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## Evaluation of Intraocular Pressure (IOP) Regarding Circadian Rhythm, Age, Sex and Eye Side in Awassi Sheep

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**SUMMARY.** Measurement of intraocular pressure (IOP) in domestic animals has become a part of routine eye examination with advent of applanation tonometer. Delayed control of high IOP may lead to permanent blindness due to retinal ganglion cells dysfunction and optic nerve degeneration. This study aimed at evaluating IOP of Awassi sheep with respect to circadian rhythm, age, sex and eye sides and finally to establish a reference (baseline, normal) IOP value for this particular species. A total of 24 healthy sheep with different ages and sexes were used. The animals were divided into 2 equal groups, <1 (6 male, 6 female, n = 12) and ≥1 (6 male, 6 female, n = 12) years old. IOP measurements were performed twice, in the morning (6:00 a.m.) and in the evening (8:00 p.m.) with Tono-pen Vet<sup>®</sup> applanation tonometer.

Mean IOP in the animals decreased from 16.21 mmHg in the morning to 12.65 mmHg in the evening with an approximately rate of 22% ( $P < 0.0001$ ). Comparison of mean IOP values of right eyes (n=12) to the left (n=12), male (n=48) to female (n=48), and ages < 1 (n=48) to ≥ 1 (n=48) showed no difference ( $P > 0.05$ ). The reference IOP for this animal was calculated as  $14.43 \pm 2.72$  mmHg notwithstanding any variable.

It was concluded that in this breed IOP values can vary significantly as far as circadian rhythm is concerned and Tono-pen Vet<sup>®</sup> can be used for sheep IOP measurement as an alternative to other applanation tonometry.

**Keywords:** Age, Awassi Sheep, Circadian Rhythm, IOP, Tono-pen Vet<sup>®</sup>

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## INTRODUCTION

Intraocular pressure (IOP) represents the balance between aqueous humor production and drainage (Park et al., 2011; Pigatto et al., 2011). Routine IOP measurement is very important for the early diagnosis and effective treatment of glaucoma and other ocular diseases associated with ocular hypertension, such as uveitis, local or generalized corneal edema, orbital trauma and lens luxation (Andrade et al., 2012; Park et al., 2011; Rusanen et al., 2010). Delayed control of high IOP may lead to permanent blindness due to retinal ganglion cells dysfunction and optic nerve degeneration (Andrade et al., 2012).

IOP values may vary according to animal breed (Barsotti et al., 2013; Ghaffari et al., 2012; Pereira et al., 2011; Pigatto et al., 2011), age (Pereira et al., 2011; Verboven et al., 2014) and sex (Ofri et al., 1998), the measurement techniques applied (Pereira et al., 2011; Pigatto et al., 2011), the examiner's practice (Pereira et al., 2011; Pigatto et al., 2011), circadian rhythms (Giannetto et al., 2009; Pereira et al., 2011; Pigatto et al., 2011), stress (Pigatto et al., 2011) and anesthetic applications (Pigatto et al., 2011).

IOP measurement is performed via two basic techniques, manometry and tonometry (Andrade et al., 2012). Manometry is an invasive technique that requires anterior camera cannulation/ paracentesis (Von Spiessen et al., 2015) and general anesthesia and thus it is not practical for clinical use (Park et al., 2011; Von Spiessen et al., 2015). Tonometry, a noninvasive and indirect measurement of IOP, works on indentation, applanation or rebound principle and today it is a method of choice for clinical practice (Jeong et al., 2007; Park et al., 2011; Von Spiessen et al., 2015).

In recent years, several noninvasive applanation tonometers such as Tonopen-XL<sup>®</sup>, Tonopen Avia<sup>®</sup> and Tono-pen Vet<sup>®</sup> have managed to find a place in veterinary clinical practice due to be portable, easy and practical to use and less influenced by the sizes and postures of animals (Andrade et al., 2012).

Ophthalmic diseases in farm animals are important because they may cause significant economic losses. Similarly, ocular studies on these animals have a research value in comparative ophthalmology (Ribeiro et al., 2014). Among farm animals the sheep has become a popular animal model in a range of diseases including steroid-induced hypertension; therefore, it is

important to know the mean intraocular pressure (Gerometta et al., 2010; Pigatto et al., 2011).

Awassi breed, a fat-tailed and combined trait sheep, is well adapted to tropical environments and widespread through the Mediterranean region and the Arab peninsula (Al-Atiyat and Aljumaah, 2014). This breed is found mainly in Eastern Mediterranean and South-east Anatolia of Turkey and constitutes 4-5% of its total sheep population (Internet).

According to our literature research no study has so far tried to measure IOP in Awassi sheep. The aim of this study was to measure this parameter with applanation tonometry, to evaluate that with respect to circadian rhythm, age, sex and eye side and finally to establish a reference (baseline, normal) IOP value for this particular species.

## MATERIALS AND METHODS

The study was carried out at Agriculture and Livestock Research Farm of Firat University after official approval from the university ethic committee. Following thorough ophthalmologic examination including direct and indirect ophthalmoscopy and slit-lamp biomicroscopy (XL-1<sup>®</sup>, Shin-Nippon, Japan), and assessments of the pupillary light reflex, Schirmer tear test (Tear Flo<sup>®</sup>, Rose Stone Enterprises, India) and fluorescein staining, a total of 24 healthy Awassi sheep aging from 6 months to 4 years were selected as the study material.

The animals were divided into 2 equal groups, <1 (6 male, 6 female, n = 12) and ≥1 (6 male, 6 female, n = 12) years old. IOP measurements were performed twice, in the morning (6:00 a.m.) and in the evening (8:00 p.m.) with an applanation tonometer (Tono-pen Vet<sup>®</sup>, Reichert, U.S.A.).



**Figure 1.** IOP measurement with Tono-Pen Vet<sup>®</sup>.

**Table 1.** Distribution of IOP data of Awassi sheep according to the various variables: measurement time points, age, sex and eye sides.

Measurement Time Points (Circadian Rhythm)	Age	Sex	Eye Sides	IOP (mmHg)
<b>Morning (08.00 a.m.)</b>	<1 years	Male	Right	17.17 ± 1.94
			Left	16.33 ± 2.58
			Mean	16.75 ± 2.22
	<1 years	Female	Right	16.67 ± 1.75
			Left	16.33 ± 3.61
			Mean	16.50 ± 2.71
	MALE AND FEMALE MEAN			16.63 ± 2.42
	≥1 years	Male	Right	14.50 ± 1.37
			Left	15.50 ± 1.51
			Mean	15.00 ± 1.47
≥1 years	Female	Right	16.33 ± 1.86	
		Left	16.83 ± 1.16	
		Mean	16.58 ± 1.50	
MALE AND FEMALE MEAN			15.79 ± 1.66	
TOTAL MEAN			16.21 ± 2.10	
<b>Evening (08.00 p.m.)</b>	<1 years	Male	Right	11.83 ± 0.75
			Left	11.67 ± 0.51
			Mean	11.75 ± 0.62
	<1 years	Female	Right	11.83 ± 0.75
			Left	11.17 ± 1.16
			Mean	11.50 ± 1.00
	MALE AND FEMALE MEAN			11.63 ± 0.82
	≥1 years	Male	Right	12.83 ± 1.72
			Left	13.33 ± 2.33
			Mean	13.08 ± 1.97
≥1 years	Female	Right	14.17 ± 3.06	
		Left	14.33 ± 2.33	
		Mean	14.25 ± 2.59	
MALE AND FEMALE MEAN			13.67 ± 2.33	
TOTAL MEAN		12.65 ± 2.01		
ALL TOTAL MEAN			14.43 ± 2.72	
SEM				0.73
ANOVA*				---P---
			Time	0.0001
			Age	0.135
			Sex	0.164
			Eye	0.959
			Time x Age	0.001
			Age x Sex	0.046

\*There is no significant interactions between others at  $P > 0.05$

All ocular examination and measurements were carried out by the same investigator (KK). Great attention was paid to avoid the animals from unnecessary stress, abnormal pressure on the head and neck, disproportional physical constraints and abnormal body posture during the measurement. Prior to the measure-

ment the tonometer was calibrated and the data were recorded using the ear tag numbers.

Thirty seconds after instilling topical anesthesia the eyelids were gently opened while the animals held in sitting position head right in midline (Figure 1). Three consecutive IOP measurements were obtained from

each side of the eyes and their average was recorded. The latex Tono-pen Vet® tip coating was changed from animal to animal and the measurements were repeated if the average rate of device measurement error was higher than 5%. All the measurements performed in the morning, were repeated in the evening.

Data are given as mean  $\pm$  standard error of the mean (SEM). The data were analyzed by general linear models (GLM) procedure of SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Sample size was calculated based on a power of 85% and a  $P$  value of 0.05.

## RESULTS

In the morning measurements, mean IOP data in male and female animals <1 years old were found to be  $16.75 \pm 2.22$  and  $16.50 \pm 2.71$  mmHg ( $P > 0.05$ ), those of the right and left eyes to be  $16.92 \pm 1.78$  and  $16.33 \pm 2.99$  mmHg ( $P > 0.05$ ), respectively. In animals  $\geq 1$  years old IOP data were  $15.00 \pm 1.47$  in males and  $16.58 \pm 1.50$  mmHg in females. Mean right and left eye data were  $15.42 \pm 1.83$  and  $16.17 \pm 1.46$ , respectively ( $P > 0.05$ ). At this point, cumulative means of IOP data were  $16.63 \pm 2.42$  mmHg in animals <1 years old and  $13.67 \pm 2.33$  in animals  $\geq 1$  years old and that was  $16.21 \pm 2.10$  mmHg regardless of age variability (Table 1).

For evening measurements, in male and female animals <1 year old mean IOP data were recorded as  $11.75 \pm 0.62$  and  $11.50 \pm 1.00$  mmHg and mean IOP data of their right and left eyes as  $11.83 \pm 0.71$  and  $11.42 \pm 0.90$  mmHg ( $P > 0.05$ ), respectively. These data were determined to be  $13.08 \pm 1.97$  in males and  $14.25 \pm 2.59$  mmHg in females;  $13.50 \pm 2.46$  in the right eyes and  $13.83 \pm 2.29$  mmHg ( $P > 0.05$ ) in the left eyes of the animals  $\geq 1$  year old. At this time point regardless of sex and eye variability, overall mean IOP data were  $11.63 \pm 0.82$  mmHg in animals <1 and  $11.63 \pm 0.82$  mmHg in animals  $\geq 1$  years old and also overall mean of  $12.65 \pm 2.01$  mmHg was recorded when sex, age and age variability were neglected (Table 1).

According to these parameters, mean IOP ratio in morning measurements ( $16.21 \pm 2.10$  mmHg) was about 22% higher than that ( $12.65 \pm 2.01$  mmHg) of the evening and the difference between these time points was found to be statistically significant ( $P < 0.0001$ ). The reference IOP for this animal was calculated as

$14.43 \pm 2.72$  mmHg notwithstanding any variable. An interaction ( $P > 0.05$ ) was found between IOP data of the variables such as age, sex, eye sides and measurement time point. This was determined to be between age x measurement time points ( $P < 0.001$ ) and age x sex ( $P < 0.05$ ) according to the statistical test (Table 1).

## DISCUSSION

The aim of this study was to determine reference IOP mean for Awassi sheep, an indigenous breed in Southeast Anatolia and Mediterranean with an applanation tonometer and to reveal an interaction of this value with the variables such as age, sex, circadian rhythm and eye side. In recent years, many studies have been performed on reference IOP values of various animal species in a variety types of applanation tonometers, i.e. of eurasian eagle owls with TonoPen XL® (Jeong et al., 2007), dogs and cats with TonoPen XL® and Perkins® (Andrade et al., 2012) and TonoPen-XL® (Park et al., 2011), rabbits with TonoPen Avia® (Pereira et al., 2011), calves and dairy cows with Mackay-Marg® and TonoPen-XL® (Gum et al., 1998), long-eared hedgehogs with Tono-Pen Vet® (Ghaffari et al., 2012), eurasian tawny and little bred owls, common buzzards, european kestrels with TonoPen-XL® (Barsotti et al., 2013), ferrets (Montiani-Ferreira et al., 2006) and koala (Grundon et al., 2011) with TonoPen-Vet®, Kapacin monkey with TonoPen-XL® (Montiani-Ferreira et al., 2008), horses with Tono-Pen Avia (Marzok et al., 2014). Some studies have measured IOP values in Texel and Santa Ines bred sheep with Tono-Pen XL® (Pigatto et al., 2011; Ribeiro et al., 2014), Sanjabi bred male sheep with Tono-Pen Vet® (Ghaffari et al., 2011) and Corriedal bred sheep by Gerometta *et al.* (2009) utilizing Perkins® tonometer. In the present study, we measured IOP in Awassi bred sheep using Tono-Pen Vet® with a mean value of  $14.43 \pm 2.72$  mmHg (Table 1). This value is higher than that ( $9.37 \pm 2.45$  mmHg) of Sanjabi sheep reported by Ghaffari *et al.* (2011), and that ( $10.6 \pm 1.4$  mmHg) of Corriedal bred sheep by Gerometta *et al.* (2009) and is near to that ( $14.56 \pm 1.14$  mmHg) of Santa Ines bred sheep by Riberio *et al.* (2014), however, it is lower than that ( $16.36 \pm 2.19$  mmHg) of Texel bred sheep reported by Pigatto *et al.* (2011). Despite the usage of the same type tonometer, these studies results show great variations, which

indicates that IOP value may vary according to different breeds.

In the present study mean IOP values of measured as  $14.42 \pm 2.61$  mmHg in the right eyes and  $14.44 \pm 2.85$  mmHg in the left eyes with a resultant of no significant difference between them ( $P > 0.05$ ) were similar to those reported previously (Ghaffari et al., 2012; Ghaffari et al., 2011; Gum et al., 1998; İşler et al., 2014; Pigatto et al., 2011). It has been reported that IOP values can be influenced markedly by stress factors including abnormal pressure on the head and neck, disproportional physical constraints and abnormal body posture during the measurement (Broadwater et al., 2007; Komaromy et al., 2006; Rusanen et al., 2010). In this study an ultimate care was paid to these remarks during the measurement. To avoid individual diversity (Gelatt and MacKay, 1998; Pigatto et al., 2011), all measurement was performed by the same investigator.

The effects of sex on IOP is arguable, while many researchers (Ghaffari et al., 2012; Grundon et al., 2011; İşler et al., 2014; Montiani-Ferreira et al., 2006; Montiani-Ferreira et al., 2008; Nuhsbaum et al., 2000; Pereira et al., 2011) deny the presence of such an effect on IOP, Ofri et al. (1998) study on lions and Wu et al. (2006) study on human have reported higher IOP value in males as compared to females. The present study results in males ( $14.15 \pm 2.51$  mmHg) and females ( $14.71 \pm 2.91$  mmHg) shows no statistical difference ( $P > 0.05$ ) in consistency with the majority opinion. The studies of Pamuk et al. (2011) in Anatolian Buffalo using TonoPen XL<sup>®</sup> and Gelatt and Mackay (1998) in dogs applying MacKay-Marg<sup>®</sup> and TonoPen XL<sup>®</sup> reported that average IOP decreased with age contrary to that of Montiani-Ferreira et al. (2006) in mountain ferrets and Montiani-Ferreira et al. (2008) in capuchin monkeys. Present study measured IOP values of Awassi sheep  $\geq 1$  ( $14.73 \pm 2.27$  mmHg) and  $< 1$  ( $14.13 \pm 3.09$  mmHg) year old with a resultant of no significant difference ( $P > 0.05$ ) between these two age groups were similar to the findings of the last two studies.

IOP is not a constant value and may vary according

to different times of a day, termed circadian rhythm (Gelatt et al., 1981; Giannetto et al., 2009; Jaen-Diaz et al., 2007; Pereira et al., 2011). In veterinary ophthalmology there is a limited number of studies investigating the relation of IOP with circadian rhythm. Studies on rabbit IOP utilizing rebound tonometer (Tonovet<sup>®</sup>) and applanation tonometer (Tono-Pen Avia<sup>®</sup>) determined a significantly higher IOP value in the morning compared to that later in the day, which has been reported to be associated with the transition from the dark phase to the light phase (Pereira et al., 2011). Schuster et al. (2015) in a study on IOP measurements of 56 dragons with rebound tonometry have set a higher value in the morning compared to that taken later in the day. Similarly to this, as well as to that of Giannetto et al. (2009) on healthy dogs and Gelatt et al. (1981) on healthy and glaucomatous beagles the present study determined IOP value in the morning ( $16.21 \pm 2.10$  mmHg) reduced about 22% when ration to that ( $12.65 \pm 2.01$  mmHg) late in the day. As a result, these findings appear to confirm the claim that IOP measurement tends to reduce during the light phase of a day.

## CONCLUSION

So far no study has investigated the effect of age, sex and circadian rhythm on IOP in sheep, the reference IOP data in Awassi sheep has also not been determined. Thus, the present study may contribute to relevant litterateur deficiency. Studies performed on the same species with even the use of the same type of tonometer, may produce different IOP values indicating breed variability in that species. The presence of circadian rhythm in IOP of the sheep as in people suggests that this species may be a proper animal model for experimental ocular studies.

## CONFLICT OF INTEREST STATEMENT

None of the authors of the present paper has a financial and personal relationship with other people or organization that could inappropriately influence their work. ■

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