Research Article

Comparison of Intraocular Pressure Measurements in 500 Calves Using Tono-Pen Vet® and Tonovet®

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Abstract | Intraocular pressure (IOP) measurement is one of the important parameters used to evaluate the development of ocular disorders. The aim of this study was to determine and compare the IOP readings in calves obtained with Tono-Pen Vet® and TonoVet® tonometry from a total of 500 calves of different breed, age, sex, degree of dehydration, eye-side and under different diurnal variation factors. Three to five minutes after two drops of 2% lidocaine ophthalmic anesthetic were applied to both eyes, a randomized tonometer protocol and eye order were used to measure the intraocular pressure in both eyes. Calculated mean IOP±SD with the Tono-Pen Vet® was 20.18±2.284 mmHg (13-27 mmHg) and 16.34±2.195 mmHg (11-23 mmHg) for TonoVet®. Readings from Tono-Pen Vet® were noticeably higher, with a mean difference of 3.84 mmHg ($P < 0.001$). A moderate connection between the devices was found using linear regression analysis ($y = 0.750x + 7.923$; $r^2 = 0.5792$), suggesting that the instruments are connected but not interchangeable. Although IOP did not show significant differences in breed, sex, and eye-side ($P > 0.05$), whereas it displayed significant differences related to age and diurnal variation ($P < 0.05$). It was determined that the eye pressure was higher during morning and afternoon measurements compared to evening. Even though there was a statistically significant difference based on hydration state, the small number of dehydrated calves necessitates careful interpretation. In conclusion, Tono-Pen Vet® consistently overestimates IOP in calves when compared to TonoVet®. Age and measurement time should be taken into considerations in clinical practice.

Keywords | Applanation tonometry, Calves, Intraocular pressure, Rebound tonometry

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INTRODUCTION

Circadian clock rhythms in mammals essentially regulate the temporal organization of biochemical and physiological functions of the body, including the function of the ocular (Dardente and Cermakian, 2007; van der Veen *et al*., 2008). The rhythm of the circadian clock is influenced by environmental factors (Pittendrigh, 1993). Zeitgeber literally as a "giver of time" refers to a whole set of external factors that synchronize the body to the circadian rhythm cycle. The daily light-dark cycle is the strongest zeitgeber in the circadian system (Devlin and Kay, 2001). Photic entrainment is thoroughly dependent on the entry of light through photoreceptors in the retina then forwarded to the suprachiasmatic nucleus (SCN) of the anterior hypothalamus of the brain (Dardente and

Cermakian, 2007; Saper *et al*., 2005). Animal's intraocular pressure (IOP) fluctuate throughout the day, and accurate measurement is crucial for identifying and monitoring eye conditions. These daily variations in intraocular pressure point to a possible connection between circadian rhythms and ocular physiology, suggesting that the time of day can affect the results of measurements. In order to create precise and reliable IOP measurements in animals, it is crucial to comprehend the mechanisms of photic entrainment and circadian control.

Measurement of intraocular pressure (IOP) with tonometry has become a common procedure in routine eye examinations. For instance, it is used to evaluate ocular health and disease in domestic animals. A high IOP level indicates the presence of glaucoma, whereas a low IOP is indicative of anterior uveitis (Del Sole *et al*., 2007). IOP is the balance that occurs between the amount of aqueous humor production and drainage of intraocular fluid (Miller, 2008).

In veterinary practice, calves are often evaluated. Ocular assessments are frequently required for determining between pathogenic alterations and normal physiological variation. Based on recent literature studies, IOP measurements have been evaluated with different tonometer devices, but there are not currently any studies comparing applanation and rebound tonometry on a large number of ±500 calves from different breed, age, sex, degree of dehydration, eye-side and diurnal variation factors.

The purpose of this study was to use Tono-Pen Vet® and TonoVet® to assess intraocular pressure in calves in a randomized manner and compare the results. Although the incidence of glaucoma in cattle is less than 1%, establishing a reliable IOP reference in calves remains clinically and scientifically important. Accurate IOP readings in healthy calves are essential for both establishing the accuracy and consistency of different tonometry devices and for the early detection of ocular disorders. Cross-species validation studies reveal systematic variations in tonometer measurements and offer a contemporary scientific framework for evaluating these instruments. To guarantee that clinical interpretations of IOP are accurate and consistent across studies and animal species, it is essential to comprehend such device-specific variances.

Various ocular diseases such as inflammatory or neoplastic conditions can lead to changes in the production of aqueous humor, which leads to an increase or decrease in IOP. The incidence of spontaneous glaucoma in cattle is rare, but the incidence of congenital, primary and secondary glaucoma has been reported in dairy cattle. Primary glaucoma has been characterized historically as an autosomal dominant condition in Friesian Holstein calves and this condition has also been found in both young and adult Jersey breeds (Mertel *et al*., 1996). Few recent studies have particularly re-evaluated this inheritance pattern in calves, despite the fact that cattle genetics has advanced significantly over the previous few decades. Calves are especially vulnerable to systemic variables that can influence ocular physiology and confuse IOP values, particularly dehydration and metabolic issues.

Considering age, hydration status, sex, and diurnal fluctuation affect calves' IOP, recording data on these factors improves in both therapeutic choices and more comprehensive comparative research. All treatment results may not always be positive due to the delayed of treatment in some ocular lesions. Therefore, it becomes more important to know the value of IOP and the factors affecting its measurement. For this reason, abnormalities detected in the early stages of the disease can be healed by intervention without the need for treatment. The purpose of this study was to determine and compare the values of IOP by using Tono-Pen Vet® and TonoVet® tonometry in calves of different breed, age, sex, degree of dehydration and eye side, and under diurnal variation conditions.

MATERIALS AND METHODS

Calves treatments

This study was carried out on a total of 500 calves of different age, sex, degree of dehydration, eye-side, diurnal variation and breed (64 Montofon, 233 Holstein, 189 Simmental and 14 Crossbreed). Calves that received any treatment after birth were not included in the study group. This was an experimental study with randomized measurement on the eye-side in calves after the administration of of 2% lidocaine ophthalmic anesthetic. This study was conducted at Aksüt and Dutpınar dairy farm from December 2021 to March 2022. Calves were kept in feeding fences fastened to halters to prevent jugular constriction, and all measurements were conducted with the same operator using the same instruments and consistent handling procedures. The tonometer was calibrated before to each session in compliance with the manufacturer's instructions to ensure comparable diurnal evaluation across sites.

Calves underwent general and ocular examinations to assess hydration status and assign ASA categories. Using common veterinary field criteria for calves, the clinical examination was used to determine the hydration status. Skin tenting, globe recession, mucous membrane moisture, and capillary refill time were used in clinical evaluations of hydration. Based on these criteria, calves were categorized as: (1) normal hydration, (2) mild dehydration, or (3) moderate dehydration. Although systemic status was indicated using ASA nomenclature, results should be interpreted with caution as classification was dependent on clinical judgment rather than a validated scoring system.

Routine eye examinations were performed on calves for pupillary light reflex, vision and threat response tests. To assess baseline tear production, the Schirmer Tear Test I (Tear Flo®, Rose Stone Enterprises, India) was conducted before the tonometer measurement. Animals producing less than 10 mm on the Schirmer tear test were excluded. Readings below this threshold may indicate decreased lacrimation in large animals. This exclusion criterion was not used to arbitrarily limit the study population, but rather to reduce the possibility of confounding effects of ocular surface abnormalities on intraocular pressure measurements.

Intraocular pressure measurement was performed randomly by using Tono-Pen Vet® (Reichert, Depew, New York, USA) or TonoVet® (Icare, Vantaa, Finland) 3-5 minutes after two drops of 2% lidocaine ophthalmic anesthetic instillation were applied to both eyes. Since topical lidocaine produces sufficient corneal anesthetic within this time range, the 3–5 minutes interval was chosen based on normal veterinary ophthalmology practice. To guarantee uniformity amongst animals, all measurements were taken throughout this predetermined time frame.

A set of randomly generated numbers was used to randomize each calf. The eye side measured first (left or right) and the order of tonometer use (Tono-Pen Vet® or TonoVet® first) were assigned at random for every calf. To reduce bias, the operator was not aware of the randomization scheme until the measurement time. This minimized possible order effects in intraocular pressure measurement and guaranteed unbiased allocation.

The axis of the probe of this device should be used in a position perpendicular to the surface of the cornea. The tonometer probe was replaced after each measurement. The mean IOPs from the right and left eye were obtained and recorded. All measurements were conducted in the morning, afternoon and evening. Calf enrollment, grouping, randomization, and measurement sequence can be seen in Figure 1, which summarizes the experimental design flowchart. 500 calves were enrolled and sorted according to their breed (Montofon, Holstein, Simmental, and crossbreed), age, sex, eye side, level of hydration, and time of day (morning, afternoon, and evening). The tonometer order (Tono-Pen Vet® or TonoVet®) and eye side (left or right) were randomly assigned. Three to five minutes before the IOP was measured, two drops of a 2% lidocaine ophthalmic anesthetic were administered. Pearson correlation, regression, ANOVA, and paired t-tests were used for data collecting and statistical analysis after measurements were conducted according to the randomized sequence.

Calves enrolled (n = 500)

↓

Grouping factors
(Breed, Age, Sex, Degree of Dehydration, Eye Side, Diurnal Variation)

↓

Randomization procedure
(Device order: Tono-Pen Vet® or TonoVet® first
Eye side: Left or Right first)

↓

Topical anesthetic
(2% lidocaine, 2 drops, 5 minutes before measurement)

↓

IOP measurement
(Assigned tonometer + randomized eye order)

↓

Data recording and analysis
(Pearson correlation, Regression, ANOVA, and Paired t-test)

Figure 1: Flowchart of experimental design.
A total of 500 calves were included in the study, comprising 64 Montofon, 233 Holstein, 189 Simmental, and 14 crossbred animals. Each calf was categorized according to breed, age, sex, hydration status, eye laterality (right or left), and time of measurement (morning, afternoon, or evening). Allocation procedures were conducted individually for each animal using a computer-generated randomization schedule. This process determined both the sequence of tonometer application (Tono-Pen Vet® or TonoVet® first) and which eye would be examined initially. The allocation sequence remained undisclosed to the operator until the time of assessment in order to reduce potential measurement bias. Three to five minutes prior to tonometry, two drops of 2% lidocaine ophthalmic anesthetic were applied topically. Intraocular pressure was subsequently measured according to the assigned device and eye order. The collected data were processed using paired t-tests, linear regression analysis, ANOVA, and Pearson correlation tests.

Statistical analysis

All statistical analysis in this study were performed using the SPSS program (IBM Company, Version 23, SPSS Inc. USA, 2015). The Shapiro-Wilk test and visual examination of Q-Q plots were used to assess the normality of continuous variables. Before inferential testing, the distribution of paired differences between Tono-Pen Vet® and TonoVet® measurements was evaluated. The distribution of differences was roughly symmetric, despite slight departures from complete normalcy. Boxplots and standardized residual evaluation were used for outlier analysis, and no extreme outliers (±3 SD) were found. The results were not significantly changed by sensitivity analysis that eliminated moderate outliers. According to the Central Limit Theorem, the test was deemed robust due to the large sample size (n= 500 calves). Statistical evaluation was performed using paired t-test and simple linear regression analysis was applied to determine the differences between the two tonometers. The degree of relationship between devices was also evaluated using Pearson correlation analysis. One-way analysis of variance (ANOVA) was used to examine intraocular pressure values by breed, age group, sex, eye side, diurnal variation, and dehydration level. Bonferroni correction was used to control the family-wise error rate in post-hoc pairwise comparisons for variables with more than two categories (breed, age group, dehydration status, and diurnal variation). Only single comparisons were made for the binary variables (sex and eye side), and multiple-comparison correction was not necessary. Statistical significance was set at $P < 0.05$.

Study period and location

This research was conducted at Dutpinar and Aksüt dairy farm during 2021-2022 in Malatya Province, Turkiye.

RESULTS

Intraocular was measured from four different breeds using both Tono-Pen Vet® and TonoVet® tonometers. Breed differences in IOP levels were not statistically significant ($P > 0.05$; Table 1). There were only 14 calves in the crossbreed subgroup, which reduced statistical power and made it necessary to interpret breed-related results with caution. Prior to ANOVA, homogeneity of variance was verified, and resampling techniques were avoided because of the tiny subgroup size, which could result in estimates that are unstable or deceptive. According to device selection, Tono-Pen Vet® readings were 3.84 mmHg higher than TonoVet® (P= 0.000). Rebound and applanation tonometry showed a moderate connection, according to linear regression analysis ($y = 0.750x + 7.923$, $r^2 = 0.5792$; Figure 2).

Using both devices, sex had no obvious effect on IOP readings ($P > 0.05$; Table 2). Calves were categorized as: (1) normal hydration, (2) mild dehydration, or (3) moderate dehydration. However, dehydration status was related to a substantial difference in IOP ($P < 0.05$; Table 3). Mild or moderately dehydrated calves presenting higher values than those in normal state, emphasizing how fluid balance affects eye pressure physiologically.

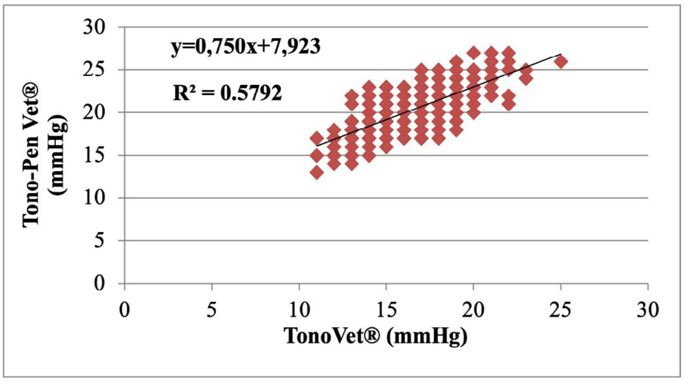

Figure 2: Regression line and distribution graph showing the relationship between Tono-Pen Vet® and TonoVet®. Each point corresponds to a pair of intraocular pressure readings obtained from the same calf using the two different tonometers. Linear regression analysis yielded the equation $y= 0.750x+7.923$, the coefficient of determination (r^2) was 0.5792, reflecting a moderate level of association between the devices. The dispersion of data points around the fitted regression line demonstrates measurement variability, which becomes more apparent at higher IOP values. This pattern indicates that although the measurements are correlated, the instruments cannot be used interchangeably. The dashed diagonal line illustrates the line of equality ($y = x$) and serves as a visual reference for perfect agreement.

IOP was similarly affected by age, with younger and older calves showing significant variations ($P < 0.05$), implying that intraocular dynamics may be influenced by developmental and maturational processes. Table 5 provides a summary of intraocular pressure readings by eye side. There were no statistically significant differences ($P > 0.05$) between the right and left eyes for both tonometers, supporting regular measurement with either eye. IOP measurements with the TonoVet® showed obvious diurnal variation, with statistically significant variations between morning, afternoon, and evening sessions ($P < 0.05$; Table 4). This pattern points to a physiological rhythm that is to be considered taken into account in both scientific and clinical contexts.

Table 1: Distribution of intraocular pressure values in calves according to breed.

Tonometer	Montofon (n=64)	Holstein (n=233)	Simmental (n=189)	Crossbreed (n=14)	Mean±SD (mmHg)	Range (mmHg)
TonoVet®	16.19±1.697	16.45±2.135	16.20±2.403	17.02±2.248	16.34±2.195*	11-23
Tono-Pen Vet®	19.89±1.824	20.30±2.331	20.05±2.357	21.25±2.146	20.18±2.284*	13-27

No significant variation in intraocular pressure was observed between breeds ($P > 0.05$). However, when the overall mean values generated by the two tonometers were compared, a statistically significant difference was identified ($P = 0.000$). The asterisk (*) denotes a significant difference between the mean IOP measurements obtained with TonoVet® and Tono-Pen Vet®. The relatively small crossbreed subgroup (n = 14) should be interpreted cautiously.

Table 2: Mean intraocular pressure values according to sex.

Tonometer	Female (n=246)	Male (n=254)	P
TonoVet®	16.37±2.207	16.31±2.188	> 0.05
Tono-Pen Vet®	20.22±2.225	20.14±2.343	> 0.05

No statistically significant differences were observed between males and females (P > 0.05).

Table 3: Mean intraocular pressure (IOP) values according to degree of dehydration.

Tono-meter	Degree of dehydration	Number of animals	Total eye	Mean IOP±SD (mmHg)	Range (mmHg)
Tono Vet®	1	493	986	16.38±2.174	11-23
	2	4	8	13.04±2.016	11-15
	3	3	6	13.83±1.453	12-15
Tono Pen Vet®	1	493	986	20.22±2.250	14-27
	2	4	8	16.13±2.401	13-19
	3	3	6	17.94±2.562	15-20

Statistically significant differences were observed among categories (P < 0.05).

Table 4: Table distribution of intraocular pressure values according to diurnal variation.

Tonometer	Morning	Noon	Evening
TonoVet®	18.01±3.165	17.06±3.398	13.94±2.521
Range (mmHg)	11-28	8-28	9-21
Tono-Pen Vet®	22.00±3.160	21.43±3.702	17.11±2.771
Range (mmHg)	14-31	12-31	8-26

Significant differences in intraocular pressure were detected across the morning, afternoon, and evening measurement periods (P < 0.05). The results indicated a clear diurnal pattern, with higher IOP values recorded during daytime hours compared to the evening.

Table 5: Table distribution of intraocular pressure values according to eye side.

Tonometer	Left Eye	Right Eye	P
TonoVet®	16.21±2.188	16.37±2.207	> 0.05
Tono-Pen Vet®	20.14±2.343	20.22±2.225	> 0.05

For both tonometers, there were no statistically significant differences between the right and left eyes.

DISCUSSION

Heredity, exercise, breed, posture changes, blood pressure, eye inflammation, age, medications, eye movements, sex, seasonal changes and diurnal variation are reported as the causes of intraocular pressure changes (Gum, 2007). In many studies, there have been reported that IOP is not a fixed parameter and may vary depending on different measurement times during the day (Garzon-Ariza *et al*., 2018). IOPs showed higher in the morning and noon compared to the evening, and they reported that glaucomatous beagles had slightly higher IOP values in the morning than in the evening. Some previous studies have also concluded that IOP is higher in the daytime of the day and lower in the evening (Garzon-Ariza *et al*., 2018).

In general, IOP values increase during the daytime (Miller, 2008). Many studies (Giannetto *et al*., 2009; Pereira *et al*., 2011) have reported that IOP is not a fixed parameter and may change depending on different measurement times during the day. Previous studies on various breeds have reported that IOP may show different results depending on the circadian cycle between breeds. In nocturnal species such as mice, cats, and rabbits, the IOP level increases at night, while in diurnal species such as monkeys and humans, peak IOP is reported during the day. Our results show higher IOP values in the morning and afternoon than in the evening, similar to (Gelatt *et al*., 1981), and slightly higher IOP values in the morning than in the evening in glaucomatous beagles. The results obtained in this study and some previous studies (Giannetto *et al*., 2009; Pereira *et al*., 2011) are similar. It was concluded that IOP was higher during daytime measurements (morning and afternoon) compared to the evening. Average IOP values vary depending on measurement time.

Species-specific validation is still emphasized in recent research (2020–2025) comparing rebound and applanation tonometry in large animals. Cross-species validation studies give tonometry research a modern methodological base. Reports of heterogeneity in intraocular pressure (IOP) readings across various species, such as cattle (Kovalcuka *et al*., 2024) and systematic measurement differences and agreement between rebound and indentation tonometers in dogs (Michaud and Lesne, 2024), have brought attention to the significance of device-specific reference ranges. These results highlight how important it is to interpret tonometer measurements in accordance with the equipment. These recent results underline the significance of device-specific reference values in veterinary ophthalmology and encourage cautious interpretation of inter-device agreement.

Cattle are diurnal creatures with circadian cycles controlled by endocrine and photoperiod. According to earlier research on cows, intraocular pressure might vary slightly during the day. All measurements in this investigation were taken during a standardized daytime period to reduce temporal variability and decrease circadian effect. Practical factors in calf handling must be taken into account, even though TonoVet® showed promising analytical qualities, such as non-contact measurement. Under farm situations, simplicity of usage was occasionally limited by positioning issues. Tono-Pen Vet®, however, can be used regardless of head position. Thus, both analytical performance and clinical practicality should be taken into consideration when choosing a device. Under regulated indoor circumstances, the ambient temperature during measurements varied between -4 to 15°C. To reduce the possible impact of temperature on ocular physiology and device calibration, environmental standardization was put into place.

In our study, IOP measurements performed in the morning, afternoon and evening. The mean IOPs with the TonoVet® were 18.01±3.165 mmHg, 17.06±3.398 mmHg and 13.94±2.521 mmHg, while the mean IOP using Tono-Pen Vet® were 22.00±3.160 mmHg, 21.43±3.702 mmHg and 17.11±2.771 mmHg, respectively. It was found that measurements differed significantly ($P < 0.05$).

Using both Tono-Pen Vet® and TonoVet® tonometers, intraocular measurements were taken from four breeds. Breed had no significant effect on IOP levels ($P > 0.05$). However, it should be noted that there were only 14 calves in the crossbreed sample, which would have limited the statistical power to detect any breed-related differences. Therefore, it should not be assumed that all breed groups are categorically equal just because there is no statistical significance.

Although there has been limited recent research specifically in cattle, age-related increases in intraocular pressure and central corneal thickness in Holstein have been reported (Kibar-Kurt and Gulendag, 2025). These results underline the significance of age stratification when establishing reference ranges. Recent studies have shown that the mean IOP values in adult calves have been reported as 26.9±6.7 mmHg with Tono-Pen XL (Gum *et al.*, 1998). The mean IOP by using Perkins applanation tonometer were 16.1±1.0 mmHg in the right eye and 16.5±1.2 mmHg in the left eye (Gerometta *et al.*, 2009). In another study with the Mackay-Marg applanation tonometer, the mean IOP was 20.0±5.5 mmHg in healthy calves (Passaglia *et al.*, 2004). Using the TonoVet® rebound tonometer, IOP was 15.2±5.2 mmHg (Tofflemire *et al.*, 2015). In the latest study, Tono-Pen Vet® tonometer was used on 24 healthy calves and the mean IOP was 16.59±2.59 mmHg (Passaglia *et al.*, 2004). IOP measurements using applanation tonometer in dogs and horses were 12.9±2.70 mmHg and 21.00±5.90 mmHg, respectively (Kumarasamy *et al.*, 2006).

In the current study, mean IOP recorded in calves using TonoVet® tonometer was 16.34±2.195 mmHg, while the mean IOP values recorded through Tono-Pen Vet® tonometer was 20.18±2.284. Thus, according to the results herein found the mean IOP values obtained by the TonoVet® were lower than those obtained by Tono-Pen Vet®. Many researchers state that even with the same working principle, the use of different methods or the use of different tonometric devices may be associated with differences in mean IOP values (Pigatto *et al.*, 2011).

TonoVet® (Rebound tonometer) provides methodological advantages in terms of speed, repeatability, and usage without topical anesthetic. However, its practical implementation in calves proved more challenging due to placement and probe stability (Verboven *et al.*, 2014). In this study, some difficulties were encountered in measuring IOP using TonoVet®, the measurement is adversely affected by the position of the animal and sudden movements of the probe. In this study we also used Tono-Pen Vet®, it is widely used in clinics for ophthalmological examinations, quite comfortable and convenient to use in large animals. There were no difficulties in measuring the intraocular pressure value with Tono-Pen Vet®. Disposable latex protector protects the tip of the Tono-Pen Vet® sensor by placing it where it comes into contact with the eye and prevents diseases that can be transmitted from one eye to the other (Ollivier, 2007; Verboven *et al.*, 2014). There is no need to keep the animal's head upright, only the probe of the instrument should be applied to the corneal surface at a right angle. However, the disadvantage of Tono-Pen Vet® is tonometer suddenly requires recalibration and takes several minutes. This may be relation with the very cold temperature at study research conducted during the peak of winter.

The primary finding of this research is that Tono-Pen Vet® continuously generated intraocular pressure (IOP) readings that were higher than TonoVet®, with a mean difference of 3.84 mmHg. Since clinical interpretation and reference ranges might differ based on the tonometer used, this systematic bias emphasizes how crucial equipment selection is in veterinary ophthalmology. Although measures are related, they are not interchangeable, as further evidenced by the moderate correlation ($r^2 = 0.5792$) between devices. While there have been noted differences between applanation and rebound tonometry, these results are consistent with earlier reports in cattle and other animals.

There are also published comparative large-animal tonometry investigations. There has been some evidence of moderate agreement between rebound and applanation tonometers in sheep (Okur *et al.*, 2024), and the authors came to the conclusion that the two instruments should not be used interchangeably. This result supports our suggestion for device-specific interpretation and is in good agreement with our discovery of a moderate correlation between Tono-Pen Vet® and TonoVet®. The two devices may be affected differently by differences in corneal biomechanics, such as corneal thickness, curvature, and hydration level. Variability may also have been caused by small head movement, sensitivity to probe alignment, and environmental factors such ambient temperature during winter measurements. As a result, even though the device showed a moderate correlation, they should not be used interchangeably in real life. The same tonometer should be used for serial monitoring, and reference ranges to the particular device should be used.

The present research found no significant relation between age and IOP (Pereira *et al*., 2011; Rusanen *et al*., 2010). On the other hand, other studies showed decreases in IOP with increasing age (Gelatt and MacKay, 1998). There are many studies examining the relationship between age and IOP in animals. Gelatt and MacKay reported that IOP decreased by approximately 2-4 mmHg between dogs younger than 2 years and those older than 6 years in healthy dogs grouped as young, mature and old (Gelatt and MacKay, 1998). Similarly, it has reported that age has an effect on IOP in clinical studies on cats and dogs, IOP decreased with age (Kroll *et al*., 2001). In this study, the age of the calves used in this study between 1-60 days, both TonoVet® and Tono-Pen Vet® were randomly compared by making measurements in 64 Montofon, 233 Holstein, 189 Simental and 14 Mixed breeds. There were significant difference between all breed ($P < 0.05$).

Different studies conducted in 1998 and 2006 reported that gender may have a significant effect on IOP in some species such as humans (Wu *et al*., 2006) and lions (Ofri *et al*., 1998). In a study conducted on humans in 2006, it was reported that the average IOP of men was higher than that of women (Wu *et al*., 2006). On the other hand, in a different study conducted in 1989, no relationship was observed between gender and IOP (Hoskins and Kass, 1989). Similar with previous experiments, it was reported that intraocular pressure was not affected by gender (Hoskins and Kass, 1989). In our study with TonoVet®, mean IOP was 16.31±2.188 mmHg in males and 16.37±2.207 mmHg in females; with Tono-Pen Vet®, corresponding values were 20.14±2.343 mmHg in males and 20.22±2.225 mmHg in females. It was determined that these values did not show a statistically significant difference ($P > 0.05$).

In many studies, it has been reported that there were no significant effects in IOP between the right or left eye (Garzon-Ariza *et al*., 2018; Wu *et al*., 2006). In addition, comparisons of IOP between the right and left eyes of each breed indicated no significant differences (Gum *et al*., 1998). In this study, the mean IOP values with the TonoVet® according to the eye side was 16.31±2.505 mmHg in the right eye and 16.49±2.395 mmHg in the left eye, respectively. Mean IOP in females was 16.37±2.411 mmHg in the right eye and 16.64±2.402 mmHg in the left eye. However, using Tono-Pen Vet® in males was 20.04±2.592 mmHg in the right eye and 20.43±2.604 mmHg in the left eye. The mean IOP in females was 20.15±2.386 mmHg in the right eye, while in the left eye was 20.38±2.458 mmHg. There were no statistically significant difference in these values ($P > 0.05$).

Blood pressure can affect episcleral venous pressure which playing a role in regulating aqueous flow from the trabecular network to the Schlemm channel (Carreon *et al*., 2017). In recent studies, it has been reported that low blood pressure is the cause of low IOP such as dehydration, hypovolemic shock and cardiogenic shock. In a study published in 2008 showed that dehydration problems caused low IOP values (Miller, 2008). In this study, according to ASA status, 493 calves were classified in the category of non-dehydration (1), 4 calves with mild dehydration due to diarrhea (2) and 3 calves with moderate dehydration due to diarrhea (3). The mean IOP with the TonoVet® according to the degree of dehydration were 16.38±2.174 mmHg, 13.04±2.016 mmHg and 13.83±1.453 mmHg whilst using Tono-Pen Vet®, IOP values were 16.13±2.401 mmHg, 17.94±2.562 mmHg and 20.22±2.250 mmHg, respectively. Statistically significant differences were found in the categories of degree of dehydration ($P < 0.05$). It can be determined that IOPs were lower in calves with dehydration than in calves without dehydration status.

Dehydration status and age showed significant associations with variations in intraocular pressure, highlighting the influence of systemic physiological conditions on ocular pressure regulation. Conversely, neither sex nor laterality had a measurable effect on IOP, which is in agreement with previous findings in cattle and other domestic species. A clear diurnal pattern was observed, with higher IOP values recorded during the morning and afternoon compared to the evening, consistent with circadian fluctuations documented across multiple species.

These observations underline the importance of accounting for physiological rhythms when establishing reference ranges or structuring experimental designs. Overall, the findings underscore two principal considerations: first, the choice of tonometer device substantially impacts IOP readings; and second, systemic and temporal factors, particularly hydration status and diurnal variation, should be carefully controlled in both clinical assessments and research protocols. Incorporating these factors into practice will enhance diagnostic precision and improve the consistency of inter-study comparisons.

CONCLUSION

To the best of authors knowledge, the current study focused, for the first time, on randomized measurement of IOPs through TonoVet® and Tono-Pen Vet® in 500 calves. Although incidence of glaucoma is rarely observed in ruminants, tonometry remains an important component of the ophthalmic examination. Tono-Pen Vet® and TonoVet® are hand-held devices that can be easily carried and used in applications.

In conclusion, the findings of this study indicate that intraocular pressure measurements obtained with the Tono-Pen Vet® are consistently higher than those recorded with the TonoVet® in calves. While the use of TonoVet® in field situations may present certain practical difficulties, the values generated by this device align more closely with previously published reference ranges and appear to offer greater clinical dependability. Practitioners should therefore interpret Tono-Pen Vet® readings with caution, as they may systematically overestimate IOP and potentially influence the clinical assessment of ocular hypertension or glaucoma. Ultimately, selection of a tonometer should consider both ease of use and measurement accuracy. In calves, TonoVet® seems to provide results that more accurately represent actual intraocular pressure. Based our study observed moderate correlation and systematic differences between devices, authors advise against employing baseline correction factors without device-specific validation. Rather, it is recommended that veterinarians utilize the same tonometer for serial monitoring and interpret values using reference ranges particular to the device.

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NOVELTY STATEMENT

This study is the first large-scale randomized comparison of Tono-Pen Vet® and TonoVet® in 500 calves, establishing device-specific reference values while assessing breed, age, sex, dehydration status, eye side, and diurnal variation in the field.

AUTHORS' CONTRIBUTION

Conceptualization, methodology and investigation: AP and MGS. Writing-original draft: AP. Writing-review and editing, english proof read: IPN. Supervision: MGS. All authors have read and agreed to the published version of the manuscript.

Generative AI and AI-assisted technology statement

Generative AI and AI-assisted technologies were utilized for grammar and style checking, as well as to refine author written paragraphs for improved clarity. The authors thoroughly reviewed, verified, and edited all AI-generated output to ensure the accuracy and integrity of the scientific content.

Conflict of interest

The authors have declared no conflict of interest.

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