

BRIEF COMMUNICATION

Intra-Rater Variability and Inter-Rater Reliability in Measuring Tear Osmolarity in Dogs Using the ScoutPro System

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ABSTRACT

Objective: To evaluate the applicability of the ScoutPro (Trukera Medical, Southlake, Texas, USA) osmolarity system for tear film osmolarity measurement in healthy dogs.

Animals Studied: Seventeen dogs (34 eyes) of dolichocephalic and mesocephalic breeds were tested.

Procedures: Tear osmolarity was tested with the ScoutPro osmolarity system. Two examiners performed three subsequent measurements of each eye. Intra-rater variability and inter-rater reliability were calculated.

Results: The technique was straightforward; instrument handling was simple. Mean tear osmolarity measured by examiner one was 323.51 ± 9.89 mOsm/L and by examiner two 326.74 ± 9.02 mOsm/L. Mean difference between examiners was 3.2 ± 11.2 mOsm/L. Paired two-tailed *t*-test was $p = 0.1$. The intra-rater variability was acceptable with a Cronbach's Alpha of 0.75 for rater one and 0.80 for rater two. There were no significant differences in the three measurements of the individual examiners. There were no significant differences in the three measurements of the individual examiners (asymptotic significance rater one was 0.56, asymptotic significance rater two was 0.11).

Conclusions: Measurements with the ScoutPro osmolarity system in dogs are easy to perform. Variability and inter-rater reliability seem to be acceptable in the small group of dogs tested. Further studies regarding the reliability of the test results and the evaluation of the tear osmolarity in ocular surface disease are warranted.

1 | Introduction

The tear film fulfills many important functions in the eye, broadly summarized as protecting the ocular surface from environmental factors, supplying nutrients to the cornea, and acting as a medium for the refractive exchange of light [1]. The tear film homeostasis is therefore of great importance for the healthy eye [1]. A disruption of this homeostasis results in the development of dry eye disease [2], a multifactorial disease in which hyperosmolarity has been identified as the underlying pathophysiological core mechanism for its development in humans

[3]. Hyperosmolarity is caused either by increased evaporation (evaporative dry eye disease (EDED)) or by insufficient tear production (aqueous-deficient dry eye disease (ADDE)) [3]. The increase in osmolarity results in direct damage to goblet cells and epithelial cells of the ocular surface, as well as indirect damage to these cells through an inflammatory response involving, among other things, inflammatory cytokines, matrix metalloproteinases, and tumor necrosis factor alpha, which ultimately leads to cell loss [3]. These ongoing pathological mechanisms reinforce one another, thereby creating a “vicious cycle” with various entry points [3, 4].

While the measurement of osmolarity is common practice in human ophthalmology [5] and is the single best metric parameter for diagnosing dry eye disease [5–7], measurement of osmolarity in veterinary ophthalmology is not common. Osmolarity values below 308 mOsm/L are considered normal for humans, while the threshold of 316 mOsm/L has been proposed as a means of differentiating between a moderate and a severe form of dry eye disease [3].

In veterinary ophthalmology, the first description of the use of an osmometer for the measurement of animal tear fluid occurred in 2009. Korth et al. used the TearLab osmometer for the measurement of tear osmolarity in 70 healthy dogs [8]. Since then, several studies have been conducted (Table 1).

Lamkin et al. described a mean osmolarity of 318.55 ± 20.82 mOsm/L in 22 healthy dogs of various breeds [9]. Similarly, Brito et al. reported a mean osmolarity of 315.27 ± 6.15 mOsm/L in 15 healthy Pugs and 15 healthy Shih Tzus, and 353.02 ± 16.58 mOsm/L in 22 Pugs and 30 Shih Tzus with signs of keratoconjunctivitis sicca [15]. In a separate study on Pugs and Shih Tzus, with five dogs of each breed in each group, Brito et al. reported a mean osmolarity of 320.15 ± 5.55 mOsm/L for their control group and 352.00 ± 8.29 mOsm/L and 348.75 ± 5.10 mOsm/L for their treatment groups of dogs with keratoconjunctivitis sicca [16]. The measurements were taken before the start of treatment. Estanho et al. observed a mean osmolarity of 299.2 ± 13.9 mOsm/L in a control group of 11 healthy dogs. In their two treatment groups of 11 dogs each undergoing treatment for keratoconjunctivitis sicca, a modest increase in osmolarity was measured. The exact values of the osmolarity for the treatment groups and for the breeds included in the study have not been explicitly stated [10]. In another study, Choi et al. reported a mean osmolarity of 305.54 ± 18.67 mOsm/L in 14 healthy dogs [11]. Armor et al. noted a mean osmolarity of 317.5 ± 21.6 mOsm/L in 44 healthy dogs [12] and Ng et al. reported a mean osmolarity of 293.6 ± 14.1 mOsm/L in 32 non-brachycephalic, healthy dogs [13]. All measurements listed above were taken using the I-PEN Vet osmometer.

Another osmometer used in veterinary ophthalmology is the TearLab. The following TearLab data was published. Conceição et al. reported a mean osmolarity of 321.0 ± 9.7 mOsm/L in a control group of 40 eyes and 314.0 ± 22.0 mOsm/L in a group of 31 eyes of dogs with keratoconjunctivitis sicca [19]. Sebbag et al. reported a mean osmolarity of 337.4 ± 16.2 in 6 healthy Beagles and a mean of 306.2 ± 18.0 mOsm/L in 5 Beagles with keratoconjunctivitis sicca [20]. Mohoric et al. measured a mean osmolarity of 337.2 ± 7.3 mOsm/L in 20 healthy dogs [18]. Leonard et al. measured a median osmolarity of 339 mOsm/L in 13 healthy West Highland White Terriers and 346 mOsm/L in 3 West Highland White Terriers with keratoconjunctivitis sicca [21]. Korth et al. reported a median osmolarity of 355.50 mOsm/L in 76 healthy dogs [8] and 356 mOsm/L in 91 healthy dogs [17]. Williams et al. reported a mean osmolarity of 339.2 ± 22.7 mOsm/L in 153 eyes of dogs with no signs of keratoconjunctivitis sicca and a mean osmolarity of 350.5 ± 26.7 mOsm/L in 47 eyes of dogs with signs of keratoconjunctivitis sicca [22]. In a separate study, Delgado et al. reported a mean osmolarity of 320.8 ± 9.6 mOsm/L in a cohort of

healthy dogs and a mean osmolarity of 340.3 ± 21.6 mOsm/L in a cohort of atopic dogs with allergic conjunctivitis [23]. In a direct comparison of the two devices in the same patient, Kim et al. recorded a mean osmolarity of 340.42 ± 15.87 mOsm/L for the TearLab and 321.58 ± 17.39 mOsm/L for the I-PEN Vet [14]. The study was conducted on 52 healthy dogs of various breeds [14].

Mean osmolarity of the tear film in healthy canines was found to be within the normal range, with values of 337–339 mOsm/L as determined by the TearLab and 315–319 mOsm/L as measured by the I-PEN Vet [24].

The objective of this study was to investigate the use of the novel ScoutPro osmolarity system and its usability in the veterinary ophthalmological examination, taking into account intra-rater variability and inter-rater reliability.

2 | Materials and Methods

The dogs examined consisted of patients presented to the practice as part of a routine hereditary eye examination. The owners were informed in advance of the additional measurement of the osmolarity of the tear fluid and gave their written consent for this to be done. All examinations were carried out within the framework of the GERVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the approval of the Berlin State Office for Health and Social Affairs under the number StN 017/23.

Only non-brachycephalic dogs that did not have any underlying systemic disease, that had not been treated with any form of eye drops for at least 7 days prior to the examination, and were considered healthy by their owners were included in the study.

Animals were excluded from the study when the ophthalmic examination revealed ocular abnormalities that could have influenced the tear film. Brachycephalic breeds are predisposed to ADDE and EDED [25], these breeds were excluded.

2.1 | Osmometry

Tear film osmolarity was measured using the Trukera Medical ScoutPro (Trukera Medical, Southlake, Texas, USA) by both examiners, consisting of a board-certified ophthalmologist and a resident in training. The measurement procedure followed the manufacturer's instructions [26]. At the beginning of each examination day, a quality check of the device was carried out by using the electronic check card provided by the manufacturer.

The measurement was performed on the tear meniscus of the lateral canthus in the area of the lower eyelid via the single use osmolarity test cards collecting 50 nanolitres of tear fluid. (Figure 1) The procedure was similar to that previously described for the TearLab [20]. (Figure 1) The device measured the osmolarity of the collected sample through a temperature-corrected impedance measurement [26]. Each

TABLE 1 | Overview of studies regarding tear osmolarity in dogs. Included in this overview are the types of osmometers used, the health status of the patients, and the breeds that have been represented.

Device used	Mean, with standard deviation, or median osmolarity (mOsm/L)	Health status	Breeds	Study
I-PEN Vet	318.55 ± 20.82	Healthy	Mixed breed (<i>n</i> = 7), Labrador Retriever (<i>n</i> = 4), Boxer (<i>n</i> = 3), Borzoi (<i>n</i> = 2), Bluetick Coonhound (<i>n</i> = 1), Border Collie (<i>n</i> = 1), Chihuahua (<i>n</i> = 1), Cocker Spaniel (<i>n</i> = 1), French Bulldog (<i>n</i> = 1), Italian Sighthound (<i>n</i> = 1)	Lamkin et al. [9]
I-PEN Vet	299.2 ± 13.9	Healthy	Breeds not specified (<i>n</i> = 11)	Estanho et al. [10]
I-PEN Vet	305.54 ± 18.67	Healthy	English Springer Spaniel (<i>n</i> = 4), Labrador Retriever (<i>n</i> = 10)	Choi et al. [11]
I-PEN Vet	317.5 ± 21.6	Healthy	Breeds not specified (<i>n</i> = 44)	Armor et al. [12]
I-PEN Vet	293.6 ± 14.1	Healthy	Mixed breed (<i>n</i> = 16), Labrador Retriever (<i>n</i> = 6), Golden Retriever (<i>n</i> = 1), Weimaraner (<i>n</i> = 1), Coonhound (<i>n</i> = 1), Blue Heeler (<i>n</i> = 1), German Shepherd (<i>n</i> = 1), Great Dane (<i>n</i> = 1), Miniature Schnauzer (<i>n</i> = 1), Beagle (<i>n</i> = 1), Siberian Husky (<i>n</i> = 1), Portuguese Water Dog (<i>n</i> = 1)	Ng et al. [13]
I-PEN Vet	321.58 ± 17.39	Healthy	Shih Tzu (<i>n</i> = 6), Maltese (<i>n</i> = 9), Bichon Frisé (<i>n</i> = 4), Poodle (<i>n</i> = 17), Yorkshire Terrier (<i>n</i> = 2), Maltipoo (<i>n</i> = 2), mixed breed (<i>n</i> = 6), Italian Sighthound (<i>n</i> = 1), French Bulldog (<i>n</i> = 1), Shetland Sheepdog (<i>n</i> = 1), German Spitz (<i>n</i> = 2), Border Collie (<i>n</i> = 1)	Kim et al. [14]
I-PEN Vet	315.27 ± 6.15	Healthy	Pug (<i>n</i> = 15), Shih Tzu (<i>n</i> = 15)	Brito et al. [15]
I-PEN Vet	353.02 ± 16.58	Keratoconjunctivitis sicca	Pug (<i>n</i> = 22), Shih Tzu (<i>n</i> = 30)	
I-PEN Vet	320.15 ± 5.55	Healthy	Pug (<i>n</i> = 5), Shih Tzu (<i>n</i> = 5)	Brito et al. [16]
I-PEN Vet	352.00 ± 8.29	Keratoconjunctivitis sicca	Pug (<i>n</i> = 5), Shih Tzu (<i>n</i> = 5)	
I-PEN Vet	348.75 ± 5.10	Keratoconjunctivitis sicca	Pug (<i>n</i> = 5), Shih Tzu (<i>n</i> = 5)	
TearLab	340.42 ± 15.87	Healthy	Shih Tzu (<i>n</i> = 6), Maltese (<i>n</i> = 9), Bichon Frisé (<i>n</i> = 4), Poodle (<i>n</i> = 17), Yorkshire Terrier (<i>n</i> = 2), Maltipoo (<i>n</i> = 2), mixed breed (<i>n</i> = 6), Italian Sighthound (<i>n</i> = 1), French Bulldog (<i>n</i> = 1), Shetland Sheepdog (<i>n</i> = 1), Pomeranian (<i>n</i> = 2), Border Collie (<i>n</i> = 1)	Kim et al. [14]
TearLab	355.50	Healthy	Breeds not specified (<i>n</i> = 70)	Korth et al. [8]
TearLab	356	Healthy	Breeds not specified (<i>n</i> = 91)	Korth et al. [17]
TearLab	337.2 ± 7.3	Healthy	Breeds not specified (<i>n</i> = 20)	Mohoric et al. [18]

(Continues)

TABLE 1 | (Continued)

Device used	Mean, with standard deviation, or median osmolarity (mOsm/L)	Health status	Breeds	Study
TearLab	321.0 ± 9.7	Healthy	Breeds and number of dogs not specified (<i>n</i> = 40 eyes)	Conceição et al. [19]
TearLab	314.0 ± 22.0	Keratoconjunctivitis sicca	Breeds and number of dogs not specified (<i>n</i> = 31 eyes)	
TearLab	337.4 ± 16.2	Healthy	Beagle (<i>n</i> = 6)	Sebbag et al. [20]
TearLab	306.2 ± 18.0	Keratoconjunctivitis sicca	Beagle (<i>n</i> = 5)	
TearLab	339	Healthy	West Highland White Terrier (<i>n</i> = 13)	Leonard et al. [21]
TearLab	346	Keratoconjunctivitis sicca	West Highland White Terrier (<i>n</i> = 3)	
TearLab	339.2 ± 22.7	Healthy	Breeds and number of dogs not specified (<i>n</i> = 153 eyes)	Williams et al. [22]
TearLab	350.5 ± 26.7	Keratoconjunctivitis sicca	Breeds and number of dogs not specified (<i>n</i> = 47 eyes)	
TearLab	320.8 ± 9.6	Healthy	Breeds not specified (<i>n</i> = 20)	Delgado et al. [23]
TearLab	340.3 ± 21.6	Atopic dogs with allergic conjunctivitis	Breeds not specified (<i>n</i> = 20)	

examiner performed three consecutive measurements on the left eye followed by three consecutive measurements on the right eye with a pause in-between measurements of around 40 s to exchange the test card. The examiners then alternated. The ophthalmic examination consisted of the following procedures, depending on the requirement of each patient: slit-lamp biomicroscopy (Kowa SL-17; Kowa, Tokyo, Japan), gonioscopy (Koepe Small Diagnostic Lens; Ocular Instruments Inc., Bellevue, USA), indirect ophthalmoscopy (Video Omega 2C; HEINE Optotechnik GmbH & Co. KG, Gilching, Germany) and Schirmer tear test 1 (Tear Touch Blu, Madhu Instruments Pvt. Ltd., New Delhi, India). The ophthalmic examination was only performed after both examiners had completed their osmolarity measurements.

All examinations were performed in the same room at a room temperature ranging from 20°C to 25°C. Humidity was not determined.

2.2 | Statistical Analysis

The statistical analysis of the data was carried out by Novostat GmbH (Wollerau, Switzerland). Intra-rater variability was assessed using both the Friedman test and Cronbach's Alpha. The Friedman test was applied to detect any systematic differences across the three repeated measurements performed by the same rater. In parallel, Cronbach's Alpha was calculated to evaluate the internal consistency of these repeated measurements, indicating how closely they varied together. A high alpha suggests that the rater produced stable and consistent results across all measurements. The coefficient ranges from 0 to 1, with higher values indicating greater agreement. An alpha value of ≥ 0.70 is considered acceptable. For the inter-rater reliability, the average of all osmolarity measurements of rater one and rater two was compared using a paired-t test.

3 | Results

Seventeen dogs (34 eyes) of various dolichocephalic and mesocephalic breeds [Golden Retriever (*n* = 6), Australian Shepherd (*n* = 3), Labrador Retriever (*n* = 2), Dachshund (*n* = 2), Elo (*n* = 1), Siberian Husky (*n* = 1), Greater Swiss Mountain Dog (*n* = 1), mixed breed (*n* = 1)] with an age range of 0.5–10 years (mean 4 ± 3.04) were included.

The technique was straightforward and the instrument was easy to use and well tolerated by the patients.

Mean tear osmolarity measured by examiner one was 323.51 ± 9.89 mOsm/L and by examiner two 326.74 ± 9.02 mOsm/L. Mean difference between examiners was 3.2 ± 11.2 mOsm/L. The paired *t*-test showed no significant difference between the two means with a *p* = 0.1. There were no significant differences (*p* = 0.4) between the mean measurements between the left and the right eye. (Figure 2).

The intra-rater variability was acceptable with a Cronbach's Alpha of 0.75 for rater one and 0.80 for rater two. There were no significant differences in the three measurements of the



FIGURE 1 | Handling of the ScoutPro.

individual examiners (asymptotic significance rater one was 0.56, asymptotic significance rater two was 0.11) (Figure 3).

4 | Discussion

The current veterinary literature contains descriptions of two osmometer devices used in ophthalmic examinations. These include the TearLab and I-PEN Vet, which have been used

in a variety of studies [8–23]. Both devices measure tear film osmolarity through electronic impedance measurement. The TearLab uses a temperature-corrected impedance measurement, while the I-PEN Vet uses a non-temperature-corrected impedance measurement. The TearLab collects the measurement sample from the tear meniscus in the temporal area of the lower eyelid, while the I-PEN Vet measures by contact with the conjunctiva on the inner side of the lower eyelid. Consequently, a temperature correction is not necessary for the I-PEN Vet, as the assumed stable temperature of the palpebral conjunctiva negates the need for correction [27].

The ScoutPro device is similar to the sample collection unit of the TearLab. It has an integrated measurement system without the need for a separate measurement station and uses the same temperature-corrected impedance measurement method as the TearLab [26]. Unlike the TearLab, the test cards for the ScoutPro do not require storage in the test card tray for 25 min prior to use [26, 28], allowing for immediate osmolarity measurement during ophthalmic examinations throughout the day and eliminating the need for temperature equalization between the device and the test cards. This reduces potential sources of error due to incorrect handling.

In the review paper by Iwashita et al. the authors reported a range from 337 to 339 mOsm/L for the TearLab and 315–319 mOsm/L for the I-Pen Vet in healthy dogs [24]. In the present study, the mean tear osmolarity measured using the ScoutPro was 323.51 ± 9.89 mOsm/L and 326.74 ± 9.02 mOsm/L for examiners one and two, respectively. These values fall within the expected physiological range and lie between those previously reported for the I-PEN Vet and the TearLab [24] (Table 1). The mean tear osmolarity values measured by the ScoutPro system are methodologically similar to the TearLab [26, 28], yet numerically closer to those reported for the I-PEN Vet. This discrepancy may be indicative of subtle variations in sensor calibration, unique measurement characteristics of the device, or differences attributable to the heterogeneous patient populations studied across different studies. The

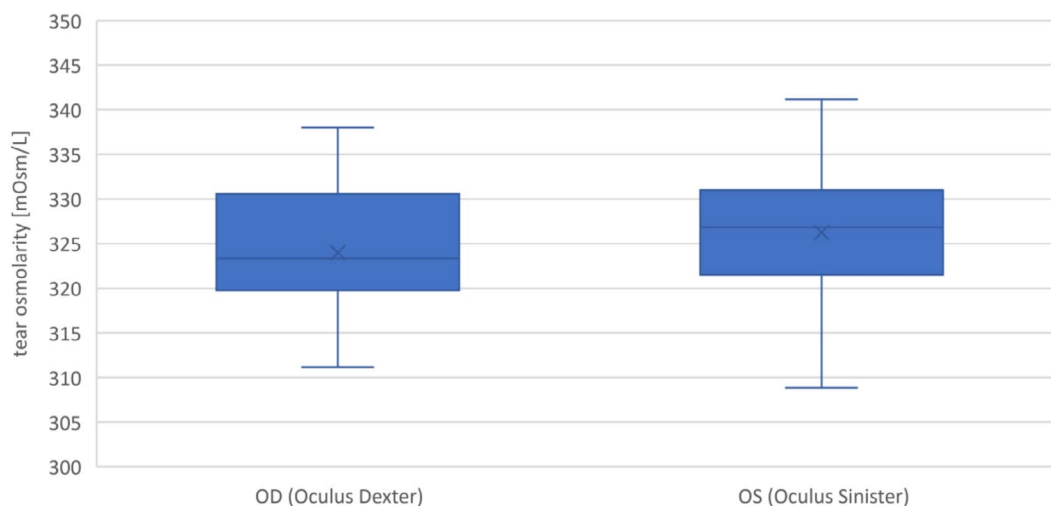


FIGURE 2 | Box and whisker plots of tear osmolarity (mOsm/L) for the right (OD) and left (OS) eye. Boxes represent the interquartile range, horizontal lines indicate the median, × symbols indicate the mean, and whiskers extend to the minimum and maximum values. Data include all measurements from both investigators. No significant difference was found between eyes (paired *t*-test, *p* = 0.398).

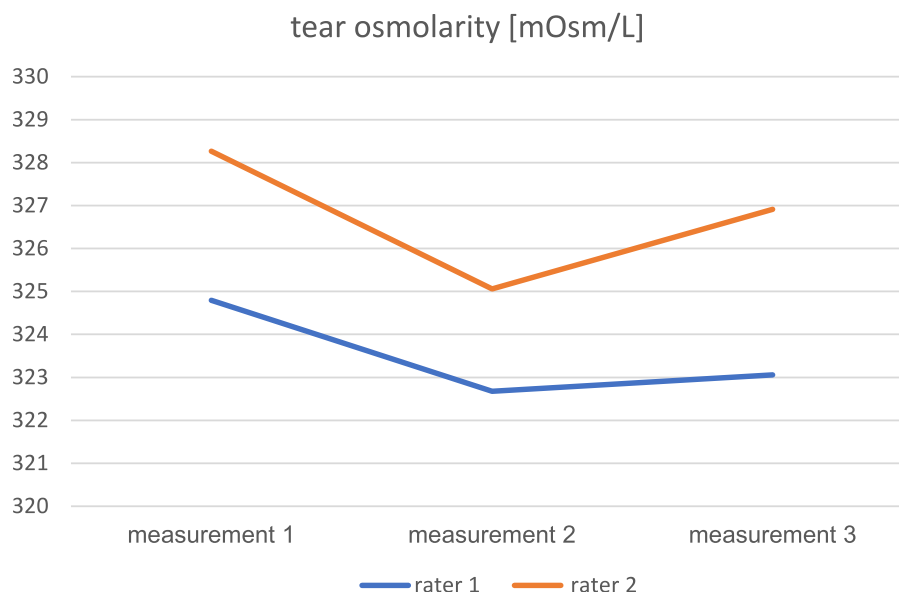


FIGURE 3 | Line graph showing the mean tear osmolarity (mOsm/L) across three measurements for rater 1 and rater 2. Differences in mean values between raters at each time point were assessed using the paired *t*-test and were not statistically significant at any time point.

intra-rater variability was acceptable, with Cronbach's Alpha values of 0.75 and 0.80 being attained by the two examiners. These values suggest moderate to good internal consistency, thus supporting the use of the ScoutPro in clinical settings. The Friedman test also confirmed that no significant differences occurred among repeated measurements from the same rater, indicating measurement stability.

The average results of the tear film osmolarity measurements vary greatly between the different devices [24] (Table 1) and potentially between different breeds. Brachycephalic breeds are predisposed to ADDE and EDED [25], thus the osmolarity measurements taken in healthy dogs of these breeds might not be comparable to normative values measured in dolichocephalic and mesocephalic breeds.

Comparison of I-PEN Vet and TearLab results shows that the I-PEN Vet almost always measures a lower osmolarity than the TearLab in healthy dogs [8–22]. With the exception of the studies conducted by Sebbag et al. [20] and Conceição et al. [19], all studies demonstrated an increase in osmolarity, though not always statistically significant, in dogs with keratoconjunctivitis sicca (ADDE) when compared to healthy dogs [15, 16, 21, 22]. From a physiological point of view, this is to be expected, as osmolarity should increase with a decrease in lacrimal fluid [20]. Sebbag et al. discussed their contrary results as being probably due to errors in the sampling process, leading to involuntary prolonged ocular contact and the introduction of air bubbles into the microchip [20]. The difference in osmolarity measured between healthy dogs and those affected by keratoconjunctivitis sicca in the study conducted by Conceição et al. was not statistically significant, and the contrary result was not discussed. However, it was noticeable that the procedure of osmolarity measurement performed poorly, especially in severe cases of keratoconjunctivitis sicca [19]. It can be assumed that the reasons given by Sebbag et al. [20] also apply to the results of Conceição et al. [19]. Comparable studies for the ScoutPro are lacking.

To the authors knowledge, this is the first study using the ScoutPro system for the measurement of canine tear osmolarity.

Limitation of this study includes the small number of dogs examined.

Further studies are needed to confirm osmolarity as an accepted part of dry eye disease assessment in dogs, taking into account not only the differences in the measurements using different osmometers, but also possible variations observed across breeds.

In conclusion, the results of this study demonstrate that the ScoutPro can be easily used to measure tear osmolarity in dogs and that it provides comparable readings in both repeated measurements and between different examiners.

Author Contributions

Joschka Spornberger: conceptualization, investigation, writing – original draft, writing – review and editing, methodology, validation, visualization, formal analysis, data curation. **Petr Soukup:** conceptualization, writing – original draft, writing – review and editing, methodology, visualization. **Andrea Meyer-Lindenberg:** writing – original draft, writing – review and editing, methodology, supervision. **Ingrid Allgoewer:** conceptualization, investigation, funding acquisition, writing – original draft, methodology, validation, writing – review and editing, formal analysis, data curation, supervision, resources, project administration.

Ethics Statement

This study was approved by the Berlin State Office for Health and Social Affairs under the number StN 017/23. Animal owners or owners' representatives provided written informed consent for enrollment in the study, procedure(s) and therapy undertaken, and publication of data and images.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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