ORAL MUCOSAL AUTOGRAFTING, LOWER PUNCTAL OCCLUSION AND TRANSPLANTATION OF PROCESSED HUMAN AMNIOTIC MEMBRANE FOR MANAGEMENT OF DRY MELANOTIC CORNEA IN DOGS

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(17-MVP-09)

THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Oral mucosal autografting, lower punctal occlusion and transplantation of processed human amniotic membrane for management of dry melanotic cornea in dogs"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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EXTERNAL EXAMINER

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Dedicated to my Guide, Family and Dept. of VSR

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INTRODUCTION

1. INTRODUCTION

Dry eye disease or keratoconjunctivitis sicca (KCS) is an inflammatory disorder of the lacrimal functional unit of eye, and is multifactorial in origin. KCS leads to chronic ocular surface disease, impaired vision, and a wide range of complications, eventually causing reduction in quality of life of the animal. Clinical features of dry eye include unstable tear film, ocular surface inflammation and epitheliopathies like pigmentary keratitis and corneal ulcerations. In most cases of dry eye disease, cornea would be either ulcerated or melanotic, and vision will get impaired. Pigmentary keratitis or non-ulcerative keratitis is the inflammation of cornea with deposition of melanin pigments. It is also most commonly associated with conditions like chronic superficial keratitis, distichiasis, trichiasis and chronic ulcerative keratitis. Brachycephalic breeds, due to their anatomical peculiarities of their head, are more prone for dry eye syndrome and pigmentary keratitis.

Various treatment modalities are reported for treatment of dry eye in humans and dogs. Transplantation of oral mucosa as an autograft into the conjunctival fornix, is one such treatment which has shown satisfactory results in humans and dogs having severe dry eye syndrome. Secretions from the minor salivary glands of the oral mucosal transplant is suggested to serve as an alternative for tears, lubricating the ocular surface and thus treating dry eye. Occlusion of the lower puncta is another treatment adopted to prevent drainage of tears through the nasolacrimal duct and to increase the tear volume in the tear well. Superficial keratectomy is suggested and practised for removal of melanotic epithelial layer. Keratoplasty using processed human amniotic membrane, following superficial keratectomy, is reported to facilitate healing of corneal epithelium and superficial stroma thereby alleviating ocular surface inflammation and epitheliopathies. Medial canthoplasty corrects the medial canthal entropion, and thereby reduces the constant irritation to the eyes.

Considering all the treatment modalities, the present study “Oral mucosal autografting, lower punctal occlusion and transplantation of processed human amniotic membrane for management of dry melanotic cornea in dogs" was undertaken with the following objective

- To study the concurrent use of autografting with oral mucosa, lower punctal occlusion along with transplantation of processed human amniotic membrane for management of dry melanotic cornea in dogs

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. LACRIMAL FUNCTIONAL UNIT

Pflugfelder *et al.* (2000) opined that the ocular surface and tear secreting glands act in concert as a functional unit.

According to Stern *et al*. (2004) tear production is controlled by lacrimal functional unit which consists of main and accessory lacrimal glands, meibomian glands, and ocular surface including cornea, conjunctiva, and its associated sensory and motor nerves.

Miller (2013) reported that the lacrimal functional unit is critical in providing a pure optical surface for light refraction, preventing bacterial or viral infections and desiccation, and maintenance of homeostasis and lubrication of surface environment.

2.1.1. Sensory and motor innervations of the lacrimal functional unit

Dartt (1986) reported that the lacrimal gland is innervated by parasympathetic and sympathetic nervous systems and is also affected by various hormones.

Dartt (1994) described that stimuli from the cornea, conjunctiva, optic nerve and brain stimulate lacrimal gland fluid secretion using parasympathetic and sympathetic efferent pathways.

According to Sullivan *et al.* (1997), the lacrimal functional unit receives impulse of the status of the ocular surface through the afferent innervation of the first (ophthalmic) division of the trigeminal ganglion.

Pflugfelder *et al.* (2000) opined that the sensory nerve endings senses the status of the ocular surface and carries the impulse to the lacrimal centre of brain stem and from there the autonomic nerves send motor impulses to the secretory glands, including meibomian gland, main and accessory lacrimal glands and the conjunctival goblet cells. The author also reported that cornea is the most densely innervated epithelial surface in the body, while the rest of the ocular surface epithelia are populated by morphologically unspecified sensory neural receptors called free nerve endings.

2.2. CORNEA

Startup (1984) reported that corneal thickness varied from 0.73 mm to 0.95 mm in different animals, and the peripheral cornea was slightly thicker than the central cornea. The author also reported that the diameter of the cornea varied from 12 mm to 17 mm depending upon the breed of the dogs.

Miller (2001) described that the anterior epithelium of cornea is composed of stratified squamous epithelial cells, 2 to 3 layers of intermediate cells and a basal columnar layer. The stromal layer formed 90 percent of the cornea and it is composed of collagen, glycosaminoglycan and water. Descemet's membrane lies next to the stromal layer, is elastic in nature, and more resistant than stroma. The endothelium is the innermost layer which is made up of a single layer of hexagonal shaped endothelial cells.

According to Janssens (2007), the cornea is composed of epithelium, basement membrane, stroma, descemet's membrane and an endothelial layer. The control of water content in the cornea and non-keratinised surface, arrangement of its collagen fibrils and the absence of the blood vessels gives cornea a unique transparent nature.

2.3. CONJUNCTIVA

According to Hartley (2006), the normal conjunctival epithelium is of non-keratinising squamous type in palpebral and bulbar conjunctiva, while it is of cuboidal stratified epithelium, six or more layers thick in the fornices. The author also opined that the fornices are having microvilli structures which act as the attachment points for mucoid component of tear film.

Doughty (2012) mentioned that the conjunctiva is made up of non-stratified cuboidal epithelium comprising a mixture of epithelial cells and mucus-secreting goblet cells.

2.4. MAIN LACRIMAL GLAND

Getty (1975) reported that the lacrimal gland is flattened in shape due to its location between the bony orbit and the eyeball. In dogs, the lacrimal glands are small, flat and lobular in structure and it lies on the medial side of the orbital ligament within the periorbital fascia.

According to Sakai (1989), the main lacrimal gland is located in the dorsolateral aspect of the orbital cavity.

According to Cormack (1996), the lacrimal glands of dogs consist of secretory units called acini which secretes through its ducts on to the conjunctival surface. The author also reported that the secretory glandular terminals of lacrimal gland consisted tubuloacinar units and acini arranged around the tubular segments.

Evans and de Lahunta (2010) reported that, in dogs the lacrimal gland is tubuloacinar gland and which approximately produce 60% of the serous portion of tears in dogs. The main lacrimal gland secretes tears through its small microscopic ducts into the dorsal conjunctival fornix.

2.5. ANATOMY AND DISTRIBUTION OF MINOR SALIVARY GLAND IN ORAL MUCOSA

Nickel *et al*. (1979) described that minor salivary glands in dogs are localised in the area demarcated from canine to third cheek tooth.

Murube (1997) reported the existence of a large number of the minor salivary glands between the quadratus labi and the lip or the buccal mucosa. He also described labial minor salivary glands as a potential alternative lubricant source for the ocular surface in humans for dry eye.

Minor salivary glands in the oral mucosa can be located in close proximity with the epithelial lining and in the interior of the lamina propria-submucosa in dogs (Dyce *et al*., 2004; Frappier, 2006).

Salivary glands are mainly classified into minor and major salivary glands. Parotid, mandibular, monostomatic and polystomatic sublingual and zygomatic glands are categorised as the major salivary glands. The labial, buccal, molar, lingual, and palatine are grouped under the minor salivary glands in canine species (Gioso and Carvalho, 2005; Evans and de Lahunta, 2013).

The minor salivary glands may be mucus, serous or mixed, and are the miniaturized forms of the major salivary glands (Frappier, 2006).

Cherry *et al*. (2018) reported that there were no minor salivary glands detected at the following locations of canine oral cavity: upper rostral labial mucosa at the midline, lower rostral labial mucosa at the midline, upper labial mucosa near the commissure, lower labial mucosa near the commissure and the buccal mucosa approximately 1 cm caudal to the commissure.

2.6. PRE CORNEAL TEAR FILM

According to Wolff (1954), the precorneal tear film is composed of three layers, the innermost mucus layer, middle aqueous layer, and outermost lipid layer.

Iwata (1983) and German *et al.* (1998) reported that aqueous layer contains lactoferrin, lysozyme, secretory immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), albumin, transferrin, ceruloplasmin, tear specific prealbumin, and glycoproteins, which participate in the defense mechanisms of the ocular surface. Authors also reported that the components like inorganic salts, glucose, oxygen, and proteins in the aqueous layer provide nutrition to the avascular cornea.

Nichols (1985) reported that mucus layer is composed of mucin, immunoglobulins, urea, salts, glucose, leukocytes, cellular debris and enzymes.

According to Adams (1986), mucus entraps material, engulfs it in a mucus thread and moves the entire thread to the lower fornix where it is expelled from the eye. This mechanism allows the eye to constantly remove cellular debris, micro-organisms and foreign material. Author also reported that the deficiency of aqueous tears, damage to the epithelium or glycocalyx, or an increase in epithelial cell loss, allows mucus to adhere to itself or to the epithelium causing mucus clumping and leads to tear film instability and corneal damage.

Dilly (1986) reported that the mucus layer is secreted mostly by the conjunctival goblet cells; however, the corneal and conjunctival epithelium also contributes to the mucus layer.

According to Aura and Tiffany (1986), the aqueous part of the tear film provides protection and lubrication of ocular surface. They opined that, the presence of soluble mucin causes decrease in the surface tension, which improves the spread and attachment of aqueous layer and provides viscosity to the tear film and the presence of antibacterial factors like immunoglobulins provides protection to the cornea.

Gilbard *et al.* (1989) reported that lack of lipid layer leads to evaporation of tear film, reduction in tear film breakup time and increase in tear film osmolarity. The authors also opined that tear film osmolarity has an important role in the pathogenesis of dry eye.

According to Driver and Lemb (1996), meibomian glands which is a type of modified sebaceous glands, secrets the lipid layer of the tear film. The lipid layer prevents evaporative loss of tears and contamination of tear film with debris, and also provides a smooth optical surface. The authors also opined that the meibomian secretions remain as fluid in tear film due to their lower melting point than sebum. As the lipids spread over the corneal surface, the surface tension of tear film decreases and helps in drawing water into the tear film increasing the film thickness.

Nichols *et al*. (2011) suggested that tear film contains more mucin than previously thought. Since then, the tear film has been considered a bilayer structure (lipid and aqueous-mucin layers).

Hirayama *et al*. (2013) reported that the tear components, in addition to the protection of the eye from pathogens, helps to maintain the health of cornea by spreading the major source of proteins and electrolytes, lubricating and moistening the bulbar and palpebral conjunctiva.

2.7. BRACHYCEPHALIC OCULAR SYNDROME

Bedford and Jones (2001) reported that large palpebral fissures are usually accompanied by shallow orbits and lead to exophthalmos and causes external injuries. The authors also mentioned that exophthalomos can lead to the failure of normal blinking of the eyes called lagophthalmos which causes the corneal drying due to the failure of spreading of protective tear film.

According to Gelatt (2001), several brachycephalic breeds have a peculiarity of the skin overlying the short muzzle where the skin is not reduced in accordance to the proportion of the facial skeleton resulting in excessive wrinkles. The hair on the nasal fold (nasal fold trichiasis) rub on to the cornea and resulting in keratitis and ulceration.

Stades and Gelatt (2008) suggested medial canthoplasty as a surgical correction for brachycephalic ocular syndrome by shortening the macropalpebral fissure by 6-8mm.

Maggs (2013) defined brachycephalic ocular syndrome in dogs as a combination of lower medial entropion, exophthalmos, macropalpebral fissure, lagophthalmos, medial caruncular or nasal fold trichiasis, and “kinking” of the nasolacrimal canaliculi.

2.8. DRY EYE SYNDROME

Ribeiro *et al.* (2008) described that dry eye is a chronic inflammatory disorder due to excessive evaporation of tear film or deficiency of the same due to lack of tear production. The authors also stated that dry eye is the most common ophthalmic condition in dogs.

Dry eye is an inflammatory condition of the cornea, sclera and conjunctiva resulting from the lack of tear output which leads to mucoid to mucopurulent ocular discharge (Best *et al., 2*014).

Masmali *et al.* (2014) reported that dry eye is a multifactorial disease condition of tear and ocular surface resulting in discomfort, tear film instability and damage to the ocular surface.

2.9. CLASSIFICATION OF DRY EYE SYNDROME

Worda *et al.* (2001) classified dry eye into two types- aqueous deficiency type and evaporative type. Aqueous deficiency type is due to lack of tear output from the lacrimal gland. It is again classified into congenital type and acquired type. Congenital type is genetic in miniature long haired breeds and dachshunds mostly associated with curly hair gene. Acquired type is multifactorial in nature. Evaporative type is due to lack of lipid layer which lead to increased evaporation and reduced tear film because of meibomian gland dysfunction.

Ribeiro *et al.* (2008) classified dry eye into two types. The qualitative type or evaporative dry eye is characterised with reduced lipid and mucous production of the tear film and reduced TFBT (Tear film breakup time). The quantitative type is characterised by reduced aqueous portion of the tear film and reduced STT value.

2.10. ETIOLOGY

Sansom and Barnett (1985) reported (i) urinary analgesic like phenozopyridine hydrochloride causing destruction of the nictitating gland and lacrimal gland, (ii) atropine causing dryness of the eye (iii) sulpha which causes irreversible damage to the lacrimal gland, (iv) trauma to the nerve supplying the lacrimal gland, leading to lack of the tear output due to damage of the parasympathetic nerve (facial nerve), (v) hypothyroidism and (vi) canine distemper and chronic bacterial infection leading to infiltration of the inflammatory cells destroying the lacrimal gland as etiologies for dry eye syndrome in dogs.

Kaswan and Salisbury (1990) categorised the etiology of dry eye into three sub types, such as, (i) Immune mediated, (ii) secretory disorder, (iii) hormone imbalance and other miscellaneous factors. In immune mediated etiologies, there were histopathological and circulating autoantibodies. The inflammatory cells get accumulated in the gland resulting in fibrosis and atrophy. The immune mediated conditions were canine atopy, hypothyroidism, hyperadrenocorticism, systemic lupous erythematosis, rheumatoid arthritis, Diabetes mellitus, chronic active hepatitis and pemphigoid disorder. The secretory disorders include seborrhoea, xerostomia/periodontitis and pancreatic exocrine insufficiency. The hormonal imbalance in females were affected because of lack of testosterone hormone and other miscellaneous factors include canine distemper, drugs like sulpha, atropine, lacrimal gland hypoplasia and injury to the nerve.

Berger and King (1998) opined that small breed dogs have inherited, reduced basal tear production.

Ribeiro *et al.* (2008) reported that removal of third eye lid gland, cyclophotocoagulation of ciliary body, hypothyroidism and diabetes mellitus lead to qualitative and quantitative deficiency in tear production. The author reported that dry eye in dogs may occur even with normal tear production.

Dodi (2015) reported hypothyroidism and diabetes mellitus as the common metabolic etiologies for dry eye in dogs.

2.11. COMMONLY AFFECTED BREEDS

Sansom and Barnett (1985) reported that brachycephalic breeds were prone to evaporative type of dry eye compared to other breeds of dogs such as Cavalier King Charles spaniel, English bulldog, Lhasa Apso, Shih Tzu, West Highland White Terrier, Pug, Bloodhound, Cocker spaniel, Pekingese, Boston terrier, Miniature schnauzer and Samoyed.

Whitley *et al.* (1991) documented that keratoconjunctivitis sicca is more common in Chinese Pugs.

Whitley *et al.* (1995) stated that breed and heritability is important for ocular diagnosis and prognosis of dry eye condition. They opined that Pugs were commonly affected with aberrant dermis, caruncle trichiasis, corneal dystrophy, distichiasis, entropion, exposure keratopathy, lagophthalmos or exophthalmos, pigmentary keratitis, progressive retinal atrophy, trichiasis and ulcerative keratitis.

Williams (2008) pointed out that brachycephalic breeds had more prominent globe and lagophthalmos and were prone to evaporation of corneal tear film in the central portion of cornea resulting in keratoconjunctivitis sicca ending up with corneal neovascularization, pigmentation, perforated ulcer and blindness.

Chinchu (2010) reported that rise in intraocular pressure and lagophthalmos were the reasons for over representation of ocular affection in brachycephalic breeds. The author stated that corneal affection was more common in young puppies in brachycephalic breed like Chinese Pugs.

Lacerda *et al*. (2017) reported that the higher incidence of dry eye in Pug may be attributed to lagophthalmos, macro palpebral fissure, blepharospasm and subsequent inability to spreading of tear over the cornea, a decrease sensitivity of cornea and subsequent abnormality of corneal affection.

Charbiwala (2019) also reported that out of 29 pugs in his study 28 pugs had corneal melanosis.

2.12. AGE

In a study conducted by Sansom and Barnett (1985) in 200 dogs over a period of 9 years observed that mean age group of dogs affected with dry eye as five years.

Kaswan *et al*. (1991) reported that loss of sex hormones like testosterone and female androgen in aged animals reduced pituitary secretion resulting in loss of tear production.

In a review article related to the etiopathogenesis and diagnosis of keratoconjunctivitis sicca in dogs, Ribeiro *et al.* (2008) reported that kerato conjunctivitis sicca (KCS) is more seen in older dogs compared to younger dogs.

Williams *et al*. (2008), in a review article pointed out that the brachycephalic breeds had an increased risk of dry eye in their old age.

Anoop *et al.* (2015) reported that in a study of 55 dogs for the presence of pigmentary keratitis, 29 animals (53%) were aged between 1 to 3 years followed by 17 animals (30.9%) below 1 year of age, 8 animals (15%) above 5 years of age and 2 animals (4%) were between 3-5 years of age.

Parulekar (2016) reported that corneal pigmentation was observed bilaterally in majority of the dogs of his study. He reported, 8, 10 and 14 dogs in the age group of < 1, 1-5 and > 5 respectively while unilaterally in 6, 11 and 6 dogs in the age group of < 1, 1-5 and > 5 respectively.

2.13. SEX

Parulekar (2016) reported that overall sex wise distribution of corneal pigmentation was more in male dogs among different brachycephalic breeds.

Charbiwala (2019) had an observation in which males showed a higher distribution (82.92%) in melanosis compared to female (17.08%).

2.14. INCIDENCE

Labelle *et al.* (2013) examined 295 pugs and detected corneal pigmentation in at least one eye of 243 (82.4%) out of 295 pugs.

In a retrospective study Krecny *et al.* (2015), correlated the ophthalmologic findings with age, gender, presenting signs and time of onset of disease. A total of 130 pugs were examined. Ocular abnormalities identified included keratoconjunctivitis sicca (n = 39), macroblepharon (n = 258), entropion (n = 258), distichiasis (n = 56), ectopic cilia (n = 8), conjunctivitis (n = 88), corneal pigmentation (n = 101), opacity (n = 63), ulceration (n = 46), vascularization (n = 35), iris–iris persistent pupillary membranes (n = 21) and cataract (n = 18). Keratoconjunctivitis sicca was significantly associated with the presence of corneal pigmentation. However corneal pigmentation was also identified in pugs (n = 61) without KCS.

2.15. COMPLICATIONS OF DRY EYE DISEASE

According to Renwick (1996) brachycephalic breeds are more susceptible to pigmentary keratitis. Progression of corneal pigmentation, as a sign of chronicity, is observed from medio-ventral quadrant to axial area of cornea.

Slatter and Dietrich (2003) stated that epithelial and stromal pigmentation is more common in chronic corneal diseases due to vascularization, chronic exposure or nasal fold trichiasis and distichiasis. The authors also stated that corneal epithelium may get reverted to skin pattern with thickening, rete peg formation, keratinisation and pigmentation in chronic exposure.

Gilger *et al.* (2008) stated that chronic inflammation results in migration and deposition of melanocytic cells from limbal and perilimbal tissues into the basal epithelial cells of the cornea and anterior stromal tissue, resulting in corneal pigmentation.

Azouly (2014) reported that pigmentary keratitis was observed in dogs of age ranging from 3-14 years with mean age of 7 years. Author stated that deposition of melanin pigments in corneal epithelium results in superficial corneal pigmentation. Melanocytes originate from the limbal conjunctiva and get deposited in the cornea through neovascularization. The author also stated that macrophages and fibroblasts accounts for melanin deposition.

Pigmentary keratitis or non- ulcerative keratitis is the inflammation of cornea with deposition of melanin pigments. It is most commonly associated with conditions like chronic superficial keratitis, KCS, distichiasis, trichiasis and in chronic ulcerative keratitis (Gelatt, 2014).

Anoop *et al.* (2015) observed the scar and peripheral pigmentation towards the limbus which slowly progress towards the central part of cornea in dogs which were under treatment for corneal ulceration. Authors also stated that during stromal wound healing keratocytes transform into fibroblast and collagen which are disorganized and result in scar formation. Neovascularization during healing carry the melanin pigment from limbal and perilimbal area and deposit them around the central scar and result in pigmentation.

2.16. DIAGNOSIS OF DRY EYE SYNDROME

2.16.1. Clinical signs

Bedford (1982) reported that mucoid to mucopurulent discharge is an important symptom of KCS in dogs.

Sansom and Barnett (1985) opined that most of the animals showing sticky, opaque mucoid to mucopurulent discharge cause the eyelid margin to stick each other. The cornea appears dry or with superficial vessels, ulcer and uneven surface. The vascularization of the cornea leads to pigmentation which was transported from the limbus. The dryness causes more friction between the bulbar and palpebral conjunctiva leading to epithelial erosion and entropion.

Kaswan and Salisbury (1990) reported common clinical signs associated with KCS which include mucoid to mucopurulent gluey discharge, conjunctivitis, keratitis, neovascularization, keratinization, hypertrophy of the cornea and pigmentary keratitis.

Gionfriddo (1995) reported that KCS was presented with mild hyperaemia followed by corneal ulceration and pigmentary keratitis.

Featherstone *et al.* (2001) reported that neovascularization is one of the beneficial mechanisms in ulcerative keratitis, but excess of the same will lead to ocular discomfort and opacity of the cornea.

The pattern of corneal blood vessels differs depending on the type of keratitis. Long branching vessels are consistent in superficial ulcerative or non-ulcerative keratitis. Corneal neovascularization appears fine and non-branching type in deep keratitis while they form a 360^{o} perilimbal pattern in intraocular disease (Moore, 2001).

Morreale (2003) reported that loss of corneal clarity is the chief sign of corneal diseases and occurs as sequelae to accumulation of noncelluar infiltrate, pigmentation, fibrosis and oedema.

Mandell and Holt (2005) opined conjunctivitis as one of the common clinical sign of dry eye which get confused with other ocular affections.

Williams (2008) opined that ocular affections like corneal ulceration, conjunctivitis, neovascularization and pigmentation were because of tear evaporation.

Ribeiro *et al.* (2008) reported that conjunctival hyperaemia, blepharospasm and keratitis with or without corneal luster were common in both quantitative and qualitative dry eye. Initially the animal showed blepharospasm which got abolished later because of deterioration of nerve endings.

Pontes *et al.* (2014) reported that water enters the stroma and results in corneal oedema, when there is loss of integrity of both corneal epithelium and endothelium.

Feizi *et al*. (2017) stated that corneal neovascularization is a nonspecific response to corneal pathologies like congenital diseases, contact lens-related hypoxia, inflammatory disorders, chemical burns, limbal stem cell deficiency, allergy, trauma, infectious keratitis, autoimmune diseases, and corneal graft rejection.

2.16.2. Blink rate and character of blink

Carrington *et al.* (1989) reported that brachycephalic dogs have incomplete blinks due to lagophthalmos condition which makes the central cornea dry.

It has been reported that the blinking rate in dogs and humans is 13.7/ min (Harmer and Williams, 2003) and 17.0/min (Bentivoglio *et al*., 2006), respectively.

Mathers (2004) opined that increased levels of meibomian secretions in dogs can be the reason of reduced blink rates compared to humans.

Nakajima *et al.* (2011) reported that the estimated typical blinking rate of a dog was 14.5 and 12.99 blinks/min.

2.16.3. Examination of eye and adnexa

Miller and Crenshaw (1988) evaluated corneal clarity, contour and symmetry, and contact between eyelid margins and cornea by direct diffuse illumination. Localization and extent of lesions were evaluated by direct focal illumination.

Moore and Constantinescu (1997) explained that a complete evaluation of the adnexa should include evaluation of the lids for its structure and deformities, abnormal hair deformities, periocular deformities and abnormal masses if any. According to the authors, the menace response and palpebral reflex can be used to evaluate the ability of the eyelids to completely close over the eye.

Felchle and Urbanz (2001) opined that any changes from the normal transparency of the cornea indicate a disease condition. The authors also opined that adequate illumination and magnification are required for the examination of the cornea.

According to Moore (2001) cornea should be examined with a good light source such as a strong penlight or Finnoff transilluminator. The author also opined that colour, location, shape and pattern of corneal lesions are useful in determining the underlying cause.

Michau *et al.* (2003) suggested techniques like fluorescein dye staining, slit lamp biomicroscopy and direct ophthalmoscopy for the diagnosis of corneal lesions.

Best *et al.* (2014) described that the eyelid margins should be examined to access the inflammation of the meibomian gland because meibum secreted from that gland helps to reduce the evaporation of the tear film.

Packer *et al.* (2015) reported that globe size, prominent eye, shallow orbit and extra-large palpebral fissure are associated with exposure of white part (sclera) which is a predictor of corneal affections.

2.16.4. Goblet cell distribution and density

Moore *et al*. (1987) reported calculation of goblet cell index from the conjunctiva of dogs by finding the ratio of goblet cells to the total epithelial cells of each site. The highest value of goblet cell index was found in two sites-lower nasal fornix and lower middle fornix with 0.30 and 0.290 respectively. The lowest value of goblet cells was found in different sites of bulbar conjunctiva (GCI < 0.035)

It is reported that reduction of goblet cell counts or their complete disappearance can be common in dogs suffering from lacrimal film production disorders (Moore and Collier, 1990; Balicki *et al*., 2011).

Fixed and processed sections of conjunctiva were mounted on glass slides and treated using periodic acid schiff reaction to identify and count the mucous cells in horses (Bourges-Abella *et al.,* 2007*)*

Balicki *et al*. (2011) in a study on mixed-breed dogs, observed reduced counts or complete absence of goblet cells at every stage of KCS prior to treatment, regardless of the severity of signs. As a result of treatment, an increase in the number of goblet cells, or their reappearance, was observed in impression cytology specimens of dogs in their first month of therapy. This increase in goblet cell counts correlated with the improvement of STT scores.

2.16.5. Neuro ophthalmic tests

2.16.5.1. Menace response test

Scagliotti (1999) described menace response as a test to evaluate visual status and eyelid functioning of the animal. In menace test, sudden threatening movement is made with the finger, against the eye being tested. A negative menace response indicates blindness.

Mitchell (2011) stated negative menace response as blindness, or due to the cerebral lesions of the animal. The author also reported that, negative menace response is noted in animals with normal vision.

2.16.5.2. Pupillary light reflex (PLR) test and consensual pupillary light reflex

Maggs (2008) stated that positive PLR does not indicate the sign of vision; it indicates a subcortical response. Decreased or absence of PLR can also be due to atrophy of iris , physical obstruction of the pupil, prior use of dilating drug, iris ischemia, or increased concentrations of circulating adrenaline in fearful animals.

According to Mitchell (2011) both PLR and consensual PLR are examined by pointing a bright light into the lateral aspect of individual eyes, one after the other. The author opined that, it does not evaluate the visual function of eye because it is observed in blind animals with cataract, while not observed in animals having normal vision with iris atrophy.

Featherstone and Heinrich (2013) examined direct PLR by showing a bright light in to the eye being examined observing constriction of ipsilateral pupil, consensual PLR by observing the constriction of contralateral pupil.

2.16.5.3. Dazzle reflex test/photic blink reflex

Martin (2001) described dazzle reflex as a bilateral narrowing of palpebral fissure in response to a very bright light stimulating retina. The author also stated that it is a subcortical response and for this response requiring an intact retina, optic nerve, optic tract, optic chaisma, supraoptic nuclei and rostral colliculi.

Mitchell (2011) stated that a strong light shown suddenly into the eye elicits a normal involuntary response of blinking and head aversion. The author suggested that a positive reflex indicates the functioning of visual pathway while a negative reflex is a poor prognostic indicator of vision.

2.16.5.4. Palpebral reflex test

Moore (2001) described palpebral reflex as a response to stimulation of ophthalmic branch of trigeminal nerve, which initiates by touching the medial or lateral canthus resulting in a blink response.

Mitchell (2011) explains normal palpebral reflex as a response when the sensory (trigeminal nerve) and motor pathways (facial nerve) are intact. Absence of blink indicates poor sensation or facial nerve paralysis.

2.16.5.5. Corneal sensitivity

Barret *et al.* (1991) defined corneal sensitivity or corneal touch threshold as the minimum stimulus required over the cornea for eliciting a blink reflex and the authors also reported that brachycephalic dogs have relatively lower corneal sensitivity which causes corneal damages.

Featherstone and Heinrich (2013) evaluated the corneal sensitivity by touching the cornea using a wisp of cotton wool which resulted in blink response and retraction of eyeball. The authors reported that, the sensory branch for the corneal reflex is the ophthalmic branch of cranial nerve V. The motor response of the reflex is mediated through cranial nerve VI (for globe retraction) and VII (for eyelid closure).

2.16.5.6. Cotton ball test

Martin (2001) described cotton ball test as a visual function test by noting the initial reactions of an animal while dropping cotton balls in to their visual field.

Beranek and Vit (2007) conducted cotton ball test to assess the visual function of dogs, by dropping cotton ball in front of their sight.

2.16.5.7. Maze test/ obstacle course test

Martin (2001) carried out maze test by placing several objects of different sizes, in a scattered manner, in the main exit pathway of an examination area and allowed dogs to move towards it. The test was done in both ambient and dim light, and also by covering individual eye to check monocular blindness. If the animal collided in to the obstacles, demonstrated an altered gait or simply refused to move, the test was graded as positive (implying compromised vision) and otherwise negative (implying normal vision).

2.16.6. Special diagnostic procedures.

2.16.6.1. Fluorescein dye tests

Renwick (1996) reported the confirmed corneal ulcers using fluorescein dye impregnated strips.

Miller (2001) described, fluorescein dye staining as a diagnostic test for corneal ulcers. The fluorescent dye strip is placed over the superior bulbar conjunctiva and a few drops of physiological saline is dropped over the strip to elute the dye on to the cornea. The animal is then allowed to blink the eyes for distributing the stain all over the cornea. The excess stain is then washed out from the eyes to identify the fluorescein stained exposed corneal stroma indicating corneal ulceration.

Fluorescein dye is used to detect corneal ulcers, precorneal tear film defects and nasolacrimal duct obstructions (Moore, 2001).

Mitchell (2011) stated that fluorescein dye is highly lipophobic and hydrophilic, due to which it does not penetrate the corneal epithelium, while in cases of corneal ulceration where the stroma is exposed, the dye will get absorbed and retained. The fluorescein dye does not stain the Descemet's membrane hence the clear area at the centre of a deep corneal ulcer indicates a descemetocele.

2.16.6.2. Rose Bengal dye test

Gelatt, (1972) stated that Rose Bengal dye is used in veterinary medicine as a diagnostic aid for the tear film disorders and breaks in corneal epithelium.

Normal tear film components (mucin and albumin) protect the corneal epithelium and prevent staining by the Rose Bengal dye. Staining indicates an abnormality in tear film (Feenstra and Tseng, 1992; Kim, 2000).

Maggs (2008) stated Rose Bengal stains dead and devitalised cells and gives positive staining for partially eroded corneal epithelium. The author also reported that it gives positive staining for altered mucin coating and squamous cells with altered structure and hence it is very crucial for the diagnosis of KCS and other tear film deficiency disorders.

2.16.6.3. Tear film break up time (TBUT)

Moore and Collier (1990) reported that the animals having mucin deficiency shown tear breakup time less than 5 seconds.

Saito and Kotani (2001) reported that the normal values of TBUT in beagles ranged from 19.7 ± 5 to 21.53 ± 7.42 seconds.

The aqueous, lipid and mucin layers of the precorneal tear film maintains the integrity of corneal and conjunctival tissues. The antimicrobial factors present in the aqueous part provides protection to the eye and the mucin part plays a role in the defence mechanism especially in cases of dry eye disease. Evaluation of the production, secretion and function of these components of tear film can aid in the treatment of ocular surface infections and dry eye syndromes (Davidson and Kuonen, 2004).

Tear film break up time is used for assessment of the stability of precorneal tear film. TBUT is the time taken for the fluoroscein dye to get dissociated from the corneal surface and hence it indirectly measures the quality of mucin and lipid layers of the precorneal tear film (Cullen *et al.,* 2005).

According to Maggs (2008), shortening of the TBUT indicates reduction in mucin quantity or quality which causes instability of the tear film.

2.16.6.4. Schirmer tear test

Rubin *et al*. (1965) conducted Schirmer tear test in dogs using the same paper used for humans. The authors reported that the STT values in normal dogs were 20 ± 4 mm/min without topical anaesthesia.

Gelatt *et al*. (1975) reported the STT values following topical anaesthesia was 11.6 ± 6.1 mm/min.

Moore (1999) suggested that STT assessment is critical in every patient with any ophthalmic issues and in animals predisposed of keratoconjunctivitis sicca.

Murphy *et al.* (2001) reported the Schrimer tear test as a crude estimation of tear production. It is performed by hooking a Schirmer tear strip in to the conjunctival sac, at the junction of lateral one third and medial two thirds of the lower eyelid, for 60 seconds. The length of tear strip getting wet is measured in millimetres and recorded.

According to Hartley (2006), the STT-2 measures the basal tear production by abolishing the reflex by application of proparacaine. The readings are usually approximately half those seen with STT-1.

According to Morreale (2003) Schrimer tear test is the most common test quantifying the aqueous part of the tear film produced. The author states that STT1 measures both the basal and reflex tears produced and STT2 measures basal tear production alone.

Morreale (2003) also reported that Schirmer's tear test values in a normal dog ranges from 14 to 25 millimeters per minute. A Schirmer tear test value less than 15 millimeters per minute should be suspicious for KCS and those less than 10 millimeters per minute is indicative of KCS.

2.16.6.5. Ophthalmoscopy

Bowersox and La Croix (2001) stated that an indirect ophthalmoscope has the capacity to examine a wider fundus area by maintaining a distance from the patient.

Closer examination of the eye in a dimly lit room provides information about size of the pupil of each eye and transparency of individual ocular layers. Direct and oblique illumination using focusing flashlight or the direct light beam of the ophthalmoscope facilitates the examination of ocular adnexa, conjunctiva and cornea (Beranek and Vit, 2007).

Featherstone and Heinrich (2014) stated that opacities of the cornea, lens and vitreous are seen as dark areas highlighted against the fundic reflection when examined by indirect ophthalmoscopy.

2.16.6.6. Slit lamp bio microscopy

The slit lamp biomicroscope allows three dimensional as well as magnified examinations of the cornea, anterior chamber, lens and vitreous along with the adnexa of the eye and therefore it is considered as an important diagnostic tool in veterinary ophthalmology (Featherstone and Heinrich, 2013).

2.16.6.7. Culture and sensitivity of conjunctival swabs

Gerding *et al.* (1993) obtained positive microbial culture in 94% of the clinically normal eyes in dogs. The authors also reported that the major conjunctival bacteria representing 42.8% of total isolates were Gram–positive organisms. The bacteria isolated were *Staphylococcus* spp. (22.9%), *Staphylococcus intermedius* (13.3%), *Corynebacterium* (10.8%), *Streptococcus* spp. (6.0%) and α–hemolytic *Streptococcus* spp. (1.8%). Gram–negative organisms isolated were *Escherichia*, *Neisseria*, *Pseudomonas*, and *Klebsiella* comprising 4.2% of the total isolates. *Fusobacterium* spp. was the only anaerobe isolated.

Kudirkiene *et al.* (2006) in a study of 92 samples of ocular discharge from 46 dogs, observed *Staphylococcus* spp. (55%), *Pseudomonas* spp. (11.4%) and *Corynebacterium* spp. (6.8%) from the eyes without any clinical signs and *Staphylococcus* spp. (58%) from those eyes with clinical signs.

Microbial culture was obtained in 63.67% of conjunctival swabs from normal healthy dogs and 71.43% in diseased dogs. The microflora in diseased conditions composed of Gram–positive microorganisms (87.18%) and while in healthy cases the predominant one was *Staphylococcus* spp. Gentamicin was the most sensitive antibiotic for treatment of ophthalmic infections in dogs (Tyagi, 2009).

Varges *et al.* (2009) in a study regarding antibiotic sensitivity of staphylococci isolated from samples of commonly occurring ocular diseases in dogs observed enrofloxacin as the most effective fluroquinolone followed by ciprofloxacin.

2.17. TREATMENT OF DRY EYE SYNDROME

Barnett and Sansom (1987) reviewed various types of medical treatment for dry eye which included replacement therapy with artificial tears, ocular inserts, lacrimogenics, antibiotics, corticosteroids, mucolytics and hormones. The authors also reported punctal occlusion, tarsorrhaphy, conjunctival flaps, contact lenses, superficial keretectomy as well as parotid duct transposition as surgical treatment for dry eye syndrome.

2.17.1. Eyelid hygiene

Korb and Greiner (1994) reported treatment of meibomian gland dysfunction syndrome in humans using cotton dipped in local anaesthetic solution. The eyelid margin was cleaned by pressing outside using digital pressure and inside using the cotton tipped applicator. The expressed sebum was cleaned by cotton tipped applicator and the eyelid margin was scrubbed with a baby shampoo.

Gillbard (2005) stated that washing of eyelid margin using a gentle soap can decrease bacterial colonisation and improve the mucous and lipid layers of tear film. The author also stated that warm compresses over eyelids will increase lipid secretion and thereby thickening of the lipid layer reducing the evaporative tear loss.

2.17.2. Artificial tear solution

Townsend (2007) suggested use of polyvinyl alcohol, hydroxy propyl methyl cellulose, dextran, chondroitin sulphate and sodium hyaluronate based artificial tears due to their similarity to normal tear until tear stimulants act to increase tear secretion.

Ribeiro *et al.* (2008) reported that tear replacers which contains hypromellose, dextran, carboxymethyl cellulose, chondroitin sulphate and polyacrylic acid were used for moistening the ocular surface.

Best *et al.* (2014) stated that artificial tears containing polyvinyl alcohol removes foreign materials from the eye. More frequent application of the artificial tears and continuous caring of the animal are required. Cellulose based solution or gels, hydroxy prophyl and hyaluronate are more viscous, and have more half-life compared to normal artificial tears. Petrolatum, mineral oil and lanolin based artificial tears are more viscous and hence provide long term lubrication, but can lead to debris accumulation because of lack of an aqueous portion in it. These products are used before sleeping or in case of lipid layer deficiency, lagophthalmos and when the owner is not available long term.

2.17.3. Antibiotics

Whitley *et al.* (1991) opined that treatment of dry eye should end the root cause, replace the tears, stimulate the tear production and control the secondary bacterial infection.

Sansom (2000) reported that topical and systemic antibiotic therapy is essential in ocular infections. If the infection enters the globe it was very difficult to treat due to the blood ocular barrier and absences of lymphatics.

Best *et al.* (2014) opined that in dry eye disease corneal ulcerations caused dehydration are more prone for secondary bacterial infection. So antibiotic is needed for the initial part of the treatment.

2.17.4. Tear stimulatants: Secretagogues

2.17.4.1. Cyclosporine

Kaswan and Salisbury (1990) reviewed and reported that T helper cells produce inflammatory mediators and cytokines. Cyclosporine is most effective in chronic inflammatory conditions where it reduces the activity of the helper cell and increases the number of suppressor cells. The lymphocyte produced prolactin like substance which inhibited the tear production. The cyclosporine reversed the action of the prolactin by which it increases the tear output. It inhibited the interleukin-2 T producing cells and cytotoxic cell production. In long term use, it reduced the pigmentation and vascularization.

Kaswan *et al.* (1995) reported that ophthalmic cyclosporin ointment resolve corneal neovascularization and pigmentation in a period of three to 12 months or more.

Williams *et al.* (1995) stated that cyclosporin is a lacrimomimetic which stimulates tear production.

Ribeiro *et al.* (2008) reported that the drug has immunosuppressive activity by inhibiting the interferon and interleukin- 2. Thereby increased the apoptosis of lymphocyte and reduced the apoptosis of the epithelial cell.

Stevenson *et al.* (2012) reported that cyclosporin combines with cyclophilin to form a complex, and blocks calcineurin phosphate pathway. Calcineurin was responsible for T cell activation. The authors also stated that corneal fungal infection is a contraindication of cyclosporine.

Cyclosporin has anti-inflammatory property and reduces cell death in the third eyelid and lacrimal gland (Wei and Asbell, 2014).

Dodi (2015) stated that cyclosporine is obtained from *Tolypocladium inflatum* and inhibits the T cell proliferation and is non-cytotoxic.

Williams (2018) reported that cyclosporin has immunomodulating, immunosuppressing and also had lacrimomimetic effect.

2.17.4.2. Tacrolimus

Ribeiro *et al.* (2008) in a review article reported that tacrolimus was 10-100 times more effective than cyclosporin. Tacrolimus is nephrotoxic if administered systemically. Weight loss, hepatotoxicity, gastrointestinal problems, infection, impaired glucose metabolism, cardiac vasculitis, myocardial necrosis and pancreatic cell degeneration are the common side effects if administered through systemically. The authors reported that tacrolimus can be used when the patient is unresponsive to cyclosporine, one can use tacrolimus.

Dodi (2015) stated that tacrolimus is obtained from the macrolides (*Streptomyces tsukubaesis*). Tacrolimus is more potent than cyclosporin and mechanism of the action is similar to cyclosporine.

2.17.5. Anti-inflammatory agents

Best *et al.* (2014) stated that NSAIDs can be used if cornea is ulcerated or congested. The authors also described that superficial ulcers are more painful than deep ulcer. Hence NSAIDs are essential in superficial ulcers.

Aragona and Pietro (2007) reported that NSAIDs (especially diclofenac) reduced corneal sensitivity, potentially contributing to corneal damage in dry eye disease (DED) by interfering with reflex tearing and blinking. Cases of corneal melting were observed especially in postoperative settings. The authors also reported that topical NSAIDs can promote corneal melting in patients with a compromised ocular surface and hence their use in DED is controversial.

2.17.6. Topical corticosteroids

Berdoulay *et al.* (2005) described that steroids increased anti-inflammatory factors and promoted the apoptosis of lymphocytes.

Topical corticosteroids are approved by the FDA for corticosteroid responsive inflammatory conditions of the conjunctiva, cornea, and anterior globe. Short-term topical corticosteroid use (as long as 4 weeks) improved signs and symptoms of DED (Anthony, 2007).

Steroid reduces the inflammation on the ocular surface but it is contraindicated in the corneal ulcer. It increase the collagenase and protease activity in the cornea and causes corneal melting (Wei and Asbell, 2014).

2.17.7. Essential fatty acids

Pinna *et al*. (2007) opined that DED are benefited by essential fatty acids which reduce the inflammation of the corneal surface and alter meibomian gland secretions.

Stevenson *et al.* (2012) reported that essential fatty acids like omega -3 (α linolenic acid) and Omega 6 (linoleic acid) has anti-inflammatory property and improves clinical signs of DED.

Wei and Asbell (2014) reported that fatty acids reduced the inflammation of ocular surface and the inflammatory cytokines production.

2.17.8. Acetyl cysteine

Absolon and Brown (1968) reported treatment of DED patients with dense mucus accumulation using topical acetylcysteine.

Anzaar *et al.* (2006) reported the usage of diluted off-label inhalational acetylcysteine (FDA approved for use as a bronchial mucolytic) as a topical ophthalmic agent for DED.

2.17.9. Hormone replacement therapy

Worda *et al.* (2001) reported that androgen plays an important role in the lipid synthesis of the tear film. Androgen promotes the differentiation and secretion of the lipid from meibomian gland. Androgen deficiency causes KCS which was mostly noticed in the auto immune disorders like Sjogren’s syndrome, rheumatoid arthritis, lupus erythematous, and aging. Androgen has anti- inflammatory effect by which it reduces the inflammatory cell accumulation on the ocular surface.

2.17.10. Diaquafosol tetrasodium

Riberio *et al.* (2008) reported that diaquafosol tetra sodium increased non-glandular secretion by water transport using chloride channels, and it increased the mucin production and lipid from the meibomian gland. It promoted ocular wound healing and increased the tear production.

2.17.11. Surgery to increase the inflow of tears

2.17.11.1. Parotid duct transposition

Rhodes *et al.* (2012) reported parotid duct transposition as an effective treatment for dry eye in dogs. Transpositioning the parotid duct from the oral cavity to conjunctival fornix was performed by two approaches as oral and facial approach. The authors reported intraoperative damage (damage to parotid duct), parotid duct failure, facial oedema after surgery, wound dehiscence, calcium deposition in the eye, epiphora, facial dermatitis, sialolithiasis, conjunctival hyperaemia, blepharitis, indolence to the saliva, stromal abscess, reduced production of saliva, permanent failure and retrograde infection from the conjunctival flora as complications of this procedure.

2.17.11.2. Submandibular gland auto transplantation

Castanho *et al.* (2013) reported submandibular gland auto transplantation as another technique for dry eye. Saliva secreted during chewing movement following submandibular gland transplantation.

2.17.11.3. Minor salivary gland transposition

Angelic *et al.* (2011) reported that the minor salivary gland secretion was biochemically and biophysically same as tears. Recipient bed was mostly the upper eye lid fornix because lower eye lid has nictitating gland and there is chance of microtrauma to the graft.

Marinho *et al*. (2010) suggested that mucus of labial salivary glands contains mucin that is thicker than that produced by lacrimal glands, which reduces water evaporation and the secretion of the labial gland is an aqua-serous mucus which is similar to tears and, therefore, can be well tolerated by the ocular surface.

Anna *et al. (*2012) implanted labial salivary glands into the superior and inferior conjunctival fornix of humans and observed improvement in Schirmer tear test values in patients who received submucosal grafts containing more than 10 glands.

Castanho *et al.* (2013) described the parameters to assess labial salivary gland transplantation as corneal luster, mucoid to mucopurulent discharge, blepharospasm, and hyperaemia of the conjunctiva and neo-vascularization of the cornea. Authors described the procedure of transplantation into three stages, viz; obtaining graft from the donor site, preparation of recipient site and suturing of the graft.

Qin *et al*. (2018) transplanted labial minor salivary glands in a Rhesus monkey model of severe dry eye in which an obvious increase in the quantity of tears was observed and foam-like secretions resembling saliva, suggesting an exocrine activity of the grafted salivary glands at the level of the ocular surface.

Grixti and Malhotra (2018) reported antibacterial properties of saliva in humans due to the presence of immunoglobulin A (IgA), lactoferrin, human beta- defensin (hBD) and lysozymes. The authors also reported that minor salivary gland secretions contain growth factors such as epidermal growth factor and transforming growth factor- β which helps in corneal reepithelialisation and healing.

2.17.12. Surgery to decrease outflow

2.17.12.1. Punctal occlusion

Ribeiro *et al.* (2008) reported that punctual occlusion blocked the drainage system of lacrimal duct and increased the presence of the tear on the ocular surface while permanent partial tarsorrhaphy reduces the evaporation of tear film.

Badawy (2017) following a study in dogs suggested surgical punctal occlusion prevent drainage of low amount of produced tear and allowed the distribution of tear over the ocular surface which could improve the prognosis and considered it a permanent replacement for medical treatment

2.17.13. Management of corneal pigmentation

Moreau and Haut (1971) suggested different treatment methods for pigmentary keratitis which includes medical management by application of cyclosporine or tacrolimus (immunosuppressant drugs), judicial application of steroids and anti-inflammatory agents. The authors also suggested other treatment methods like beta - irradiation, carbondioxide laser ablation, lamellar keratectomy, nasal canthopalsty and cryotherapy of melanocytes for pigmentary keratits in dogs.

Cryosurgical treatment for pigmentary keratitis lead to ice ball formation inside the cell and alters osmatic activity ending up in cell death. Since cornea is avascular and dehydrated, cryotherapy causes less damage when compared to that in pigmentary cells. Corneal oedema and blepharospasms were the complications of cryosurgery. Epithelial thinning and separation of corneal epithelium and stroma were the histopathological changes observed (Holmberg *et al.*, 1986).

Hersh and Kenyon (1991), used keratome blades for superficial keratectomies in corneal degenerative and dystrophic conditions in humans.

Corneal epithelial debridement or cauterization can be performed using swabs impregnated with different chemical agents such as trichloroacetic acid, phenol liquid, tincture of iodine or diluted povidone iodine (Whitley and Gilger, 2014)

Da Silva *et al*. (2011) evaluated corneal changes immediately after debridement of corneal epithelium using diamond burr in superficial corneal wounds of dogs and it was concluded that diamond burr method of superficial keratectomy is a safe method.

According to Gunderson (2013), the purpose of majority treatments for pigmentary keratitis is to control the progression of pigmentation by removal of the cause of irritation .The author suggests that facial fold resection and medial canthoplsty helps in correcting the medial canthal entropion, nasal fold trichiasis and reduction in the palpebral fissure and thereby reduces constant irritation to eyes.

Brachycephalic breeds are more susceptible to pigmentary keratitis. Surgical removal of pigmentaed corneal layers has been suggested after inciting causes have been corrected. However, frequent recurrence of the pigment and coneal scarring, despite appropriate therapy, generally limits the success of the procedure (Ledbetter and Gilger, 2013).

Anoop *et al*. (2015) suggested superficial keratectomy for removal of pigmented corneal layer as therapy for pigmentary keratis in dogs.. Authors stated basement membrane and a part underlying pigmented epithelium are surgically removed in this procedure. Scar formation and re-pigmentation were the main complication following the procedure. The authors also suggested lamellar keratoplasty with collagen based biomaterials for corneal remodelling.

Charbiwaala (2019) observed perilimbal pigmentation of bulbar conjunctiva and it was excised during surgical intervention because perilimbal conjunctiva act as a major potential source of pigmentation and vascularisation of superficial cornea during an inflammatory process. The author suggested that removal of the conjunctiva will significantly reduce the chances of recurrence of corneal vascularisation and concurrent corneal pigmentation which is usually observed during corneal healing after keratectomy.

2.17.14. Human amniotic membrane grafting

Wichayacoop *et al.* (2005) used preserved human amniotic membrane to cover induced corneal stromal wound in dogs and observed complete corneal epithelialization in seven days, and reported that all dogs regained their vision.

Meller *et al*. (2011) reported that processed human amniotic membrane grafts have been increasingly used to treat a wide variety of ocular surface conditions like persistent corneal epithelial defects, acute chemical burns, and cicatrizing conditions such as Stevens–Johnson syndrome and ocular cicatricial pemphigoid in humans.

According to Janson and Sikder (2014), human amniotic membrane grafts were thought to promote epithelial and stromal healing, suppression of transforming growth factor β (TGF-β) signaling, and fibroblast proliferation, and had good anti-inflammatory properties.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Six dogs irrespective of age, breed and sex diagnosed with dry eye and having melanotic cornea formed the subjects for the study.

3.1. SELECTION OF DOGS

Dogs of various breed, age and either sex presented to the Teaching Veterinary Clinical Complex, Pookode, Wayanad, Kerala with ocular conditions and having symptoms of dry eye were subjected to detailed ophthalmic investigations. Among these, six dogs with basal tear production less than 5mm/min, diagnosed by Schirmer tear test, and having melanotic cornea were selected for the study. The selected dogs were numbered D1, D2, D3, D4, D5 and D6.

3.2. ITEMS OF OBSERVATIONS

Signalment and anamnesis were recorded on the day of presentation. General condition of the patient, physiological parameters, nature of the ocular discharge, conjunctival changes, results of visual function tests, corneal sensitivity, number of blinks per minute, direct and indirect ophthalmoscopic examination findings, results of Schirmer tear test, findings of Fluorescein dye test, Rose Bengal dye test, Slit lamp biomicroscopic findings, Tear film break up time, depth and degree of corneal melanosis and vascularisation were recorded on day 0 and on 3rd, 7th, 14th ,21st, 30th and 60th post-operative days.

3.3. PRE-OPERATIVE OBSERVATIONS

3.3.1. Signalment and anamnesis

The age, breed, sex, symptoms, duration, concurrent illness if any, and details of any previous treatment and medications were recorded in all the animals studied.

3.3.2. General body condition

The general conditions of all the animals were visually assessed on the day of presentation and also on the day of surgery and categorized as excellent, good, fair or poor. The body weight (kg) was also recorded.

3.3.3. Physiological parameters

Physiological parameters like heart rate (beats/min), respiration rate (breaths/min), rectal temperature (⁰C) and colour of visible mucus membrane (CMM) were assessed and recorded on the day of presentation and post-operatively on day 3, 7, 14, 21, 30 and 60.

In all cases, samples were collected for bacterial culture and sensitivity tests.

3.4. OPHTHALMIC EXAMINATION

3.4.1. Gross eye examination

Distant and close examinations were carried out for both eyes and adnexa. Facial and ocular symmetry, resting eyelid position and conformation of the eyelids, size of the eye, movement and direction of gaze, presence and nature of ocular discharge and overt signs of ocular pain were observed and recorded.

Following distant examination, a hands-on examination with minimal manipulation of adnexal tissue were carried out. The eyelid margins were examined for the status of meibomian glands, eyelid oedema, entropion, ectropion, medial caruncular trichiasis, macropalpebral fissure, trichiasis and distichiasis or ectopic cilia. Animals were also observed for nasal fold trichiasis.

Cornea was checked for lustre, opacities or surface irregularities, scar formation, oedema, pigmentation, perforation, vascularisation and ulceration. Anterior segment was also examined for abnormalities.

Corneal clarity was noted as 0 (no corneal haze), 1(iris details visible), 2(pupillary margin visible, iris details not visible), 3 (pupillary margin not visible), 4 (cornea totally opaque).

Corneal luster was noted as absent or present.

Corneal edema was noted as 0 (no signs), 1(mild corneal haze), 2(marked corneal opacity, anterior chamber still visible), 3(severe corneal opacity, anterior chamber not visible).

3.4.2. Nature of ocular discharge

The eyes of the animals were observed for lacrimation and nature of ocular discharge. The nature of ocular discharge was recorded as serous, mucoid, mucopurulent or purulent.

3.4.3. Conjunctival changes

All the animals were observed for conjunctival changes and recorded as generalized congestion (mild, moderate, severe), pigmented or oedematous.

3.4.4. Results of visual function tests

Visual function was assessed for the affected eye by way of menace response test, cotton ball test and maze test (Azoulay, 2014) and vision was scored 2 (positive to all three tests and graded normal), 1 (insufficient response to one or two tests and graded decreased) or 0 (negative response to all three test and graded absent). Pupillary light reflex and dazzle reflex were also noted and recorded.

3.4.5. Rate of blink and character of blink

The blinking of eyelids was noted in each animal upon rest and number of blinks per minute was recorded. The character of blink was also noted and counted as complete and incomplete blinks based on the closure of eyelids.

3.4.6. Indirect ophthalmoscopic examination

Indirect ophthalmoscopic examination was done using 20D Volk lens (Double Aspheric) to visualise the fundus and findings were recorded.

3.4.7. Direct ophthalmoscopic examination

Direct ophthalmoscopy was done using hand held ophthalmoscope (Welch Allyn, 11770 Inc.) and the findings were recorded. Corneal clarity, corneal oedema, vascularization of cornea and extent of pigmentation were examined and recorded with the help of direct ophthalmoscope.

3.4.8. Vascularisation of cornea

All the affected eyes were examined for vascularization of cornea and graded as 0 (no visible vessels), 1 (mild superficial vascularisation, thin vessels visible with magnification), 2 (profuse superficial vascularisation visible to naked eye) and 3 (extensive vascularisation with thick vessels originating from all quadrants).

3.4.9. Pigmentation of cornea

The cornea was schematically divided into 24 sectors for documenting the position and extent of melanotic pigmentation. The central sectors were made smaller than the periphery considering the greater importance of central portion of cornea than the periphery in vision. Each corneal sector was evaluated for extent of corneal pigment. Corneal pigment was graded within the sectors from 0-3 (0 = No pigmentation, 1 = pigmented area < 30% of the sector area, 2 = pigmented area > 30% - 60% of the sector area, 3 = pigmented area > 60% of the sector area). Pigmentation grading of the particular cornea was calculated by adding all the sector grades. The maximum grading is 72 for a totally pigmented eye (Allgoewer and Hoecht, 2010).

The density of pigmentation was recorded as mild, moderate and severe.

3.4.10. Pigmentation depth

Categorisation of the depth of the pigment deposition and concurrent vascularization was done by gross and magnified, focal examination of the cornea under direct as well as retro-illumination. Visualisation of the anterior segment or fundic reflex through the curtain of corneal pigment was considered as superficial pigmentation, and lack thereof as deep.

Cornea was examined under magnification using the hand held slit-lamp light bio microscope to get a better judgement of the pigmentation as well as any active vascularisation not visible grossly. Different light intensities and aperture sizes were used to illuminate and retro-illuminate the cornea and anterior chamber, to get a better idea about pigment depth.

The cornea was examined by selecting an aperture with the highest intensity and angled directly and perpendicular to the corneal surface, to help visualisation of the anterior segment through the pigmentation if possible. This position retro-illuminate the cornea and provide a detailed comprehensive image of the melanotic patch and any concurrent vascularisation embedded in it.

In the next examination the beam was pointed lateral to the corneal bulge and the aperture was changed to a fine slit with maximum intensity at this position. The slit beam was focused directly on the pigment patch being examined to have a clear view of the slit passing through the different layers of cornea, the aqueous and its image on the iris, pupil and anterior capsule of the lens. If a break in the slit beam or shadow of the melanotic patch was visible or the reflection of the slit beam in the anterior chamber was not visible, the melanin was considered deep and otherwise recorded as superficial. Categorization of the depth of pigmentation was done under three levels: only superficial, only deep and superficial as well as deep (Charbiwala, 2019).

3.5. NEURO- OPHTHALMOLOGIC EXAMINATION

3.5.1. Pupillary light reflex (PLR)

Direct PLR was recorded and noted as absent, sluggish or present.

3.5.2. Dazzle reflex

This test was conducted by directing a beam of light into each eye separately eliciting a normal involuntary avoidance response comprising of complete or partial blink and/or head aversion from the light source. The source of light used was of a high intensity and this test was performed in a darkened room. It was noted as positive or negative.

3.5.3. Palpebral reflex

The palpebral reflex was tested by lightly touching the lateral and medial canthi of the animal's eye with the tip of the finger or cotton bud. A positive response resulted in complete closure. It was noted as absent, incomplete or present

3.5.4. Corneal sensitivity

The corneal sensitivity was tested by stimulating the cornea from the periphery to the centre with a fine wisp of cotton wool that resulted in closure of the eyelids and in some animal's retraction of the globe. The eye was approached from the lateral aspect to avoid menace reflex. It was noted as absent, sluggish or present.

3.6. SPECIAL DIAGNOSTIC PROCEDURES

3.6.1. Schirmer tear test (Fig: 1a)

Schirmer tear test was done using sterile Schirmer tear test strip (Tear Touch, Madhu Instruments Pvt. Ltd) with printed millimetre scale on it and the observations were recorded. The strip was placed into the ventral conjunctival *cul-de-sac* for one minute and the distance of wetness was measured immediately to measure the reflex tear production (STT-1) and compared against the normal range.

Cornea was then desensitised using proparacaine and STT-2 was measured by gentle drying of the lower conjunctival sac after 1 minute, and then placement of a test strip as for STT-1.

3.6.2. Flourescien dye test

Fluorescein dye test was done using sterile dye impregnated strips (Fluoro Touch, Madhu Instruments Pvt. Ltd). The fluorescein dye impregnated tip was held against the bulbar conjunctiva and two drops of sterile normal saline was instilled on it for the dye to elute and fall on the eye. The animal was allowed to blink to distribute the fluorescein dye across the ocular surface. The excess stain was flushed out with sterile normal saline. The eye was then examined under cobalt blue light and the positive or negative staining on cornea was noted.

3.6.3. Rose Bengal dye test

The test was done in the similar manner as described for fluorescein dye test. The positive or negative staining on corneal epithelium and conjunctival cells were noted.

3.6.4. Slit lamp biomicroscopy (Fig: 1b)

A hand held portable slit lamp bio-microscope was used for elaborate magnified examination of the adnexa, anterior segment and anterior surface of the lens of the eye. Diffuse forms of lighting were used to examine a large area for gross abnormalities and to obtain an initial overview. To gain more detailed information and highlight subtle lesions, more focal slit forms of illumination were used.

3.6.5. Tear film breakup time (Fig: 1c)

The qualitative assessment of pre-corneal tear film was performed by tear film breakup time, in all the animals, on the day of presentation. Fluorescein dye was applied to ocular surface and the animal was allowed to blink. Eyelids were opened and examined under the silt lamp bio microscope to observe the first break point of the tear film. The test was repeated post operatively on day 3, 7, 14, 21, 30 and 60 to assess quality of the tear film (Ribeiro *et al.*, 2008).

3.7. CULTURE AND ANTIBIOTIC SENSITIVITY TEST OF CORNEAL SWAB

The samples for antibiotic sensitivity test were collected using sterile swabs before the instillation of any medication. The eyelids were gently retracted and the sides of sterile swabs were rolled over the cornea without touching eyelid margin or eyelashes. A primary isolate was made out of the sample in Brain Heart infusion agar and from there it was inoculated in to Muller Hinton agar. Antibiotic discs (Himedia) were placed on inoculated Muller Hinton agar surface at about two to three cm apart

PLATE 1

Special diagnostic procedures

Fig: 1a, Schirmer tear test (STT)

Fig: 1b, Slit lamp bio microscopy

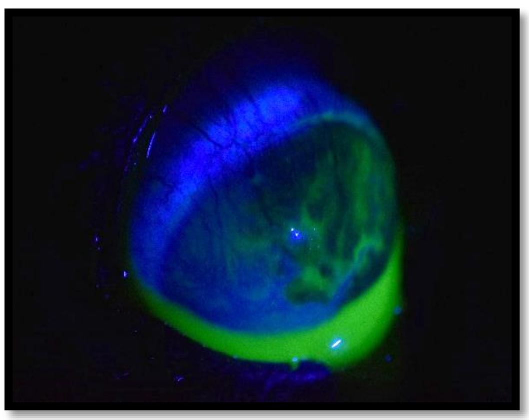


Fig: 1c, Breaking of tear film over the cornea with gentle pressure and incubated for overnight at 37 °C. Antibiotic discs used were enrofloxacin, gentamicin, tetracycline, amoxicillin, ciprofloxacin, ofloxacin, norfloxacin, chloramphenicol and streptomycin. The zone of inhibition diameter was measured for each antibiotic. The obtained data was compared with interpretative chart furnished by the manufacturer and the sensitivity of the organism was recorded as (S) sensitive, (I) intermediate and (R) resistant.

3.8. PRE-OPERATIVE MEDICAL THERAPY

Moxifloxacin eye drops were instilled pre-operatively for three days in all cases. Instilling of artificial tears was also advised if dryness was severe.

3.9. SURGICAL TREATMENT

3.9.1. Preoperative preparation

3.9.1.1. Patient preparation

Topical instillation of antibiotic eye drops three times daily was commenced three days prior to surgery. Withholding of solid food for twelve hours and liquid food for six hours was advised before the day of surgery in all the animals.

3.9.1.2. Preoperative preparation of eye and oral cavity

The affected dry eye was thoroughly irrigated with sterile normal saline and the periocular area was cleaned with sterile cotton to remove accumulated ocular discharge, dirt and tissue debris. The periocular area and intraocular area was thoroughly washed with dilute povidone iodine solution (0.5%). The oral cavity was irrigated with normal saline. Donor site was aseptically prepared by painting 5% povidone iodine solution.

3.9.2. Anaesthetic protocol

All the dogs prepared for surgery were premedicated with meloxicam at the rate of 0.2 mg/kg body weight intra muscularly followed by xylazine hydrochloride at the rate of 0.2 mg/kg body weight and butorphanol at the rate of 0.2 mg/kg bodyweight, both given intravenously. Following sedation, general anaesthesia was induced with ketamine hydrochloride at the rate of 2 mg/kg body weight and midazolam at the rate of 0.2 mg/kg body weight, both given intravenously “to effect”. The animals were intubated with endotracheal tube of appropriate size and anaesthesia was maintained using 1-1.75% isoflurane in oxygen.

3.9.3. Patient positioning

The animal was placed on lateral recumbency with the affected eye above.

3.9.4. Surgical technique (Plate: 3)

The surgical technique was performed in four stages

1. Collection of oral mucosal autograft

2. Grafting of oral mucosa autograft in conjunctival fornix

3. Superficial keratectomy and transplantation of processed human amniotic membrane

4. Lower puncta occlusion followed by medial canthoplasty

3.9.4.1. Collection of oral mucosal autograft

A 10 mm long full thickness oral mucosa at lower canine to 3rd premolar area, encircling 3-4 duct openings, was harvested as the autograft. A small section of the graft was taken for histological examination while the rest was kept soaked in normal saline for quick grafting. The mucosal wound was immediately apposed with 5-0 polyglactin 910 in interrupted suture pattern.

3.9.4.2. Autografting of oral mucosa

The eyelid of the selected eye was kept retracted using a speculum. A fusiform wound was created to the size approximating the graft in the superior conjunctival fornix and a portion of conjunctiva was taken for histological examination. The autograft was placed in the wound created and retained by suturing using polyglactin 910 size 5-0 in simple interrupted pattern (Castanho *et al.*, 2013).

3.9.4.3. Superficial keratectomy and transplantation of processed human amniotic membrane

The eye ball was positioned central using stay sutures. Superficial keratectomy was done to remove the pigmented epithelium. Keratome blades (Fig: 2a) were used to remove the deep pigmentation and a 3 mm non-cutting diamond burr (Shafire) (Fig: 2b) was utilised for removing the superficial pigmentation and polishing the cornea after the keratectomy using keratome blades (Fig: 3f).

A leading edge of the corneal epithelium was made by gentle scraping of pigmentation using the keratome blade. After an edge of the sub epithelial fibrous membrane was identified, the tissue was stripped and peeled with the aid of colibri forceps, as a continuous sheet by the to and fro movement of keratome blades under the epithelium. The cleared corneal surface was then polished with a diamond burr or lightly scraped with a stromal scrubber (Hersh and Kenyon, 1991) (Fig: 3g to 3i).

For superficial pigmentation, the diamond burr was fixed on a dental hand piece and rotated at maximum RPM setting on a portable dental micromotor (Marathone-4 Saeyang Microtech Co. Ltd; Korea) (Fig: 2d), the cornea was lubricated adequately with normal saline and the rotating burr was gently scraped over the pigmented epithelium to debride it. Constant flushing with normal saline was carried out to remove scraped corneal material from the surgical site and avoid damage to surrounding normal tissue due to heat generated by the rotating burr. Utmost care was taken while scarping near the limbus to avoid accidental involvement and entrapment of the conjunctiva.

The final step comprised of partial conjunctivectomy of pigmented adjacent perilimbal conjunctiva. The conjunctiva was resected as close to the limbus as possible.

After the superficial keratectomy procedure, an ear bud (Johnson and Johnson India Pvt Ltd) dipped in tri-chloro acetic acid (Fig: 3j) was held in contact with the corneal surface and vascularisations present on the cornea for debridement. The cornea was flushed with normal saline immediately. The procedure was repeated again, once.

Decellularised, processed human amniotic membrane (Amnio-care™) (Fig: 2c) was resized with scissors to obtain a diameter greater than the corneal defect. The membrane was placed over the prepared corneal surface, with the stromal side of the membrane facing the cornea and secured in place as an onlay graft using 10-0 polyamide suture.

3.9.4.4. Lower punctal occlusion followed by medial canthoplasty

The lower puncta was identified and occluded using fine tip of the electrocautery by inserting it in to the lower puncta (Fig: 3m).

Medial canthoplasty was resorted for cases which had medial canthal entropion (Fig: 3n to 3o). A no. 11 scalpel blade was used to make an incision that ran from the center of the medial canthus to the upper lacrimal punctum along the eyelid margin, superficial to the meibomian gland openings. A second incision was made on the lower eyelid in the same fashion. The superficial part of the medial palpebral ligament was cut with tenotomy scissors, and the circumscribed skin was undermined to free it from its orbital attachment. An arrow-shaped wedge of skin near the medial canthus was removed. The resulting skin wound was closed with 6-0 polyglactin 910, employing a figure-of-eight pattern. One or two simple interrupted sutures were also applied medial to the figure-of-eight suture to enhance skin closure.

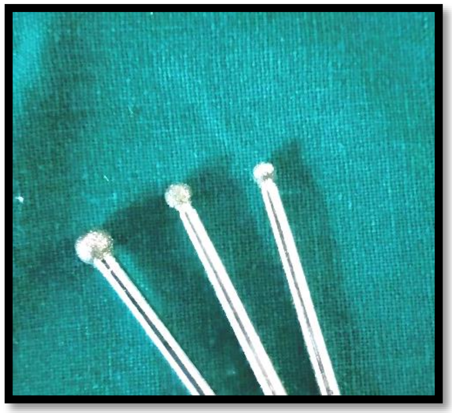
3.9.5. Post-operative care

An Elizabethan collar was advised for all animals to prevent self-mutilation, till complete healing of cornea and the graft. Intravenous administration of Ceftriaxone @ 20 mg/kg (Intacef) body weight and Meloxicam @ 0.2 mg/kg (Melonex) was given on the day of surgery, followed by oral administration of Ceftriaxone @ 25 mg/kg (sporidex) body weight and meloxicam @ 0.2 mg/kg once daily for five days was advised to all the animals. Topical ciporofloxacin eye drop was advised for two weeks. Based on results of fluorescein dye test, non- steroidal anti- inflammatory drugs and

PLATE 2

Materials used for keratectomy and grafting

Fig: 2a, Keratome blades



Fig; 2b, Non – cutting diamond burrs

Fig: 2c, Processed human amniotic membrane

Fig: 2d, Dental micromotor and handle

PLATE 3

Surgical procedure

Fig: 3a - Donor site

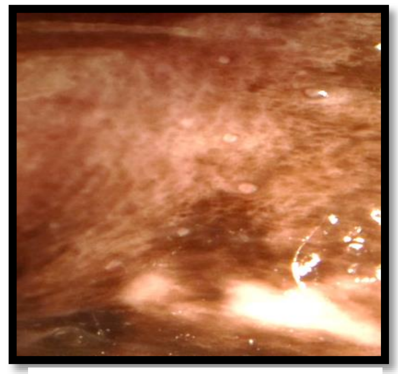


Fig: 3b - Duct openings of minor salivary glands

Fig: 3c - Autograft

Fig: 3d - Recipient site

Fig: 3e - Sutured autograft

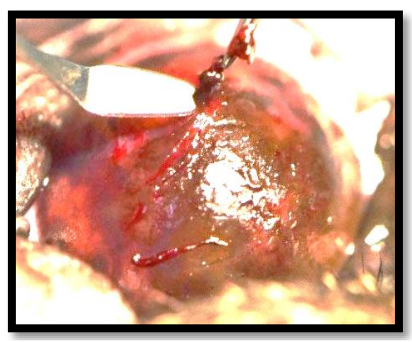


Fig: 3f - Keratectomy of deep pigmented layer using keratome blades

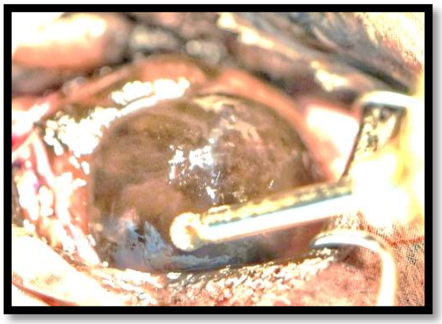


Fig: 3g - Removal of superficial pigmentation using diamond burr

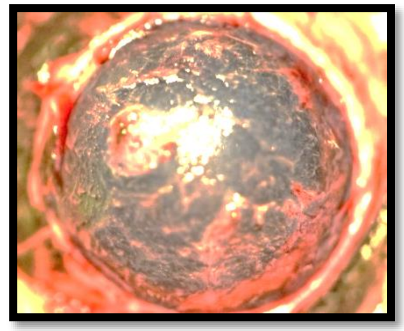


Fig: 3h - Cornea after removal of pigmentation

Fig: 3i - Cornea after diamond burr polishing

Fig: 3j - Cornea after treatment of trichlo acetic acid

Fig: 3k - Sutured conjunctiva after perilimbal conjunctivectomy

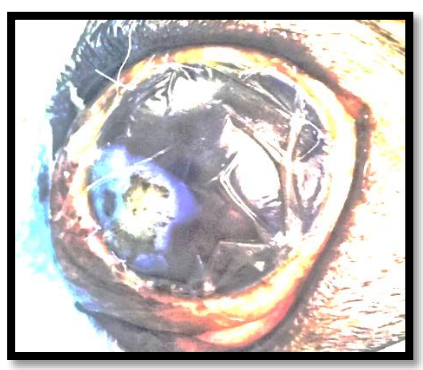


Fig: 3l - Human amniotic membrane grafted cornea

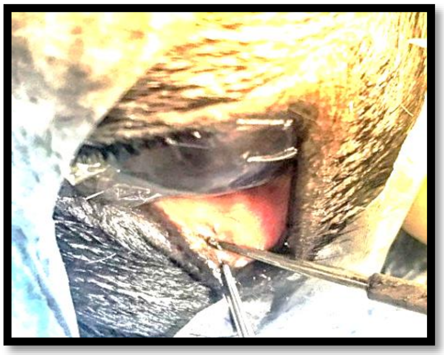


Fig: 3m - Punctal occlusion using electro cautery

Fig: 3n - Medial canthoplasty

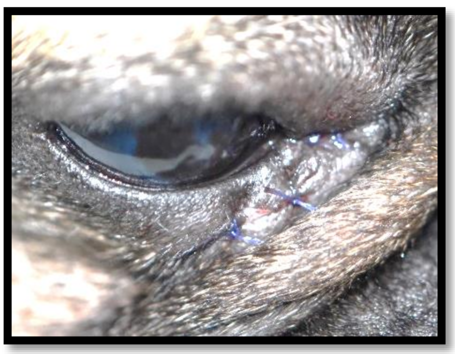


Fig: 3o - Sutured eyelid

Fig: 3p - Sutured donor site

topical ocular medications like autoserum fortified with antibiotics, EDTA eyedrops and tacrolimus eye ointment were used, as and when required. The observations on 30th and 60th days were taken after withdrawal of tacrolimus for minimum of 4 days before the reading.

Post-operative observations of autograft (recipient site and donor site) and cornea were recorded on days 3, 7, 14, 21, 30 and 60.

3.10. HISTOLOGICAL EXAMINATION

The small portion of oral mucosal autograft was preserved in ten per cent neutral buffered formalin till histological evaluation. The tissue samples were processed by routine paraffin embedding technique (Sheehan and Hrapchak, 1980). The paraffin embedded tissues were sectioned at five-micron thickness and stained with Haematoxylin and Eosin stain (Bancroft and Cook, 1984). The stained section was subjected to detailed examination under light microscope for presence of minor salivary glands and its characterisation.

The portion of conjunctiva obtained from the fornix was also preserved and processed in the same manner and the slides were stained using PAS technique to calculate goblet cell index. Goblet cell indices was determined by counting GC profiles versus all epithelial cell profiles, i.e., GCs plus squamous, polygonal, and basal cells. Goblet cells were identified by the presence of PAS-positive intracellular material. Crescent-shaped nuclei associated with PAS-positive material were considered nuclei of GCs and were not included in the remaining epithelial cell counts. The ratio of GC profiles to total epithelial cell profiles for each site was computed as an index of GC density or goblet cell index (GCI). 500 total epithelial cells were counted using the X40 objective and a standard light microscope. Five foci of 100 cells/focus were selected along the length of each linear section (Moore *et al.* 1987).

3.11. HISTOPATHOLOGICAL EXAMINATION

The pigmented epithelium obtained after superficial keratectomy was preserved in ten per cent neutral buffered formalin till histopathological evaluation. The tissue samples were processed by routine paraffin embedding technique (Sheehan and

Hrapchak, 1980). The paraffin embedded tissues were sectioned at five-micron thickness and stained with Haematoxylin and Eosin stain (Bancroft and Cook, 1984). The stained section was subjected to detailed examination under light microscope.

3.12. COMPLICATIONS

Complications, if any were recorded and managed during the course of observation period.

3.13. STATISTICAL ANALYSIS

The data was statistically analysed. The tests adopted were repeated measures of ANOVA. The level of significance was fixed at 5% level ($p < 0.05$).

RESULTS

4. RESULTS

Six dogs irrespective of age, breed and sex, having an eye with basal tear production less than 5 mm/min and having melanotic cornea formed the subjects of the study. The selected dogs were numbered D1, D2, D3, D4, D5 and D6. These dogs underwent oral mucosal autografting, punctal occlusion, superficial keratectomy and processed human amniotic membrane transplantation. Medial canthoplasty was also done in those cases which required correction of medial canthal entropion. The observations of the study are presented below.

4.1. SIGNALMENT AND ANAMNESIS (Table 1)

The age of the dogs ranged from eight months to five years. All the dogs were Chinese Pugs. Among the six dogs, two (33.33%) were females while the rest (66.66%) were males. Both eyes were affected in all the dogs. The symptoms noticed were thick ocular discharge in all dogs, while three dogs (D1, D5 and D6) had blackish colouration of eye and impaired vision. Duration of the condition ranged from 7 months to 24 months. Four dogs (D1, D2, D3 and D4) had been on topical ocular antibiotics and artificial tears for a period of one month. Two dogs (D5 and D6) were on artificial tears for a period of one year. The eyes (OD, n=5 and OS, n=1) having basal tear production less than 5 mm/min were selected for the study.

4.2. GENERAL CLINICAL EXAMINATION

4.2.1. General body condition

General body condition was good in all the dogs on all days of observation. Body weight of the dogs ranged from 8 kg to 12 kg.

4.2.2. Physiological parameters (Table 2)

4.2.2.1. Rectal temperature

The mean rectal temperature (°C) on the day of presentation and post-operatively on days 3, 7, 14, 21, 30 and 60 were 38.15 ± 0.57, 38.08 ± 0.57, 38.3 ± 0.50, 38.2 ± 0.44, 38 ± 0.54, 37.95 ± 0.39 and 38.1 ± 0.42 respectively. The values were within the normal physiological range and variation in rectal temperature noticed during the observation period was not significant at 5% level.

4.2.2.2. Respiration rate

The mean respiration rate (per min) of the dogs on day of presentation and post-operatively on days 3, 7, 14, 21, 30 and 60 were 28.83 ± 1.47, 31.17 ± 1.60, 29.33 ± 1.03, 28.83 ± 1.17, 30.17 ± 2.86, 28.17 ± 2.04 and 30.67 ± 3.01 respectively. The values were within the normal physiological range and variation in respiration rate noticed during the observation period was not significant at 5% level.

4.2.2.3. Pulse rate

The mean pulse rate (per min) of the dogs on day of presentation and post-operatively on days 3, 7, 14, 21, 30 and 60 were 97.17 ± 8.77, 98.17 ± 7.33, 98.83 ± 2.71, 97.17 ± 2.48, 101.67 ± 5 .82, 97 ± 3.79 and 92.67 ± 4.89 respectively. The values were within normal physiological range and variation noticed during observation period was not significant.

4.2.2.4. Appearance of visible mucous membrane

The conjunctival mucous membrane was congested while oral mucous membrane was pigmented in all the dogs studied. The tongue was pink in colour. The conjunctival mucous membrane colour during the observation period varied according to the inflammatory changes. Colour of tongue remained pink throughout the observation period.

4.3. OPHTHALMIC EXAMINATION

4.3.1. Nature of ocular discharge

On day 0, all the dogs had thick mucoid ocular discharge while on 3rd day discharge was mucopurulent. On 7th day, the discharge became serous in nature in two dogs (D3 and D4) while remained mucopurulent in the rest. On 14th day, the ocular discharge was observed serous in all dogs and continued so on 21st, 30th and 60th day of observation except in one dog, D5. D5 had mucopurulent discharge on 21st day of

Table 1: Signalment and anamnesis of the case presented

Dog	BREED	AGE	SEX	EYE AFFECTED	EYE SELECTED	DURATION OF ILLNESS	PREVIOUS MEDICATION
D1	Pug	48 m	M	OU	OD	24 months	Ciprofloxacin eyedrops and artificial tears
D2	Pug	12 m	F	OU	OS	12 months	Ciprofloxacin eyedrops and artificial tears
D3	Pug	8 m	M	OU	OD	7 months	Ciprofloxacin eyedrops and artificial tears
D4	Pug	12 m	M	OU	OD	12 months	Moxifloxacin eyedrops and artificial tears
D5	Pug	54 m	M	OU	OD	18 months	Artificial tears
D6	Pug	60 m	F	OU	OD	24 months	Artificial tears

OU- both eyes, OS – left eye, OD - right eye, M – male, F - female

Table 2 : Mean values of physiological parameters

Physiological parameters	Days of observation						
	0	3	7	14	21	30	60
Rate of respiration (per minute)	28.83 ± 1.47^{a}	31.17 ± 1.60^{a}	29.33 ± 1.03^{a}	28.83 ± 1.17^{a}	30.17 ± 2.86^{a}	28.17 ± 2.04^{a}	30.67± 3.01^{a}
Rectal temperature (ºC)	38.15 ± 0.57^{a}	38.08 ± 0.57^{a}	38.3 ± 0.50^{a}	38.2 ± 0.44^{a}	38 ± 0.54^{a}	37.9 5 ± 0.39^{a}	38.1± 0.42^{a}
Pulse rate (per minute)	97.17 ± 8.77^{a}	98.17 ± 7.33^{a}	98.83 ± 2.71^{a}	97.17 ± 2.48^{a}	101.67 ± 5.82^{a}	97 ± 3.79^{a}	92.67 ± 4.89^{a}

Means bearing same superscript in the row do not differ significantly at 5 % level observation and become serous on 30th day and remained same on 60th day of observation.

4.3.2. Appearance of conjunctiva (Table 3)

On day 0, conjunctiva appeared mildly congested in three dogs (D2, D3 and D4) while it was moderately congested in the rest (D1, D5 and D6). Bulbar conjunctiva was pigmented around the limbus in all the dogs and was denser on the medial side (Fig: 12a). On the 3rd day of observation, the conjunctiva appeared severely congested in all the dogs. On 7th day of observation, conjunctiva was severely congested in two dogs (D5 and D6) with granulating appearance (Fig: 16c and Fig: 17c). In three dogs (D2, D3 and D4), congestion reduced and conjunctiva became normal, while in one dog (D1) a cherry red coloured granulation tissue growth (Fig: 12c), confluent to an adjacent growth over the cornea, was noticed over the medial bulbar conjunctiva. On the 14th day of observation conjunctiva was normal in two dogs (D2 and D4). The granulation tissue growth in dog (D1) appeared flattened with the conjunctiva grown over the cornea and having normal pink colour (Fig: 12d). In the rest three dogs (D3, D5 and D6) the conjunctiva had a granulation tissue growth on its medial side (Fig: 14d, Fig: 16d and Fig: 17d). On the 21st and 30th days of observation, the conjunctiva appeared normal in three dogs (D2, D3 and D4) while in one dog (D1) it remained the same with pigmentation over the conjunctival over growth (Fig: 12f). In the rest of the dogs (D5 and D6), granulation got extended to temporal peri-limbal area on 21st day of observation (Fig: 15e and Fig: 16e) and subsided by 30th day of observation. On 60th day of observation, bulbar conjunctiva was normal in all the dogs. In one dog (D1), conjunctiva which had grown over the cornea appeared transparent along with dispersed pigmentation over it, when compared to its 30th day of observation (Fig: 12g).

4.3.3. Eyelid deformities

On the day of presentation, five dogs (D1, D2, D3, D5 and D6) had medial canthal entropion (Fig: 14a) and medial canthoplasty was performed. On the 3rd day of observation, inflammatory changes were observed on the medial side of the eyelid.

Table 3: Conjunctival changes

	Days of observation						
DOG	0	3	7	14	21	30	60
D1	Moderate congestion, Pigmented perilimbal conjunctiva	Severely congested	Granulation -medially	Growth flattened	Conjunctiva grown over the cornea	Pigmentati on appeared over overgrown conjunctiv a	Overgrown conjunctiva appeared transparent with dispersed pigmentation
D2	Mild congestion, Pigmented perilimbal conjunctiva	Severely congested	Normal	Normal	Normal	Normal	Normal
D3	Mild congestion, Pigmented perilimbal conjunctiva	Severely congested	Normal	Granulation	Granulation subsided	Normal	Normal
D4	Mild congestion, Pigmented perilimbal conjunctiva	Severely congested	Normal	Normal	Normal	Normal	Normal
D5	Moderate congestion, Pigmented perilimbal conjunctiva	Severely congested	Severely congested - granulation	Granulation - medially	Extended to temporal perilimbal area	Subsided	Normal
D6	Moderate congestion, Pigmented perilimbal conjunctiva	Severely congested	Severely congested - granulation	Granulation- medially	Extended to temporal perilimbal area	Subsided	Normal

Healing of the wound was observed in all the dogs within 7 days of observation. There was no rubbing observed over the cornea by the sutures or the part of eyelid during the period of healing or after the healing.

4.3.4. Results of Visual Function Tests (Table 4)

On the day 0, the result of visual function tests was scored 2 (positive to all three tests and vision graded normal) in three dogs (D2, D3 and D4) while it was scored 0 (negative response to all three tests and vision graded absent) in the rest (D1, D5 and D6). On 3rd and 7th days of observation, the result of visual function tests was scored 1 (insufficient response to one or two test and vision graded decreased) in one dog (D1) while it was scored 2 in the rest (D2, D3, D4, D5 and D6). On 14th day of observation the score was 0 (negative response to all three tests and vision graded absent) in two dogs (D1 and D6) while it was either 1(insufficient response to one or two test and vision graded decreased) or 2 (positive to all three tests and vision graded normal) in the rest. On 21st, 30th and 60th days of observations visual function tests scored 2(positive to all three tests and vision graded normal) in three dogs (D2, D3, and D4) while it scored 0 (negative response to all three tests and vision graded absent) in the rest (D1, D5 and D6).

The observations of visual function tests and their results are presented in table No: 5

4.3.5. Rate and character of blink (Table 13)

The mean number of blinks per minute of all the dogs on 0th, 3rd, 7th, 14th, 21st, 30th and 60th days of observation were 14.83 ± 1.94, 18.67 ± 2.42, 14.17 ± 1.47, 14.67 ± 1.86, 15.17 ± 0.75, 14.5 ± 2.17 and 14.83 ± 0.75 respectively. The mean number of blinks per minute on postoperative observation days differed significantly at 5% level when compared to 0th day.

The character of blinks observed were complete and incomplete in which majority of the blinks observed were incomplete.

The mean number of complete blinks per minute on 0th, 3rd, 7th, 14th, 21st, 30th and 60th days of observation were 2.5 ± 1.05, 8.67 ± 1.37, 7.33 ± 1.03, 7.33 ± 1.03, 7.5 ± 1.05, 7.17 ± 1.17 and 7.5 ± 1.05 respectively**.** The mean number of complete blinks

Table 4: Visual function score

Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	0	1	1	0	0	0	0
D2	2	2	2	2	2	2	2
D3	2	2	2	2	2	2	2
D4	/2	2	2	2	2	2	2
D5	0	2	2	1	0	0	0
D6	0	2	2	0	0	0	0

Score 0 – Absent (negative to all the three tests), Score 1 – Decreased (insufficient response to one or two tests), Score 2 – Normal (positive to all the three tests

Tests: menace test, maze test, cotton ball test per minute on postoperative observation days differed significantly at 1 % level when compared to 0^{th} day.

4.3.6. Indirect ophthalmic examination

Fundic reflection was not visible in three dogs (D1, D5 and D6) on the day of presentation. Pigmentation was noticed as a black opaque curtain against the fundic reflection in three dogs (D2, D3 and D4). On 3^{rd} day of observation, fundic reflection was observed in two dogs (D2 and D3) while in the rest (D1, D4, D5 and D6) it was not visible. On 7^{th}, 14^{th} and 21^{st} day of observation fundic reflection was observed in one dog (D2) while in the rest it was not visible. On the 30^{th} and 60^{th} day of observation, fundic reflection was visible in three dogs (D2, D3, D4) along with pigmentation as a black opaque curtain against it, while in the rest it was not visible.

4.3.7. Direct ophthalmoscopic examination

4.3.7.1. Corneal luster (Table: 5)

Corneal luster was present in all the dogs on all days of observations. Post operatively artificial tears was stopped but the luster maintained throughout the observation period.

4.3.7.2. Corneal Clarity (Table: 6) (Plate: 4)

On day 0, corneal clarity was graded 4 (completely opaque) (Fig: 4d) in three dogs (D1, D5 and D6) while it was 1 (iris details visible) (Fig: 4a) in the rest of the dogs (D2, D3 and D4). On 3^{rd} day of observation, clarity was graded 1 (iris details visible) in two dogs (D2 and D3) while it was either grade 2 (pupillary margin visible, iris details not visible) (Fig: 4b) or 3 (pupillary margin visible, iris details not visible) (Fig: 4c) in rest of the four (D1, D4, D5 and D6) dogs. On 7^{th} day of observation the clarity was graded 2 (pupillary margin visible, iris details not visible) in three dogs (D3, D5 and D6) while it was graded 3 (pupillary margin not visible) in two dogs (D4 and D1) and 1 (iris details visible) in rest (D2). The clarity on 14^{th} day of observation was graded 1 (iris details visible) and 2 (pupillary margin visible, iris details not visible) in D2 and D3 respectively, while it was either 3 (pupillary margin visible, iris details not visible) or 4 (completely opaque) in rest of the dogs. On 21^{st} day of observation clarity, was graded 4 (completely opaque) in three dogs (D1, D5 and D6) while 3 (pupillary margin not visible), 2 (iris details visible) and 1 (iris details visible) in D3, D4 and D2 respectively. On 30th and 60th day of observation, the clarity was graded 4 (completely opaque) in three dogs (D1, D5 and D6) while 1 (iris details visible) in rest of the dogs (D2, D3 and D4) except D1. On 60th day of observation the clarity had become grade 3 in D1.

4.3.7.3. Corneal oedema (Table: 7) (Plate: 5)

Corneal oedema was absent (grade 0) (Fig: 5a) in all dogs on day zero. On 3rd day of observation, there was mild corneal haze (grade 1) (Fig: 5b) in two dogs (D2 and D3), marked corneal opacity (grade 2) (Fig: 5c) in one dog (D1) and severe corneal opacity (grade 3) (Fig: 5d) in three dogs (D4, D5 and D6) . On 7th day of observation severe corneal opacity (grade 3) was observed in four dogs (D1, D4, D5 and D6) and marked opacity (grade 2) in two dogs (D2 and D3). On day 14 there was no corneal oedema (grade 0) in one dog (D1) , mild corneal haziness (grade 1) in one dog (D2), marked opacity (grade 2) in one dog (D4) and severe corneal opacity (grade 3) in three dogs (D3, D5 and D6). On day 21 there was no corneal oedema in two dogs (D1 and D6), mild corneal haziness in one dog (D2), marked opacity (grade 2) in one dog (D4) and severe corneal opacity (grade 3) two dogs (D3 and D5). On day 30, there was mild corneal haze (grade 1) in two dogs (D3, and D4) while all other dogs had no signs of corneal oedema (grade 0). On day 60 mild corneal haziness (grade 1) was observed in one dog (D4) while there was no signs of corneal oedema (grade 0) in any other dogs.

4.3.7.4. Vascularisation of Cornea (Table: 8) (Plate: 6)

On day 0 there was mild superficial vascularisation (grade 1) (Fig: 6b) in three dogs (D2, D3 and D4) while in one dog (D6) vascularisation was extensive with thick vessels originating from all quarters (grade 3) (Fig: 6d). In two dogs (D1 and D5) ophthalmoscopic examination did not reveal any vascularisation (grade 0) (Fig: 6a) but, extensive vascularisation with thick vessels originating from all quarters (grade 3) was noticed against fundic reflection, intraoperatively, following removal of the

Table 5: Corneal luster

CORNEAL LUSTER							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	+	+	+	+	+	+	+
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+
D5	+	+	+	+	+	+	+
D6	+	+	+	+	+	+	+

+ : Present - : Absent

Table 6: Grades of corneal clarity

CORNEAL CLARITY							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	4	2	3	4	4	4	3
D2	1	1	1	1	1	1	1
D3	1	1	2	2	3	1	1
D4	1	3	3	3	2	1	1
D5	4	2	2	3	4	4	4
D6	4	2	2	4	4	4	4

Score 1 – iris details visible,
Score 2 – pupillary margin visible, iris visible not visible,
Score 3 – pupillary margin not visible,
Score 4 – cornea totally opaque

Table 7: Grades of corneal oedema

CORNEAL OEDEMA							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	0	2	3	0	0	0	0
D2	0	1	2	1	1	0	0
D3	0	1	2	3	3	1	0
D4	0	3	3	2	2	1	1
D5	0	3	3	3	3	0	0
D6	0	3	3	3	0	0	0

Score 0 – no signs
Score 1 – mild corneal haze
Score 2 – marked corneal opacity, anterior chamber still visible
Score 3 – severe corneal opacity, anterior chamber not visible

PLATE 4

Corneal clarity – Grading

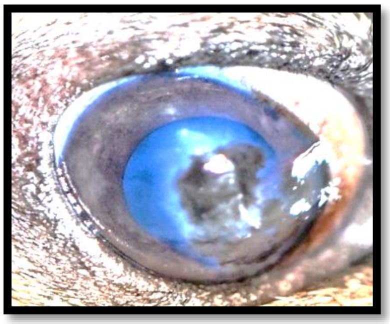


Fig: 4a – Grade 1, iris details visible, D4

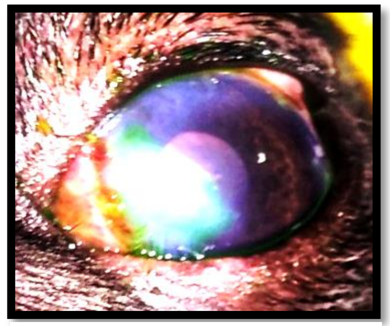


Fig: 4b - Grade 2, pupillary margin visible, iris details not visible, D2

Fig: 4c - Grade 3, pupillary margin not visible, D5

Fig: 4d - Grade 4, cornea totally opaque, D5

PLATE 5

Corneal oedema – Grading

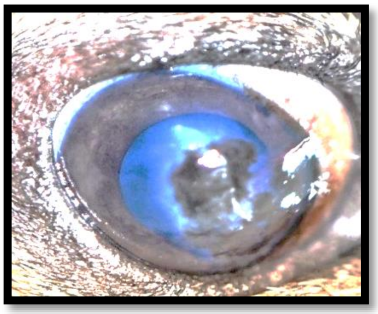


Fig: 5a - Grade 0, no signs, D4

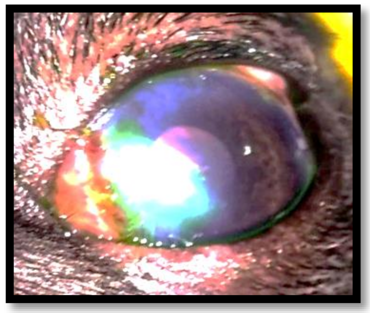


Fig: 5b - Grade 1, mild corneal haze, D2

Fig: 5c - Grade 2, marked corneal opacity, anterior chamber still visible, D3

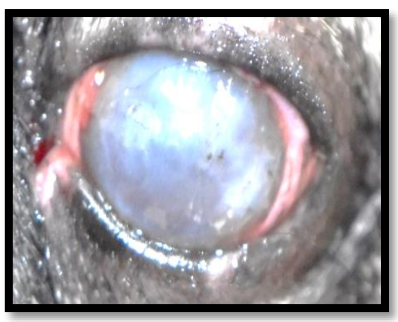


Fig: 5d – Grade, severe corneal opacity, anterior chamber not visible, D6 superficial pigmented layer. On day 3, there was profuse vascularisation visible to naked eye, (grade 2) (Fig: 6c) in one dog (D1) while in the rest there was no vascularisation (grade 0). On day 7 profuse superficial vascularisation (grade 2) was observed in two dogs (D1 and D3) on medial side while in the rest of the dogs (D2, D4, D5 and D6) vascularisation was mild superficial (grade 1). On the 14th day of observation, vascularisation was extensive with thick vessels originating from all quarters (grade 3) in one dog (D1), while in the rest of the dogs, profuse vascularisation visible to naked eye (grade 2) was observed around a granulation tissue. On 21st day of observation, extensive vascularisation with thick vessels orginating from all quarters (grade 3) was observed in two dogs (D1 and D5) while two dogs (D3 and D6) had profuse vascularisation visible to naked eye (grade 2). One dog (D2) revealed mild superficial vascularisation (grade 1) while another dog (D4) did not reveal any vascularisation (grade 0). On 30th day of observation, profuse vascularisation visible to naked eye (grade 2) was observed in three dogs (D1, D5 and D6) while the rest of the dogs did not had any vascularisation (grade 0). On 60th day, remnants of vessels were observed over the cornea in D1 while no other dogs showed any signs of vascularisation on cornea (grade 0).

4.3.7.5. Pigmentation score of cornea (Table: 9)

On day 0, pigmentation score was 72 (complete pigmentation of cornea) in two dogs (D1 and D5) (Fig: 12a and Fig: 16a) while it was 42 (Fig: 13a), 30 (Fig: 14a), 20 (Fig: 15a) and 64 (Fig: 17a) in dogs D2, D3, D4 and D6 respectively. Pigmentation score was 0 on day 3, 7 and14 in all dogs. Even though the pigmentation score was 0, granulation was noticed in one dog (D1) on medial side of the cornea on day 7, which got flattened and conjunctiva started growing over the cornea on day 14. On day 14, there was granulation tissue noticed either in the medial side of cornea or in the nasal and temporal regions of the limbus in four dogs (D3, D4, D5 and D6) while there was no growth over the cornea in one dog (D2). On 21st day of observation, corneal

PLATE 6

Corneal vascularisation – Grading

Fig: 6a - No vessels visible-grade 0, D3

Fig: 6b - Mild superficial vascularisation-grade 1, D2

Fig: 6c - Profuse superficial vascularisation visble to naked eye-grade 2, D3

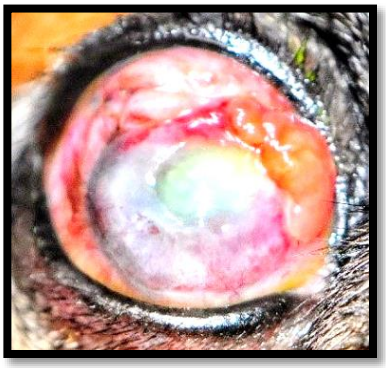


Fig: 6d - Extensive vascularisation with thick vessels originating from all quarters-grade 3, D5 pigmentation was noticed in five dogs (D1- score 6, D2- score 10, D4- score 30, D5- score 4, D6-pigmentation score 18).The pigmentation was mostly noticed on the medial side of the cornea. There was no pigmentation in one dog (D3). On day 30 pigmentation was seen dispersed in one dog (D1) with score 9 while it was extended from medial side with pigmentation score 32 in D2, 20 in D5 and 50 in D6. In one dog (D3) recurrence of pigmentation started along with a scar on the same day of observation with pigmentation score 8 while in one dog (D4) the pigmentation score remained same with no extension. On day 60, pigmentation score 72 in two dogs (D5 and D6) whilein dogs D1, D2, D3 and D4 pigmentation score was 26, 40, 8 and 30 respectively.

4.3.7.6. Pigmentation depth (Plate 7) (Table: 10)

On day 0, the pigmentation depth was superficial as well as deep in three dogs (D1, D5 and D6) while it was superficial alone in the rest (D2, D3 and D4). On day 3, 7 and 14 there was no pigmentation observed in any dogs. On 21st day of observation superficial pigmentation was present in three dogs (D1, D2 and D4) while it was superficial as well as deep in two dogs (D5 and D6). On 30th day, pigmentation was superficial in four dogs (D1, D2, D3 and D4) while it was superficial as well as deep in two dogs (D5 and D6). The Depth of pigmentation remained same on 60th day of observation in all dogs.

4.3.7.7. Pigmentation density (Plate 7) (Table: 11)

On day 0, pigmentation density was severe in five dogs (D1, D2, D3, D4 and D5) while one dog (D6) had moderate density. On day 3, 7 and 14 there was no pigmentation for any of the dogs. On day 21, D3 did not had any pigmentation while four dogs (D1, D2, D4 and D5) had mild pigmentation density and one dog (D6) had severe. On day 30, the pigmentation density was mild in three dogs (D1, D3 and D4), moderate in one dog (D2), and severe in two dogs (D5 and D6). On day 60, the density has become moderate for D2 and D4 while it remained same as that on previous day of observation for the rest.

Table 8: Grades of corneal vascularisation

CORNEAL VASCULARISATION							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	0 (on ophthalmic examination) 3 (intra-operatively)	2	2	3	3	2	0
D2	1	0	1	2	1	0	0
D3	1	0	2	2	2	0	0
D4	1	0	1	2	0	0	0
D5	0 (on ophthalmic examination) 3 (intra-operatively)	0	1	2	3	2	0
D6	3	0	1	2	2	2	0

Score 0 – no visible vessels
Score 1 – mild superficial vascularisation, thin vessels visible with magnification
Score 2 – profuse superficial vascularisation visible to naked eye
Score 3 – extensive vascularisation with thick vessels originating from all quadrants

Table 9: Pigmentation score

PIGMENTATION SCORE							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	72	0	Granulation No pigmentation	Granulation flattened. Overgrown conjunctiva. No pigment	6	Pigmentation dispersed-9	26
D2	42 medially	0	0	0	10 (medial)	32	40
D3	30 medially	0	0	Granulation (medial)	0 (granulation subsided)	8 pigmentation along with a scar	8
D4	20 medially	0	0	Granulation (medial)	30 medially	30	30
D5	72	0	0	Granulation (nasal and temporal limbus)	4	20 medially	72
D6	64	0	0	Granulation (medial)	18	50	72

Score 0 – no pigmentation, Score 1 – pigmented area < 30% of the sector area, Score 2 – pigmented area 30% - 60% of the sector area, Score 3 – pigmented area > 60% of sector area

Table 10: Pigmentation depth

PIGMENTATION DEPTH							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	Superficial as well as deep	-	-	-	Superficial	Dispersed superficial pigmentation	Superficial
D2	Superficial	-	-	-	Superficial	Superficial	Superficial
D3	Superficial	-	-	-	-	Superficial	Superficial
D4	Superficial	-	-	-	Superficial	Superficial	Superficial
D5	Superficial as well as deep	-	-	-	Superficial and deep	Superficial and deep	Superficial and deep
D6	Superficial as well as deep	-	-	-	Superficial and deep	Superficial and deep	Superficial and deep

Table 11: Pigmentation density

PIGMENTATION DENSITY							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	Severe	-	-	-	Mild	Mild	Mild
D2	Severe	-	-	-	Mild	Moderate	Moderate
D3	Severe	-	-	-	-	Mild	Mild
D4	Severe	-	-	-	Mild	Mild	Moderate
D5	Severe	-	-	-	Mild	Severe	Severe
D6	Moderate	-	-	-	Severe	severe	Severe

PLATE 7

Pigmentation in D1, D2, D3, D4, D5 and D6

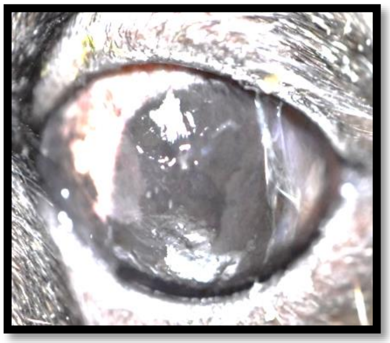


Fig: 7a - Severe density and Superficial as well as deep, D1 pigmentation,D1

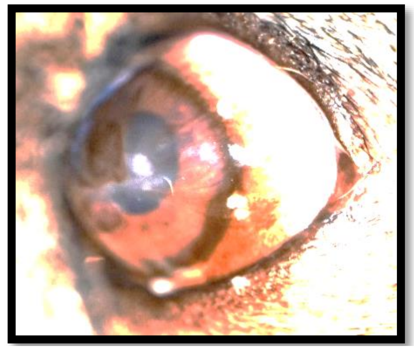


Fig: 7b - Severe density and Superficial pigmentation, D2

Fig: 7c - Severe density and Superficial pigmentation, D3

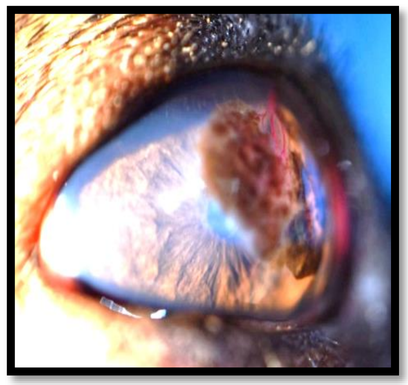


Fig: 7d - Severe density and Superficial, D4

Fig: 7e - Severe density and Superficial as well as deep pigmentation, D5

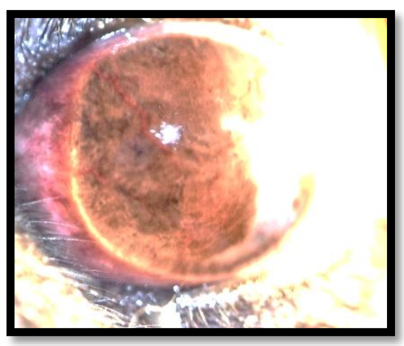


Fig: 7f - Severe density and moderate, D6

4.4. NEURO-OPHTHALMIC TEST RESULTS (Table: 12)

4.4.1. Menace reflex

On day 0, menace test was positive in three dogs (D1, D5 and D6) indicating impaired vision while the rest of the dogs (D2, D3 and D4) showed negative menace test implying normal vision. On day 3 and 7 positive menace test (impaired vision) was observed in one dog (D1) while, rest of the dogs (D2, D3, D4, D5 and D6) showed negative menace test (normal vision). From day 14 to day 60 positive menace test (impaired vision) was observed in three dogs (D1, D5 and D6) while the rest of the dogs (D2, D3 and D4) showed negative menace test (normal vision).

4.4.2. Cotton ball test

On day 0, positive response to cotton ball test was observed in three dogs (D2, D3 and D4) while the rest (D1, D5 and D6) showed negative response. On day 3, positive response to cotton ball test was observed in four dogs (D2, D3, D4 and D5), while two dogs (D1 and D6) showed negative response. On day 7, positive response to cotton ball test was observed in five dogs (D2, D3, D4, D5 and D6) while one dog (D1) showed negative response. On day 14, positive response to cotton ball test was observed in four dogs (D2, D3, D4 and D5) while two dogs (D1 and D6) showed negative response. On 21^{st}, 30^{th} and 60^{th} day negative response of cotton ball test was observed in three dogs (D1, D5 and D6) while the rest of the dogs (D2, D3 and D4) showed positive response.

4.4.3. Maze test

On day 0, maze test was positive in three dogs (D1, D5 and D6) indicating impaired vision while the rest of the dogs (D2, D3 and D4) showed negative maze test implying normal vision. On day 3 and 7 positive maze test (impaired vision) was observed in one dog (D1) while, rest of the dogs (D2, D3, D4, D5 and D6) showed negative maze test (normal vision). From day 14 to day 60 positive maze test (impaired vision) was observed in three dogs (D1, D5 and D6) while the rest of the dogs (D2, D3 and D4) showed negative maze test (normal vision).

4.4.4. Dazzle reflex

On day 0, positive dazzle reflex was observed in four dogs (D2, D3, D4 and D6) while, the rest (D1and D5) had negative reflex. On day 3 and day 7 positive dazzle reflex was observed in all the dogs. On the 14th day, negative dazzle reflex was observed in one dog (D1) while, the rest of the dogs (D2, D3, D4, D5 and D6) showed positive reflex. On 21st day, negative dazzle reflex was observed in two dogs (D1 and D6) while the rest of the dogs (D2, D3, D4 and D5) showed positive dazzle reflex. On 30th and 60th day of observation positive reflex was observed in three dogs (D2, D3 and D4), while the rest dogs (D1, D5 and D6) showed negative reflex.

4.4.5. Pupillary light reflex

On day 0, PLR was observed in three dogs (D2, D3 and D4) and it was not observed in rest of the dogs (D1, D5 and D6). On the 3rd day of observation, PLR was observed in all the dogs. On 7th day of observation, PLR was observed in all the dogs except D1. On 14th day, PLR was observed in four dogs (D2, D3, D4 and D5), while in two dogs (D1 and D6) PLR was not observed. On 21st, 30th and 60th day of observation PLR was observed in three dogs (D2, D3 and D4) while in rest of the dogs (D1, D5 and D6) PLR was not observed.

4.4.6. Corneal sensitivity

On day 0, the corneal sensitivity was absent in all parts of cornea in three dogs (D1, D5 and D6) while it was present medially (sluggish), laterally, dorsally, ventrally and centrally in the rest (D2, D3 and D4). On 3rd day, corneal sensitivity was observed in all parts of the cornea in all the dogs. On 7th day, corneal sensitivity was observed in lateral part of cornea alone in one dog (D1), while it was present in all parts of cornea in all other dogs. On 14th day, sluggish corneal sensitivity was observed in lateral part of cornea in two dogs (D1 and D6) while, the rest (D2, D3, D4 and D5) were having normal sensitivity. On 21st day, corneal sensitivity was lost in two dogs (D1 and D6) while, four dogs (D2, D3, D4 and D5) were having normal corneal sensitivity. By 30th day, corneal sensitivity was lost in three dogs (D1, D5 and D6) and it remained same on 60th day. D2, D3 and D4 had normal corneal sensitivity throughout the observation period.

4.5. SPECIAL DIAGNOSTIC PROCEDURE

4.5.1. Schirmer Tear Test (STT) (Table 13)

Mean values for Schirmer tear test (STT-1) (mm/min) on the day of presentation and post-operatively on day 3, 7, 14, 21, 30 and 60 were 7±3.95, 21.33±2.07, 20.83±1.17, 18.5±1.22, 18±0.63, 16.83±0.75 and 16.5±2.07 respectively. STT-1 was increased during the observation period compared to the pre-operative values and the increase in values were significant at 1% level.

Mean values of Schirmer tear test (STT-2) (mm/min) on the day of presentation and post-operatively on day 3, 7, 14, 21, 30 and 60 was 0.83±1.6, 11.33±1.97, 10.33±1.03, 8.33±1.03, 8.5±1.05, 6.83±1.94 and 6.33±2.34 respectively. STT-2 was increased during the observation period compared to the pre-operative value and the increase in values were significant at 1% level.

4.5.2. Fluorescein Dye Test (FDT) (Table 14)

On day 0, FDT was negative for all the dogs. On 3rd day, FDT was positive for all the dogs. On 7th day of observation, FDT was negative in one dog (D1) alone while, the rest all showed positive for FDT. On 14th day of observation, FDT was negative for all the dogs. On 21st day of observation, FDT was positive for one dog (D5) alone while, rest all showed FDT negative. On 30th and 60th day of observation, all dogs were FDT negative.

4.5.3. Rose Bengal dye test

On 0th day of observation, cornea and conjunctiva of all dogs were stained positive for rose Bengal dye test.

4.5.5. Tear film breakup time (TBUT) (Table 13)

The mean values for the tear film breakup time (sec) on the day of presentation and post-operatively on day 3, 7, 14, 21, 30, and 60 were 4.83 ± 1.17, 8.67 ± 2.06, 9.83 ± 3.06, 10 ± 3.03, 9.33 ± 1.53, 9.33 ± 2.31 and 9 ± 2.19 respectively. TBUT observed

Table 12: Neuro - ophthalmic tests

MENACE TEST							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	-	+	+	-	-	-	-
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+
D5	-	+	+	+	-	-	-
D6	-	+	+	-	-	-	-
+ : Present - : Absent							
COTTON BALL TEST							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60-
D1	-	-	-	-	-	-	-
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+
D5	-	+	+	+	-	-	-
D6	-	-	+	-	-	-	-
+ : Present - : Absent							
MAZE TEST							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	+	+	+	+	+	+	+
D2	-	-	-	-	-	-	-
D3	-	-	-	-	-	-	-
D4	-	-	-	-	-	-	-
D5	+	-	-	+	+	+	+
D6	+	-	-	+	+	+	+
+ : Hitting the objects (impaired vision) - : Not hitting the objects (normal vision)							
CORNEAL SENSITIVITY							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	-	+	+laterally	Sluggish laterally	-	-	-
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+
D5	-	+	+	+	+	-	-
D6	-	+	+	Sluggish laterally	-	-	-
+ : Present - : Absent							
PUPILLARY LIGHT REFLEX							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	-	+	-	-	-	-	-
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+
D5	-	+	+	+	-	-	-
D6	-	+	+	-	-	-	-
+ : Present - : Absent							

DAZZLE REFLEX							
Dog	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	-	+	+	-	-	-	-
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+
D5	-	+	+	+	+	-	-
D6	+	+	+	+	-	-	-
+ : Present - : Absent							

Table 13: Mean values of ophthalmic tests

Tests	Day 0	Day 3	Day 7	Day 14	Day 21	Day 30	Day 60
STT1 (mm/min)	7 ± 3.95 [a]	21.33 ± 2.07 [a]	20.83 ± 1.17 [a]	18.5 ± 1.22 [b]	18 ± 0.63 [b]	16.83 ± 0.75 [b]	16.5 ± 2.07 [b]
STT2 (mm/min)	0.83 ± 1.6 [a]	11.33 ± 1.97 [a]	10.33 ± 1.03 [a]	8.33 ± 1.03 [b]	8.5 ± 1.05 [b]	6.83 ± 1.94 [b]	6.33 ± 2.34 [b]
TBUT (sec)	4.83 ± 1.17 [a]	8.67 ± 2.07 [b]	9.83 ± 3.06 [a]	10 ± 3.03 [a]	9.33 ± 1.53 [b]	9.33 ± 2.31 [b]	9 ± 2.19 [b]
Blink rate (per min)	14.83 ± 1.94 [a]	18.67 ± 2.42 [b]	14.17 ± 1.47 [a]	14.67 ± 1.86 [a]	15.17 ± 0.75 [a]	14.50 ± 2.17 [a]	14.83 ± 0.75 [a]
Complete blinks (per min)	2.5 ± 1.05 [a]	8.67 ± 1.37 [b]	7.33 ± 1.03 [a]	7.33 ± 1.03 [a]	7.5 ± 1.05 [a]	7.17 ± 1.17 [a]	7.5 ± 1.05 [a]

Means bearing different superscript in the row differ significantly at 1% level

Table 14: Special staining tests

Fluorescein dye staining test							
Dogs	DAY 0	DAY 3	DAY 7	DAY 14	DAY 21	DAY 30	DAY 60
D1	-	+	-	-	-	-	-
D2	-	+	+	-	-	-	-
D3	-	+	+	-	-	-	-
D4	-	+	+	-	-	-	-
D5	-	+	+	-	+	-	-
D6	-	+	+	-	-	-	-

+: staining present - : staining absent

on post-operative days were significantly high at 1 % when compared to those of pre-operative values.

4.5.6. Culture and antibiotic sensitivity

Culture of corneal swabs in three dogs (D4, D5 and D6) revealed presence of gram positive cocci with colony characters of *staphylococcus spp.* while there was no growth observed in the rest. The cultures had maximum sensitivity to ciprofloxacin followed by norfloxacin, ofloxacin, gentamicin and ceftriaxone. They were resistant to tetracycline, chloramphenicol, streptomycin and amoxicillin. Based on the antibiotic sensitivity, topical ciprofloxacin eye drops were used in all the cases.

4.6. HISTOLOGY (Plate: 8)

Sections from oral mucosa revealed mucosal, submucosal and the muscularis layers. Mucosa was lined by non-keratinised stratified squamous epithelium, while submucosa was composed of well vascularised dense irregular connective tissue. The oral mucosal autograft were having abundant number of mixed salivary glands. The glands were well encapsulated by connective tissue. The septa from the capsule divided the gland into lobules which enclosed secretory acini. Both serous and mucous secretory acini were present. The glands were observed in the submucosal layer very close to the epithelium.

4.7. GOBLET CELL INDEX (Plate: 9) (Table: 15)

The goblet cell indices of dogs D1, D2, D3, D4, D5 and D6 were, 0.09, 0.16, 0.16, 0.10, 0.02 and 0.07 respectively on day of presentation.

4.8. HISTOPATHOLOGY (Plate: 10)

Out of six dogs, pigmented epithelium of only three dogs (D1, D5 and D6) were submitted for histopathology, because the dogs in which diamond burr superficial keratectomy was performed, the pigmented epithelium got grinded and was not enough for processing.

Table 15: Goblet cell index

Dogs	Goblet cell index
D1	0.09
D2	0.16
D3	0.16
D4	0.10
D5	0.02
D6	0.07

$$\text{Goblet cell index} = \frac{\text{Number of goblet cells}}{\text{Number of epithelial cells}}$$

In dog D1, melanin pigments were observed in basal cells and in a few superficial cells. Corneal stroma contained large number of variably sized blood vessels. Most of them were located very close to the epithelium. Majority of the pigment laden cells were seen in the upper part of the stroma, close to the epithelium.

In dog D5, histopathological examination revealed mildly hyperplastic corneal epithelium. Corneal stroma contained melanin laden cells with minimal variation in shape and newly formed blood vessels. The normal architecture of the stromal collagen was lost.

In dog D6, histopathologic examination revealed moderately hyperplastic corneal epithelium. The melanin laden cells were mainly spindiloid in shape and were chiefly centered around newly formed blood vessels.

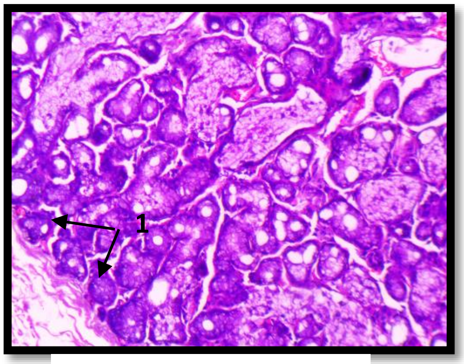
All the three conditions were diagnosed as melanosis.

4.9. INCORPORATION OF ORAL MUCOSAL AUTOGRAFT INTO CONJUNCTIVAL FORNIX (Plate 11)

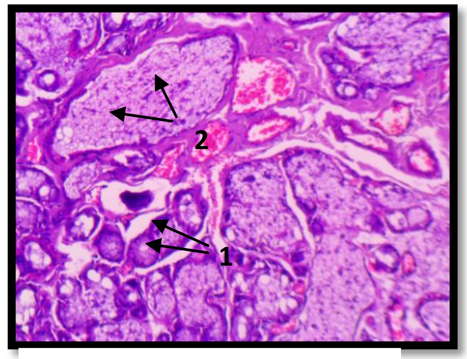
Harvested autografts had pigmented mucosal surface with minimum four or five duct openings over it. Glandular appearance was observed on the cut surface of the autograft. All the oral mucosal autografts fixed to the conjunctival fornix had healed completely and merged into the surrounding conjunctival surface without any complication in the post-operative period. On 3rd day of observation, all the autografts were elevated from the surface with hyperaemia around the conjunctiva and the sutured surface of the graft. On 7th day of observation, the colour of the graft started fading and inflammation was present and the grafts were elevated from the sutured surface. By 14th day the colour of the graft was faded and the graft started getting integrated in to the recipient bed. By 21st day of observation, all grafts were fully integrated in to the recipient bed and surrounding conjunctival mucosa of the fornix. By 30th day of observation, the colour of the graft became almost similar to the surrounding

PLATE: 8

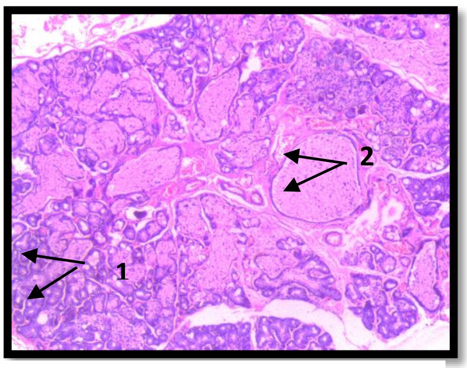
Photomicrograph of oral mucosal autograft showing minor salivary glands (MSG)



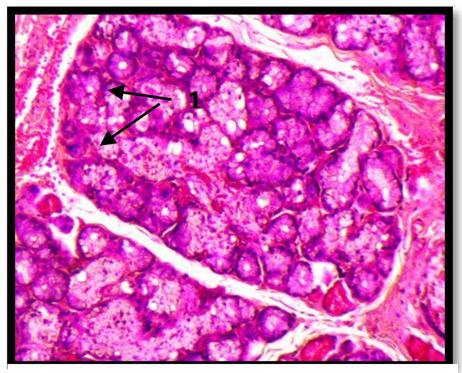
D1 - H and E, X 100



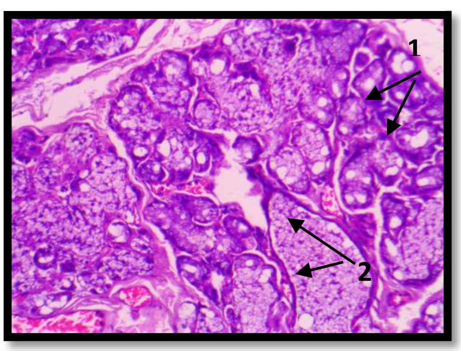
D2 – H and E, X 100



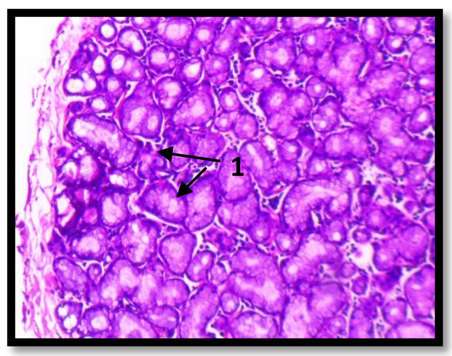
D3 – H and E, X 40



D4 – H and E, X 100



D5 – H and E, X 100



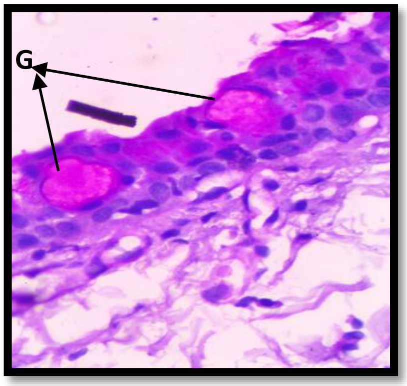
D6 – H and E, X 100

1 – Serous glands, 2 – Mucus glands

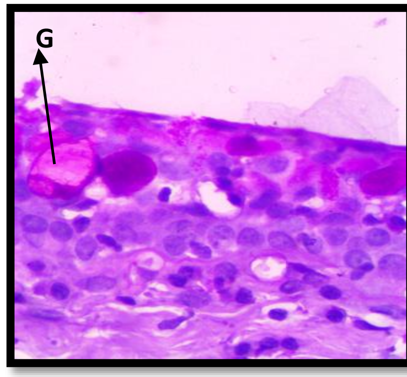
PLATE: 9

Photomicrograph of conjuctival mucosa for goblet cell counting. H and E, X 400

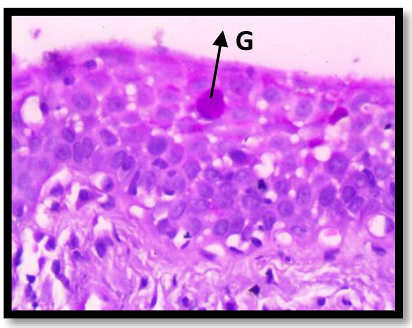
G – Goblet cell



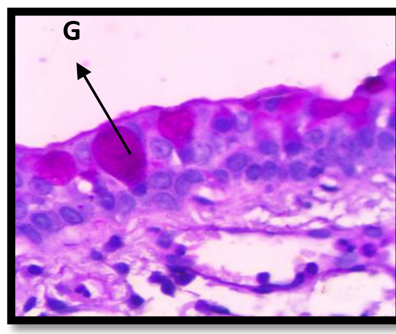
Dog - D1



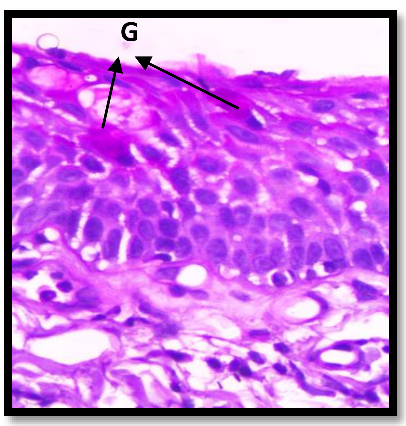
Dog – D2



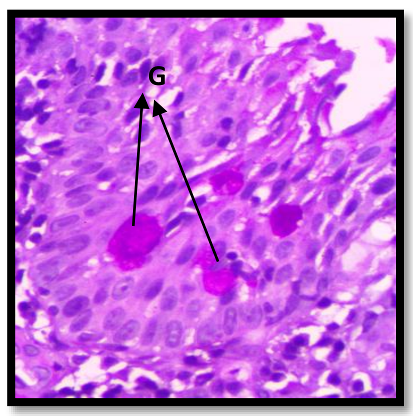
Dog – D3



Dog – D4



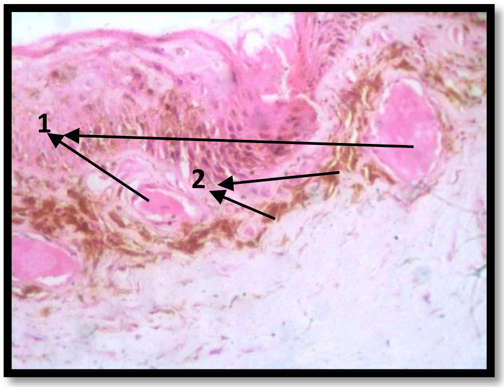
Dog – D5



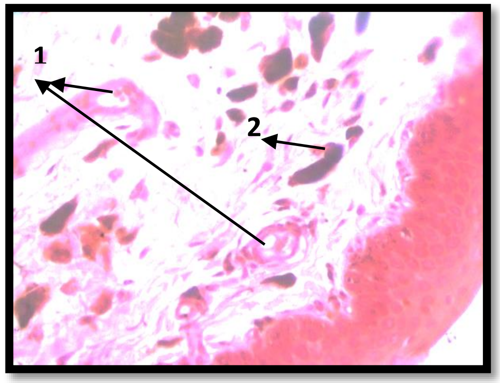
Dog – D6

PLATE: 10

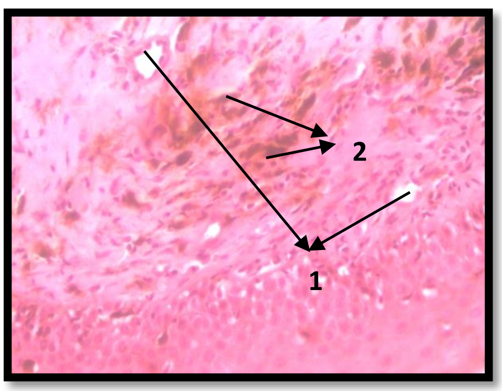
Photomicrograph of histopathology sections of pigmented corneal epithelium, H and E, x 400, (1) Blood vessels, (2) Melanin laden cells



D1, Corneal tissue- stroma -melaning laden cells around blood vessels and melanin pigment in basal epithelial cells



D5, Corneal tissue - stroma with neovascularisation and melanin laden cells



D6, Corneal tissue – stroma with spindle shaped melanin laden cells around newly formed blood vessels conjunctival tissue and it remained the same for 60th day of observation. In dog D2, D3 and D4, the surface of graft had mild elevation noticed after the healing process, however the surface of graft didnot affect the free movement of eyelid and graft or the sutures were not rubbing over the cornea throughout the observation period.

4.10. COMPLICATIONS

In D2 and D5, corneal melting was observed on 7th and 21st day of observation respectively (Fig: 13c and Fig: 16e). Auto serum fortified with amikacin and EDTA eye drops were administered in these cases. Within one week both the ulcers were healed. On 21st day of observation, there was recurrence of pigmentation in five dogs (D1, D2, D4, D5 and D6) while, in one dog (D3) the recurrence of pigmentation was observed on 30th day. Gradually the pigmentation started extending from the medial surface in all dogs while in three dogs D1, D5 and D6 the cornea was completely covered with pigmentation.

Conjunctiva was grown over the cornea in D1. It was initiated on 14th day and covered completely by 21st day of observation and recurred pigmentation was appeared dispersed over it.

PLATE 11

Incorporation of autograft in conjunctival fornix in D1, D2, D3, D4, D5 and D6

Fig: 11a, D1

Fig: 11b, D2

Fig: 11c, D3

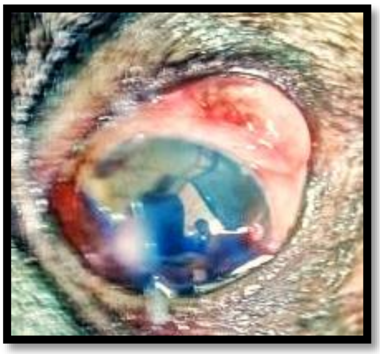


Fig: 11d, D4

Fig: 11e, D5

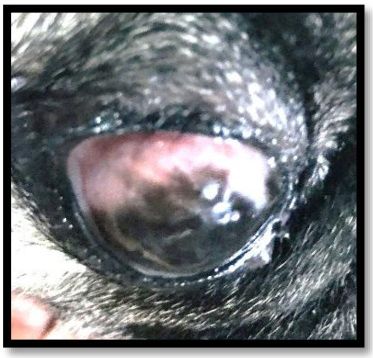


Fig: 11f, D6

PLATE 12

Observations of dog D1 on day 0, 3, 7, 14, 21, 30 and 60.

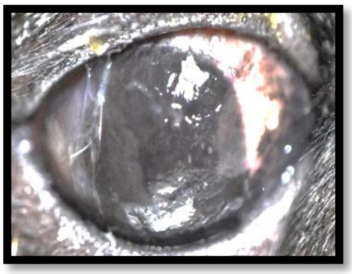


Fig: 12a - Day 0

Fig: 12b - Day 3

Fig: 12c - Day 7

Fig: 12d - Day 14

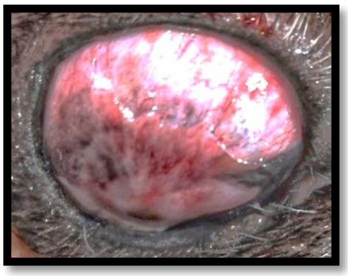
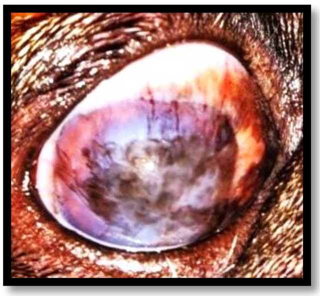


Fig: 12e - Day 21

Fig: 12f- Day 30



Reduced pigmentation score, depth and density compared to 0^{th} day

Fig: 12g- Day 60

PLATE 13

Observations of dog D2 on day 0, 3, 7, 14, 21, 30 and 60.

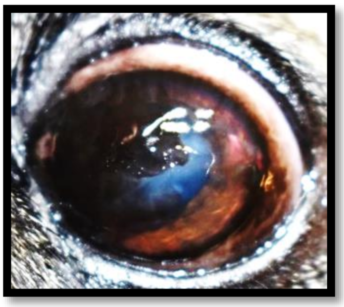


Fig: 13a - Day 0

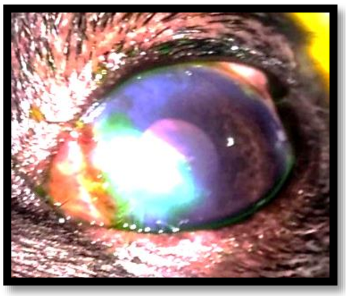


Fig: 13b - Day 3

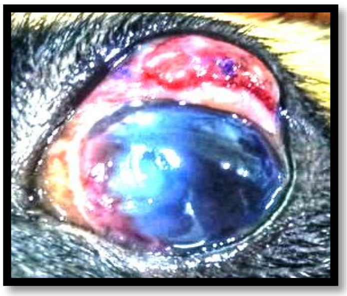


Fig: 13c - Day 7

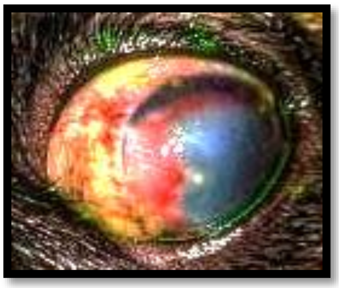


Fig: 13d - Day 14

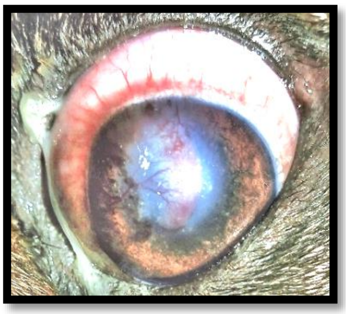


Fig: 13e - Day 21

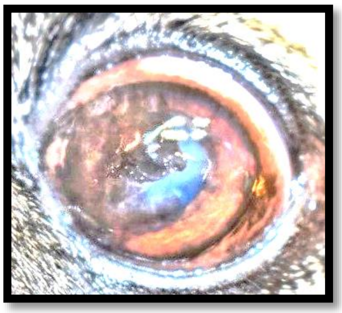


Fig: 13f-Day 30

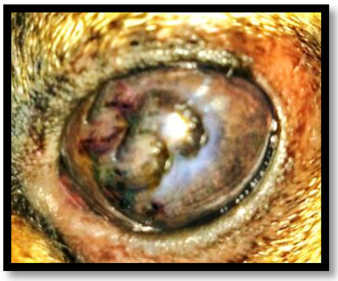


Fig: 13g -Day 60

Reduced density of pigmentation compared to 0th day

PLATE 14

Observations of dog D3 on day 0, 3, 7, 14, 21, 30 and 60.

Fig: 14a - Day 0

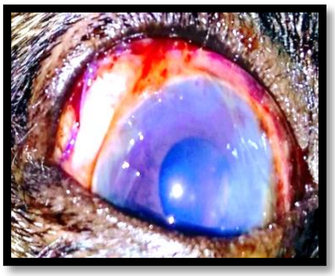


Fig: 14b - Day 3

Fig: 14c - Day 7

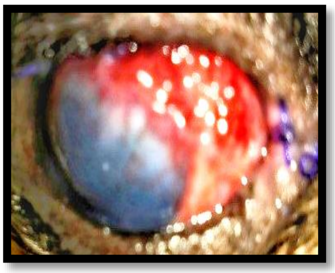


Fig: 14d - Day 14

Fig: 14e - Day 21

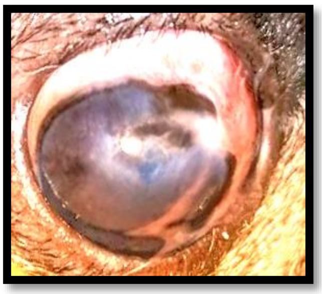


Fig: 14f- Day 30

Fig: 14g - Day 60

Reduced pigmentation score and density of pigmentation compared to 0^{th} day

PLATE 15

Observations of dog D4 on day 0, 3, 7, 14, 21, 30 and 60.

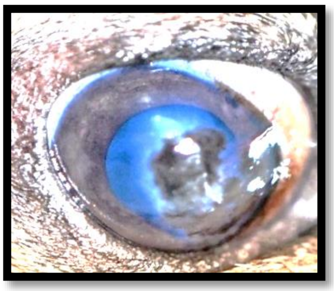


Fig: 15a - Day 0

Fig: 15b - Day 3

Fig: 15c - Day 7

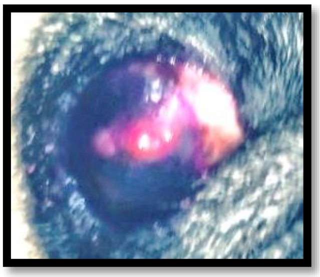


Fig: 15d - Day 14

Fig: 15e - Day 21

Fig: 15f - Day 30

Fig: 15g - Day

Reduced pigmentation score and density compared to 0th day

PLATE 16

Observations of dog D5 on day 0, 3, 7, 14, 21, 30 and 60.

Fig: 16a - Day 0

Fig: 16b - Day 3

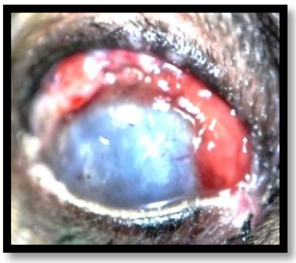


Fig: 16c - Day 7

Fig: 16d - Day 14

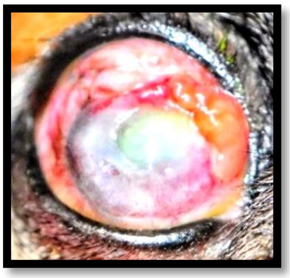


Fig: 16e - Day 21

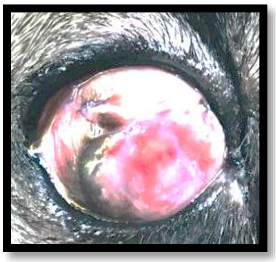


Fig: 16f – Day 30

Fig: 16g – Day 60

Complete recurrence of pigmentation

PLATE 17

Observations of dog D6 on day 0, 3, 7, 14, 21, 30 and 60.

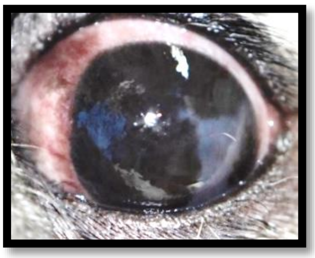


Fig: 17a - Day 0

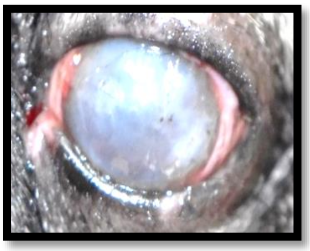


Fig: 17b - Day 3

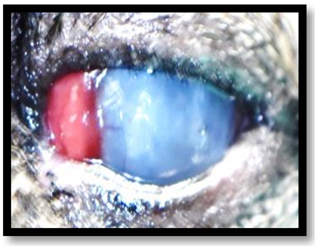


Fig: 17c - Day 7

Fig: 17d - Day 14

Fig: 17e - Day 21

Fig: 17f –Day 30

Complete recurrence of pigmentation

Fig: 17g – Day 60

DISCUSSION

5. DISCUSSION

Six dogs irrespective of age, breed and sex, having an eye with basal tear production less than 5 mm/min and having melanotic cornea formed the subjects of the study. The selected dogs were numbered D1, D2, D3, D4, D5 and D6. These dogs underwent oral mucosal autografting, punctal occlusion, superficial keratectomy and processed human amniotic membrane transplantation. Medial canthoplasty was also done in cases which required correction of medial canthal entropion. The results of the study are discussed below.

5.1. SIGNALMENT AND ANAMNESIS

The age of the dogs in this study ranged from eight months to five years. The observation in this study was similar to that of Anoop *et al.* (2015) where, the age of majority of the dogs studied were in between 1-3 years. All the dogs in the present study were Chinese Pugs. This could be due to the anatomical peculiarities of chineese pugs which make them susceptible for dry eye. This observation was in accordance to that of Sansom and Barnett (1985), Whitley *et al.* (1991), Whitley *et al.* (1995), Williams (2008), Chinchu (2010), Lacerda *et al*. (2017) and Charbiwala (2019). Out of the six dogs studied, majority were males which could be attributed to the preference of owners towards male dogs. Parulekar (2016) and Charbiwala (2019) had similar observations where in males showed higher distribution in corneal melanosis compared to females. Both the eyes were affected in all animals in the present study. This could be attributed to the anatomical peculiarities of brachycephalic dogs. This finding was in accordance to Parulekar (2016) where the corneal pigmentation was observed bilaterally in majority of the dogs of his study. The symptoms noticed were thick ocular discharge in all dogs, while three dogs had blackish colouration of eye and impairment in vision. Similar observations were reported by Bedford (1982), Sansom and Barnett (1985) and Kaswan and Salisbury (1990). The common clinical signs reported were mucoid to mucopurulent gluey discharge, conjunctivitis, keratitis, neovascularization, keratinization, hypertrophy of the cornea and pigmentary keratitis. Duration of the condition in the dogs of this study ranged from 7 months to 24 months. The eyes (OD,n=5 and OS, n=1) having basal tear production less than 5mm/min were selected for the study, since the efficiency of minor salivary gland autografting can be appreciated only when the basal tear production is minimum.

5.2. GENERAL CLINICAL EXAMINATION

5.2.1. General body condition

General body condition was good in all the dogs on all days of observation, suggesting that the procedures did not affect any general physical status of the dogs studied.

5.2.2. Physiological parameters

5.2.2.1. Rectal temperature

The mean rectal temperature (°C) on 0, 3, 7, 14, 21, 30 and 60 days of observation were 38.15 ± 0.57, 38.08 ± 0.57, 38.3 ± 0.50, 38.2 ± 0.44, 38 ± 0.54, 37.95 ± 0.39 and 38.1 ± 0.42 respectively. The values were within the normal physiological range and variation in rectal temperature noticed during the observation period was not significant.

5.2.2.2. Respiration rate

The mean respiration rate (per min) of the dogs on 0, 3, 7, 14, 21, 30 and 60 days of observation were 28.83 ± 1.47, 31.17 ± 1.60, 29.33 ± 1.03, 28.83 ± 1.17, 30.17 ± 2.86, 28.17 ± 2.04 and 30.67 ± 3.01 respectively. The values were within the normal physiological range and variation in respiration rate noticed during the observation period was not significant.

5.2.2.3. Pulse rate

The mean pulse rate (per min) of the dogs on 0, 3, 7, 14, 21, 30 and 60 days of observation were 97.17 ± 8.77, 98.17 ± 7.33, 98.83 ± 2.71, 97.17 ± 2.48, 101.67 ± 5.82 and 97 ± 3.79 and 92.67 ± 4.89 re[illegible]vely. The values were within normal physiological range and variation noticed during observation period was not significant.

5.2.2.4. Appearance of visible mucous membrane

The conjunctival mucous membrane was congested. The conjunctival congestion is attributed to the inflammation of conjunctiva associated with the kerato conjunctivitis sicca (Mandell and Holt, 2005), oral mucous membrane was pigmented in all the dog studied and can be attribute to the breed peculiarity. Colour of tongue was pink throughout the observation period. The colour of conjunctival mucous membrane during the days of observation varied as to the stages of healing.

5.3. OPHTHALMIC EXAMINATION

5.3.1. Nature of ocular discharge

All the dogs studied had thick mucoid ocular discharge on day 0 which is a symptom of dry eye (Bedford, 1982; Sansom and Barnett, 1985). The discharge had become mucopurulent on 3rd day of observation and which could be attributed to the accumulation of white blood cells as a result of surgical trauma. The discharge had become serous in nature in two dogs (D3 and D4), while it remained mucopurulent in the rest on 7th day of observation. The nature of discharge can be attributed to the extent of surgical trauma and the time required for the healing process. In D1, D5 and D6 the cornea was completely pigmented for which complete superficial keratectomy and trichloro acetic acid debridement had to be performed. But in D2, D3 and D4 the extent of pigmentation was less, for which, extent of superficial keratectomy, trichloro acetic acid debridement and thereby the surgical trauma was comparatively less. Anti-inflammatory, antibacterial and regenerative properties (Janson and Sikder, 2014) of the processed human amniotic membrane and topical antibiotics could have contributed for the improvement of ocular discharge. D2 had corneal melting on the respective day, which would have been an additional reason for its muco-purulant discharge. Ocular discharge was observed serous in nature in all dogs on 14th day of observation and continued so on 21st, 30th and 60th day of observation. In D5, mucopurulant discharge was observed on 21st day and is attributed to corneal melting observed on the same day. Later on the discharge had become serous. The normal serous nature of ocular discharge towards last days of observations can be attributed to the reduction in the inflammation, healing of cornea and resumption of minor salivary gland secretions. Anti-inflammatory, antibacterial and regenerative properties of saliva have already been reported earlier (Grixti and Malhotra, 2018). The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

5.3.2. Appearance of Conjunctiva

Conjunctiva appeared mildly congested in three dogs (D2, D3 and D4) on day 0, while it was moderately congested in the rest. This could be attributed to the severity of inflammatory changes associated to the condition and is in accordance to that reported earlier (Mandell and Holt, 2005). Bulbar conjunctiva was pigmented around the limbus which was denser on the medial side in all the dogs. The lagophthalmos nature of the eye exposing the bulbar conjunctiva and resultant dryness would have resulted in chronic irritation and thereby pigmentation. The denser pigmentation on the medial side might be due to the constant rubbing of medial canthal entropion over the bulbar conjunctiva (Packer *et al*., 2015). Similar peri-limbal pigmentation of bulbar conjunctiva was observed by Charbiwaala (2019). The conjunctiva appeared severely congested in all dogs on 3rd day of observation. This is attributed to the surgical trauma associated with peri-limbal conjunctivectomy. Peri-limbal conjunctiva act as a major potential source of pigmentation and vascularisation of superficial cornea during an inflammatory process. Therefore removal of the conjunctiva will significantly reduce the chances of reccurance of corneal vascularisation and concurrent corneal pigmentation which happens during corneal healing after keratectomy. The observation are in accordance to that of Charbiwaala (2019). Conjunctiva was severely congested with granulating appearance in two dogs (D5 and D6) on 7th day of observation. The granulation can be attributed to the healing of the conjunctiva.

Gradually the conjunctival hyperaemia and granulation reduced in three dogs (D1, D2, D4) while the rest three dogs (D3, D5 and D6) had a granulation tissue growth on its medial side and which extended towards the temporal limbal area. Conjunctiva appeared normal in three dogs (D2, D3 and D4) on 21st and 30th day of observation while in one dog (D1) conjunctiva remained the same with pigmentation over the conjunctival growth. In rest of the dogs (D5 and D6) granulation got extended to temporal peri-limbal area and central cornea on 21st day of observation and subsided by 30th day of observation. These conjunctival changes can be attributed to the extend of surgical trauma induced on conjunctiva or the area of pigmentation excised and sutured. As the extend of trauma increases, the time taken for healing increases and the granulation and other inflammatory changes also prolongs. On 60th day of observation, bulbar conjunctiva was normal in all the dogs. The anti-inflammatory, antibacterial and regenerative properties of saliva (Grixti and Malhotra, 2018) could have assisted in conjunctival healing. The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

In one dog (D1), conjunctiva which had grown over the cornea appeared transparent along with dispersed pigmentation over it, when compared to its 30th day of observation.

5.3.3. Eyelid deformities

Medial canthal entropion was noticed in five dogs studied, which required medial canthoplasty. Medial canthoplasty was performed in all these five dogs as per

Gunderson (2013) and Stades *et al*. (2007). Medial canthal entropion is a component of brachycephalic ocular sydome which is common in pugs. Where the eyelid folded inwards continuously rub the cornea and results pigmentary keratitis (Packer *et al*., 2015). Hence surgical correction of medial canthoplasty was opted in these five cases to reduce the irritation. Further rubbing of cornea was not noticed following medial canthoplasty on subsequent days of observation. Gunderson (2013), suggested that, medial canthoplsty helped correcting the medial canthal entropion, and thereby reduced constant irritation to eyes. The medial canthoplasty also reduces the macro palpebral fissure and there by reduces lagophthalmos condition of eye and helps in complete blinking of eyelids. This is in accordance to Stades *et al*. (2007) who suggested medial canthoplaty as a surgical correction for brachycephalic ocular syndrome by shortening the macropalpebral fissure.

5.3.4. Results of Visual Function Tests

The results of visual function tests were graded normal (positive to all the three tests and score 2) in three dogs on day 0. The degree of pigmentation was less in these three dogs which helped these dogs to score high for their visual function tests. The results of visual function tests were graded absent (negative to all the three tests and score 0) in rest of the three dogs (D1, D5 and D6) because they had complete pigmentation of the cornea, which made the cornea completely opaque. The results of visual function tests on 3rd and 7th days of observation were graded normal (positive to all the three tests and score 2) in five dogs (D2, D3, D4, D5 and D6) while vision graded decreased (insufficient response to one or two tests and score 1) in one dog (D1). The complete removal of pigmented layer of cornea in those five dogs helped them to grade their vision normal on these days of observations. Vision was graded absent (score 0) in two dogs (D1 and D6) on 14th day of observation while it was decreased (score 1) in one dog (D5) but graded normal (score 2) in three dogs (D2, D3 and D4). On 21st, 30th and 60th days of observations vision graded normal (score 2) in three dogs (D2, D3, and D4) while it was graded absent (score 0) in the rest (D1, D5 and D6) due to complete pigmentation of cornea. The reduction or absence of vision noticed is due to obstruction of the visual axis by corneal oedema, granulation growth or pigmentation.

Degree of oedema and pigmentation had played a role in the vision of dogs in the present study. The dogs with deep, extensive and severe degree of pigmentations showed negative result in menace, cotton ball and maze test which was similar to the observations of Charbiwala (2019).

5.3.5. Rate and character of blink

The mean number of blinks per minute of the dogs on 0, 3, 7, 14, 21, 30 and 60 days of observation were 14.83 ± 1.94, 18.67 ± 2.42, 14.17 ± 1.47, 14.67 ± 1.86, 15.17 ± 0.75, 14.50 ± 2.17 and 14.83 ± 0.75 respectively. The mean number of blinks per minute on 3rd day of observation was significantly higher when compared to the 0th day at 1% level. It could be due to increased sensitivity of cornea due to superficial keratectomy and the exposed nerve endings by keratectomy. The increase in the rate of blink in the initial days of postoperative observation is due to the blepherospasms caused by exposed corneal nerve endings. This is similar to the observation of the Ribeiro *et al.* (2008) who also reported that reduction in number of blinks could be due to deterioration of nerve endings.

Majority of the blinks were incomplete, in the dogs studied (Carrington *et al*., 1989). This is attributed to the lagophthalmos and macropalpebral fissure of the dog. Dog would not be able to close the eyelids completely due to their wide opened eyelids and the boophthalmous eyeball (Bedford and Jones, 2001).

The mean number of complete blinks per minute on 0, 3, 7, 14 ,21, 30 and 60 days were 2.5 ± 1.05, 8.67 ± 1.37, 7.33 ± 1.03, 7.33 ± 1.03, 7.5 ± 1.05, 7.17 ± 1.17 and 7.5 ± 1.05 of observation respectively. The mean number of complete blinks per minute on postoperative observation days were significantly higher when compared to 0th day at 1% level. Preoperatively, number of complete blinks are alarmingly less. The reduction in the palpebral fissure length following medial canthoplasty could have reduced the lagophthalmos condition and helped to increase the number of complete blinks. This is in accordance to the study of Stades *et al*. (2007). Postoperatively all the blinks did not turned to be complete blinks because in brachycephalic breeds exophthalmos is a combined effect of shallow orbits and large palpebral fissure (Bedford and Jones, 2001). The increase in number of complete blinks would help in spreading of tears over the cornea and thereby preventing dryness.

5.3.6. Indirect ophthalmoscopic examination

Fundus could not be visualized in three dogs (D1, D5 and D6) on day 0, because the cornea was completely pigmented preventing visualization of the fundus. Incomplete or partial corneal pigmentation was noticed as a black opaque curtain against the fundic reflection in three dogs (D2, D3 and D4) because the image of incomplete or partial pigmentation on cornea will cast a black shadow against the reflected light from the fundus. Featherstone *et al.* (2014) had also reported similar observations. Fundic reflection could be seen in two dogs (D2 and D3) on 3rd day while it could not be seen in the rest. The postoperative oedema of cornea had prevented the visualisation of fundus in these four dogs (D1, D4, D5 and D6). On 7th, 14th and 21st day of observation, fundic reflection was observed in one dog (D2), while in the rest it was not visible. Post-operative oedema and granulation tissue during these periods prevented the fundic reflection. In addition to this, D2 and D5 had corneal melting on 7th and 21st day respectively. On 30th and 60th day of observation, fundic reflection was visible in three dogs (D2, D3, and D4) along with pigmentation as a black opaque curtain against it, while in the rest it was not visible. This can be attributed to the extent of recurrence of the pigmentation.

5.3.7. Direct ophthalmoscopic examination

5.3.7.1. Corneal luster

Corneal luster was present in all dogs on 0th day of observation. This could be due to the fact that all dogs were on artificial tears for a long period. Post operatively artificial tears was stopped but the luster was maintained throughout the days of observation. This could be due to the combined effect of inflammatory fluids and lacrimomimetic effects of tacrolimus in the initial healing period, and the saliva produced from the autograft after the healing period. The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

5.3.7.2. Corneal Clarity

Cornea was completely opaque (grade 4) in three dogs (D1, D5 and D6) on day 0, because these dogs had complete pigmentation over the cornea. Corneal clarity was graded 1 (iris details visible) in the rest of the dogs (D2, D3 and D4) because the degree of pigmentation was less in these three dogs.

On 3rd day of observation, clarity had improved in four dogs (D1, D5 and D6). This attributed to the complete removal of pigmentation. In two dogs, the clarity remained same as that of 0th day and in one dog the clarity worsened. This is attributed to the post-operative oedema. On 7th day of observation the clarity was graded 2 (pupillary margin visible, iris details not visible) in three dogs (D3, D5 and D6) while it was graded 3 (pupillary margin not visible) in two dogs (D4 and D1) and 1 (iris details visible) in rest (D2). The reduction in the clarity is attributed to the granulation tissue growth over the cornea. In addition, there was corneal melting in D2 which resulted in reduction in corneal clarity. The corneal clarity in three dogs (D2, D3 and D4) improved when examined on 14th and 21st day of observation while it was worsened in three dogs (D1, D5 and D6). The reduction of the clarity is attributed to the extension of the granulation area over the cornea, postoperative oedema and recurrence of the pigmentation. The improvement in clarity in D2, D3 and D4 could be due to the reduction in the oedema, healing of melting area (D2) and the reduction of granulation. On 30th day the corneal clarity was graded 4 (completely opaque) in three dogs (D1, D5 and D6), while it was graded 1(iris details visible) in the rest (D2, D3 and D4). The improvement in corneal clarity in three dogs can be attributed to the anti-inflamatory and healing properties of saliva (Grixti and Malhotra, 2018), processed human amniotic membrane (Janson and Sikder, 2014). The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

On 60th day of observation the clarity remained same as that on 30th day of observation in five dogs, while in one dog (D1) there was mild improvement in the corneal clarity (grade 3). The observations on corneal clarity in the five dogs (D2, D3, D4, D5 and D6) is attributed to the different degrees of recurrence of corneal pigmentation. The improvement of corneal clarity in D1 could be attributed to the improvement in transparency of the conjunctival layer and dispersal of pigmentation. These observations are similar to that reported by Morreale (2003), where loss of corneal clarity was reported as chief sign of corneal diseases, occurring as sequelae to accumulation of non-cellular infiltrate, pigmentation, fibrosis and oedema.

5.3.7.3. Corneal oedema

Corneal oedema was absent (grade 0) in all the dogs on day zero. There was mild corneal haze (grade 1) in two dogs (D2 and D3), marked corneal opacity (grade 2) in one dog (D1) and severe corneal opacity (grade 3) in three dogs (D4, D5 and D6) on 3rd day of observation. These changes are attributed to the inflammation due superficial keratectomy, where water enters in to the stroma when corneal epithelium loss its integrity. This observation is in accordance with Pontes *et al.* (2014) who reported that water enters the stroma and results in corneal oedema, when there is loss of integrity of both corneal epithelium and endothelium. There was severe corneal opacity (grade 3) in four dogs (D1, D4, D5 and D6) and marked opacity (grade 2) in two dogs (D2 and D3) on 7th day of observation. This increase in opacity is attributed to the inflammatory changes and the infiltrates over the cornea. On day 14 and 21, there was reduction of corneal oedema and improvement of the condition. On day 30, there was no signs of corneal oedema in four dogs (D1, D2, D5 and D6), while cornea had mild haziness (grade 1) in the rest (D3 and D4). On day 60, mild corneal haziness (grade 1) was observed in one dog (D4), while there was no signs of corneal oedema (grade 0) in any other dogs. The superficial keratectomy and acid debridement required might be one reason for the corneal oedema to persist long in the dogs studied.

Anti-inflammatory, antibacterial and regenerative properties of saliva (Grixti and Malhotra, 2018) and processed human amniotic membrane (Janson and Sikder, 2014) could have assisted in the reduction of oedema and enhancement of the healing. The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

5.3.7.4. Vascularisation of Cornea

Cornea had mild superficial vascularisation (grade 1) in three dogs (D2, D3 and D4) and extensive vascularisation with thick vessels originating from all quarters (grade 3) in one dog (D6) on day 0. This could be due to the keratitis as a result of dryness and exposure of cornea along with irritation from the medial canthal entropion. The severity of corneal vascularisation depends upon the severity of the associated etiologies. The corneal vessels originating from the limbus and carries melanin pigments which later deposits on cornea leading to corneal pigmentation (Sansom and Barnett, 1985 and Featherstone *et al.*, 2001)

Two dogs (D1 and D5) did not reveal any vascularisation (grade 0), but extensive vascularisation with thick vessels originating from all quarters (grade 3) was noticed against fundic reflection, intraoperatively, following removal of the superficial pigmented layer. The dense corneal pigmentation had masked the corneal vascularisation, which became visible following removal of the pigmented layer. On day 3, profuse vascularisation visible to naked eye (grade 2) in one dog (D1), while in there was no vascularisation (grade 0) in the rest. This could be sign that the cornea started healing from day 3. Various degree of corneal vascularisation was noticed on day 7, 14 and 21. The vascularisation depend upon the extent of surgical trauma (Charbiwala, 2019). Corneal vascularisation started subsiding from 30th day of observation. The disappearance of the vascularisation can be taken as a sign of completion of corneal healing or the vessels might have masked by the pigment that had recurred during the healing period.

On 60th day, remnants of vessels were observed over the cornea in D1 while no other dogs did not show any vascularisation on cornea (grade 0). This could be attributed to the complete healing of the cornea in all dogs due to combined effect of anti-inflammatory, antibacterial and regenerative properties of saliva (Grixti and Malhotra, 2018), and processed human amniotic membrane (Janson and Sikder, 2014) immunomodulatory and lacrimomimetic effect of tacrolimus. The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

In D1, there was only remnants of vessel as the conjunctiva grown over the cornea and healed completely. The remnant of vessels were seen as pigmented areas like vessels over the conjunctiva. This could be due to the deposition of melanocytes transported toward the cornea (Charbiwala, 2019).

5.3.7.5. Pigmentation score of cornea

Pigmentation score was 72 (complete pigmentation of cornea) in two dogs (D1 and D5) on day 0 while it was 42, 30, 20 and 64 in dogs D2, D3, D4 and D6 respectively. This could be attributed to the chronicity, degree of exposure, dryness and medial canthal entropion of the eyes. This is in accordance to Slatter and Dietrich (2003). Pigmentation score was 0 on day 3, 7 and 14 in all dogs since the complete removal of pigmentation was done by superficial keratectomy. Even though the pigmentation score was 0, vascularization and granulation was noticed in all the dogs in this period. This could be due to the healing process of the cornea. On 21st day of observation, corneal pigmentation was noticed in five dogs (D1- score 6, D2- score 10, D4- score 30, D5- score 4, D6- score 18). This could be attributed due to the melanocytes carried by the blood vessel from the limbus and its deposition over the cornea. This is in accordance with Gilger *et al.* (2008), Azouly (2014), Anoop *et al.* (2016) and Charbiwala (2019). The pigmentation was mostly noticed on the medial side of the cornea since most of the vascularization occurred in the medial side of the cornea. This is in accordance to Renwick (1996) who reported progression of corneal pigmentation from medio-ventral quadrant to axial area of cornea. There was no pigmentation in one dog (D3) on day 21. This could be due to effect of human amniotic membrane grafting and destruction of vessels using trichloro acetic acid leading to reduced vascularization during the healing. On day 30, pigmentation was seen dispersed in one dog (D1) with score 9 and one dog (D4) the pigmentation score remained same with no extension. This could be due to combined effect of anti-inflammatory, antibacterial and regenerative properties of saliva (Grixti and Malhotra, 2018), and immunomodulatory and lacrimomimetic effect of tacrolimus. The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

In few dogs, the pigmentation got extended from medial side and in D3, recurrence of pigmentation started along with a scar. This could be due to deposition of melanocytes over the cornea by blood vessels. On day 60, pigmentation score was 72 in two dogs (D5 and D6) while in dogs D1, D2, D3 and D4, pigmentation score was 26, 40, 8 and 30 respectively. The reason for recurrence of pigmentation could be attributed to the severity of the pigmentation which was present preoperatively. As the preoperative severity of pigmentation is high, the success rate of keratectomy and grafting reduces and there are chances of complete recurrence of pigmentation. This observation is similar to that of Ledbetter and Gilger (2013).

5.3.7.6. Pigmentation depth

The pigmentation was superficial as well as deep in three dogs (D1, D5 and D6), while it was superficial alone in the rest (D2, D3 and D4) on day 0. This could be attributed to the chronicity, degree of exposure, dryness and medial canthal entropion of the eyes involved. There was no pigmentation, on day 3, 7 and 14, in any dogs. This can be attributed to the complete removal of pigmentation by superficial keratectomy. On 21st day, pigmentation was noticed superficial in three dogs (D1, D2 and D4), while it was superficial as well as deep in two dogs (D5 and D6). The blood vessels originated from the limbus would have carried melanocytes and deposited on the cornea. This is in accordance with Gilger *et al.* (2008), Azouly (2014), Anoop *et al.* (2016) and Charbiwala (2019). On 30th day, pigmentation was superficial in four dogs (D1, D2, D3 and D4) while it was superficial as well as deep in two dogs (D5 and D6). The depth of pigmentation, on 60th day remained same on all dogs. The reason for recurrence of pigmentation and its depth could be attributed to the severity of the pigmentation which was present preoperatively. As the preoperative severity of pigmentation is high, the success rate of keratectomy and grafting reduces and there are chances of complete recurrence of pigmentation. This observation is similar to that of Ledbetter and Gilger (2013).

5.3.7.7. Pigmentation density

The density of pigmentation was severe in five dogs (D1, D2, D3, D4 and D5) while it was moderate in one dog (D6) on day 0. This could be attributed to the chronicity, degree of exposure, dryness and medial canthal entropion of the eyes involved. There was no pigmentation on day 3, 7 and 14 in any dogs. This can be attributed to the complete removal of pigmentation by superficial keratectomy. On day 21, the density of pigmentation was mild in four dogs (D1, D2, D4 and D5) while it was severe in one dog (D6). On day 30, the density of pigmentation was mild in three dogs (D1, D3 and D4), moderate in one (D2), and severe in two (D5 and D6). On day 60, the pigmentation has become moderately dense for two dogs (D2 and D4) while for the rest it remained same as that on previous day of observation. Post operatively, there was improvement in the density of pigmentation in four dogs which could be due to combined effect of anti-inflammatory and healing properties of saliva (Grixti & Malhotra, 2018), and immunomodulatory and lacrimomimetic effect of tacrolimus. The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017). .

5.4. NEURO-OPHTHALMIC TEST RESULTS

5.4.1. Menace reflex

On day 0, menace reflex was positive in three dogs (D2, D3 and D4) because the degree of pigmentation was less in these three dogs, while it was negative for the rest (D1, D5 and D6) due to the complete pigmentation of the cornea. Menace reflex was positive in all the dogs on day 3 and 7 because of the complete removal of pigmented layer of cornea in all dogs. On 14th day of observation, menace reflex was negative in two dogs (D1 and D6) while the rest (D2, D3, D4 and D5) showed positive response. On 21st, 30th and 60th day of observation menace reflex was negative in three dogs (D1, D5 and D6) while the rest (D2, D3 and D4) showed positive response. The absence of menace reflex noticed is due to obstruction of the visual axis by corneal oedema, granulation growth or pigmentation.

The dogs with deep, extensive and severe degree of pigmentations showed negative maze test which was similar to the observations of Charbiwala (2019).

5.4.2. Cotton ball test

On day 0, positive response to cotton ball test was observed in three dogs (D2, D3 and D4) because the degree of pigmentation was less in these three dogs, while the rest (D1, D5 and D6) showed negative response due to the complete pigmentation of the cornea. On day 3, positive response to cotton ball test was observed in four dogs (D2, D3, D4 and D5), while two dogs (D1 and D6) showed negative response. The complete removal of pigmented layer of cornea might have helped in positive response of four dogs while in two dogs the postoperative oedema would have affected the result.

On day 7, positive response to cotton ball test was observed in five dogs (D2, D3, D4, D5 and D6) while one dog (D1) showed negative response. On day 14, positive response to cotton ball test was observed in four dogs (D2, D3, D4 and D5) while two dogs (D1 and D6) showed negative response. The reduction in the oedema would have resulted in positive response while in the granulation tissue growth in the rest could have hindered the vision and affected the result. On 21st, 30th and 60th days of observation the response to cotton ball test was negative in three dogs (D1, D5 and D6) while it was positive in the rest (D2, D3 and D4). Degree of oedema and pigmentation affected the vision of dogs in the present study. The dogs with deep, extensive and severe degree of pigmentations showed negative cotton ball test which was similar to the observations of Charbiwala (2019).

5.4.3. Maze test

Maze test was positive in three dogs (D1, D5 and D6) indicating impaired vision, while the rest of the dogs (D2, D3 and D4) showed negative maze test implying normal vision. This could be attributed to the degree of pigmentation over the cornea. Positive maze test (impaired vision) was observed in one dog (D1) while, rest of the dogs (D2, D3, D4, D5 and D6) showed negative maze test (normal vision) on day 3 and 7. This could be attributed to the complete removal of pigmentation and postoperative oedema of the cornea which affects the vision. On day 14, 21, 30 and 60 positive maze test (impaired vision) was observed in three dogs (D1, D5 and D6) while the rest of the dogs (D2, D3 and D4) showed negative maze test (normal vision). Degree of oedema and pigmentation had played a role in the vision of dogs in the present study. The dogs with deep, extensive and severe degree of pigmentations showed negative maze test which was similar to the observations of Charbiwala (2019).

5.4.4. Dazzle reflex

Positive dazzle reflex was observed in four dogs (D2, D3, D4 and D6) while, the rest (D1 and D5) had negative reflex on day 0. This could be attributed to the degree of pigmentation over the cornea. On day 3 and day 7, positive dazzle reflex was observed in all the dogs since the complete removal of pigmentation was performed. Negative dazzle reflex was observed in one dog (D1) while, the rest of the dogs (D2, D3, D4, D5 and D6) showed positive reflex on 14th day. On 21st day, negative dazzle reflex was observed in two dogs (D1 and D6) while, the rest of the dogs (D2, D3, D4 and D5) showed positive dazzle reflex. The varying degrees of postoperative oedema, granulation growth and occurrance of pigmentation can be the reason for varied responses for the dazzle reflex by the dogs in this study.

5.4.5. Pupillary light reflex (PLR)

On day 0, PLR was observed in three dogs (D2, D3 and D4) while, it could not be observed in rest of the dogs (D1, D5 and D6). The complete opacity of the cornea in the three dogs (D1, D5 and D6) had prevented visualising the pupillary response. On 3rd day, PLR could be observed in all the dogs due to complete removal of pigmentation. This indicated that neurologically vision was intact in all the dogs in this study. On 14th day, PLR could be observed in four dogs (D2, D3, D4 and D5) while, it could not be observed in the rest (D1 and D6). This could be due to the granulation tissue growth which hindered the visualisation of the pupil. On 21st, 30th and 60th days of observation PLR was observed in three dogs (D2, D3 and D4) while it was not observed in the rest (D1, D5 and D6). The degree of pigmentation and growth of granulation had affected visualising the pupillary light response in these dogs studied even though the vision was neurologically normal.

5.4.6. Corneal sensitivity

On day 0, the corneal sensitivity was absent in all parts of cornea in three dogs (D1, D5 and D6) while, it was present medially (sluggish), laterally, dorsally, ventrally and centrally in the rest (D2, D3 and D4). Chronic exposure and the pigmentary keratopathy might have masked the nerve endings on the surface of the corneas and reduced the corneal sensitivity in the respective dogs. On 3rd day, corneal sensitivity was observed in all the parts of the cornea in all dogs. This could be due to the nerve endings getting exposed following keratectomy. On 7th day, corneal sensitivity was observed in lateral part of cornea alone in one dog (D1) while it was present in all parts of cornea in all other dogs. The granulation tissue growth on the medial part would have resulted the cornea being less sensitive. On 14th day, sluggish corneal sensitivity was observed in lateral part of cornea in two dogs (D1 and D6) while, the rest (D2, D3, D4 and D5) had normal sensitivity. On 21st day, corneal sensitivity was lost in two dogs

(D1 and D6) while, others had normal sensitivity. Corneal sensitivity was lost in three dogs (D1, D5 and D6) from 30th day of observation. D2, D3 and D4 had normal corneal sensitivity throughout the observation period. The observations in the corneal sensitivity noticed in this study were in accordance to Barret *et al.* (1991). As healing progressed the pigmentation progressed and there will be gradual masking of the nerve endings and reduced corneal sensitivity. Similar observations in reduction in corneal sensitivity following corneal ulcers were reported by Ribeiro *et al.* (2008).

5.5. SPECIAL DIAGNOSTIC PROCEDURE

5.5.1. Schirmer Tear Test (STT)

Mean values (mm/min) for Schirmer tear test (STT-1) on 0, 3, 7, 14, 21, 30 and 60 were 7 ± 3.95, 21.33 ± 2.07, 20.83 ± 1.17, 18.5 ± 1.22, 18 ± 0.63, 16.83 ± 0.75 and 16.5±2.07 respectively. Values for STT-1 was found significantly increased during the observation periods when compared to the pre-operative values at 1% level. The inflammatory fluids could have contributed to the significant increase of the schirmer tear test values in the initial post-operative days. Lacrimomimetic effect of tacrolimus and secretions from the minor salivary glands of the transplanted oral mucosa could have contributed to the increased STT values during the later days of observation. The consistent, but increased values of schirmer tear test noticed on later days of observation, even after withdrawal of tacrolimus four days prior to the test, can be taken as an indication of resumption of secretions by the minor salivary glands of the transplanted oral mucosa. The similar observations were made by Anna *et al.* (2012), Castanho *et al.* (2013) and Qin *et al*., (2018) in humans, dogs and Rhesus monkeys respectively.

The punctal occlusion performed might have helped in retaining the secretions of minor salivary glands of the transplanted oral mucosa with in the tear well and augmented the values for Schirmer tear test.

Mean values (mm/min) for Schirmer tear test (STT-2) on 0, 3, 7, 14, 21, 30 and 60 were 0.83 ± 1.6, 11.33 ± 1.97, 10.33 ± 1.03, 8.33 ± 1.03, 8.5 ± 1.05, 6.83 ± 1.94 and 6.33 ± 2.34 respectively. Values for STT-2 was found significantly increased compared to the pre-operative values at 1% level. The resumption of secretions from the minor salivary glands of the transplanted oral mucosa and improvement in basal tear production due to lacrimomimetic effect of tacrolimus would be the reasons for the significant increase in the STT 2 values of the dogs studied.

5.5.2. Fluorescein Dye Test (FDT)

On day 0, FDT was negative for all the dogs studied. This can be attributed to the intact corneal epithelium. FDT was positive for all the dogs on 3rd day, because superficial keretectamy had exposed the corneal stroma. On 7th day of observation FDT was negative in one dog (D1) while, it remained positive in the rest. The persistence of corneal wound following extensive keratectamy and acid debridement can be the reason for the FDT remaining positive in five dogs. On 14th day of observation, FDT had become negative in all the six dogs. The epitheliogenesis properties of the processed human amniotic membrane (Janson and Sikder, 2014) and saliva (Grixti and Malhotra, 2018), secreted from the minor salivary gland of the oral mucosal autograft could have contributed in the healing of cornea and resultant negative FDT. The punctal occlusion performed would have augmented the effects and helped in retaining the secretions of the minor salivary glands of the transplanted oral mucosa (Badawy, 2017).

FDT was positive in D5 on day 21 due to the melting of the cornea while all the other dogs showed FDT negative. FDT was negative in all dogs on 30th and 60th day of observation which indicated that keratectomy wound had healed completely cornea. Fluorescein dye is highly lipophobic and hydrophilic, due to which it does not penetrate the corneal epithelium, while in cases of corneal ulceration where the stroma is exposed, the dye will get absorbed and retained (Moore, 2001 and Mitchell, 2011).

5.5.3. Rose Bengal dye test

On 0th day of observation cornea and conjunctiva of all dogs were stained positive for Rose Bengal dye test. Rose Bengal dye stains the corneal surface and bulbar conjunctival surface when there is tear film deficiency and resultant death of epithelial cells. According to Maggs (2008), Rose Bengal dye stains dead and devitalised cells and gives positive staining for partially eroded corneal epithelium. The author also reported that it gives positive staining for altered mucin coating and squamous cells with altered structure.

5.5.4. Tear film breakup time (TBUT)

The mean values for the tear film breakup time (sec) on the day of presentation and post-operatively on day 3,7, 14, 21, 30, and 60 were 4.83 ± 1.16, 8.66 ± 2.06, 9.8 ± 3.06, 10 ± 3.03, 9.33 ± 2.3 and 9 ± 2.19 respectively. TBUT observed on post-operative days were significantly high when compared to those of pre-operative values at 1% level. Similar observation was made by Moore and Collier (1990) and Maggs

(2008). The inflammatory fluids or immunomodulators like tacrolimus could have assisted in the improvement of TBUT in the initial period of time. Lacrimomimetic effect of tacrolimus and secretions from the minor salivary glands of the transplanted oral mucosa could have contributed to the increased TBUT values during the later days of observation. The consistent, but increased values of TBUT noticed on later days of observation, even after withdrawal of tacrolimus four days prior to the test, can be taken as an indication of resumption of secretions by the minor salivary glands of the transplanted oral mucosa. The saliva from MSG contains mucin which gives stability to the tear film over cornea (Marinho *et al*., 2010). Histology study of a section of the autografts revealed plenty of mixed salivary glands which clearly indicates the presence of mucin in the saliva produced. So the rough surface of the cornea after the recurrence of pigmentation might be the reason of reduced TBUT even after the resumption of salivary secretions. Castanho *et al.* (2013) also reported improvement in TBUT after MSG autografting.

5.5.5. Culture and antibiotic sensitivity

Culture of corneal swabs in three dogs (D4, D5 and D6) revealed presence of gram positive cocci with colony characters of *staphylococcus spp.* while there was no growth observed in the rest. The cultures had maximum sensitivity to ciprofloxacin followed by norfloxacin, ofloxacin, gentamicin and ceftriaxone. They were resistant to tetracycline, chloramphenicol, streptomycin and amoxicillin. Based on the antibiotic sensitivity, topical ciprofloxacin eye drops were used in all the cases. The observations in the present study was similar to those reported earlier (Gerding *et al.,* 1993, Kudirkiene *et al.,* 2006 and Varges *et al.,* 2009)

5.6. HISTOLOGY

The oral mucosal autograft were having abundant number of well – encapsulated mixed salivary glands as shown in the section. The glands and ducts were observed in the submucosal layer very close to the epithelium. This was in accordance to Murube (1997), Dyce *et al*. (2004) and Frappier (2006).

5.7. GOBLET CELL INDEX

The goblet cell indices of dogs D1, D2, D3, D4, D5 and D6 were, 0.09, 0.16, 0.16, 0.10, 0.02 and 0.07 respectively on day of presentation. Goblet cell index of the section of conjunctiva was less when compared to the normal data reported earlier. Reduction of the goblet cells indices indicates mucin deficiency which is associated with KCS (Moore and Collier, 1990; Balicki *et al*., 2011).

5.8. HISTOPATHOLOGY

Out of the six dogs studied, histopathology results of three dogs are discussed below. In three dogs, histopathology samples could not be accessed due to the diamond burr superficial keratectomy procedure. Pigmented epithelium of three dogs (D1, D5 and D6) were submitted for histopathology after keratome blade keratectomy.

All the three cases were diagnosed as melanosis. Histopathologic examination revealed corneal stroma enclosing melanoma laden cells with minimal variation in shape and newly formed blood vessels and moderately hyperplastic corneal epithelium. The melanin laden cells were mainly centered around newly formed blood vessels. This could be attributed to the transpotation of melanin laden cells through the newly formed vessels and deposition of it around the vessels during the healing process happening over the cornea due to chronic irritation and dryness (Charbivala, 2019).

5.9. INCORPORATION OF ORAL MUCOSAL AUTOGRAFT IN TO THE CONJUNCTIVAL FORNIX

Harvested autografts had pigmented mucosal surface with minimum four or five duct openings over it. Glandular appearance was observed on the cut surface of the autograft. All the oral mucosal autografts transplanted in the conjunctival fornix healed completely and merged into the surrounding conjunctival surface without any complication in the post-operative period. However the surface of graft didn't affect the free movement of eyelid and graft or the sutures were not rubbing over the cornea throughout the observation period. (Castanho *et al.*, 2013)

5.10. COMPLICATIONS

Two dogs (D2 and D5) had corneal melting noticed on 7th and 14th day of observation. Auto serum fortified with amikacin as eyedrops along with EDTA eyedrops were effective for healing of these ulcers within a week.

On 21st day of observation, there was recurrence of pigmentation in five dogs (D1, D2, D4, D5 and D6) while, in one dog (D3) the recurrence of pigmentation was observed on 30th day. Gradually the pigmentation started extending from the medial surface in all dogs while in three dogs (D1, D5 and D6) the cornea was completely covered with pigmentation (Charbiwala, 2019). In D2, D3 and D4 the pigmentation was controlled in the medial aspect of cornea with comparatively less density.

Conjunctiva was grown over the cornea in D1. It was initiated on 14th day and covered completely by 21st day of observation and the pigmentation recurred and was dispersed over it by 60th day of observation.

SUMMARY

6. SUMMARY

Dry eye disease (KCS) is an inflammatory disorder of the lacrimal functional unit. This leads to chronic ocular surface disease, and impaired vision, eventually causing a reduction in quality of life of the animals. Brachycephalic breeds are more prone for dry eye. Clinical features of dry eye include unstable tear film, ocular surface inflammation and various epitheliopathies like pigmentary keratitis and corneal ulcerations. Pigmentary keratitis is the inflammation of cornea with deposition of melanin pigments.

Various treatment modalities are reported for treatment of dry eye. Transplantation of oral mucosa as an autograft has shown satisfactory results in humans and dogs having severe dry eye syndrome. Secretions from the minor salivary glands of the oral mucosal transplant is suggested to serve as an alternative for tears. Occlusion of the lower puncta is also adopted to prevent drainage of tears through the nasolacrimal duct. Superficial keratectomy is practised for removal of melanotic epithelial layer. Keratoplasty using processed human amniotic membrane, following superficial keratectomy, facilitates healing of corneal epithelium and superficial stroma thereby alleviating ocular surface inflammation and epitheliopathies. Medial canthoplasty corrects the medial canthal entropion, and thereby reduces the constant irritation to the eyes.

Considering these points the study was carried out in six dogs having an eye with basal tear production less than 5 mm/min along with melanotic cornea, presented to the Department of Veterinary Surgery a diology, Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode.

All the dogs were subjected to detailed examinations for general body condition, physiological parameters, nature of the ocular discharge, conjunctival changes, visual function tests, corneal sensitivity, rate and character of blinks, direct and indirect ophthalmoscopy, Schirmer tear tests (STT1 and STT2), fluorescein dye test, Rose Bengal dye test, slit lamp bio microscopy, tear film break up time, depth, degree and score of corneal melanosis, grade of corneal vascularisation, corneal oedema and clarity. Culture and antibiotic sensitivity tests of corneal swabs were done on all cases.

All the dogs were treated with topical ciprofloxacin eye drops prior to surgery. Oral mucosal autografting, lower punctual occlusion, superficial keratectomy and human amniotic membrane transplantation were done in all the dogs. A 10 mm long full thickness oral mucosa, encircling 3-4 duct openings, at the level of lower canine to 3^{rd} premolar area was harvested and transplanted into the superior conjunctival fornix of the selected eye. Keratectomy was performed. Perilimbal conjuctivectomy was also performed in all cases to remove the pigmented conjunctiva around the limbus.

Following removal of the pigmented layer of cornea, tri-chloro acetic acid debridement was done in all cases. All the cornea were immediately flushed with normal saline. Corneal surface was then grafted with a processed human amniotic membrane (Amnio-care^{TM}), as an onlay graft. Medal canthoplasty was performed in those cases which required correction of medial canthal entropion. Systemic antibiotic and NSAID were then followed along with topical ciporofloxacin eye drop and tacrolimus eye ointment. All the dogs were examined and observed on day 0 and on 3^{rd}, 7^{th}, 14^{th}, 21^{st}, 30^{th} and 60^{th} days post-operatively. Tacrolimus was withdrawn four days prior to the examinations made on 21^{st}, 30^{th} and 60^{th} days.

Values of Schirmer tear tests (STT1 and STT2), tear film break up time, rate of blinks and number of complete blinks improved significantly in the post-operative days. The keratectomy wounds healed in majority of the animals by 14^{th} day. The pigmentation score and density reduced in three animals towards later days of observation while the pigmentation depth remained same in all animals. Pigmentation recurred with same severity, as on day 0, in three animals towards the last day of observation and thereby significant reduction in corneal clarity and vision occurred in these animals. All the oral mucosal autografts got integrated into the conjunctival fornix. The symptoms of KCS disappeared in the late post-operative days.

Plenty of minor mixed salivary glands were visible submucosally on histological examination of the oral mucosal autograft. Histopathology of the pigmented corneal epithelium revealed melanosis.

From the present study it could be concluded that,

- The oral mucosa at the level of lower canine to 3^{rd} premolar area in pugs have plenty of minor salivary glands, which are mixed in nature, and hence is suitable as an autograft for management of dry eye in dogs.
- When the oral mucosal autografts gets incorporated into the conjunctival fornix, they resume the secretions from their minor salivary glands and significantly improves the nature of ocular discharge and appearance of the eye, and thereby address one of the main concern of the owners regarding dry eye.

- Secretions of the minor salivary glands of the transplanted autograft augments corneal and conjunctival healing.
- Occlusion of the lower puncta prevents the drainage through nasolacrimal duct and retain tears and secretions, in the tear well, and moistens the eye.
- Decellularised, sterilised, processed human amniotic membrane is efficient in healing of cornea following superficial keratectomy, and it treats dry melanotic cornea to a certain extent with varying degrees of recurrence.
- Medial canthoplasty increases the number of complete blinks and helps spreading the tears across the cornea thereby reduces corneal drying.
- Schirmer tear test 2 and tear break up time (TBUT) can be taken as non-invasive tests that forecast dry eye and pigmentary keratopathy in pugs. Early detection followed by autografting of oral mucosa along with its minor salivary glands combined with surgical occlusion of lower puncta could prevent the eyes becoming dry and the cornea becoming melanotic in these dogs.
- Oral mucosal autografting along with lower punctal occlusion, transplantation of processed human amniotic membrane and medial canthoplasty is effective for management of dry melanotic cornea in dogs at an earlier stage.

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ABSTRACT

Dry eye disease (KCS) is an inflammatory disorder of the lacrimal functional unit. This leads to chronic ocular surface diseases like pigmentary keratitis and impaired vision eventually causing a reduction in quality of life of the animals. Pigmentary keratitis is the inflammation of cornea with deposition of melanin pigments. .

Various treatment modalities are reported for treatment of dry eye in dogs. Transplantation of oral mucosa as an autograft into the conjunctival fornix helps in lubricating the ocular surface. Occlusion of the lower puncta is another treatment adopted for retaining tears by preventing drainage through the nasolacrimal duct. Superficial keratectomy is suggested for removal of melanotic epithelial layer. Keratoplasty using processed human amniotic membrane, following superficial keratectomy, is reported to facilitate healing of corneal epithelium thereby alleviating ocular surface inflammation and epitheliopathies. Medial canthoplasty corrects the medial canthal entropion.

Considering these points the study was carried out in six dogs having an eye with basal tear production less than 5 mm/min along with melanotic cornea, presented to the Department of Veterinary Surgery and Radiology, Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode.

All the dogs were subjected to detailed examinations for their general body condition, physiological parameters, nature of the ocular discharge, conjunctival changes, visual function tests, corneal sensitivity, rate and character of blinks, direct and indirect ophthalmoscopy, Schirmer tear tests (STT1 and STT2), fluorescein dye test, Rose Bengal dye test, slit lamp biomicroscopy, tear film break up time, depth, degree and score of corneal melanosis, grade of corneal vascularisation, corneal oedema and clarity. Culture and antibiotic sensitivity tests of corneal swabs were done on all cases.

All the dogs were treated with topical ciprofloxacin eye drops prior to the surgery. Oral mucosal autografting, lower punctual occlusion, superficial keratectomy

and human amniotic membrane transplantation were done in all the dogs. Medial canthoplasty was performed in those cases which required correction of medial canthal entropion. Systemic antibiotic and NSAID were then followed along with topical ciporofloxacin eye drop and tacrolimus eye ointment. All the dogs were examined and observed on day 0 and on 3rd, 7th, 14th, 21st, 30th and 60th days postoperatively. Tacrolimus was withdrawn four days prior to the examinations made on 21st, 30th and 60th days.

Values of Schirmer tear tests (STT1 and STT2), tear film break up time, rate of blinks and number of complete blinks improved significantly in the post-operative days in all the dogs. The keratectomy wounds healed in majority of the animals by 14th day. Pigmentation recurred with same severity, as on day 0, in three animals towards last day of observation while it reduced in rest. All the oral mucosal autografts got integrated in to the conjunctival fornix and started secreting, which was evident from the improvement in nature ocular discharge and moistness of eye. The symptoms of KCS disappeared in the late post-operative days.

Plenty of minor salivary glands were visible submucosally on histological examination of a section of the oral mucosal autograft. Histopathology of the pigmented corneal epithelium revealed melanosis.

From the present study of oral mucosal autografting, lower punctual occlusion, superficial keratectomy and processed human amniotic membrane grafting for managing dry melanotic cornea in these dogs, it could be concluded that,

- Medial canthoplasty increases the number of complete blinks and helps spreading the tears across the cornea thereby reduces corneal drying.
- Schirmer tear test 2 and tear break up time (TBUT) can be taken as non-invasive tests that forecast dry eye and pigmentary keratopathy in pugs. Early detection followed by autografting of oral mucosa along with its minor salivary glands combined with surgical occlusion of lower puncta could prevent the eyes becoming dry and the cornea becoming melanotic in these dogs.

ANNEXURE

OPHTHALMIC EXAMINATION SHEET

Case number:	Date:
Owner's details:	Ph no:
Animal:	Breed:
Age:	Sex:
General condition:	Hydration status of the animal

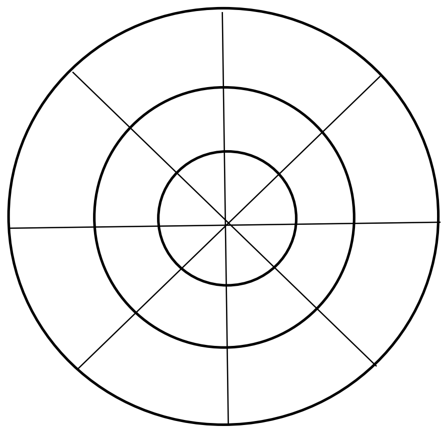
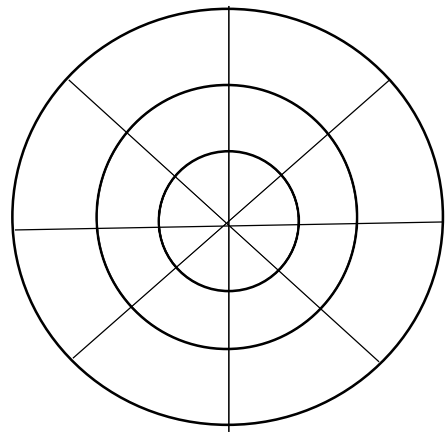
Physiological parameters: Respiratory rate: Rectal temperature:
Colour of mucous membrane: Pulse rate:

History

Symptoms

PARAMETERS	OD	OS
Nature of ocular discharge	Serous / Seromucoid / Mucoid / Mucopurulant	Serous/ seromucoid/mucoid/ mucopurulant
Blepherospasm	Present/Absent	Present/absent
Conjunctival hyperemia	Mild/Moderate/ Severe/ Absent	Mild/Moderate/ Severe/ Absent
Medial canthal entropion	Present/absent	Present/absent
Medial caruncular trichiasis	Present/absent	Present/absent
Obstacle course test	+ve (hitting objects) / -ve (not hitting objects)	+ve (hitting objects) / -ve (not hitting objects)
Pupillary light reflex	Present/absent	Present/absent
Menace reflex	Present/absent	Present/absent
Dazzle reflux	Present/absent	Present/absent
Visual function grading (Menace, Maze, Cotton ball test)	Score 0 – Absent (negative to all the three tests) Score 1 – Decreased (insufficient response to one or two tests) Score 2 – Normal (positive to all the three tests)	Score 0 – Absent (negative to all the three tests) Score 1 – Decreased (insufficient response to one or two tests) Score 2 – Normal (positive to all the three tests)
Blinks (character)	Complete/incomplete	Complete/incomplete
Blinks (no: of blinks / min)		

Conjunctival swab- culture and sensitivity		
Direct ophthalmoscopy findings		
Indirect ophthalmoscopy findings		
Corneal clarity	0-no corneal haze 1-iris details visible 2-pupillary margin visible,iris details not visible 3-pupillary margin not visible 4-cornea totally opaque	0-no corneal haze 1-iris details visible 2-pupillary margin visible,iris details not visible 3-pupillary margin not visible 4-cornea totally opaque
Corneal lustre	Present/absent	Present/absent
Corneal oedema	Score 0 – no signs Score 1 – mild corneal haze Score 2 – marked corneal opacity, anterior chamber still visible Score 3 – severe corneal opacity, anterior chamber not visible	Score 0 – no signs Score 1 – mild corneal haze Score 2 – marked corneal opacity, anterior chamber still visible Score 3 – severe corneal opacity, anterior chamber not visible
Corneal vascularisation	Score 1 – mild superficial vascularisation, thin vessels visible with magnification Score 2 – profuse superficial vascularisation visible to naked eye Score 3 – extensive vascularisation with thick vessels originating from all quadrants	Score 1 – mild superficial vascularisation, thin vessels visible with magnification Score 2 – profuse superficial vascularisation visible to naked eye Score 3 – extensive vascularisation with thick vessels originating from all quadrants
Corneal sensitivity Central cornea Lateral cornea Medial cornea Dorsal cornea Ventral cornea	 Present/absent Present/absent Present/absent Present/absent Present/absent	 Present/absent Present/absent Present/absent Present/absent Present/absent

Pigmentation score of cornea 0 = no pigmentation, 1 = pigmented area <30% of the sector area 2 = pigmented area 30%–60% of the sector area 3 = pigmented area >60% of the sector area		
Total score		
Pigmentation density	Mild / moderate / severe	Mild / moderate / severe
Pigmentation depth	Deep / superficial / superficial as well as deep	Deep / superficial / superficial as well as deep
STT I	1) Above 15mm/min: normal tear production 2) 11-14 mm/min: incipient KCS 3) 6-10 mm/min: moderate KCS, 4) 0-5 mm/min: severe KCS.	1) Above 15mm/min: normal tear production 2) 11-14 mm/min: incipient KCS 3) 6-10 mm/min: moderate KCS, 4) 0-5 mm/min: severe KCS.
STT II readings (mm/min)		
Fluorescein dye test		
TBUT (sec)		
Rose Bengal dye test		

Acad. Form No. 19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY
Faculty of Veterinary and Animal Sciences
PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS DEGREE

1. Title of thesis:

Oral mucosal autografting, lower punctal occlusion and transplantation of processed human amniotic membrane for management of dry melanotic cornea in dogs

2. (a) Title of the departmental/ KVASU research project of which this forms a part:

Nil

(b) Code No. if any, and order by which the departmental / KVASU research project is approved:

Not applicable

3. a) Name of the student:

Gisha G. Nair

b) Admission No:

17-MVP-09

c) Name of the Discipline:

Veterinary Surgery and Radiology

4. a) Name of the Major Advisor (Guide):

Dr. Sooryadas S.

b) Designation:

Assistant Professor
Department of Veterinary Surgery and Radiology,
College of Veterinary and Animal Sciences, Pookode, Lakkidi P.O.,Wayanad- 673576

5. Objective of the study:

Study the concurrent use of autografting with oral mucosa, lower punctal occlusion along with transplantation of processed human amniotic membrane for management of dry melanotic cornea in dogs

6. Practical / Scientific utility:

Dry eye is a multifactorial disorder of the tears and ocular surface, associated with discomfort and visual disturbance. Clinical features of dry eye include an unstable tear film, ocular surface inflammation and epitheliopathy. Drug therapy is not always effective. Hence, new treatment alternatives are needed.

Transplantation of oral mucosa as an autograft into the conjunctival fornix to treat severe dry eye syndrome has shown satisfactory results in experiments in humans and dogs. The secretions from the minor salivary glands of the oral mucosal transplant will serve as an alternative for tears, lubricating the ocular surface and thus treating dry eye syndrome. The lower punctal occlusion prevents the drainage of secretions of the mucosal autograft and tears through the nasolacrimal duct and thus helps to restore moistness on the eye. Cornea, in most cases of dry eye disease would either be melanotic or ulcerated and vision will get impaired. Processed human amniotic membrane transplantation, following superficial keratectomy of the melanotic cornea, would facilitate healing of corneal epithelium and superficial stroma and also alleviate ocular surface inflammation and epitheliopathy.

7. Important publications on which the study is based:

Nickel *et al*. (1979) opined that the minor salivary glands are localized in the buccal area extending from the canine tooth to the third cheek tooth.

Wichayacoop *et al.* (2005) used preserved human amniotic membrane as overlay graft on induced corneal stromal wound in dogs and observed complete corneal epithelialization in seven days, and reported that all dogs regained vision.

Marinho *et al*. (2010) reported that the mucin produced from labial salivary glands is thicker than that of lacrimal glands, and reduces water evaporation. The authors also reported that labial minor salivary gland secretes aqua-serous mucus, similar to tears, which can be well tolerated by the ocular surface.

Meller *et al*. (2011) reported that processed human amniotic membrane grafts have been increasingly used to treat a wide variety of ocular surface conditions like persistent corneal epithelial defects, acute chemical burns, and cicatrizing conditions such as Stevens–Johnson syndrome and ocular cicatricial pemphigoid in humans.

Anna *et al.* (2012) implanted labial salivary glands into the superior and inferior conjunctival fornix of humans and observed improvement in schirmer tear test values in patients who received submucosal grafts containing more than 10 glands.

Castanho *et al.* (2013) performed oral mucosal transplantation in dogs and reported recovery of ocular surface luster and significant decrease in irritation, photophobia, and blepharospasm. The authors also reported that the saliva from the labial glands had four times the concentration of IgA than that produced by the parotid gland.

Janson and Sikder (2014) reported that human amniotic membrane grafts promotes epithelial and stromal healing, suppression of transforming growth factor β (TGF-β) signaling, and fibroblast proliferation, and has good anti-inflammatory properties.

Badawy (2017) following a study in dogs reported that surgical punctal occlusion prevented drainage of the low amount of tear produced and allowed the distribution of tear over the ocular surface which in turn improved the prognosis. The authors considered this as permanent replacement for medical treatment.

Qin *et al*. (2018) transplanted labial minor salivary glands in a Rhesus monkey model for severe dry eye and observed obvious increase in the quantity of tears and foam-like secretions resembling saliva, suggesting an exocrine activity of the grafted salivary glands at the level of the ocular surface.

8. Outline of technical programme:

The study will be conducted in minimum of six dogs diagnosed with dry melanotic cornea presented to the teaching veterinary clinical complex, Pookode. Detailed clinical and ophthalmic examination will be carried out (Gelatt, 2014). Under general anaesthesia, oral mucosa removed from the inner portion of buccal region (canine to third premolar) will be grafted into a fusiform wound created in the fornix of the upper eyelid. A portion of the graft will be subjected to histological analysis. Melanotic areas of cornea that had resulted from dry eye disease will be treated by superficial keratectomy and processed human amniotic membrane grafting. Lower puncta will be surgically occluded. After the superficial keratectomy, based on results of fluorescein dye test, non- steroidal anti- inflammatory drugs and topical ocular medications like fortified amikacin eyedrops in artificial tears, steroidal ocular medications, atropine eye drops and cyclosporine ointment will be used, as required.

Healing of the cornea and clinical effects of the autograft and punctal occlusion will be evaluated based on corneal clarity, luster, fluorescein dye test, tear break up time, schirmer tear test I & II, conjuctival changes and presence and nature of ocular discharges on day 0, 3, 7, 14, 21, 30 and 60 postoperatively.

All observations will be recorded and the data collected will be statistically analysed (Snedecor and Cochran, 1994).

9. Main items of observations to be made:

1) Signalment and anamnesis
2) General condition
3) Physiological parameters
    a. Respiration rate (breaths per minute)
    b. Pulse rate (beats per minute)

c. Rectal temperature (°C)
d. Color of mucous membrane

4) Nature of eyeball in relation to orbit
5) Presence and nature of ocular discharge
6) Results of visual function tests

   a. Pupillary light reflex
   b. Menace reflex
   c. Dazzle reflex

7) Blinks
   a. Character (complete/incomplete)
   b. Number of blinks per minute
8) Conjunctival changes
9) Findings of direct and indirect ophthalmoscopic examination
10) Corneal clarity and luster
11) Corneal sensitivity
12) Pigmentation of cornea
13) Findings of Schirmer tear test I and II
14) Findings of Flourescine dye test
15) Tear break up time (TBUT) (seconds)
16) Goblet cell count in bulbar conjunctiva (number of cells/field)
17) Result of histological examination of graft

10. Facilities:

(a) Existing:

Existing facilities in the Department of Veterinary Surgery and Radiology, and other departments at the College of Veterinary and Animal Sciences, Pookode, will be utilized.

(b) Additional facilities required:

Biologicals and diagnostic kits

11. Duration of study:

Six semesters

12. Financial estimate:

Cost of graft , diagnostic reagents and kits	**Rs. 20,000/-**
Miscellaneous	**Rs. 5,000/-**
Total	**Rs. 25,000/-**

Signature of student

Project coordination group to which the proposal is to be placed:

Animal diseases II

Signature of the Major Advisor:

Pookode,

Name and Signature of Members of Advisory Committee:

1. Dr. Sooryadas S.
   Assistant Professor

2. Dr. Dinesh P.T.
   Assistant Professor

3. Dr. George Chandy
   Assistant Professor

4. Dr. Rajani C.V.
   Assistant Professor

APPENDIX-1:

References –

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APPENDIX II:

Time frame of work:

Semester III

1. Collection of literature
2. Planning of the programme for research
3. Preparation of synopsis

Semester IV

1. Collection of literature
2. Starting research in clinical cases

Semester V

1. Research work continued

Semester VI

1. Continuation of research work
2. Analysis of data
3. Preparation and submission of thesis

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to Animals Act (Amendment, 1998).

Place: Pookode   Dr. Sooryadas S.

Date: Major Advisor

CURRICULUM VITAE

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UNIVERSITY EDUCATION

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BVSc & AH	College of Veterinary and Animal Sciences, Pookode	Kerala Veterinary and Animal Sciences University, Pookode	2011 - 2017	7.9

Memberships : Kerala State Veterinary Council