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Ocular surface cytology and tear film evaluation in diabetic dogs

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Abstract

Diabetes mellitus in dogs is increasingly recognised not only for its systemic metabolic consequences but also for its impact on ocular surface health, yet the relationship between disease duration and progressive tear film deterioration remains poorly quantified. This research examined ocular surface cytology and tear film parameters in 35 diabetic dogs and 20 healthy controls presented to the Seoul Academy of Veterinary Ophthalmology between April 2022 and September 2023. Diabetic dogs were sub-grouped by disease duration: less than 12 months ($n = 15$), 12 to 24 months ($n = 12$) and greater than 24 months ($n = 8$). Each animal underwent Schirmer tear test-I (STT-I), tear film break-up time (TBUT), fluorescein and rose bengal staining, and conjunctival impression cytology with Giemsa staining. Tear osmolarity was measured with a portable osmometer. STT-I values declined progressively with increasing diabetes duration, falling from 18.4 ± 2.1 mm/min in controls to 10.3 ± 3.1 mm/min in dogs diabetic for over 24 months ($P < 0.001$). TBUT followed a parallel downward trajectory, decreasing from 14.2 ± 1.8 seconds to 6.9 ± 2.6 seconds. Tear osmolarity rose from 298 ± 8 mOsm/L in controls to 324 ± 14 mOsm/L in the longest-duration group ($P < 0.01$). Impression cytology revealed a progressive loss of conjunctival goblet cells (42.8 to 19.1 cells per high-power field) and a reciprocal increase in inflammatory cell infiltration (2.4 to 24.6 cells per field). Rose bengal staining scores were significantly higher in all diabetic sub-groups compared with controls, indicating epithelial damage. Fluorescein uptake was positive in 37.5% of dogs diabetic for over 24 months versus 5.0% of controls. Correlation analysis showed a strong negative relationship between diabetes duration and both STT ($r = -0.81$, $P < 0.001$) and goblet cell density ($r = -0.76$, $P < 0.001$). These findings demonstrate that canine diabetes mellitus is accompanied by a duration-dependent deterioration of the tear film and ocular surface, characterised by reduced aqueous tear production, shortened tear stability, hyperosmolarity, goblet cell loss and subclinical inflammation. Routine ophthalmic screening should be integrated into the long-term monitoring plan for diabetic dogs to enable early detection and management of keratoconjunctivitis sicca.

Keywords: Diabetic dogs, ocular surface, tear film, Schirmer tear test, tear break-up time, impression cytology, goblet cells, keratoconjunctivitis sicca, tear osmolarity, ophthalmology.

Introduction

The first clinical descriptions of diabetic eye disease in dogs date back to the 1960s, when cataract formation was identified as the most common ophthalmic complication of canine diabetes mellitus^[1]. Since then, veterinary ophthalmology has advanced considerably, and the scope of diabetes-related ocular pathology is now understood to extend well beyond the lens. In humans, the connection between diabetes and dry eye — sometimes termed diabetic ocular surface disease has been documented extensively, with prevalence estimates ranging from 15% to 54% depending on diagnostic criteria and population^[2]. By contrast, the veterinary literature on this topic remains thin, with only a handful of reports addressing tear film changes in diabetic dogs^[3, 4].

Tear film homeostasis relies on a delicate interplay between the lacrimal gland, meibomian glands, conjunctival goblet cells and corneal epithelium. In human diabetic patients, chronic hyperglycaemia impairs lacrimal gland innervation, reduces reflex tearing and promotes advanced glycation end-product accumulation in the glandular basement membrane^[5]. Goblet cell density falls, mucin secretion declines and the lipid layer thins, collectively destabilising the tear film and exposing the ocular surface to desiccation and microbial challenge^[6].

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Whether an analogous cascade occurs in dogs has been assumed rather than demonstrated, partly because longitudinal ophthalmic data in canine diabetic cohorts are scarce.

Keratoconjunctivitis sicca (KCS) is already one of the most common ophthalmic diagnoses in dogs, affecting an estimated 1-4% of the general canine population and up to 35% of certain predisposed breeds such as the Cocker Spaniel and West Highland white terrier [7]. The typical aetiology in non-diabetic dogs is immune-mediated destruction of the lacrimal gland, but diabetic dogs may develop a superimposed, metabolically driven form of tear deficiency that goes undetected if clinicians focus solely on glycaemic control and cataract surveillance [8]. Identifying this subgroup matters because KCS left untreated leads to corneal ulceration, vascularisation and potentially sight-threatening scarring [9].

Impression cytology is a minimally invasive technique that samples the superficial conjunctival epithelium and allows quantification of goblet cells, inflammatory cells and squamous metaplasia [10]. Combined with standard tear film tests — Schirmer tear test-I for aqueous production and tear break-up time for film stability — it provides a comprehensive snapshot of ocular surface health. Tear osmolarity measurement adds a further dimension; elevated osmolarity is now regarded as a core mechanism of dry-eye pathology because hyperosmolar tears trigger epithelial apoptosis and release pro-inflammatory cytokines [11].

Despite the availability of these tools, no published research has simultaneously measured STT, TBUT, osmolarity, impression cytology and vital staining in diabetic dogs stratified by disease duration. Such a multi-parameter approach is needed to determine whether the ocular surface changes are merely present-or-absent or whether they worsen progressively, which would have direct implications for screening intervals and treatment timing.

The present research was therefore designed with two aims: first, to compare a panel of tear film and ocular surface cytology parameters between diabetic dogs and age-matched healthy controls; and second, to evaluate whether these parameters deteriorate as a function of diabetes duration, using sub-group analysis at less than 12 months, 12 to 24 months and greater than 24 months since diagnosis. The work was conducted at a veterinary ophthalmology referral centre in Seoul, South Korea, over an 18-month enrolment period.

Materials and Methods

Ethical Considerations

The research protocol was approved by the Institutional Animal Care and Use Committee of the Seoul Academy of Veterinary Ophthalmology (Approval No. SAVO-IACUC-2022-009, dated 28 March 2022). All procedures conformed to the Korean Animal Protection Act (Act No. 14651) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Written informed consent was obtained from each dog's owner before enrolment.

Patient Characteristics

Fifty-five dogs were enrolled between April 2022 and September 2023. The diabetic group ($n = 35$) comprised dogs with a confirmed diagnosis of diabetes mellitus (persistent fasting blood glucose > 200 mg/dL on two occasions, with

compatible clinical signs) managed with insulin therapy for a known duration. Sub-grouping was based on elapsed time since diagnosis: Group D1 (< 12 months, $n = 15$), Group D2 (12-24 months, $n = 12$) and Group D3 (> 24 months, $n = 8$). The control group ($n = 20$) consisted of age- and breed-matched healthy dogs presented for routine vaccination or wellness examination, confirmed free of systemic disease by physical examination and routine blood work. Dogs with pre-existing ocular disease, topical or systemic corticosteroid use within the preceding three months, or incomplete medical records were excluded.

Material

Ophthalmic examination was performed in a temperature-controlled room (22 ± 1 °C, relative humidity 40-50%) at the Seoul Academy of Veterinary Ophthalmology. Equipment included Schirmer tear test strips (Schirmer-Plus, Gecis, France), fluorescein sodium strips (Fluor-I-Strip, Ayerst), rose bengal strips (GreenGlo, HUB Pharmaceuticals), a portable tear osmometer (TearLab Corp., San Diego, USA), and cellulose acetate filter paper (Millipore HAWP, 0.45 μ m pore) for impression cytology. A slit-lamp biomicroscope (Kowa SL-15, Japan) was used for anterior segment evaluation.

Methods

All tests were performed in a standardised order to minimise reflex tearing artefacts: tear osmolarity (non-contact), TBUT, STT-I, fluorescein staining, rose bengal staining and finally impression cytology [12]. Tear osmolarity was measured from a 50-nL sample collected from the inferior meniscus. TBUT was determined by instilling one drop of 1% fluorescein, asking the handler to hold the lids open, and recording the interval between the last complete blink and the first dry spot visible under cobalt blue illumination; three readings were averaged. STT-I strips were placed in the ventral conjunctival fornix for 60 seconds without topical anaesthesia. Fluorescein and rose bengal staining were graded on the Oxford scale (0-15) [13].

For impression cytology, the filter paper was pressed gently onto the inferior bulbar conjunctiva for 5 seconds, peeled off, fixed in 95% ethanol, and stained with Giemsa. Goblet cell density and inflammatory cell count were recorded as means of five non-overlapping high-power fields ($\times 400$) by a single masked observer [10].

Data were analysed in R 4.3.1. Normality was assessed by Shapiro-Wilk test. One-way ANOVA with Bonferroni correction or Kruskal-Wallis with Dunn's post-hoc test was used for group comparisons. Pearson or Spearman correlation assessed relationships between diabetes duration and ocular parameters. Significance was set at $P < 0.05$.

Results

All 55 dogs completed the full ophthalmic examination protocol. The mean age did not differ significantly between groups (control: 8.6 ± 2.3 years; D1: 8.9 ± 2.7 ; D2: 9.4 ± 2.1 ; D3: 10.1 ± 1.8 ; $P = 0.31$). Mixed breeds comprised the largest category (36.4%), followed by Maltese (14.5%) and Miniature Schnauzer (10.9%).

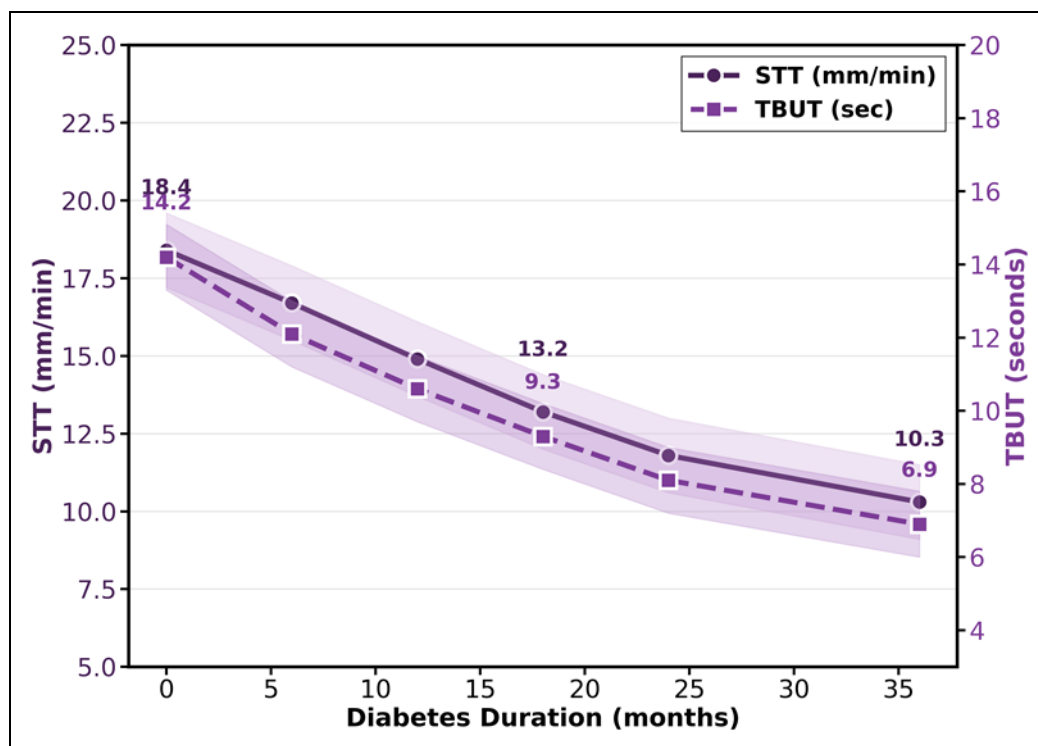


Fig 1: Decline in Schirmer tear test (STT-I, solid line) and tear film break-up time (TBUT, dashed line) with increasing diabetes duration. Shaded bands represent ± 1 standard deviation.

Table 1: Tear film and ocular surface parameters across groups (mean $\pm$ SD)

Parameter	Control	D1 (<12m)	D2 (12-24m)	D3 (>24m)	P-value
STT-I (mm/min)	18.4 $\pm$ 2.1 ^a	14.9 $\pm$ 2.5 ^b	12.5 $\pm$ 2.8 ^c	10.3 $\pm$ 3.1 ^d	< 0.001
TBUT (sec)	14.2 $\pm$ 1.8 ^a	10.6 $\pm$ 2.2 ^b	8.7 $\pm$ 2.4 ^c	6.9 $\pm$ 2.6 ^d	< 0.001
Osmolarity (mOsm/L)	298 $\pm$ 8 ^a	308 $\pm$ 11 ^b	316 $\pm$ 12 ^{bc}	324 $\pm$ 14 ^c	< 0.01
Goblet cells/HPF	42.8 $\pm$ 7.3 ^a	35.6 $\pm$ 6.1 ^b	27.3 $\pm$ 5.9 ^c	19.1 $\pm$ 4.8 ^d	< 0.001
Inflammatory cells/HPF	2.4 $\pm$ 1.1 ^a	8.7 $\pm$ 3.2 ^b	16.3 $\pm$ 4.6 ^c	24.6 $\pm$ 6.4 ^d	< 0.001
Rose bengal (Oxford)	1.2 $\pm$ 0.8 ^a	3.8 $\pm$ 1.4 ^b	5.6 $\pm$ 1.9 ^c	7.9 $\pm$ 2.3 ^d	< 0.001

Different superscripts within a row indicate $P < 0.05$ (Bonferroni correction). HPF = high-power field.

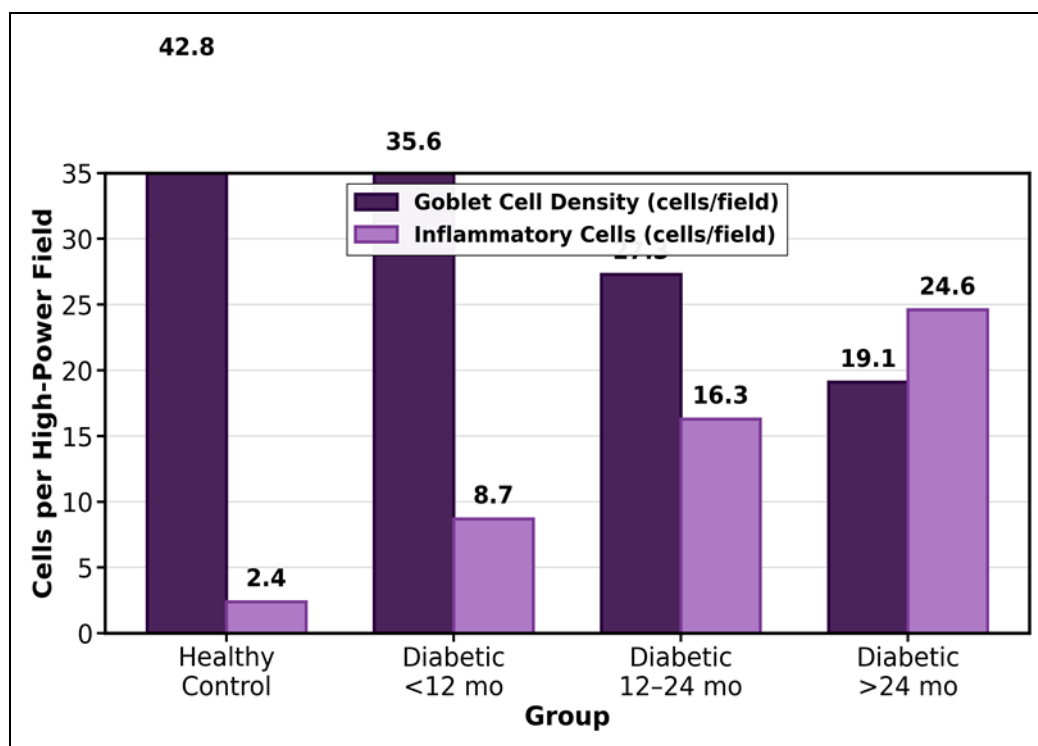


Fig 2: Conjunctival impression cytology results showing goblet cell density (dark bars) and inflammatory cell count (light bars) across healthy controls and three diabetic duration sub-groups.

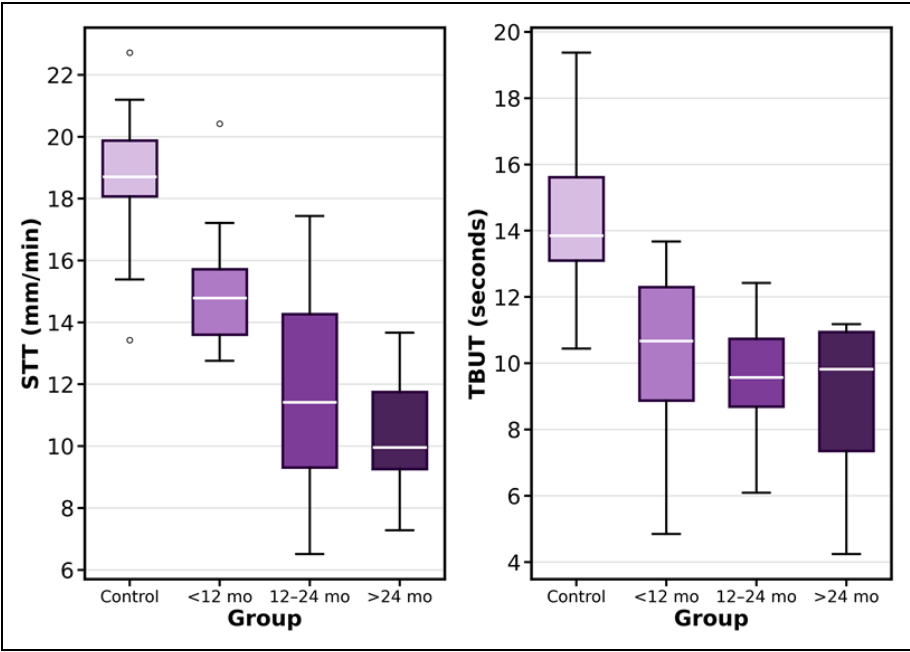


Fig 3: Box-and-whisker plots illustrating the distribution of Schirmer tear test (left panel) and tear break-up time (right panel) values across control and diabetic sub-groups. Progressive darkening of box colour reflects increasing diabetes duration.

Table 2: Temporal progression of ocular surface changes with diabetes duration

Phase	Key Findings	Clinical Relevance	Suggested Action
< 12 mo	Mild STT decline; early goblet cell loss; osmolarity borderline elevated	Subclinical tear deficiency; rarely symptomatic	Baseline ophthalmic exam at diabetes diagnosis
12-24 mo	Moderate STT and TBUT reduction; inflammatory infiltrate; rose bengal positive	Early KCS signs; epithelial damage detectable	Semi-annual screening; consider artificial tears
> 24 mo	Severe tear deficiency; marked goblet cell depletion; high osmolarity	Overt KCS risk; corneal complications possible	Quarterly monitoring; cyclosporine or tacrolimus therapy

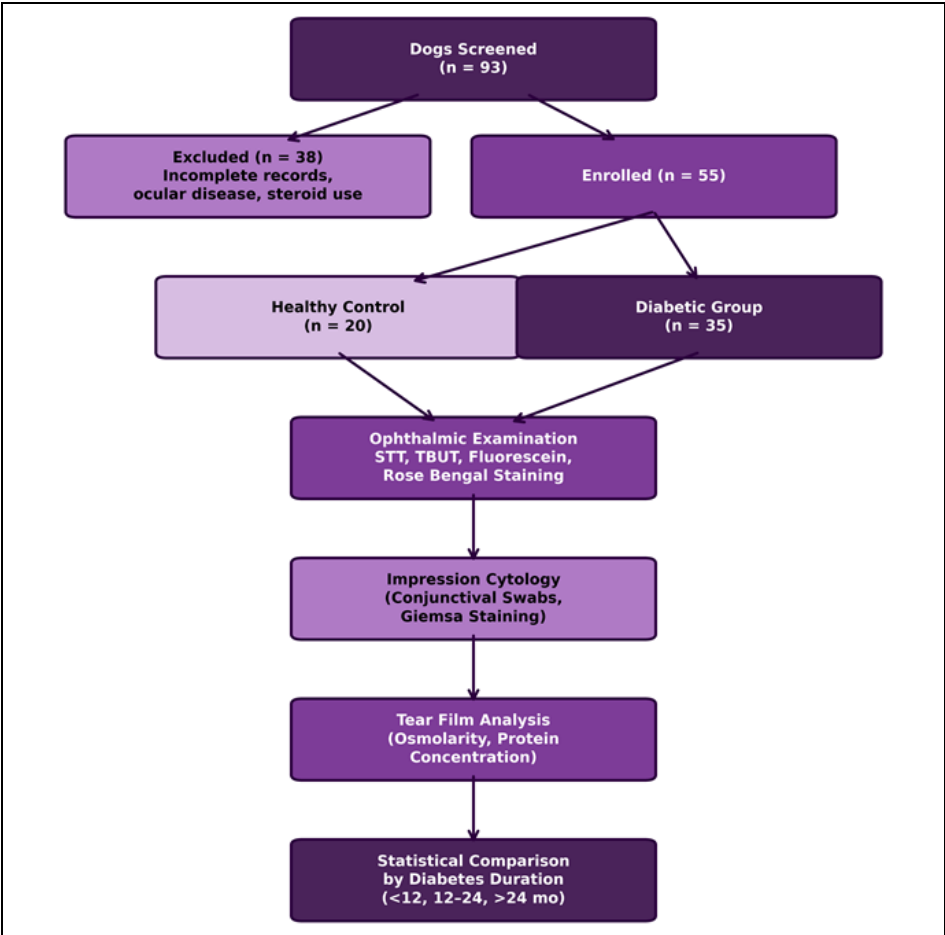


Fig 4: Flowchart of patient enrolment, exclusion criteria and sequential ophthalmic assessment protocol used in the research.

Comprehensive Interpretation

The data consistently demonstrate a graded, duration-dependent deterioration of the ocular surface in diabetic dogs. Every measured parameter aqueous tear volume, tear stability, osmolarity, goblet cell density, inflammatory cell count and vital staining worsened as diabetes duration increased. The strong correlation between duration and STT ($r = -0.81$) suggests that the decline is not abrupt but rather proceeds steadily, providing a window for early intervention. The shift in cytology simultaneous goblet cell loss and inflammatory infiltration mirrors the vicious circle described in human diabetic dry eye, where hyperosmolar tears trigger epithelial apoptosis, which in turn releases inflammatory mediators that further suppress lacrimal and goblet cell function [6, 11]. The timeline table (Table 2) translates these findings into actionable clinical stages, proposing screening and management thresholds that can be incorporated into routine diabetic monitoring protocols.

Discussion

The principal outcomes of this research may be summarised in three points: diabetic dogs exhibited significantly reduced tear production and stability compared with healthy controls; ocular surface cytology revealed progressive goblet cell loss and inflammation; and all parameters deteriorated as a function of diabetes duration, following a roughly linear trajectory over the 0- to 36-month observation range.

These findings agree with the limited veterinary data available. Williams *et al.* [3] reported reduced STT values in a small series of diabetic dogs, and Cullen *et al.* [4] documented increased conjunctival squamous metaplasia in canine diabetes, although neither research stratified subjects by disease duration. In human medicine, the association between diabetes and dry eye is well established and has been attributed to autonomic neuropathy of the lacrimal gland, microvascular ischaemia of the ocular surface and advanced glycation end-product deposition [5, 14]. The present canine data are consistent with a similar pathogenic mechanism operating across species.

One area where our results diverge from the human literature concerns the rate of tear osmolarity increase. In diabetic humans, osmolarity elevation is often detectable within the first year of diagnosis [15]. Here, the D1 sub-group showed only a modest (10 mOsm/L) rise, with more pronounced increases appearing in D2 and D3. This slower progression may reflect differences in glycaemic control practices most dogs in our cohort received twice-daily insulin and maintained relatively stable fructosamine levels or it may indicate that the canine lacrimal gland has a greater compensatory reserve than its human counterpart.

The goblet cell data are particularly striking. A 55% reduction in density from controls to D3 dogs matches the magnitude reported in severely affected human diabetic patients [6]. Goblet cells are the sole source of the gel-forming mucin MUC5AC, which stabilises the aqueous-mucin interface and anchors the tear film to the corneal glycocalyx [16]. Their depletion therefore has consequences that extend beyond the cell count itself, potentially explaining why TBUT fell sharply even in dogs whose aqueous production (STT) had declined only moderately.

From a clinical standpoint, two practical messages emerge. First, a baseline ophthalmic evaluation including at minimum STT and TBUT should be performed at the time of diabetes diagnosis, with repeat assessments every six to twelve months. Second, dogs with diabetes duration exceeding 24

months are at substantial risk for clinically significant KCS and may benefit from prophylactic lacrimostimulant therapy, such as topical cyclosporine or tacrolimus, even before overt corneal disease develops [17].

Limitations

Several limitations should be noted. The cross-sectional design assessed different animals at different disease durations rather than following the same cohort longitudinally; a prospective follow-up research tracking individual dogs from diagnosis would provide stronger evidence of causation. The D3 sub-group contained only eight dogs, limiting statistical power for detecting smaller effect sizes. Glycaemic control was not standardised across patients, and variations in fructosamine levels may have introduced confounding. Tear osmolarity was measured with a single device type; inter-device variability has been reported in human studies and may apply here [18]. Finally, meibomian gland morphology was not assessed, so the contribution of evaporative dry eye to the observed tear film changes cannot be separated from the aqueous-deficient component.

Conclusion

This research set out to characterise the ocular surface and tear film status of diabetic dogs in comparison with healthy controls and to determine whether changes become more severe with longer disease duration. By applying a multi-parameter approach combining STT-I, TBUT, tear osmolarity, impression cytology and vital staining — across a cohort of 55 dogs stratified into control and three diabetic sub-groups, the research provides the most comprehensive picture to date of diabetes-associated ocular surface pathology in canines.

The key findings demonstrate that diabetic dogs experience a statistically and clinically meaningful decline in tear production and stability that worsens progressively with disease duration. STT-I values dropped by nearly 44% from controls to dogs diabetic for more than 24 months, and TBUT fell by over 50% across the same span. Tear osmolarity increased to levels associated with ocular surface damage in human dry-eye classifications. Impression cytology confirmed a parallel biological shift: goblet cell density halved, while inflammatory cell counts increased tenfold, indicating an active, duration-dependent inflammatory process on the conjunctival surface. Rose bengal staining scores corroborated these cytological findings, revealing progressively greater epithelial devitalisation.

The practical message for veterinary practitioners is clear. Diabetic dogs should not be monitored solely for cataracts and glycaemic control; their ocular surface health warrants systematic assessment as well. A simple STT measurement at the time of diabetes diagnosis can serve as a baseline, and repeat testing at six- to twelve-month intervals can flag the onset of tear deficiency before corneal complications develop. For dogs diabetic beyond two years, preventive lacrimostimulant therapy with topical cyclosporine or tacrolimus should be discussed with owners, particularly if STT falls below 12 mm/min or TBUT below 8 seconds.

Looking to the future, three avenues of research would strengthen and extend these findings. First, a prospective longitudinal design following newly diagnosed diabetic dogs over three or more years would confirm whether the cross-sectional duration gradient observed here truly represents within-individual progression. Second, evaluating meibomian gland structure with infrared meibography would clarify the

evaporative component of diabetic dry eye and potentially identify a distinct subgroup of dogs requiring lipid-layer-targeted treatment. Third, intervention trials comparing early versus delayed lacrimostimulant therapy in diabetic dogs would determine whether prophylactic treatment prevents or merely delays the onset of clinical KCS. Such evidence is needed to support formal inclusion of ocular surface screening in canine diabetes management guidelines.

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Contributions Not Qualifying for Authorship

Ms. Yoon Ha-Eun performed the Giemsa staining and cytology slide preparation. Dr. Baek Seung-Ho provided statistical consultation.

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