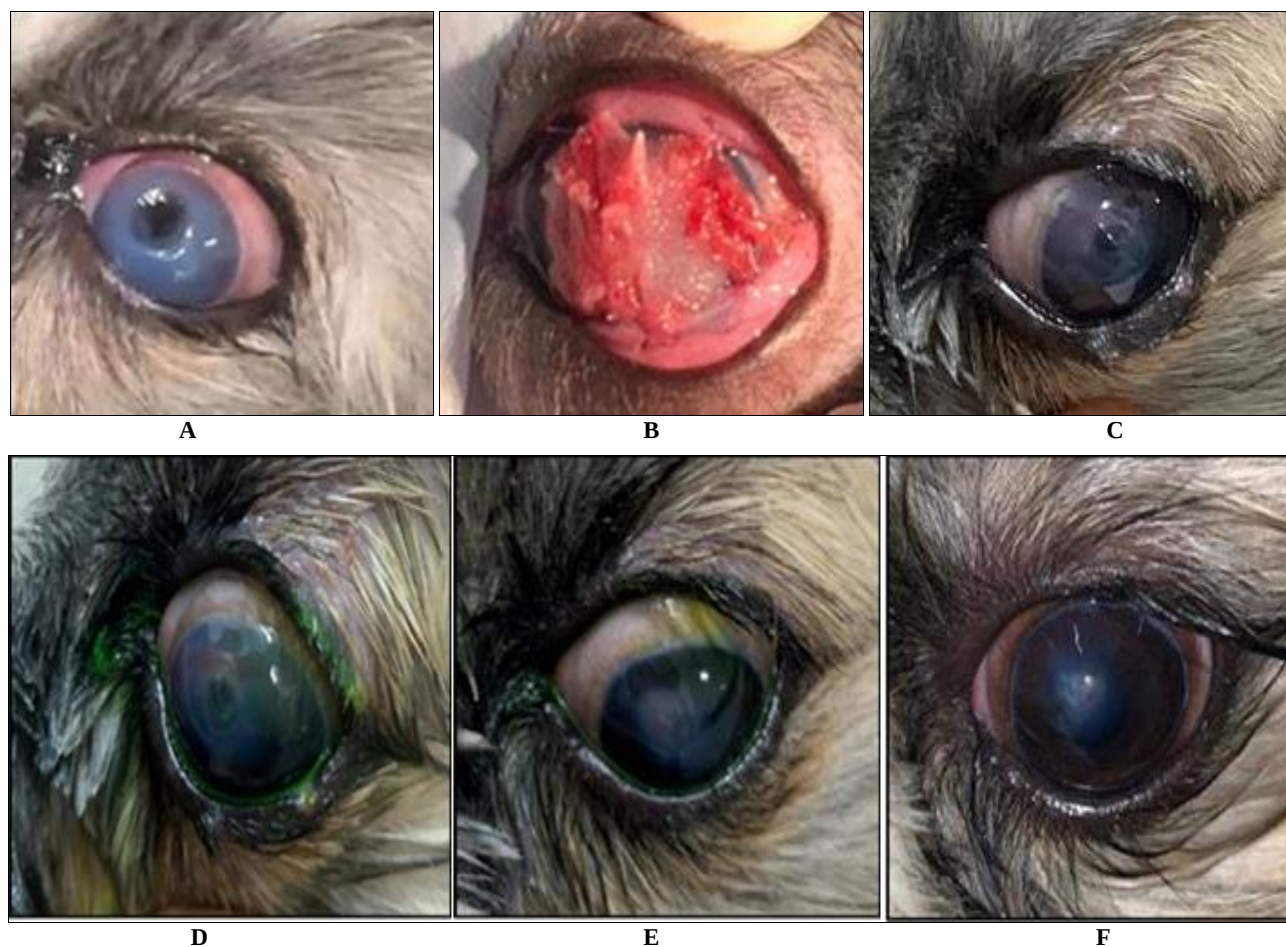


Fig 7: Pre and post-operative images of Case 3, A: Melting stromal ulcer, B: On the day of surgery, C: Neovascularisation and edema, D: Neovascularisation, E: Scar tissue formation, F: Minimal corneal opacity

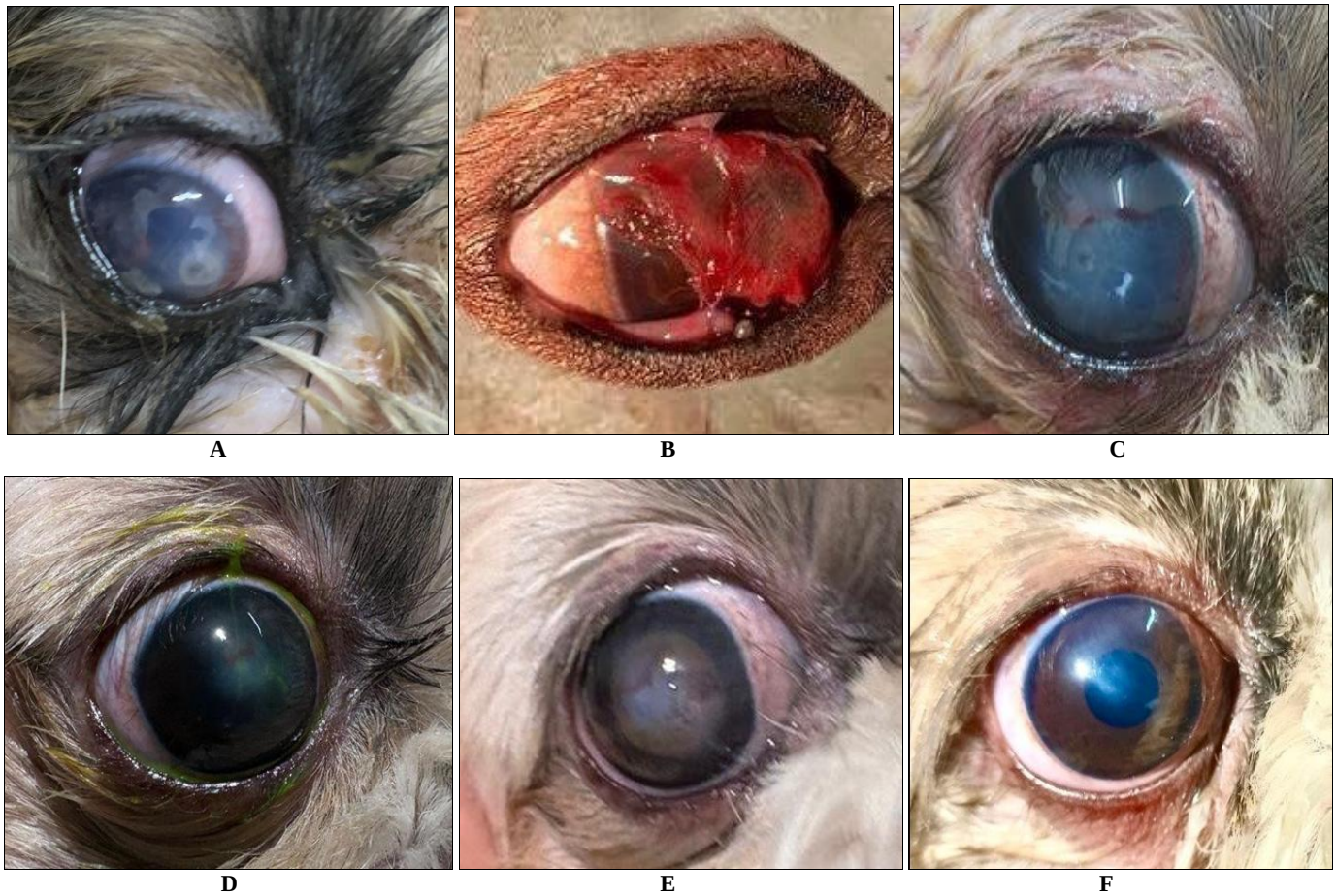


Fig 8: Pre and post-operative images of Case 4, A: Deep stromal ulcer, B: On the day of surgery, C: Neovascularisation and edema, D and E: Scar tissue formation, F: Clear cornea

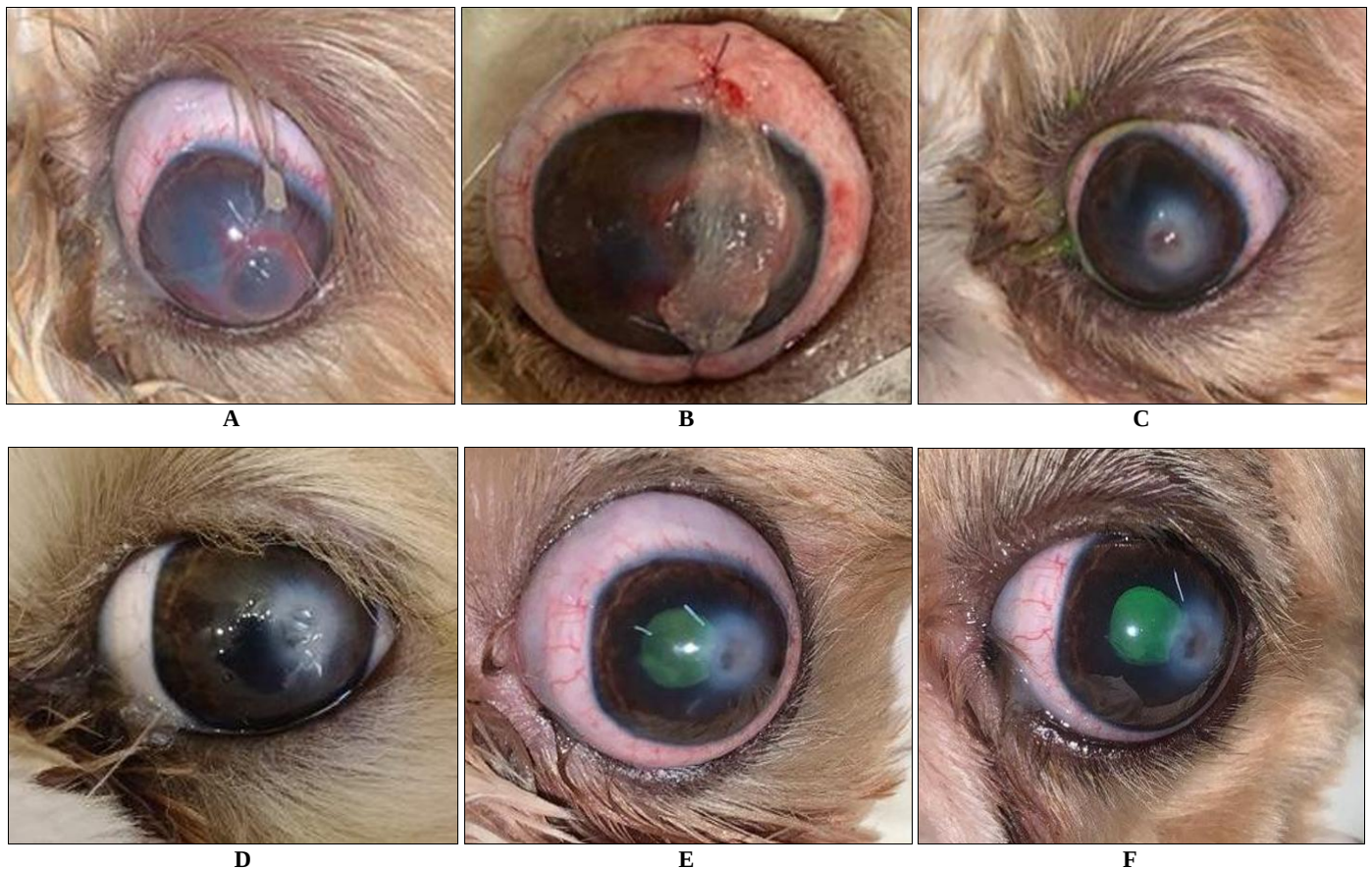


Fig. 9: Pre and post-operative images of Case 5, A: Deep stromal ulcer with bulla formation, B: On the day of surgery, C: granulation and edema, D and E: Scar tissue formation, F: Minimal scar tissue

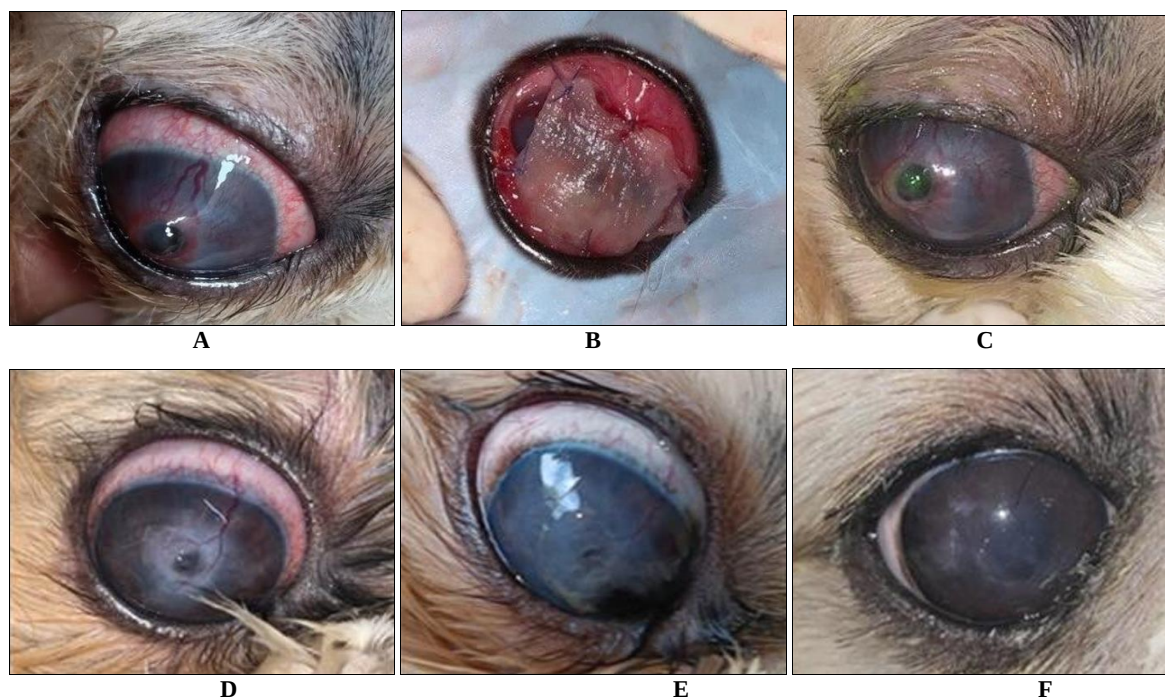


Fig 10: Pre and post-operative images of Case 6, A: Staphyloma, B: On the day of surgery, C: Neovascularisation and edema, D and E: Scar tissue formation, F: Minimal scar tissue

Conclusion

The autologous CGFM prepared as per Sacco's protocol provided a good tectonic and chemotactic support which in turn resulted in early epithelialisation and healing with minimal scarring which consequently lead to restoration of complete vision function in all the cases under present study. Timely diagnosis and appropriate treatment with canine autologous CGFM were effective in successful surgical management of deep corneal ulcers, melting ulcer, descemetocoele and staphyloma in dogs.

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