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Investigation of ocular surface parameters in dogs with different cephalic conformations using veterinary ocular surface analyzer (OSA-VET)

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Abstract

Objective: To compare ocular surface parameters in dogs with different cephalic conformations and evaluate correlations among tests.

Animals Studied: Sixty-eight privately owned dogs.

Procedures: The study categorized canine eyes into three groups based on the craniofacial ratio (CFR): brachycephaly (≤0.52), mesocephaly (>0.52 to <0.67), and dolichocephaly (≥0.67). All eyes were examined using an ocular surface analyzer (OSA-VET) to determine lipid layer thickness (LLT) of the tear film, tear meniscus height (TMH), non-invasive tear breakup time (NIBUT), and meibomian gland loss rate of the lower eyelids (MGLRL). Schirmer tear test 1 (STT-1) and tear film breakup time (TBUT) were also performed. Statistical analyses involved one-way ANOVA, Kruskal–Wallis *H* test, post hoc Holm–Sidak test, and Pearson correlation coefficient.

Results: While STT-1 showed no significant difference among dog groups, brachycephalic dogs had significantly lower values in TBUT, NIBUT, and LLT, and a higher TMH, compared to mesocephalic and dolichocephalic dogs. Additionally, brachycephalic dogs exhibited a significantly higher MGLRL than dolichocephalic dogs. Correlations among tests were generally weak to moderate ($r<.6$) except for a strong correlation between CFR and LLT ($r=.641$, $p<.001$), and between TBUT and NIBUT ($r=.899$, $p<.001$).

Conclusions: Brachycephalic morphology predisposes dogs to a significantly thinner lipid layer and diminished tear film stability, likely due to factors such as impaired meibomian gland function and increased ocular exposure compared to other cephalic conformations, thereby increasing their risk of keratoconjunctivitis sicca (KCS). OSA-VET shows a valuable tool to provide more comprehensive and precise diagnosis for canine ocular surface disorders.

KEYWORDS

brachycephalic, canine, interferometry, meibography, qualitative KCS, tear meniscus

1 | INTRODUCTION

Brachycephalic dog breeds, characterized by their distinctive shortened skulls and muzzles, have surged in popularity recently. However, their unique facial features are associated with a range of health issues, including a spectrum of eye-related problems termed brachycephalic ocular syndrome (BOS).[1–4] Notably, these breeds face an increased risk of developing keratoconjunctivitis sicca (KCS), commonly referred to as dry eye. This complex ocular surface disorder, characterized by deficiencies in the tear film and issues with the ocular surface, poses diagnostic and treatment challenges due to its multifaceted nature, encompassing both quantitative and qualitative tear film deficiencies.[2,5]

Traditional diagnostic tools like the Schirmer tear test-1 (STT-1) and tear film breakup time (TBUT) test have limitations in accurately assessing the tear film's complexity.[6,7] To overcome this, the ocular surface analyzer for veterinary use (OSA-VET) has been introduced as a reliable method for evaluating various tear film parameters, offering a more comprehensive understanding of tear film stability and quality.[8]

Despite the recognized risks associated with brachycephalic breeds, there remains a notable gap in research, particularly regarding the qualitative differences in tear film parameters between brachycephalic and non-brachycephalic dogs. This study aimed to bridge this gap using OSA-VET to compare ocular surface parameters among brachycephalic, mesocephalic, and dolichocephalic breeds. By identifying significant differences in tear film characteristics across these groups, this study intends to deepen our understanding of the ocular health implications related to brachycephalic conformation. The insights gained could contribute to more effective diagnostic and management strategies, ultimately improving the quality of life for these increasingly popular breeds.

2 | MATERIALS AND METHODS

2.1 | Animals

Approved by the Institutional Animal Care and Use Committee (IACUC) and adhering to Association for Research in Vision and Ophthalmology's (ARVO) statement on the use of animals in ophthalmic and vision research, our study, conducted from October 2022 to June 2023, involved dogs voluntarily enrolled by informed-consenting owners. We collected detailed data on each dog, including age, breed, sex, reproductive status, and medical history, via an online owner questionnaire before their scheduled appointments.

To ensure homogeneous and representative samples, the study excluded dogs under 6 months of age, as well as those presenting with STT-1 <10 mm/min or KCS symptoms, prior ophthalmic treatment (solutions or ointments), systemic diseases that may affect the tear film (like diabetes or hypothyroidism), eye surgery or ocular trauma history, ocular surface disorders such as corneal ulceration, cilia or eyelid abnormalities. Dogs with excessive aggression or severe anxiety during examinations were also excluded.

2.2 | Experiments

Upon arrival at the hospital, each dog had a 10-min rest while the research process was explained to the owner, reducing the impact of transportation on the dog's ocular surface. All tests were performed by the same examiner (a veterinarian from the Ophthalmology Department) without the aid of sedation. Owners or assistants gently restrained the dogs, either on a table or the floor for larger breeds, in a quiet room to minimize stress.

The examination sequence was conducted on both eyes of each dog and consisted of two main phases. The first phase utilized the non-invasive OSA-VET, while the second phase included a series of basic ophthalmology examinations in a specific order as described below.

2.2.1 | Ocular Surface Analyzer for Veterinary use (OSA-VET)

Non-invasive tear film analysis was conducted using the portable OSA-VET (SBM Sistemi, Torino, Italy) device with infrared and white LED lights, positioned 5 cm from the dogs' eyes in a dark room to reduce reflections.[9,10] The examiner used a USB-connected foot pedal to control image and video capture. No pre-examination eyedrops or stains were needed.[9] Examinations were conducted alternately on each eye, with measurements taken sequentially to minimize the impact on tear film stability.[9]

Lipid layer thickness (LLT)

Initially, an interferometry examination was conducted with a plain cone placed on the OSA-VET device. After inducing the palpebral reflex, the corneal surface was photographed and videotaped. Using the six-grade Video Grading Scale (VGS) outlined in the OSA-VET Clinical Atlas,[8] and also referring to different categories described by Guillon,[11] the interferometric patterns were classified into six grades (Figure 1), ranging from a gray, transparent pattern with minimal reflection (Grade 1) to distinct, vibrantly multicolored fringes (Grade 6).[10]

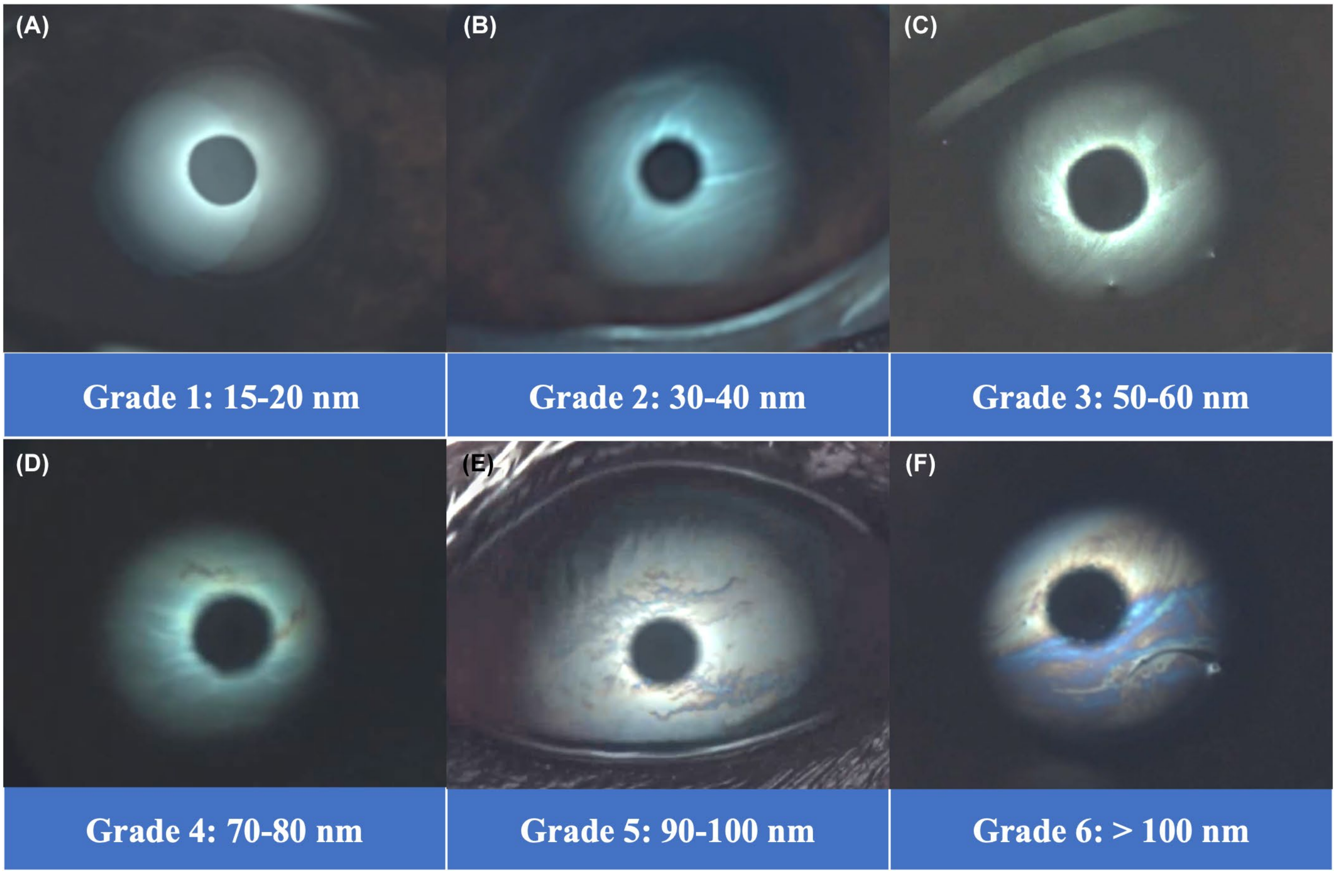


FIGURE 1 Lipid layer thickness (LLT) assessment with the interferometry grading patterns observed by OSA-VET in this study. (A) Grade 1: Gray, very transparent, little reflection pattern (right eye of Case 3). (B) Grade 2: Gray, denser and more reflective, knitted pattern (right eye of Case 53). (C) Grade 3: Reflective, with horizontal and vertical waves (left eye of Case 46). (D) Grade 4: Blue-whitish appearance with predominantly horizontal waves (left eye of Case 35). (E) Grade 5: Yellow, blue, purple, and brown interference fringes on a gray transparent background (right eye of Case 61). (F) Grade 6: Distinct regions with vibrant and diversely colored fringes (right eye of Case 63).

Tear meniscus height (TMH)

After inducing the palpebral reflex again, photographs of the area between the lower eyelid and the cornea were taken using the OSA-VET with a plain cone to capture interferometric reflections of the tear reservoir.[8] Following the imaging process, the examiner manually aligned a millimeter caliper at the upper and lower borders of the tear meniscus on the software. The TMH was then automatically calculated (Figure 2A). To ensure accuracy, measurements were taken at a minimum of three points with the highest value being recorded.[8]

Non-invasive breakup time (NIBUT)

The non-invasive breakup time (NIBUT) involves utilizing a Placido grid measurement cone on the OSA-VET device to project a series of concentric circles onto the interferometric image of the tear film.[8] During the examination, video recording began immediately after a blink and continued until the first involuntary blink occurs. NIBUT was then calculated as the time elapsed from the blink until the blurring of the projected lines was observed (Figure 2B).[8] Each eye of each dog was tested twice, and the results were averaged.

Non-contact Infrared Meibography (NCIM)

NCIM was performed by pulling down the lower eyelid of the dog gently to expose the meibomian gland area and then use the built-in infrared camera of OSA-VET to capture the image of meibomian glands without a cone. The software enabled manual selection of the central two-thirds of the eyelid for clear gland visibility,[12] while avoiding melanin-rich areas to prevent image interpretation issues. It calculates the meibomian gland loss rate by comparing the gland loss area to the selected region, providing a quantitative value for the meibomian gland loss rate of the lower eyelid (MGLRL) (Figure 2C).[8]

After completing the OSA-VET examinations, a 15-minute rest period was provided to each dog.

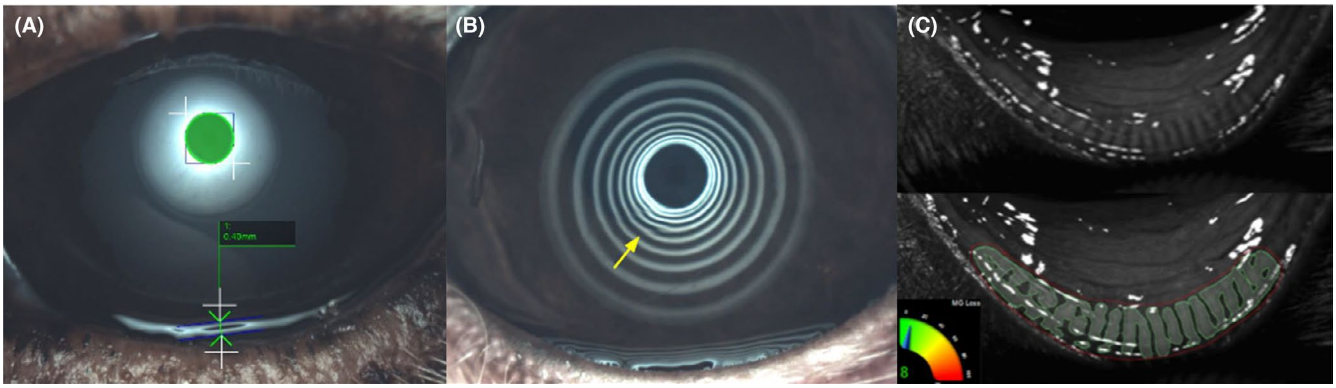


FIGURE 2 Measurement of tear meniscus height (TMH), non-invasive breakup time (NIBUT), and meibomian gland loss rate for the lower eyelid (MGLRL). (A) The OSA-VET software calculates TMH by marking the tear meniscus edges (green arrowheads) around a central reference circle. (B) Post-blink Placido disk patterns reflected on the tear film reveal initial distortion, which determines the non-invasive breakup time (NIBUT). (C) The non-contact infrared camera clearly displays the meibomian gland, with software enabling manual selection of the lower eyelid's central two-thirds for optimal gland visibility. It then quantifies the meibomian gland loss rate (MGLRL) by measuring gland loss area against this region.

2.2.2 | Basic ophthalmology examinations

The observations and tests were carried out in a specific sequence to prevent one procedure from affecting another. Apart from the slit-lamp examination, all other tests were conducted in a well-lit environment.

Schirmer tear test-1 (STT-1)

A standard STT-1 was performed to evaluate both basal and reflex tear production in all dogs. This test involved placing a standard sterile STT strip (Omni Schirmer, Omni Lens Pvt. Ltd., India) in the lateral lower conjunctival fornix of each eye and keeping the patient's eyes closed during the examination. The wetting of the strip was measured in millimeters over a 1-minute period, providing the final value recorded in mm/min.[13]

Tear film breakup time (TBUT)

The TBUT was measured using fluorescein sodium strips (Omni Fluro, Omni Lens Pvt. LTD., India) diluted with 1 mL of sterile saline to achieve a 0.1% concentration, which has been shown to be highly fluorescent in previous studies.[14,15] After applying a drop to the eye and reopening the eyelids, the dorsolateral portion of the cornea was examined under a cobalt blue filter with a slit-lamp biomicroscope (Sl-17, KOWA, Tokyo, Japan). The TBUT was then measured, indicating the time interval between the opening of the eyelid and the first appearance of a dry spot on the cornea. The result was recorded in seconds.[14,15]

Other ophthalmic examinations

Subsequently, each dog underwent further standard ophthalmic examinations. These included neuro-ophthalmic tests (including menace response, dazzle reflex, pupillary light reflexes, and palpebral reflexes), slit-lamp biomicroscopy (Sl-17; KOWA, Tokyo, Japan) for the adnexa, anterior segment, lens and anterior vitreous, and applanation tonometry (Tono-Pen® Vet, Reichert, NY, USA). These tests were conducted to ascertain whether any additional eye abnormalities were present.

2.2.3 | Morphometric measurements

Morphometric data for each dog were collected following established measuring protocols.[16,17] Palpebral fissure length (PFL) was measured first using a Castroviejo caliper or a standard 1-m soft measuring tape, extending from the medial to lateral canthi, recorded in millimeters with a precision of 0.1 mm. Muzzle length (ML) and cranial length (CL) were then measured with the same soft tape, from the nose tip to its base and from the base of the nose to the occipital protuberance, respectively (Figure 3).[17,18]

These measurements were used to calculate the craniofacial ratio (CFR) and relative palpebral fissure length (RPFL). The CFR was computed as the ratio of ML to CL, aiding in the evaluation of brachycephaly and distinguishing various cephalic conformations.[19,20] To assess the impact of PFL across dogs of varying sizes and ensure comparability, the RPFL was standardized by dividing the absolute PFL by CL, calculated as RPFL = (PFL in millimeters/CL in millimeters) × 100%.[17]

Finally, photographs of each dog's eyes, front, and side profiles were captured using a smartphone camera. Additionally, an artificial tear solution containing 0.25% viscoadaptive hyaluronan (I-Drop® Vet Plus; I-MED Animal Health, Canada) was administered to each eye to preserve the moisture of the dogs' ocular surface following the examination.

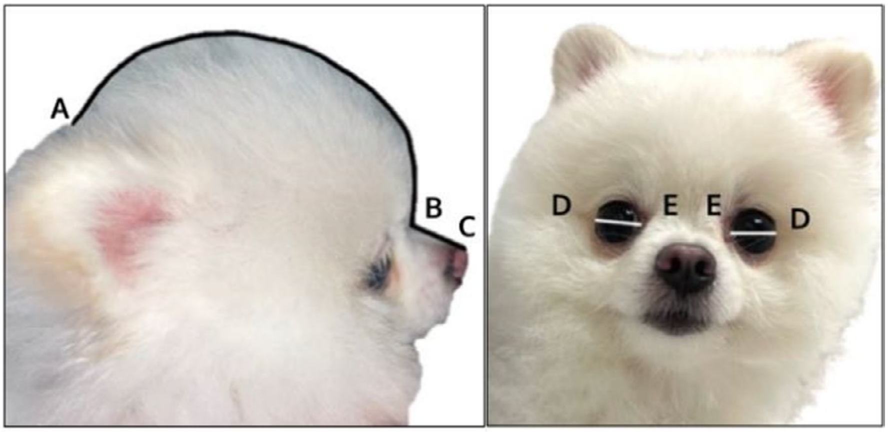


FIGURE 3 Measurement of cranial length (A, B), muzzle length (C, D), and palpebral fissure length (D, E). In the left image, cranial length (CL) is measured as the distance from the occipital protuberance (A) to the base of nose (B), and muzzle length (ML) is from the base of nose (B) to the nasal planum tip (C). In the right image, palpebral fissure length (PFL) measures from the medial to lateral canthus. For example, in a Pomeranian (Case 64), the craniofacial ratio is 0.26 (25 mm ML / 95 mm CL), and the relative palpebral fissure length is 17.9% (17 mm PFL/95 mm CL × 100%).

2.2.4 | Statistical analysis

Dogs were categorized into three groups based on their craniofacial ratio (CFR): brachycephaly (≤0.52), mesocephaly (>0.52 to <0.67), and dolichocephaly (≥0.67).[21] Data were organized using Microsoft Excel 2021 for Mac ver. 16.73 (Microsoft, Redmond, Washington, USA) and analyzed with SPSS software ver. 26.0 for Mac (IBM SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to describe the distribution of various data, and normality was checked by the Shapiro–Wilk test. One-way ANOVA and post hoc Holm–Sidak test were used to compare all continuous parameters among groups. The Kruskal–Wallis test assessed lipid layer thickness (LLT) grades. Pearson's correlation test evaluated correlations between diagnostic tests and anatomical measurements, interpreted according to guidelines described by Campbell and Swinscow[11]: very weak (0–0.19), weak (0.2–0.39), moderate (0.40–0.59), strong (0.6–0.79), and very strong (0.8–1.0). The significance level for all tests was set at .05.

3 | RESULTS

3.1 | Demographics

A total of 68 client-owned dogs ($n = 130$ eyes) were included in the study after 14 eyes were excluded. All the included dogs were categorized into three groups: brachycephalic, mesocephalic, and dolichocephalic (Figure 4). No significant variance ($p > .05$) was observed in the age among brachycephalic dogs (9.14 ± 4.03 years), mesocephalic dogs (7.23 ± 3.63 years), and dolichocephalic dogs (7.90 ± 3.63 years). Similarly, there were no significant differences ($p = .66$) in terms of sex distribution among the groups.

3.2 | Ophthalmic findings

Thirty-three eyes out of 130 displayed ophthalmic findings. The observed conditions included nuclear sclerosis (27 eyes), incipient to mature cataract (five eyes), iris atrophy (three eyes), lens subluxation (two eyes), vitreous degeneration (three eyes), and retinal degeneration (two eyes) in the included dogs under investigation. Despite these findings, these dogs were included in the study, as these conditions are intraocular changes that, theoretically, should not affect the tear film on the ocular surface.

3.3 | Diagnostic test results

The raw data for each test are comprehensively detailed in appendix 1 for dogs in the brachycephalic group, appendix 1 for the mesocephalic group, and appendix 1 for the dolichocephalic group. Results from the total subject pool, along with comparisons among these groups, are presented in Table 1 and Figure 5.

3.3.1 | Schirmer Tear Test-1 (STT-1)

The STT-1 values were 18.2 ± 3.9 for brachycephalic dogs, 18.3 ± 3.1 for mesocephalic dogs, and 16.8 ± 3.4 for

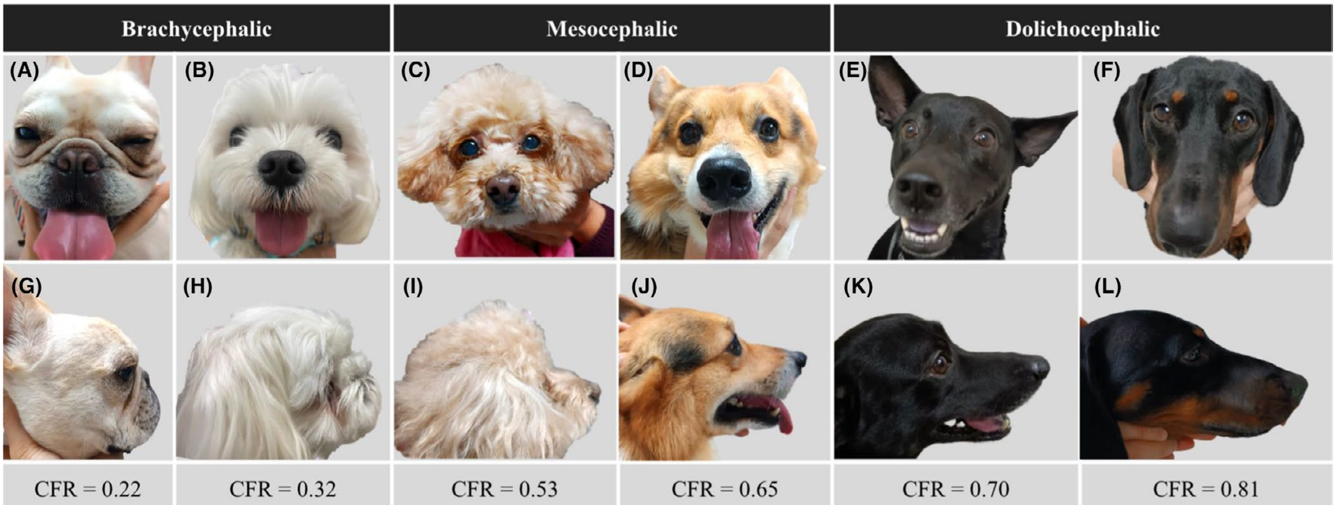


FIGURE 4 Images of both front and side profiles of representative dogs from the three categories. The breeds are French bulldog (A, G), Maltese (B, H), Poodle (C, I), Corgi (D, J), Mixed (E, K), and Dachshund (F, L).

TABLE 1 Mean ± standard deviation (range) values of tear film parameters in 130 eyes, including 49 eyes in brachycephalic group, 40 eyes in mesocephalic group, and 41 eyes in dolichocephalic group.

	All ($n=130$)	Brachycephalic ($n=49$)	Mesocephalic ($n=40$)	Dolichocephalic ($n=41$)	p-Value between groups
STT-1 (mm/min)	17.8 ± 3.6 (10–25)	18.2 ± 3.9 (11–25)	18.3 ± 3.1 (10–23)	16.8 ± 3.4 (10–24)	.075
TBUT (s)	11.3 ± 6.1 (1–25)	7.2 ± 5.1 (1–20)	14.7 ± 5.6 (3–25)	13.0 ± 4.9 (1–24)	<.001***
NIBUT (s)	13.4 ± 7.0 (0.6–29.7)	8.7 ± 5.4 (0.6–23.2)	16.9 ± 6.0 (4.3–29.7)	15.6 ± 6.7 (0.8–28.1)	<.001***
MGLRL (%)	16.2 ± 10.0 (4–58)	19.3 ± 12.6 (8–55)	15.2 ± 8.9 (4–58)	13.6 ± 5.9 (6–25)	.019*
TMH (mm)	0.32 ± 0.17 (0.12–1.02)	0.40 ± 0.23 (0.12–1.02)	0.27 ± 0.12 (0.12–0.52)	0.26 ± 0.09 (0.13–0.55)	<.001***
PFL (mm)	19.5 ± 3.5 (10.0–32.0)	19.3 ± 4.3 (13.5–32.0)	19.3 ± 3.3 (10.0–25.0)	20.2 ± 2.2 (16.5–25.0)	.389
RPFL (%)	19.4 ± 2.7 (14.0–25.2)	21.9 ± 2.3 (17.7–25.2)	17.4 ± 1.6 (15.2–22.1)	18.5 ± 1.6 (14.0–21.8)	<.001***

Note: A statistically significant difference between groups is denoted by asterisk (* for $p<.05$, ** for $p<.01$, *** for $p<.001$).

Abbreviations: MGLRL, Meibomian gland loss rate of the lower eyelids; NIBUT, Non-invasive tear breakup time; PFL, Palpebral fissure length; RPFL, Relative palpebral fissure length; STT-1, Schirmer tear test-1; TBUT, Tear film breakup time; TMH, Tear meniscus height.

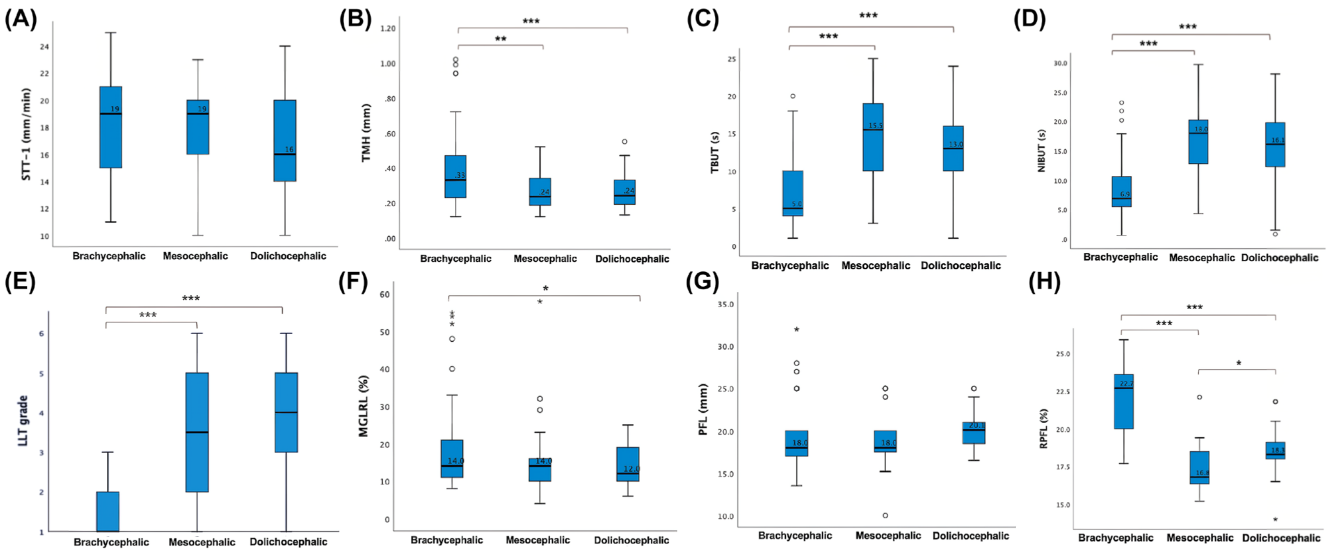
dolichocephalic dogs. No significant difference among the three groups was observed ($p=.075$) (Figure 5A).

3.3.2 | Tear Meniscus Height

The mean TMH values were 0.40 ± 0.23 mm for brachycephalic dogs, 0.27 ± 0.12 mm for mesocephalic dogs, and 0.26 ± 0.09 mm for dolichocephalic dogs. Brachycephalic dogs had a significantly higher mean TMH compared to both mesocephalic dogs ($p=.001$) and dolichocephalic dogs ($p<.001$). No significant difference was observed between mesocephalic and dolichocephalic dogs ($p=.98$) (Figure 5B).

3.3.3 | Tear Film Breakup Time (TBUT) and Non-Invasive Breakup Time (NIBUT)

The mean TBUT values were 7.2 ± 5.1 s in brachycephalic dogs, 14.7 ± 5.6 s in mesocephalic dogs, and 13.0 ± 4.9 s in dolichocephalic dogs, while mean NIBUT values were 8.7 ± 5.4 s, 16.9 ± 6.0 s, and 15.6 ± 6.7 s, respectively. Brachycephalic dogs exhibited significantly



Note: A statistically significant correlation is denoted by asterisk (* for $p < .05$, ** for $p < .01$, *** for $p < .001$)

FIGURE 5 Boxplots comparing ocular surface diagnostics in brachycephalic, mesocephalic, and dolichocephalic dogs. (A) Schirmer tear test-1 (STT-1), (B) tear meniscus height (TMH), (C) tear breakup time (TBUT), (D) non-invasive tear breakup time (NIBUT), (E) lipid layer thickness (LLT) grade, (F) meibomian gland loss rate of the lower eyelid (MGLRL), (G) palpebral fissure length (PFL), and (H) relative palpebral fissure length (RPFL).

lower TBUT and NIBUT values compared to mesocephalic and dolichocephalic groups ($p < .001$ for both), with no significant difference between the latter two (Figure 5C,D).

3.3.4 | Lipid Layer Thickness (LLT)

Brachycephalic dogs exhibited the majority of LLT data concentrated in Grade 1 (15–20 nm) and fewer observations in higher grades. Mesocephalic dogs, on the other hand, displayed a more diverse distribution across all grades, and dolichocephalic dogs predominantly fell within grades 3 (50–60 nm) to Grade 6 (>100 nm). Following statistical analysis, the results indicated that brachycephalic dogs had significantly lower LLT grades compared to both mesocephalic and dolichocephalic dogs ($p < .001$ for both comparisons). However, there was no significant difference observed between mesocephalic and dolichocephalic dogs ($p = .224$) (Figure 5E).

3.3.5 | Meibomian Gland Loss Rate of the Lower Eyelid (MGLRL)

Mean MGLRL values were 19.3 ± 12.6% for brachycephalic dogs, 15.2 ± 8.9% for mesocephalic dogs, and 13.6 ± 5.9% for dolichocephalic dogs. Brachycephalic dogs had a significantly higher mean MGLRL compared to dolichocephalic dogs ($p = .019$). However, no significant differences were observed between brachycephalic and mesocephalic dogs ($p = .13$), or between mesocephalic and dolichocephalic dogs ($p = .74$) (Figure 5F).

3.3.6 | Palpebral Fissure Length (PFL)

Mean PFL values were 19.1 ± 4.2 mm for brachycephalic dogs, 19.3 ± 3.3 mm for mesocephalic dogs, and 20.2 ± 2.2 mm for dolichocephalic dogs. No significant difference were observed among the three groups ($p = .39$) (Figure 5G).

3.3.7 | Relative Palpebral Fissure Length (RPFL)

Mean RPFL values for brachycephalic dogs, mesocephalic dogs, and dolichocephalic dogs were 21.8 ± 2.3%, 17.4 ± 1.6%, and 18.5 ± 1.6% respectively. There were significant differences between any two groups among the three studied groups. Specifically, brachycephalic dogs displayed significantly higher mean RPFL values compared to both mesocephalic and dolichocephalic dogs ($p < .001$), while mesocephalic dogs exhibited significantly lower mean RPFL values compared to dolichocephalic dogs ($p = .045$) (Figure 5H).

3.4 | Inter-test correlations

Results of the Pearson correlation testing between evaluations are described in Table 2.

3.4.1 | Craniofacial Ratio (CFR)

CFR had a strong positive correlation with LLT (r=.632; p<.001), a strong negative correlation with RPFL (r=−.625; p<.001), a moderate positive correlation with TBUT (r=.417; p<.001), NIBUT (r=.423; p<.001), a weak negative correlation with TMH (r=−.353; p<.001), and a very weak negative correlation with STT-1 (r=−.184; p<.05) and MGLRL (r=−.174; p<.05). There was no statistically significant correlation observed between CFR and PFL (r=−.086; p>.05) (Table 2).

3.4.2 | Relative Palpebral Fissure Length (RPFL)

RPFL demonstrated a strong negative association with CFR (r=−.625; p<.001), a moderate negative association with LLT (r=−.578; p<.001), a moderate negative correlation with TBUT (r=−.488; p<.001) and NIBUT (r=−.442; p<.001), and a weak positive correlation with MGLRL (r=.211; p<.05) and TMH (r=.293; p<.001). No significant association between STT-1 and RPFL (r=.133; p>.05) (Table 2).

3.4.3 | Other diagnostic tests

The correlations among the various diagnostic tests are presented in Table 2. It is worth noting that most of the correlations observed between the tests were generally weak to moderate (r<.60), except for a strong correlation between TBUT and NIBUT (r=.922; p<.001). The diagnostic tests specifically designed to assess aqueous tearing, such as STT-1 and TMH, did not exhibit significant associations with TBUT and NIBUT. However, lipid layer thickness (LLT) displayed the strongest correlations with both TBUT and NIBUT.

4 | DISCUSSION

In the current study, the OSA-VET, a non-invasive diagnostic tool, was utilized to assess tear film stability and ocular surface parameters in different skull types. Dogs were grouped based on their craniofacial ratio (CFR). Unlike previous research[19,20,22] that primarily divided dogs into brachycephalic and non-brachycephalic categories, our study further distinguishes between mesocephalic and dolichocephalic dogs. This distinction helped evaluate differences between these groups and explore potential correlations between ocular surface parameters and cephalic conformations.

NCIM was performed only on the lower eyelid in this study. Our decision to examine the lower eyelid was based on findings from our pilot study. When we attempted to evert the upper eyelid of dogs, they often exhibited noticeable discomfort, resistance, and even aggression. Additionally, some dogs experienced increased tear production following the procedure. In contrast, gently pulling down the lower eyelid without excessive eversion allowed us to perform meibography clearly and effectively without causing undue stress to the dogs. Although there are no animal studies in this field to the author's knowledge, a previous human study[23] revealed that the lower eyelid reliably represents the condition of the meibomian

TABLE 2 Pearson's correlation coefficients (r) describing the association between tear film diagnostics and morphological evaluations in dogs.

	STT-1 (mm/min)	TBUT (s)	NIBUT (s)	LLT (grade)	MGLRL (%)	TMH (mm)	RPFL (%)	PFL (mm)
CFR	−.184*	.417***	.423***	.632***	−.174*	−.353***	−.625***	−.086
PFL (mm)	.226*	.227**	.215**	.088	−.219*	.051	.118	
RPFL (%)	.133	−.488***	−.442***	−.578***	.211*	.293***		
TMH (mm)	.448***	−.162	−.128	−.326***	−.006			
MGLRL (%)	−.220*	−.378***	−.401***	−.216**				
LLT (grade)	−.077	.545***	.520***					
NIBUT (s)	.109	.922***						
TBUT (s)	.105							

Note: A statistically significant correlation is denoted by asterisk (* for p<.05, ** for p<.01, *** for p<.001).

Abbreviations: CFR, Cranial facial ratio; MGLRL, Meibomian gland loss rate of the lower eyelids; NIBUT, Non-invasive tear breakup time; PFL, Palpebral fissure length; RPFL, Relative palpebral fissure length; STT-1, Schirmer tear test-1; TBUT, Tear film breakup time; TMH, Tear meniscus height.

gland. More research is needed to verify the representativeness or variability of meibomian gland in the upper and lower eyelids in healthy and those with meibomian gland dysfunction (MGD) in the future.

During the examination, all tests were alternately performed between the two eyes, allowing each eye to blink normally for at least 1 min before the next test. This practice helped minimize the impact of the dryness caused by prolonged exposure of the ocular surface on the test data. Moreover, after completing the OSA-VET examinations, a 15-min rest period was provided to each dog. This break helped mitigate reflex tearing or lipid expression from eyelid manipulation and allowed for complete tear turnover,[24] ensuring more accurate evaluations in subsequent basic ophthalmic examinations.

4.1 | Diagnostic test findings

In the diagnostic test findings for the overall population, it is noteworthy that the TBUTs measured in this study were shorter compared to previous studies on healthy dogs (≥20 s[22,25,26]). The observed discrepancy may be attributed to a variety of factors, such as environmental conditions, variations in the volume or concentration of fluorescein, and the potential unreliability of TBUT measurements. The dogs in this study resided in a city known for its sultry climate, especially in the summer months. This climate could potentially contribute to the shortened TBUT observed in the dogs. Upon reviewing the existing literature, the average TBUT values for healthy dogs were noted to be approximately 5.9–8.6 s,[22,27] which aligns with our findings.

In a recent pilot study on normal beagles,[10] notable differences in TMH, NIBUT, and LLT were observed compared to our study. For instance, the average TMH in our study was 0.31 ± 0.17 mm, versus 0.41 ± 0.21 mm in the beagle study.[10] This suggests potential variations in data among different dog breeds, which may be one of contributing factors.

In comparing examination values across groups, there was no significant difference in STT-1 values among the three groups ($p = .075$), aligning with a previous study.[22] However, the average STT-1 values for brachycephalic and mesocephalic dogs were higher than those for dolichocephalic dogs, despite the absence of statistical significance. A similar pattern was also observed in TMH, with brachycephalic dogs having significantly higher values than their mesocephalic and dolichocephalic counterparts ($p < .001$). This similarity is logical because an increase of TMH indicates that there are more tears in the lower conjunctival sac, potentially leading to higher STT values. This finding aligns with a study on aqueous tear secretion in cats of diverse cephalic conformations, which found STT-1 values to be generally higher in brachycephalic breeds in comparison to the non-brachycephalic Sphynx breed in cats.[21] This was supported by findings from a study by Rajaei and colleagues,[28] which showed significantly higher STT-1 values in Persian cats versus domestic shorthair cats. However, these findings contradict a previous study in dogs that suggested lower STT-1 values for brachycephalic dogs,[19] marking a unique discrepancy.

While the reason for this discrepancy remains unclear, potential explanations could include differences between dogs and cats, individual variations, specific breeds included in this study, or environmental factors.

Considering that other test parameters suggest brachycephalic dogs exhibit significantly lower results in TBUT, NIBUT, and LLT grading than non-brachycephalic dogs, this suggests a greater susceptibility to evaporative or qualitative KCS. Consequently, there might be a compensatory reflex increase in aqueous tear secretion, leading to a new equilibrium with elevated tear osmolarity and volume (see Figure 6).[29] This hypothesis aligns with findings from Faghihi et al.'s study,[22] which reported a compensatory increase in STT-1 values in dogs with reduced TBUT. In human medicine, similar observations were made by Mathers[30] and Shimazaki,[31] who reported increased tear production in patients with meibomian gland dysfunction (MGD) compared to those without MGD. Notably, Mathers et al.'s study revealed that patients with meibomian gland dropout and normal STT results did not show an increase in tear osmolarity, despite increased evaporation. A significant increase in tear osmolarity was observed only in patients with both MGD and quantitative KCS, compared to the control group. The authors proposed that the patients with only MGD might experience increased tear flow to compensate for their higher evaporative loss.[30] This mechanism could also explain why canine patients with solely qualitative KCS often exhibit excessive tear staining but less keratitis. Conversely, the frequent occurrence of severe KCS in breeds like French Bulldogs and Pugs may be attributed to the simultaneous presence of poor tear film quality and inadequate tear production.

In comparing TBUT results, the brachycephalic group exhibited significantly shorter TBUT compared to the mesocephalic and dolichocephalic groups. This finding aligns with results from previous studies.[22] Furthermore, some studies have suggested that TBUT in brachycephalic dogs can be shortened by about 10 s,[32] aligning with our statistical results.

In the investigation of LLT and MGLRL, our study revealed that brachycephalic group exhibited a significantly lower LLT compared to others and a higher MGLRL than the dolichocephalic group. The latter finding contrasts

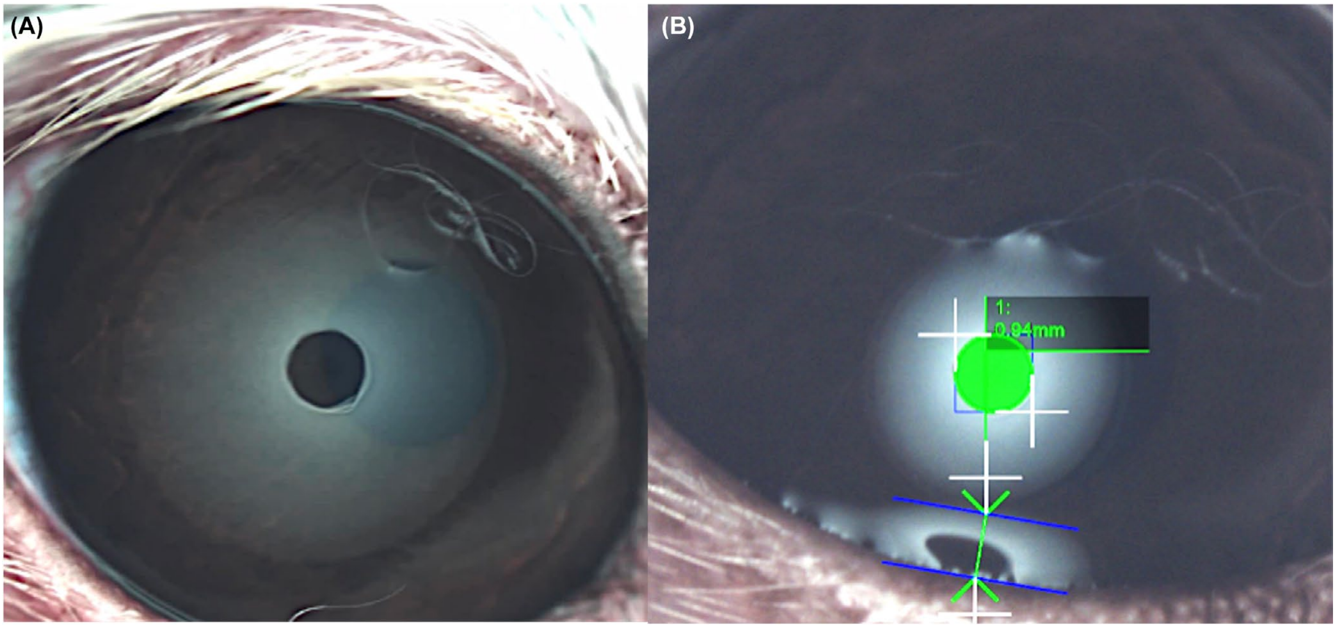


FIGURE 6 OSA-VET analysis of lipid layer thickness (LLT) and tear meniscus height (TMH) for the right eye in Case 64. This figure presents two distinct results from the OSA-VET examination of Case 64. (A) Displays an interferometry image, indicating a lipid layer thickness classified as Grade 1 (15–20 nm). (B) Demonstrates the TMH results with a measured value of 0.94 mm. This value markedly exceeds the established canine reference of 0.41 ± 0.21 mm[10]. The increased TMH may suggest a reflective compensatory increase in the aqueous tear component, underscoring the importance of an integrated perspective in evaluating these results.

with a previous study indicating no difference in MG loss between brachycephalic and non-brachycephalic dogs.[33] It is important to note that the categorization method in the previous study was not clearly detailed, suggesting that it might have relied on stereotypical characteristics rather than objective criteria, which could account for the differing results. The increased MGLRL observed in brachycephalic breeds in our study suggests that these dogs may be more susceptible to meibomian gland dysfunction, potentially due to their unique morphological traits. For instance, the compressed skull may lead to malposition of the eyelids (such as entropion or ectropion), which disrupts the normal function of the meibomian glands. Additionally, the protruding eyes commonly seen in these breeds may lead to increased exposure and evaporation of the tear film, causing the meibomian glands to work harder and potentially resulting in dysfunction. Furthermore, the abnormal skull structure may place mechanical stress on the eyelids and associated structures, leading to chronic irritation and inflammation of the meibomian glands. These factors may make brachycephalic breeds more susceptible to meibomian gland issues. Further research is needed to explore this susceptibility in more detail.

Statistical analysis of PFL and relative RPFL among brachycephalic, mesocephalic, and dolichocephalic dogs revealed interesting insights. While the absolute PFL did not show variance in these groups, RPFL varied significantly, with brachycephalic dogs showing longer RPFL values compared to the other groups, which aligned with previous research.[17] This underscores the importance in ocular surface discussions across cranial types in dogs, as mentioned in previous literature.[17]

4.2 | Inter-test correlations

Upon examining the correlation between numerical values, we initially discovered a significant and strong correlation between CFR and LLT, surprisingly more pronounced than the correlation between MGLRL and LLT. This indicates that the reduced LLT in brachycephalic dogs may not be exclusively due to variations in meibomian gland functionality.

Additionally, some brachycephalic dogs exhibited low LLT despite a low rate of meibomian gland loss (Figure 7), similar to findings in a study on rabbits using the OSA-VET.[34] The low blink rate in rabbits, averaging three to four times per hour, was proposed as a potential cause.[34] This parallels previous research indicating a lower blink rate[6,35,36] and increased incomplete blinking[6] in brachycephalic dogs, which may also contribute to lower LLT in these dogs.

Moreover, a comparative study[37] revealed that brachycephalic dogs have significantly smaller eye muscles, including the orbicularis oculi muscles and retractor anguli

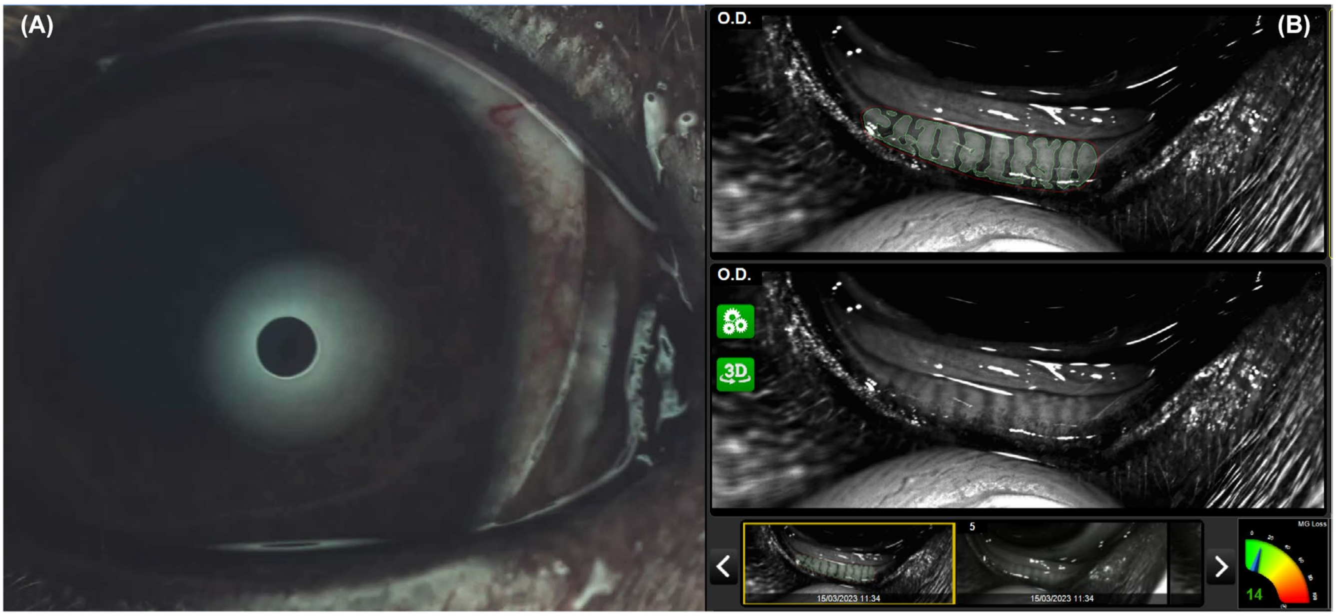


FIGURE 7 Analysis of lipid layer thickness (LLT) and non-contact infrared meibography (NCIM) using OSA-VET for a French Bulldog (Case 19). This figure elucidates two specific OSA-VET results for a French Bulldog (Case 19). (A) Presents an interferometry image where the lipid layer thickness is identified as Grade 1 (15–20 nm). (B) Displays the outcome from non-contact infrared meibography, highlighting a clear structure of the meibomian gland. The calculated meibomian gland loss rate is only 14%, indicating minimal meibomian gland dropout. The visible clarity of the meibomian gland, juxtaposed with the LLT grade, suggests that apart from the meibomian gland's structure and function, other variables may play a role in influencing the lipid layer thickness.

oculi muscles (both of which modulate meibomian gland secretion[13]), relative to their head length and cephalic index (CI) than dolichocephalic dogs. We hypothesize that the relatively smaller eye muscles could also reduce the strength of muscle contraction during blinking in brachycephalic dogs, thereby decreasing the compression and expression of meibum by a single blink.

Our study also found a moderate negative correlation between RPFL and LLT ($r = -.578$, $p < .001$) (see Table 2). This could be because a longer PFL in animals means a larger exposed eye surface area, requiring meibomian gland secretion to cover a greater area, possibly thinning the overall tear film lipid layer.

Consequently, the reduced LLT in brachycephalic dogs and the strong positive correlation between CFR and LLT may be related to several factors: meibomian gland dysfunction, lower blinking rates, proportionally smaller eye muscles, and longer RPFL in these dogs. Further research with a larger sample size is needed to confirm these findings and understand the mechanisms behind these differences.

With respect to the weak negative correlation observed between LLT and MGLRL, our results align with the findings of the study conducted by Viñas et al. in dogs,[33] as well as the aforementioned rabbit study.[34] The correlation between the two parameters was weaker than anticipated. According to Bron and Tiffany,[29] this could be because the quantity of lipid spread onto the tear film potentially remains somewhat independent of the reservoir volume until it decreases to a certain threshold, potentially due to obstructive meibomian gland disease. This implies that a reduction in reservoir size owing to meibomian gland disease, up to a certain limit, might not significantly impact the amount delivered to the tear film lipid layer, and consequently, the rate of evaporative loss.

Regarding other inter-test correlations, we expected and observed a strong correlation between TBUT and NIBUT. The main distinction between these two parameters lies in their evaluation methods: TBUT uses a fluorescent dye for subjective judgment, while NIBUT utilizes an instrument to project light for an objective image evaluation. Both techniques can undoubtedly be employed to evaluate tear film stability.

Another noteworthy finding was the absence of significant correlations between aqueous tear assessment measures (STT-1 and TMH) and tear film stability evaluations (TBUT and NIBUT). This aligns with previous studies in both dogs[22] and cats,[38] where no correlation was found between TBUT and STT, even in cats intentionally infected with feline herpesvirus-1 (FHV-1). This suggests that the aqueous tear secretion does not directly influence tear film stability. Besides tear volume, several other factors can influence tear film dynamics, including the secretion of mucin and lipids, as well as corneal exposure. The latter is associated with the RPFL and can lead to tear evaporation. Our results indicated a moderate negative correlation between the RPFL and both TBUT ($r = -.488$; $p < .001$) and NIBUT ($r = -.442$; $p < .001$), suggesting that a larger RPFL is associated with a shorter TBUT.

The correlations among other tests in this study ranged from weak to moderate, indicating the distinct importance of each test. For a comprehensive tear film assessment, multiple tests are needed instead of just relying on any single item. This recommendation is supported by recent research[39] on West Highland White Terriers with spontaneous aqueous deficiency dry eye. By implementing an array of diagnostic methods, such as the integrated and multifunctional OSA-VET used in this study, clinicians can obtain a more accurate assessment of tear film stability. Consequently, this facilitates the development of more precise treatment plans for patients with ocular surface disease.

Our study had certain limitations. The relatively small sample size (68 dogs; 130 eyes) was a primary limitation, partially due to stringent inclusion criteria such as no systemic disease history, no topical medication use, and the exclusion of brachycephalic dogs with keratitis or ocular ulceration. Despite this, the sample size was sufficient to yield statistically significant findings for our primary objectives.

Environmental control during examinations posed another limitation. We tried to maintain the indoor temperature at around 24°C using an air conditioning system, but we lacked the equipment to control ambient humidity. Consequently, we could not entirely eliminate the potential impact of humidity variations on ocular surface data. Studies have indicated that relative humidity can significantly affect the STT, albeit not to a clinically significant degree.[39] Although our analysis did not account for these factors, they may have influenced our results. Future studies should aim to conduct research under more rigorously controlled environmental conditions, incorporating equipment capable of precisely regulating humidity to enhance data accuracy and reliability.

Another limitation was our focus on comparisons among brachycephalic, mesocephalic, and dolichocephalic dogs, potentially overlooking individual variances within each group. Additionally, while we gathered dogs' medical histories and conducted basic physical examinations, the lack of bloodwork means that the potential impact of undiagnosed systemic disorders could not be entirely ruled out.

Moreover, the OSA-VET tool is unable to directly assess mucin levels in the tear film. Consequently, our study could not evaluate the correlation between mucin and other tear film components or parameters. Lastly, examination with OSA-VET is challenging with agitated or aggressive patients, and the use of sedatives, which could interfere with tear secretion, was not an option in this study. This may have led to biased subject selection based on availability and accessibility.

Future research on these aspects is recommended to gain a more comprehensive understanding of ocular surface homeostasis and the crucial role of qualitative tear deficiency in the ocular health of brachycephalic dogs.

5 | CONCLUSION

This study sheds new light on the intricate relationship between cranial morphology and tear film function in dogs, specifically highlighting the vulnerability of brachycephalic breeds to qualitative tear film deficiencies in addition to their well-documented prevalence of quantitative KCS. This is manifested by notably shorter TBUTs, reduced lipid layer thickness, and higher meibomian gland loss rates compared to mesocephalic and dolichocephalic counterparts. The observed correlations between cranial features (CFR and RPFL) and tear film stability parameters (TBUT, NIBUT, and LLT) reinforce these findings. Factors such as larger exposed ocular surface areas, incomplete blinking, and weaker ocular muscle function in brachycephalic breeds likely contribute to their tear film instability. The study also proves the versatility of OSA-VET, demonstrating its potential to enhance diagnostic capacity for ocular surface disorders, thereby allowing clinicians to make more accurate diagnoses and develop tailored treatment plans to ultimately improve canine ocular health.

AUTHOR CONTRIBUTIONS

Yan-Hui Li: Data curation; investigation; methodology; writing – original draft. **Bianca Martins:** Validation; visualization; writing – review and editing. **Chung-Tien Lin:** Conceptualization; resources; supervision; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors have declared that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in DOIs.

ETHICS STATEMENT

This study was approved by National Taiwan University Veterinary Hospital (Clinical Research No.

NTUVH111016) and the Institutional Animal Care and Use Committee of National Taiwan University (IACUC No. B202200098). Animal owners or owners' representatives provided written informed consent for enrollment in the study, procedures undertaken, and publication of data and images.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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