

SCHIRMER TEAR TEST IN DIFFERENT RABBIT BREEDS

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Farklı tavşan ırklarında Schirmer gözyaşı testi

ÖZET

Bu araştırmada farklı üç tavşan ırkında normal gözyaşı sekresyonlarının Schirmer gözyaşı Test 1 (STT-1) yöntemiyle belirlenmesi amaçlandı. Çalışma sağlıklı gözlerle sahip 15'i New Zealand, 10'u Angora ve 11'i Melez ırktan oluşan, 19'u dişi, 17'si erkek toplam 36 tavşanda yapıldı. Tavşanlar ırk özelliklerine göre üç gruba ayrıldı. Schirmer gözyaşı Test 1 (STT-1) ölçümleri, test kâğıdının (Schirmer Tear Test Paper, Clement Clarke International Limited) alt göz kapağı konjunktival kesesinin anteromedial üçte birlik kısmına yerleştirilmesiyle yapıldı. Test kâğıdı bir dakika sonra konjunktival keseden uzaklaştırıldı ve test kâğıdındaki ıslaklık milimetre olarak ölçüldü. STT-1 ölçümleri ilk yedi gün süresince ve 14., 21. ve 28. günlerde sabah saat 8 ile 10, öğleden sonra saat 3 ile 5 arasında ölçüldü. New Zealand, Angora ve Melez ırkı tavşanlardaki ortalama STT-1 değerleri sırasıyla 7.91±3.56, 7.22±2.94 ve 9.05±3.29 mm/dk olarak belirlendi. New Zealand ve Angora ırkı tavşanlar ile Melez tavşanlar arasında ve hem erkek ve dişilerinin hem de günlük ve haftalık STT-1 ölçümlerinde istatistiksel olarak önemli farklılıklar ($P<0.05$) saptandı. Sonuç olarak, üç tavşan ırkında da STT-1 değerlerinin normal sınırlar içerisinde olduğu ve bu değerlerin cinsiyet ve ölçüm zamanlarına göre farklılık gösterdiği belirlendi. Ayrıca tavşanlardaki gözyaşı sekresyonu ölçümlerinin doğru olarak değerlendirilmesi için, ırk, yaş ve cinsiyet ile içinde bulunduğu ekolojik koşullar da dikkate alınmalıdır.

ANAHTAR KELİMELER: Tavşan, Schirmer gözyaşı testi

SUMMARY

The aim of this study was to determine the normal values for the Schirmer tear test (STT-1) of the tear production in three different rabbit breeds. Thirty-six healthy adult rabbits, 19 females and 17 males, without any known ocular disease were used in this study. The rabbits of 15 New Zealand, 10 Angora and 11 mixed breed were divided into three groups. The Schirmer tear Test 1 (STT-1) was measured by inserting a bent portion of the test paper (Clement Clarke International Limited) into the antero-medial one-third of the conjunctival sac. The test paper was removed after the eyelids were closed for 60 seconds and the wet portion of the test paper was measured as mm. The STT-1 measurements were performed between 8 and 10 am and again between 3 and 5 pm twice daily for seven consecutive days and twice daily for 14th, 21th, and 28th days (weekly) on each eye of each rabbit. The mean STT-1 values in New Zealand, Angora and Mixed breed rabbits were 7.91±3.56, 7.22±2.94 and 9.05±3.29 mm/min, respectively. There were significantly different daily and weekly fluctuations for STT-1 values between Mixed breed and New Zealand or Angora rabbit breeds ($P<0.05$). There were significant differences in STT-1 values between morning and afternoon ($P<0.05$), as well as females and males fluctuations for STT-1 values ($P<0.05$). This study has shown that the STT-1 and values in rabbits are normally distributed and are significantly affected by sex and by measurement sequences. In additions data on sex, age, breed and ecological conditions should be taken into consideration together with all STT-1 measurements so that tear production can be accurately determined.

KEY WORDS: Schirmer tear test, rabbit

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INTRODUCTION

Precorneal tear film is essential in maintaining normal corneal health. It is composed of three distinct layers. The most superficial layer is the lipid layer, which is produced by the meibomian glands (Izci 1995, McLaughlin et al. 1988, Slatter 1990, Books 2003). Functions of the lipid layer include limiting the evaporation of the aqueous component of the tear film, allowing for an even spread of the tear film and limiting tear overflow from the lid margins (Kern 1992, Wilkie 1993, Alkan et al. 2004a,b). The middle aqueous layer comprises the majority of the precorneal tear film. It is produced principally by the lacrimal gland and the gland of the third eyelid. A smaller portion of the aqueous layer is also produced by the accessory lacrimal glands and mucus-secreting cells (Izci 1995, McLaughlin et al. 1988, Kern 1992, Berger and King 1998). The aqueous layer is responsible for providing oxygen and nutrients to the avascular cornea as well as removing metabolic wastes from the corneal surface. Antibacterial protection is provided by locally produced immunoglobulins (principally IgA), lysozyme, which dissolves bacterial cell walls, and lactoferrin. It is also responsible for maintaining hydration of the cornea and conjunctival surfaces and flushing away particulate debris (Hamor et al. 2000, Books 2003). The innermost mucoprotein layer, or mucin layer, is produced by the conjunctival goblet cells and corneal epithelial cells. The conjunctival goblet cells are located in highest concentrations in the regions of the lower nasal fornix, lower middle fornix and lower nasal tarsal regions (Kern 1992, Kaswan et al. 1998). Precorneal tear film production is under the control of the autonomic nervous system which controls both basal and reflex tear production. The trigeminal nerve plays an important role in the neurogenic control of lacrimation (Hamor et al. 2000, Books 2003).

The Schirmer tear test (STT) is an important component of the complete ophthalmic examination and should be performed routinely, especially for evaluating corneal conditions as an aid to their early recognition and better therapeutic results (Slatter 1990, Kern 1992, Izci 1995, Kaswan et al. 1998). There are two types with or without topical anesthesia of STT (STT-1 and STT-2). STT-1 is the most commonly used test which is performed by placing a filter paper strip in the medioventral palpebral cul-de-sac of an unanesthetized eye for one minute (Slatter 1990, Hakanson and Arnesson 1997). The STT-1 not only measures basal tear production but also reflex tear production through the stimulation of the trigeminal nerve endings by local irritation. The STT-2 is less commonly employed and measures only basal tear production as reflex lacrimation is eliminated by a topical anesthetic applied to the eye (McLaughlin et al. 1988, Berger and King 1998, Hamor et al. 2000, Alkan et al. 2004a,b).

Rabbits are widely used in ophthalmic researches. Rabbits may experience a variety of ophthalmic diseases (Abrams et al. 1990, Keil, 2004). These ophthalmic conditions are often different than eye diseases of the dog, cat, or other pet rodents. The diagnosis of the condition is usually made by the clinical signs, clinical experience and confirmed with the STT-1. The purpose of this study was (1) to determine the normal tear production in clinically normal New Zealand, Angora and Mixed breed rabbits, (2) to determine whether the sex of rabbits have any effect upon the STT-1 values, (3) to determine whether there are significant daily, weekly or diurnal fluctuations in STT-1 values, and (4) to attempt to identify the variables that might influence these findings.

Table 1. The mean values of STT-1 in clinically normal animals of five species.

Species	STT-1(mm/min)
Dogs	17.1 ±2.8
Cats	16.2 ±3.8
Rabbits	5.30±2.9
Elephants	34.3 ±1.7
Monkeys	15.1 ±4.7

Data from Jaax et al. 1984, McLaughlin et al. 1988, Abrams et al. 1990, Tuntivanich et al. 2002, Alkan et al. 2004b.

MATERIALS and METHODS

Animals: Thirty-six healthy adult rabbits, 19 females and 17 males, without any known ocular disease were used in this study. The rabbits of 15 New Zealand, 10 Angora and 11 Mixed breed were divided into three groups. Age, body weight and sex were recorded for each rabbit within each groups. All rabbits were housed under standard conditions and had free access to food and water during the experiment.

Experimental design: The Schirmer tear test (STT-1) was performed without topical anesthesia. The Schirmer tear testing paper (Clement Clarke International Limited) was used for the STT-1. One sterile Schirmer tear test paper was inserted carefully into the lower conjunctival fornix of each eye at the antero-medial one-third of the eyelid. The test paper was left for 60 seconds and removed. The amount of wetness of the test paper was immediately measured against a standard scale calibrated in millimeters. The test results were recorded. The STT-1 measurements were performed between 8 and 10 a.m. and again between 3 and 5 p.m. twice daily for seven consecutive days and twice daily for 14th, 21th and 28th days on each eye of each rabbit. All tear test papers were performed by the same investigators.

Statistical analyses: The experimental analyses of the data were carried out by one-way analyses of variance (ANOVA). The differences between groups were compared using the Duncan's multiple range test. Statements of statistical significance are based on $P < 0.05$. These analyses were accomplished by using statistical analyses system configured for computer (SPSS, Release 10.0, SPSS.Inc).

RESULTS

New Zealand rabbits (7 females and 8 males) ranged from 9 months to 2 years old (mean, 14 ± 2.3 months), and the mean body weight was 3.6 ± 0.7 kg. Angora rabbits (5 females and 5 males) ranged from 10 months to 1.5 years old (mean, 12 ± 3.2 months), and the mean body weight was 2.8 ± 0.3 kg. Mixed breed rabbits (6 females and 5 males) ranged from 12 months to 2.2 years old (mean, 16 ± 4.1 months), and the mean body weight was 3.2 ± 0.4 kg. The mean \pm standart deviation for the 1-minute Schirmer

tear test (STT-1) without topical anesthesia for all 72 clinically normal eyes was 8.09 ± 3.4 mm/min. The mean STT-1 values in New Zealand, Angora and Mixed breed rabbits were 7.91 ± 3.56 , 7.22 ± 2.94 and 9.05 ± 3.29 mm/min, respectively (Table 2). There were significantly different daily and weekly fluctuations for STT-1 values between Mixed breed and New Zealand or Angora rabbit breeds ($P < 0.05$). On the other hand, there was no significant difference in the STT-1 values between New Zealand and Angora rabbit breeds ($P > 0.05$) (Table 2).

The means STT-1 values in the morning and afternoon in New Zealand, Angora and Mixed breed rabbits are shown in Table 3. There were significant differences at over time in STT-1 values for each breed ($P < 0.05$) (Table 3).

The means STT-1 values in the males and females of New Zealand, Angora and Mixed breed rabbits are shown in table 3. There were significant differences in STT-1 values between males and females in each breed ($P < 0.05$), (Table 3).

Table 2. Values (mean \pm SD) for the Schirmer tear test (STT-1) in clinically normal rabbits of three breeds (mm/per minute).

Days	New Zealand	Angora	Mixed breed
	STT-1	STT-1	STT-1
1	7.62 ± 13.19^{ab}	6.81 ± 2.58^{abc}	9.48 ± 3.39^{ab}
2	6.23 ± 3.07^{bc}	8.33 ± 2.39^{ab}	10.18 ± 3.16^a
3	8.87 ± 3.54^{ab}	5.44 ± 2.42^c	7.70 ± 2.74^{ab}
4	8.35 ± 3.47^{ab}	8.42 ± 3.00^{ab}	8.11 ± 2.46^{ab}
5	9.18 ± 3.57^a	8.28 ± 3.05^{ab}	10.45 ± 3.15^a
6	4.87 ± 3.03^c	8.89 ± 2.69^a	10.61 ± 3.17^a
7	7.68 ± 2.75^{ab}	5.75 ± 2.42^{bc}	8.27 ± 2.99^{ab}
14	9.18 ± 3.68^a	8.67 ± 2.86^a	9.61 ± 4.01^{ab}
21	7.38 ± 3.46^{ac}	6.44 ± 2.81^{abc}	6.82 ± 2.38^b
28	9.73 ± 3.31^a	5.19 ± 2.31^c	9.25 ± 3.46^{ab}
Mean values	7.91 ± 3.56^b	7.22 ± 2.94^b	9.05 ± 3.29^a

All mean values: STT-1 8.09 ± 3.40 mm/min.

abc values in same column with different superscript are statistically different ($P < 0.05$).

Data expressed as mean \pm SEM mm wetting per minute.

Table 3. Values (mean \pm SD) for the Schirmer tear test (STT-1) of daytime and sex in clinically normal rabbits of three breeds.

Groups	New Zealand	Angora	Mixed breed
	STT-1	STT-1	STT-1
Morning	7.03 \pm 3.20 ^b	6.76 \pm 2.69 ^b	8.21 \pm 2.96 ^b
Afternoon	8.79 \pm 3.70 ^a	7.69 \pm 3.12 ^a	9.89 \pm 3.40 ^a
Males	6.88 \pm 3.33 ^b	6.68 \pm 3.13 ^b	8.34 \pm 2.92 ^b
Females	8.82 \pm 3.52 ^a	7.66 \pm 2.71 ^a	9.64 \pm 3.47 ^a

^{ab}values in same column with different superscript are statistically significantly different ($P < 0.05$). Data expressed as mean \pm SEM mm wetting per minute.

DISCUSSION

The lacrimal gland and the gland of the membrana nictitans appear to be the major source of tears in the animals (Slatter 1990, Kern 1992, Izci 1995). Clinical estimation of tear production is performed by the Schirmer tear test. The Schirmer tear test performed without topical anesthesia is most frequently used in the clinical setting to determine baseline and reflex lacrimation (Hakanson and Arnesson 1997). The conventional clinical test used is the STT-1.

Deficiency in tear production results in inflammation of the conjunctiva and cornea known as keratoconjunctivitis sicca (KCS) or "dry eye". Clinical signs include mucoid discharge, blepharospasm, conjunctival hyperemia and thickening and corneal ulceration, vascularization and pigmentation (Wilkie 1993, Izci 1995, Wyman et al. 1995, Kaswan et al. 1998). According to previous reports, the mean normal values for the STT-1 have been described for Rhesus Monkeys (15.1 \pm 4.7 mm/min, Jaax et al. 1984), cats (16.2 \pm 3.8 mm/min, McLaughlin et al. 1988), Asian Elephants (34.3 \pm 1.7 mm/min, Tuntivanich et al. 2002) and dogs (17.1 \pm 2.8 mm/min, Alkan et al. 2004b). The mean values for the STT-1 without use of topical anesthesia on totals 142 without any known ocular disease; fifty-four rabbits represented 9 pure breeds, and 17 rabbits were of mixed breed, rabbits eyes have been reported as 5.30 \pm 2.96 mm/min (Abrams et al. 1990). The same authors have reported the mean provided a normal clinical range of 0 to 11 mm/min for inclusion of 95% of the rabbit population. However, in our study, we determined that the all mean values for STT-1 in clinically normal rabbits were 8.09 \pm 3.40 mm/min (Table 2). The study has shown that STT values in apparently healthy rabbits are a bit higher than values reported by Abrams et al. (1990).

The STT-1 is inexpensive, easy to perform, and used to confirm KCS (low values) or to correlate with other signs of ocular irritation (high values) in small animal practice. Naturally acquired KCS has not been reported in rabbits. However, with the increasing popularity of rabbits, the number of rabbits with corneal disease is expected to increase. It was previously reported that there is a diurnal variations

in STT-1 values in clinically healthy dogs (Hamor et al. 2000; Alkan et al. 2004a,b). In those studies, investigators reported a diurnal fluctuation with values the lowest at morning and the highest in the afternoon. However, it has not been reported that there is diurnal fluctuations in STT-1 values in clinically normal rabbit breeds. The results of our study indicate that the rabbits of three breed have typical diurnal fluctuation with the lowest values of morning and the highest values in the afternoon. It seems possible that such fluctuations also may influence different measurements made on the same day. The highest values in the afternoon when compared with at morning must be associated with ocular irritation, stress, physical conditions, or the postprandial parasympathetic influence on STT-1.

The STT-1 values between the sexes of healthy dogs have been reported that they are higher in females than those in males (Barnett 1988, Hamor et al. 2000, Alkan et al. 2004a,b). It has not been reported that there is fluctuation in STT-1 values in clinically normal rabbit breeds. The results of our study indicate that these values are higher in females than in males, as dogs. The difference between females and males can be attributed to the female sex hormones, which have a stimulating effect on lacrimation. Deficiency in female sex hormones can contribute to the dysfunction of tear production glands and can lead to KCS.

In conclusion, this study has shown that the STT-1 values with nonanesthetized cornea in clinically normal rabbit breeds are normally distributed and are significantly affected by breed, sex, or measurement sequences. A low value of tear production in rabbits was a sign of keratoconjunctivitis sicca, while a high value was correlated with signs of ocular pain or lacrimal drainage problems. Knowledge of the normal STT-1 values in the different rabbit breeds is a prerequisite for the use of this diagnostic test in the clinical setting for diagnostic procedures on ocular abnormalities in rabbit breeds. We think that further studies are needed to properly appreciate the differences among the rabbits of various breeds and to determine the reference value for the STT-2 in rabbits, and to compare differences between STT-2 values in clinically normal rabbits.

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