












The Agreement of Intraocular Pressure Measurement in Healthy Merinos Sheep Using Rebound Tonometer (Tonovet®) and Applanation Tonometer (Tono-Pen Vet™)

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ABSTRACT

The assessment of intraocular pressure (IOP) holds significant importance in ophthalmology as a crucial diagnostic tool for various ocular disorders. This study aimed to evaluate the agreement between rebound (TonoVet®, TV) and applanation (Tono-Pen Vet™, TPV) tonometers in measuring IOP in healthy Merino sheep. 155 healthy Merinos (80 males, 75 females) with a mean weight of 54.4±8.7 kg, aged 24±6 months, were included in the study. IOP was measured between 9:00 and 11:00 am using both the rebound and applanation tonometers. The rebound tonometer was used first, followed by the applanation tonometer. A total of 620 readings (310 readings for each tonometer) were obtained from the two devices. No statistical differences were noted between the mean IOP measurements of the right and left eyes for both tonometers ($p>0.05$). However, there was a significant difference in the mean IOP measurements between the TV (11.8±2.3 mmHg) and the TPV (13.9±2.9 mmHg) tonometers ($p<0.001$). The concordance correlation coefficient indicated weak agreement strength ($\rho_c=0.319$, CI 95% = -0.169 to 0.455) between the TV and TPV. The mean difference in bias and the 95% limits of agreement for the differences between TV and TPV were -2.1 mmHg (-9.0 to 3.5 mmHg). The regression equation derived from a Bland-Altman plot, describing the relationship between the two tonometers, was $Y = 1.43 - 0.33X$ ($Y = TV$ and $X = TPV$). In conclusion, the TPV measured higher IOP values compared to the TV, and due to the significant bias and limits of agreement, the two tonometers should not be used interchangeably for IOP measurement in Merino sheep.

Keywords: Agreement, Intraocular pressure, Ophthalmology, Sheep, Tonometer.

öz

Sağlıklı Merinos Koyunlarında Rebound Tonometre (Tonovet®) ve Applanasyon Tonometresi (Tono-Pen Vet™) Kullanılarak Yapılan Göz İçi Basıncı Ölçümlerinin Uyumu

Göz içi basıncının (GİB) değerlendirilmesi, çeşitli oküler hastalıklar için önemli bir teşhis aracı olarak oftalmolojide büyük önem taşımaktadır. Bu çalışmanın amacı, sağlıklı Merinos koyunlarında GİB ölçümünde rebound (TonoVet®, TV) ve applanasyon (Tono-Pen Vet™, TPV) tonometreleri arasındaki uyumu değerlendirmektir. Ortalama 54.4±8.7 kg ağırlığında, 24±6 aylık, 155 sağlıklı Merinos (80 erkek, 75 dişi) koyunu çalışmaya dahil edilmiştir. GİB sabah 9:00 ile 11:00 arasında hem rebound hem de applanasyon tonometreleri kullanılarak ölçülmüştür. IOP ölçümleri için önce rebound, ardından applanasyon tonometresi kullanıldı. İki cihazdan toplam 620 okuma (göz başına 310 okuma) elde edilmiştir. Her iki tonometre için sağ ve sol gözlerin ortalama GİB ölçümleri arasında istatistiksel bir fark kaydedilmemiştir ($p>0.05$). Ancak, TV (11.8±2.3 mmHg) ve TPV (13.9±2.9 mmHg) tonometreleri arasında ortalama GİB ölçümleri açısından anlamlı bir fark belirlendi ($p<0.001$). Çalışma verileri, korelasyon katsayısının TV ve TPV arasında zayıf uyum gücü ($\rho_c=0.319$, CI %95 = -0.169 ila 0.455) olduğunu göstermiştir. TV ve TPV arasındaki farklar için ortalama yanlılık farkı ve %95 uyum sınırları -2.1 mmHg (-9.0 ila 3.5 mmHg) olarak belirlendi. İki tonometre arasındaki ilişkiyi tanımlayan Bland-Altman grafiğinden elde edilen regresyon denklemi $Y = 1.43 - 0.33X$ ($Y = TV$ ve $X = TPV$) olarak tanımlandı. Sonuç olarak, TPV, TV'ye kıyasla daha yüksek GİB değerleri ölçmüştür ve önemli sapma ve uyum sınırları nedeniyle, iki tonometre Merinos koyunlarında GİB ölçümü için birbirinin yerine kullanılmamalıdır.

Anahtar Kelimeler: İntraoküler basınç, Koyun, Oftalmoloji, Tonometre, Uyum.

INTRODUCTION

Ciliary activity is responsible for the production of aqueous humor, which occurs through active secretion, as

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well as passive processes, such as plasma diffusion and ultrafiltration. The balance between the production and outflow of aqueous humor is a determinant of intraocular pressure (IOP). Measurement of IOP is an essential component of ophthalmic examinations, serving as an important indicator of ocular diseases (Gum et al. 2007; Broadwater et al. 2008). The study of ocular function in sheep has considerable relevance within comparative ophthalmology research. Sheep serve as a suitable model for investigating the mechanisms underlying glaucoma, specifically corticosteroid-induced glaucoma (Gerometta et al. 2009; Gerometta et al. 2010). Additionally, the size and volume of the sheep's eye are comparable to that of humans, for instance, with an anterior-posterior axis of approximately 27 mm and an equatorial axis of approximately 30 mm (Gerometta et al. 2010). Although manometry is the only method to achieve correct IOP measurements, it is invasive and unsuitable for clinical examination. For this reason, tonometry, which is an indirect non-invasive measuring method, is commonly used as a device for measuring IOP obtained using indentation, applanation, or rebound methods (Bertens et al. 2021). The most widely used commercial tonometers in veterinary ophthalmology are the TonoVet® (rebound, TV) and Tono-Pen Vet™ (applanation, TPV) tonometer models (Lewin and Miller 2017). The TV tonometer included three different modes as follows; 'h' for horses, 'd' for dogs and cats, and 'p' for other species (Pereira et al. 2011). The TPV tonometer measures the force necessary to flatten a constant area of the central corneal surface, which indirectly measures IOP (Ahn et al. 2012), while the TV tonometer measures IOP depending on the deceleration of a moving magnetized probe as it comes into contact with the cornea. The magnet action in the probe induces a voltage in the solenoid; simultaneously, the moving parameters of the object are monitored as a digital signal (Chihara 2008). Previous studies compared the IOP measurement of TV and TPV in cats (von Spiessen et al. 2015), dogs (Kulualp et al. 2018), farm animals (Peche and Eule 2018), rabbits (Gloe et al. 2019), and porcine (Lewin and Miller 2017). However, to the author's knowledge, there have been no reported studies comparing the IOP measurement obtained by TV and TPV in Merinos sheep. Although spontaneous glaucoma is uncommon in sheep, they were used as a model of glaucoma (Gerometta et al. 2009). Therefore, it is important to assess the agreement of the tonometers for IOP measurement in Merinos sheep and to develop strategies for reducing potential biases in clinical and research assessments. The aim of the current study was to use the Bland-Altman limits of agreement technique to assess and compare the IOP measurement TV with TPV in healthy Merinos sheep.

MATERIAL AND METHODS

The protocol (2022/15) was approved by Atatürk University Local Board of Ethics Committee for Animal Experiments. The experimental study was carried out under the guidelines of the Association for Research in Vision and Ophthalmology Statement for the use of animal research in ophthalmic and vision research.

Animals

This study enrolled one hundred fifty-five (80 males and 75 females) healthy Merinos sheep, which were randomly assigned from the Food and Livestock Application and Research Center of Atatürk University. All of sheep in the study were investigated concerning non-pregnancy and were housed in the same pen under the same

environmental, nutritional, and management conditions. Sheep were considered healthy based on physical examinations, complete blood counts, and ophthalmic examinations, including direct and indirect ophthalmoscopy (Aesculap AC635 C, Braun, Tuttlingen, Germany), rebound tonometry (Tonovet, Icare, Vantaa, Finland), Schirmer's tear test, and fluorescein test.

Study Design

The right and left eyes selected for the current study were randomly determined by a randomizer (Excel, Microsoft cooperation, Redmond, WA, USA) and the same eye measured first in all subsequent reading with both tonometers. The IOP measurement of each eye in sheep was first used with the rebound tonometer (TonoVet® [TV], using the "d" setting), which was programmed to achieve six successive rebounds and demonstrate the mean value of IOP. The TV tonometer was retained in a vertical position and the distance between the tip of probe and the central cornea was kept close to 4-8 mm as possible for each sheep. After the TV measurements, two drops of the local anesthetic agent 0.5% proparacaine hydrochloride (Alcaine®; Alcon-Couvreur, Puurs, Belgium) were administered to each eye. The applanation tonometer (Topo-Pen Vet™ [TPV], Reichert Technologies, Depew, New York, USA) was used to measure IOP in each eye following two min local anesthetic agent instilled. The probe tip of TPV was touched gently with the central cornea to obtain IOP measurement. All measurements IOP were recorded between 09:00 and 11:00 a.m. to minimize diurnal variations. Both tonometers were performed by the same operator to minimize individual variations. During the study, all measurements were employed with the animals in a sternal position and their heads and necks were gently restrained upright ahead to avoid potential incorrect readings in IOP measurement. The IOP measurements in both tonometers were repeated an error sign was encountered due to excessive deviation. If a third attempt to measure the animals' IOP on either tonometer were unsuccessful, then the animal was excluded from the study. Only readings with a maximum 5% deviation were collected with both tonometers. Three sequential readings (The average IOP measurement obtained after six rebounds for TV and the IOP measurement obtained by corneal touching for TVP were counted as one reading) were recorded in each eye using both tonometers. Tonometers were calibrated as instructed in the manufacturer recommendation and a new probe for TV and an ocu-film tip cover for TPV were replaced prior to use of each animal.

Statistical Analyses

A power calculation (PS-Power and Sample Size Calculation, Version 3.1.2, Vanderbilt University, TN, USA), conducted based on information reported by previous study (Peche and Eule, 2018), determined that a total of 310 readings for each tonometer (Type I error [α] of 5%, Type II [Power, β] of 95%) to detect a 20% difference (Standard deviation of ± 3 mmHg) in the IOP between tonometers.

All data were analyzed using the Medcalc version 20.015 (Medcalc Software, Ostend, Belgium). The normality distribution of the data was evaluated by a Kolmogorov-Smirnov test. A paired Student's t-test was performed to detect differences between the IOP measurement of the right and left eyes and the mean difference of IOP measurement in TV and TPV. The result of the data was presented as mean \pm standard deviation. The concordance correlation coefficient was tested for the IOP measurement

of the tonometers, to evaluate relationship power. Agreement between TV and TPV was assessed using the Bland-Altman plot, in which the differences between tonometers were plotted against their mean IOP measurements and the limits of agreement (mean ± 1.96 × SD) (Bland and Altman 1986). The level of statistical significance was set at $p < 0.05$.

RESULTS

The mean weight of the sheep was 54.4±8.7 kg and they were aged 24±6 months. All the measurements of IOP with both tonometers were completed successfully. No signs of ocular discomfort or pain were noted throughout the study. A total of 310 readings for each tonometer were recorded. All data were stated in units based on mmHg. There were no statistical differences between the IOP measurements of right and left eyes in both tonometers ($p > 0.05$, Table 1). Hence, the IOP measurements were recorded bilaterally on each sheep and their average measurement were used.

Table 1: The mean intraocular pressure (IOP, mmHg) measurements in the right and left eyes of sheep were measured using the rebound tonometer, TonoVet® (TV), and the applanation tonometer, Topo-Pen Vet™ (TPV).

Device	Eye	n	Mean± Standard Deviation (IOP, mmHg)	95% CI for the mean	p
TV	Right	155	11.1±2.45	10.7 to 11.5	0.460
	Left	155	11.2±2.86	10.8 to 11.7	
TPV	Right	155	13.9±3.51	13.4 to 14.5	0.742
	Left	155	13.8±3.31	13.3 to 14.4	

Data were expressed as mean ± standard deviation. CI; confidence interval

A significant difference was observed between TV (11.8±2.3 mmHg) and TPV (13.9±2.9 mmHg) tonometers in the mean IOP measurements ($p < 0.001$, Fig. 1). The lowest (7 mmHg) IOP was measured by TV, while the highest (24 mmHg) was measured by TPV in sheep. The concordance correlation coefficient determined that poor strength of agreement was observed between tonometers. ($\rho_c = 0.319$, CI 95% = -0.169 to 0.455, Fig. 2).

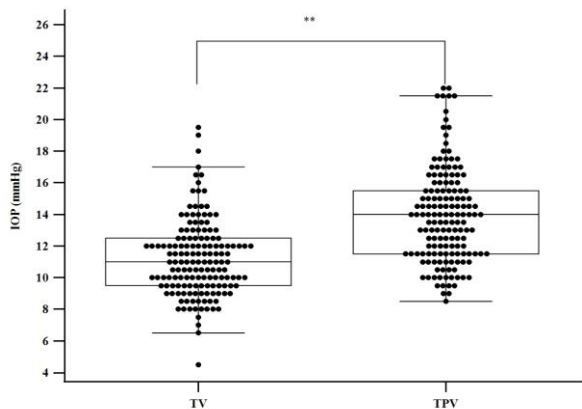


Figure 1: The minimum, mean, and maximum IOP measurements were ascertained differences between the rebound tonometer, TonoVet® (TV), and the applanation tonometer, Topo-Pen Vet™ (TPV). ** indicated significantly different between tonometer ($p < 0.001$).

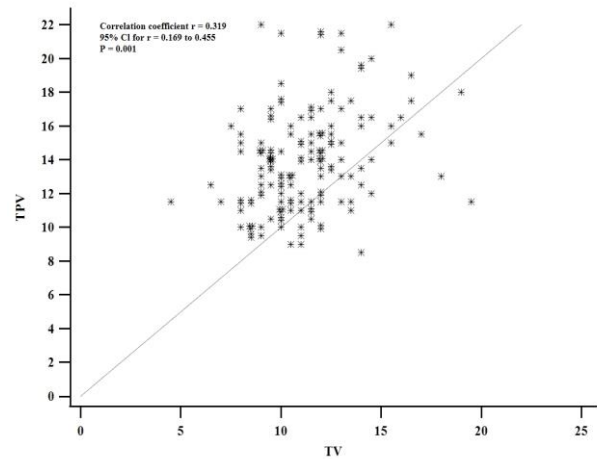


Figure 2: Correlation analysis of the rebound tonometer, TonoVet® (TV), and the applanation tonometer, Topo-Pen Vet™ (TPV). CI; confidence interval.

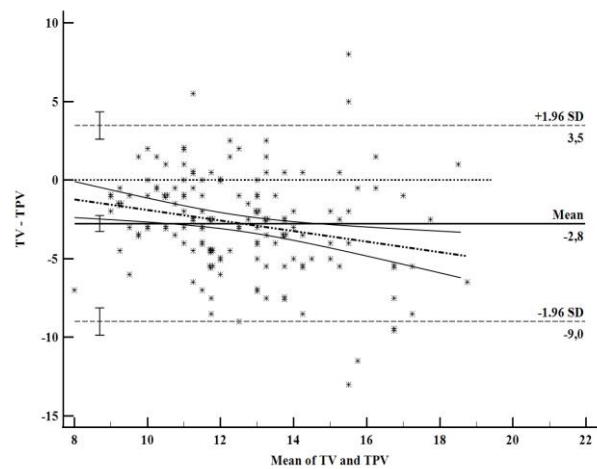


Figure 3: Bland-Altman limits of agreement plots comparing the rebound tonometer, TonoVet® (TV), and the applanation tonometer, Topo-Pen Vet™ (TPV). The solid black line is the mean difference in intraocular pressure (IOP, mmHg) with its 95% confidence interval (CI) marked by the dotted black lines. The dotted horizontal line account for zero difference. The dashed-dot sloping black line depict the slope of the regression line for the linear relationship between IOP difference and mean of IOP with its 95% CI.

Bland-Altman analysis for IOP measurement in the TV and TPV was depicted in Fig. 3. We found a significant difference in bias between the TV and TPV tonometers ($p < 0.001$). Over the range of IOP measurement reported in the current study, the model expected that the mean IOP measure in the TPV was higher TV with the mean bias and 95% CI for tonometer difference demonstrated in Fig. 3. According to the Bland-Altman plots, the differences in IOP (TV-TPV) are not near to zero, indicating that the studied methods do lack agreement. The mean difference and 95% limits of agreement for the differences between TV and TPV were -2.8 mmHg (-9.0 – 3.5 mmHg). The analysis of the regression relationship between the IOP difference and the mean IOP measurement, suggested that as the mean IOP increased, hence the IOP in the TPV increased relative to TV (Table 2). The models estimate that the IOP measured in TPV was mostly higher than TV, but this difference decreased as IOP increased.

Table 2: Bland-Altman analysis comparing intraocular pressure (IOP, mmHg) measured using the rebound tonometer, TonoVet® (TV), and the applanation tonometer, Topo-Pen Vet™ (TPV) and results of regressing the IOP difference TV (Y) and TPA (X) to quantify changes in bias.

Parameter estimate	Difference TPV (X) compared to TV (Y) TV - TPV
Mean Difference (95% CI)	-2.76 (-3.26 to -2.25)
Limit of agreement	-9.0 to 3.5
SE _{y-x}	0.114
Lower / Upper Limit (95% CI)	-8.9 (-9.8 to -8.1) / 3.5 (2.6 to 4.3)
Regression equation	Y=1.43-0.33X
Slope coefficient (95% CI)	-0.33 (-0.56 to -0.10)
p*	<0.001

*Significance test for difference in slope from zero. CI; confidence interval. SE_{y-x}; standard error of estimate.

DISCUSSION AND CONCLUSION

The main focus of this study was to compare TV with TPV tonometer in healthy Merinos sheep. Although the result of the current study indicated that both tonometers provided consistent results of IOP measurement individually, our findings suggested that both tonometers cannot be used interchangeably for IOP measurement in Merinos sheep due to the high bias and limit of agreement, and the concordance correlation coefficient indicated that the TV and TPV showed weak strength of agreement. As previous studies have reported that the position of the head and body has a substantial effect on the IOP (Broadwater et al. 2008; Ghaffari and Gherekhloo 2018), all readings of the IOP were performed in a sternal position with the head and neck gently restrained in a normal and upright position for each sheep. In a previous study carried out sheep reported that TV provided more valuable readings on IOP with calibrated 'd' setting (Peche and Eule 2018). Thus, all measurement IOP in TV was used 'd' setting in the current study due to lack of specific setting for sheep. In addition, no topical anesthetic drug was used for the IOP measurement of TV without discomfort or corneal pain, in agreement with previous studies on other species (Leiva et al. 2006; Yanmaz et al. 2016; Lewin and Miller 2017; Bertens et al. 2021). The IOP measurement of TV was obtained prior to TPV, as a previous study indicated that rebound tonometry might be altered by topical anesthesia (Baudouin and Gstaad 1994). For both TV and TPV tonometers, the first measurement attempt failed because of blinking or error signs in five readings and a second attempt was performed to obtain readings. However, a repeated measurement may cause underestimation of IOP (Morris et al. 2006), and the first measurement attempt was noted to avoid or reduce this false reading of IOP. A previous study reported that differences in distance between the probe tip of TV and the cornea surface could be affected the IOP reading (Rodrigues et al 2021). For this reason, during the IOP measurement of TV was maintained the same distance between the probe tip and cornea surface by the same operator. In this study, the IOP measurement of TPV tended to be higher than TV (mean difference [95% CI] = 2.8 mmHg). A similar result was previously reported in rabbits (Pereira et al. 2011), and dogs (Leiva et al. 2006) comparing rebound and applanation tonometer. This may

be due to the instillation of topical anesthesia for IOP measurement of TPV. Although the effect of topical anesthetic on IOP is unknown in sheep, a previous study demonstrated that proparacaine hydrochloride caused a slight increase in IOP immediately after administration of the drug in dogs (Sarchahi and Eskandari 2019). Our results were contradictory with the research of Peche and Eule (2018), who found that the IOP measurement of applanation tonometer (Tono-Pen AVIA®) tended to be lower than TV. Although the TV measurements in both studies were consistent with each other (in our study; 11.8 ± 2.3 mmHg, Peche and Eule (2018); 12.2±3.1 mmHg), the applanation tonometers in both studies were inconsistent with each other (in our study; 13.9±2.9 mmHg, Peche and Eule (2018); 10.1±2.5 mmHg). This inconsistency could be due to the type of applanation tonometer that previously reported significant variability between IOP measured using different tonometers (Guresh et al. 2021). Additionally, Peche and Eule (2018) administered a different topical anesthetic drug (oxybuprocaine hydrochloride) compared with our study (proparacaine hydrochloride) prior to the measured IOP of the applanation tonometer. A previous study has reported that the different topical anesthetics have an effect on IOP measurement (Sarchahi and Eskandari 2019). Moreover, a study carried out in humans reported that oxybuprocaine significant decreases IOP (Almubrad and Ogbuehi 2007). Although spontaneous glaucoma is uncommon in sheep, experimental animal researches are still an essential part of developing new treatment procedures in glaucoma studies. Many different tonometers are presently utilized in animal experiments, the accuracy of which has previously been established in many species (Bertens et al. 2021; Lewin and Miller 2017; Kulualp et al. 2018). In this study, both tonometers were easily used for IOP measurement and well-tolerated in sheep. The TV tonometer was more rapid reading IOP compared with the TPV tonometer due to did not require administration of the topical anesthetic prior to IOP measurement. The disadvantage of the TV tonometer required to be held in a vertical position to the cornea with the tip of the probe parallel to the cornea surface, while the TPV can be utilized regardless of device position. In the present study, the Bland-Altman bias plot demonstrated that the level of agreement between IOP measurements using two tonometers was high in sheep. Additionally, we found that the correlation coefficient between TV and TPV was poor agreement strength. This incompatibility between the two tonometers can be explained in the same order of tonometers (first TV, second TPV) for IOP measurements in each sheep. During the study, the first IOP measurement was performed with TV by handling the animal which might have been caused by elevated mental stress levels in the sheep. Therefore, IOP measurements performed secondly to TPV may tend to be higher than TV in sheep. In addition, physical stress due to immobilization increased IOP, as reported in a study conducted with rabbits (Miyazaki et al. 2000). Another possible explanation is that both tonometers have different working mechanisms (rebound vs applanation). The main limitation of this study was not evaluating manometry, which is the most accurate method of IOP measurement, due to instrumental limitations. The IOP measurement could be influenced by central corneal thickness (CCT), as reported in previous studies (Yanmaz et al. 2016; Martin-suarez et al. 2014). Thus, the lack of CCT measurement can be considered as a limitation. A further limitation is that the repeatability and inter-operator variability were not evaluated in both tonometers. Another limitation of the study is that topical

anesthetic were used during TPV measurements, while no topical anesthetic was used during TV measurements. This may be a factor affecting the agreement between each other. In conclusion, given the data obtained in the current study, it is suggested by the authors that although both tonometers were easy to employ and consistent results individually, they cannot be used interchangeably for IOP measurement in healthy sheep due to the high bias and poor agreement strength according to the correlation coefficient.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: SO, LEY, AG

Supervision / Consultancy: SO, MGS, ÖTO, YK, FT

Data collection: SO, AG, UE, EM

Writing the Article: SO, AG

Critical Review: LEY, MGS, AG, FT

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