

University of PISA

**LACRIMAL SECRETION VARIATION AND MENACE RESPONSE APPEARANCE IN
HEALTHY STANDARDBRED FOALS FROM BIRTH TO FOUR WEEKS OF AGE**

Samanta Nardi, Martina Nuti, Irene Nocera, Micaela Sgorbini, Paola Marmorini, Giovanni Barsotti

Published in:

Journal of Equine Veterinary Science

DOI:

<https://doi.org/10.1016/j.jevs.2022.104050>

Publication date:

2022

Document Version

Peer reviewed version (post-print)

Citation for published version:

Nardi, S., Nuti, M., Nocera, I., Sgorbini, M., Marmorini, P. & Barsotti, G. (2022). Lacrimal secretion variation and menace response appearance in healthy Standardbred foals from birth to four weeks of age. *Journal of Equine Veterinary Science*, 116, Article 104050. <https://doi.org/10.1016/j.jevs.2022.104050>

This is the peer reviewed version of the following article: "Lacrimal secretion variation and menace response appearance in healthy Standardbred foals from birth to four weeks of age". *Journal of Equine Veterinary Science* (2022) which has been published in final form at <https://doi.org/10.1016/j.jevs.2022.104050>. This article may be used for non-commercial purposes in accordance with Elsevier Terms and Conditions for Self-Archiving.

Journal of Equine Veterinary Science

LACRIMAL SECRETION VARIATION AND MENACE RESPONSE APPEARANCE IN HEALTHY STANDARDBRED FOALS FROM BIRTH TO FOUR WEEKS OF AGE

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research paper
Keywords:	Standardbred; foal; Schirmer tear test; menace response; horse
Corresponding Author:	Irene Nocera Universita degli studi di Pisa Dipartimento di Scienze Veterinarie ITALY
First Author:	Samanta Nardi
Order of Authors:	Samanta Nardi Martina Nuti Irene Nocera Micaela Sgorbini Paola Marmorini Giovanni Barsotti
Abstract:	<p>This study assesses lacrimal secretion in healthy foals at birth and evaluates their variations in lacrimal secretion and menace response appearance during the first month of life. A total of 31 Standardbred foals were evaluated. Foals were housed with their dams in 4x4 m boxes throughout the study. A complete ophthalmic examination was performed for each animal enrolled in the study, including the foals' dams which were used as a control group to compare the lacrimal secretion of foals at birth with that of adult horses. In foals, the Schirmer Tear Test I (STT I) and menace response were evaluated within 48 hours after birth (T0) and weekly for a total of four times (T1, T2, T3, and T4). In mares, the STT I was evaluated once, before delivery. The Schirmer Tear Test I results of 11 out of 31 dams were excluded because of signs of ocular adnexa inflammation which might otherwise have influenced lacrimal secretion. At birth STT I values in foals (19.11 ± 2.49 mm/min) were lower than those of their dams (23.20 ± 1.83 mm/min). In addition, the mean STT I in foals decreased during the study period and reached its nadir at T3 (16.84 ± 2.33 mm/min). None of the foals showed a menace response at any observation point.</p>
Suggested Reviewers:	Francesca Freccero francesca.freccero2@unibo.it DVM, PhD, ECEIM, equine specialist Barbara Lamagna blamagna@unina.it
Opposed Reviewers:	

Cover Letter

Dear Editor,

Attached is our paper entitled “LACRIMAL SECRETION VARIATION AND MENACE RESPONSE APPEARANCE IN HEALTHY STANDARD BRED FOALS FROM BIRTH TO FOUR WEEKS OF AGE” by Nardi et al. The paper is original and has not been submitted or published elsewhere. All the authors have given their approval for submission to your journal. The paper has also been edited by a native English speaking editing agency (certificate uploaded in the supporting information). We hope you will find the paper suitable for publication in your journal.

Yours sincerely,

Dr Irene Nocera

(Corresponding author)

HIGHLIGHTS

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- At birth healthy Standardbred foals show a lower lacrimal secretion than adults
- Tear secretion seems to decrease during the first month of life in foals
- Foals show no evident menace response during the first month of life
- Poor lacrimation and no menace response might predispose foals to corneal ulcers

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

LACRIMAL SECRETION VARIATION AND MENACE RESPONSE APPEARANCE IN HEALTHY STANDARD BRED FOALS FROM BIRTH TO FOUR WEEKS OF AGE

Samanta Nardi^{1°}, Martina Nuti^{1,2°}, Irene Nocera^{1*°}, Micaela Sgorbini¹, Paola Marmorini³ and

Giovanni Barsotti¹

¹ Department of Veterinary Sciences, Veterinary Teaching Hospital, via Livornese snc, San Piero a

Grado (PI), Italy;

² Private practitioner, Livorno, Italy;

³ Private practitioner, Pisa, Italy;

[°] Equal first author

* Correspondence: irene.nocera@vet.unipi.it; telephone +39.050.2210151

Running title: Schirmer tear test and menace response in foals

ABSTRACT

1
2
3
4
5 This study assesses lacrimal secretion in healthy foals at birth and evaluates their variations in
6
7 lacrimal secretion and menace response appearance during the first month of life. A total of 31
8
9 Standardbred foals were evaluated. Foals were housed with their dams in 4x4 m boxes throughout
10
11 the study. A complete ophthalmic examination was performed for each animal enrolled in the study,
12
13 including the foals' dams which were used as a control group to compare the lacrimal secretion of
14
15 foals at birth with that of adult horses. In foals, the Schirmer Tear Test I (STT I) and menace
16
17 response were evaluated within 48 hours after birth (T0) and weekly for a total of four times (T1, T2,
18
19 T3, and T4). In mares, the STT I was evaluated once, before delivery. The Schirmer Tear Test I
20
21 results of 11 out of 31 dams were excluded because of signs of ocular adnexa inflammation which
22
23 might otherwise have influenced lacrimal secretion. At birth STT I values in foals (19.11 ± 2.49
24
25 mm/min) were lower than those of their dams (23.20 ± 1.83 mm/min). In addition, the mean STT I
26
27 in foals decreased during the study period and reached its nadir at T3 (16.84 ± 2.33 mm/min). None
28
29 of the foals showed a menace response at any observation point.
30
31
32
33
34
35

36 In conclusion, at birth healthy Standardbred foals show a lower lacrimal secretion than their dams,
37
38 and tear secretion does not seem to increase during the first month of life. In this period, poor
39
40 lacrimation associated with the absence of an evident menace response might be predisposing factors
41
42 for developing corneal ulcers.
43
44
45
46
47
48

49 **Key words:** Standardbred, neonatal, foal, Schirmer tear test, menace response, horse
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1. INTRODUCTION

1
2 The ocular surface is covered by the tear film which, in association with the ocular adnexa, is
3
4 necessary for optical integrity, maintenance of the cornea, and normal eye function [1]. Tears
5
6 provide nutrients, oxygen, and leukocytes to the avascular cornea. Inadequate tear production can
7
8 induce conjunctivitis, superficial keratitis, and corneal ulceration [1]. Tears consist of a triple-layered
9
10 film: the mucin layer, aqueous tear fluid layer, and oily layer. The mucin layer is the innermost layer,
11
12 which is adsorbed from the corneal epithelial surface and distributed evenly during normal blinking.
13
14 The mucin provides a hydrophilic surface over which the aqueous tear fluid spreads evenly. The
15
16 aqueous tear fluid layer is the middle layer, which guarantees the oxygen necessary for normal
17
18 corneal metabolism. The oily layer is the superficial layer, which reduces evaporation of the
19
20 underlying aqueous tears and forms a barrier along the lid margin which prevents the overflow of
21
22 tears on the face [1,2].
23
24
25
26
27

28
29 The standard method to assess the aqueous component of the tear film is the Schirmer tear test (STT)
30
31 [3]. STT I measures trigeminal/facial reflex tearing in addition to basal secretion, and is performed
32
33 without topical anesthesia. STT II, with topical anesthesia, only measures basal tearing [3,4].
34
35

36 Although there is abundant literature on this issue in adult horses, papers on neonatal foal tear
37
38 secretion are scarce [5] and, to the best of our knowledge, the variation over time in the first month of
39
40 life has never been evaluated.
41
42

43 Healthy neonatal foals, as well as sick foals, have been reported to show a lower tear secretion than
44
45 adults. Sick foals also present a weak corneal sensitivity. It has been speculated that both weak
46
47 corneal sensitivity and low tear secretion volumes may contribute to an increase in the frequency of
48
49 ulcerative keratitis in foals [5], and more serious disease such as keratomycosis [6].
50
51

52 The Menace Response (MR) and palpebral reflexes are routinely evaluated during ophthalmic
53
54 examinations. The MR consists of making an unexpected hand motion near the eye. The response
55
56 elicited should be quick together with repeated blinking and possibly retraction of the globe or an
57
58
59
60
61
62
63
64
65

1 avoidance movement of the head [7,8]. The MR is a learned behaviour, which is not present at birth.
2 Studies have also found that in foals, the MR is often not present from the first days of life up to
3 three weeks of age [9-11]. Foals may also occasionally have a partial or an asymmetrical response
4 before showing a complete response [9-11].
5
6
7

8
9 The aim of the present study was to evaluate STT I values at birth and lacrimal secretion variation
10 and menace response appearance from birth to 4 weeks of age in a cohort of healthy Standardbred
11 foals. We also compared STT values of foals at birth with those of a control group of adult horses
12 composed of the foals' dams. The null hypothesis was that foals would have a similar lacrimal
13 secretion to findings reported in adult horses.
14
15
16
17
18
19
20
21
22
23

24 **2. MATERIALS AND METHODS**

25
26 The present study was approved by the Institutional Animal Care and Use Committee of the
27 University of Pisa (D. Lgs. 116/1992) and the owner's written consent was obtained for the
28 ophthalmic examination and STT I tests for all the animals. The study population consisted of 31
29 healthy Standardbred neonatal foals and their dams. All the foals were born on the same stud farm in
30 Tuscany (Italy) and were kept under similar management conditions. Foals were housed with their
31 dams in 4x4 m boxes throughout the study. Boxes were provided with one outside window and one
32 main door. Wood shavings were used as bedding.
33
34
35
36
37
38
39
40
41
42

43 Foals were considered healthy based on physical examinations, which were performed weekly
44 throughout the study. Mares were considered healthy based on physical examination performed at
45 the time of the ophthalmic evaluation.
46
47
48
49
50

51 A complete ophthalmic examination of the foals was performed within 48 hours (T0) of delivery. All
52 foals were examined on the dam's side in the stall and they were only manually restrained during the
53 procedure. The Schirmer tear test I was performed for both eyes as the first step of the examination
54 using commercially available test strips (Dina strip Schirmer-Plus®, GECIS srl, France) as follows:
55
56
57
58
59
60
61
62
63
64
65

1 the test strip was bent at the notch while the strip was still in its packaging to prevent oil being
2 absorbed by the examiner's skin. The folded end was inserted in the lower conjunctival fornix near
3
4 the junction of the middle and temporal part of the eyelid for one minute. Tear production was
5
6 recorded in millimeters of wetting per minute immediately at removal. The Schirmer tear test I was
7
8 performed in the morning (9:00-11:00 am) in the spring. The test was executed first on the right eye
9
10 and then on the left eye. Palpebral, corneal, direct, and indirect pupillary light reflexes and the MR
11
12 were performed. Intraocular pressure was assessed by applanation tonometry (Tonopen-XL, Mentor,
13
14 Norwell, MA, USA) following the topical administration of 0.4% oxibuprocaine chlorhydrate
15
16 (Benoxinato chlorhydrate INTES®, Alfa Intes, Industria Terapeutica Splendore S.r.l., Naples, Italy).
17
18 The adnexa and anterior segments of both eyes were examined with a slit-lamp (Kowa SL-14, Kowa
19
20 Company, Tokyo, Japan) to exclude any ocular abnormalities that could influence lacrimal secretion.
21
22 Mydriasis induction was performed in both eyes of each foal with topical 1% tropicamide
23
24 (Visumidriatic 1%, Visufarma Srl, Italy), and foals were re-examined 30 minutes after the
25
26 instillation. The ocular fundus was examined using a binocular indirect ophthalmoscope (Omega
27
28 180, Heine, Berlin, Germany) with a 20 or 30 D lens. Fluorescein staining was performed after
29
30 completion of the fundoscopy.
31
32 The Schirmer tear test I and MR were also evaluated weekly (7-day intervals) for a total of four
33
34 times in foals (T1, T2, T3, and T4). Biomicroscopy of the adnexa and anterior segment of the eye
35
36 was executed at each STT I assessment.
37
38 Before the delivery, a complete ophthalmic examination was also performed in mares, following the
39
40 same procedure described for the foals. The Schirmer tear test I and MR were performed once in
41
42 both eyes, and during the procedure, the mares were restrained in a stock.
43
44 Data distribution was evaluated by the Shapiro-Wilk normality test. Since the data distribution was
45
46 gaussian, the results were expressed as mean (X) and standard deviation (SD). The 95% confidence
47
48 intervals (CIs) of the mean for each sampling time were also calculated.
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 A paired t test was used to compare the STT I value of the right eye with that of the left eye of each
2 foal at each measurement time. The same test was used to compare the STT I value of the right eye
3 with that of the left eye of each mare. Results indicated that the STT I did not differ significantly
4 between the right and left eyes of individual animals. The mean STT I was therefore calculated for
5 the right and left eyes at each measurement time in the foals, and the same was calculated in mares.
6
7 The mean STT I values were then considered as per-foal and per-adult values, and used for all
8
9 subsequent analyses, as recommended by others [12].
10
11

12 A two-tailed unpaired t test was applied to compare STT I values obtained in mares vs STT I values
13 obtained in foals at T0.
14

15 A two-tailed paired Student t test was used to compare the mean STT I between T0 and each
16 subsequent measurement time (T1, T2, T3, and T4). A two-tailed paired Student t test was also used
17 for pairwise comparison of the mean STT I between T1, T2, T3, and T4.
18

19 Statistical significance was set at $p < 0.05$. Commercial software was used (GraphPad Prism, 6.0,
20 USA).
21

22 3. RESULTS

23 In all the 31 foals included in the study (16 fillies and 15 colts), no ocular abnormalities were found
24 at T0 and at the subsequent timeframes. In contrast, 11 out of 31 mares showed signs of ocular
25 adnexa inflammation that might otherwise have influenced lacrimal secretion, thus their STT values
26 were excluded from the study. In the remaining 20 mares (median age 9 years; range 5-13 years), the
27 STT I value was 23.20 ± 1.83 mm/min (22.34-24.06 mm/min 95% CIs). Statistical differences were
28 observed between STT I value in mares vs T0 measurement in foals ($p < 0.0001$), with higher values
29 in adult horses.
30

31 Results of STT I values in foals at all measurement times are reported in Table 1.
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Table 1

The STT I values obtained in foals at birth (T0), and then at 1 (T1), 2 (T2), 3 (T3) and 4 (T4) weeks of age. The results are reported as mean (X)±standard deviation (SD). The 95% confidence intervals (CIs) are also reported.

STT I (mm/min)	T0	T1	T2	T3	T4
X±SD	19.11±2.49	18.53±2.86	17.66±2.82	16.84±2.33	17.65±2.05
95% CIs	18.20-20.02	17.48-19.58	16.63-18.69	15.98-17.69	16.89-18.40

STT I: Schirmer tear test I; T0: time at birth; T1: time at one week; T2: time at two weeks; T3: time at three weeks; T4: time at four weeks; X: mean; SD: standard deviation; CIs: confidence intervals.

Statistical differences were detected between T0 and T2 (p=0.0062), T3 (p=0.0008) and T4 (p=0.0078), and between T1 and T3 (p=0.0094). The mean STT I decreased over time and reached its observed nadir at T3 (Fig. 1).

None of the foals showed a MR at any observation timeframe in the study.

4. DISCUSSION

Tear production is important for the health of the anterior segment of the globe. Normal tear production values have been well documented in adult horses [3,4,13-15], while the literature is scarce on neonatal foals [5] and variations over time have not yet been evaluated. The main aim of the present study was thus to assess STT I variations and menace response appearance during the first month of life in a cohort of healthy Standardbred foals. In addition, we compared STT values of foals at birth with those of a control group of adult horses composed of the foals' dams.

Our mean STT I values in healthy mares (23.20±1.83 mm/min) are similar to studies on adult horses [4,13,14,16]. Some studies have evaluated how age, season [13,14], breed [14], gender, daily

1 exposure to light [16], and eyelid position [16] influence STT I values in adult horses. According to
2 Beech and colleagues (2003) [13], no statistically significant changes in STT I values were found
3
4 with age, season, environment, sex, time of day and placement of the strips. In contrast, Piccione and
5
6 colleagues (2008) [16] reported statistically significant differences comparing the left and right eye,
7
8 and between genders. They also showed a daily rhythmicity in horse tear production. Trbolova and
9
10 Ghaffari (2017) [16] reported that the mean STT I value performed with the eye open was
11
12 significantly lower than the STT I performed with the eye closed.
13
14

15
16
17 In our study, all the adult horses evaluated were mares, and their STT I values were assessed in the
18
19 morning with the eyes open. STT I values did not differ significantly between the right and left eyes.
20
21 It was therefore not possible to compare genders, and to evaluate the variation in tear production
22
23 during the day. This could be considered a limitation of our study, although our main aim was to
24
25 evaluate normal STT I values in healthy foals at birth and during the first month of life and to
26
27 compare the foals' values at birth with STT I values in healthy adult horses. Despite this possible
28
29 limitation, the STT I values obtained in mares are in line with those previously reported [4,13,14,16].
30
31

32
33
34 To best of our knowledge, this is the first report on the assessment of STT I values in a population of
35
36 healthy foals homogeneous in terms of breed and age. Our results at T0 (19.11 ± 2.49 mm/min) are
37
38 not in line with values reported in a previous study [5] in which the lacrimal secretion value was
39
40 lower (12.8 ± 2.4 mm/min) than ours at all measurement times. This difference might be due to the
41
42 number ($n=5$) and non-homogeneous age of the foals enrolled in [5], in which the authors examined
43
44 foals aged less than 7 days, without stating the exact time of the ophthalmic examination [1]. On the
45
46 other hand, our results at T0 are in line with another study [17] (mean of 19 mm/min in both eyes), in
47
48 which the foals enrolled were also non-homogeneous in terms of breed and age (≤ 30 days), and they
49
50 were affected by systemic, and sometimes ophthalmic diseases [17].
51
52

53
54
55 In our study, Schirmer Tear Test I values in foals at T0 were lower than in mares. Moreover, values
56
57 in foals decreased over time up to the third week of life. This could be explained by an unstable tear
58
59

1 film in the foals at birth, probably due to an imbalance between the components of the triple-layered
2 film [18]. If the tear film is unstable, a higher amount of the aqueous portion may collect at the lower
3
4 conjunctival sac, resulting in an overestimation of tear production in foals at birth. No specific
5
6 studies on this have been performed on horses. However, in an experimental study on 5-day-old
7
8 mouse pups, it was demonstrated that the tear film has no precipitable ocular mucus, thus
9
10 determining its instability [19].
11
12

13
14 In our study, the stability of the tear film in foals was not evaluated by the tear film break-up time
15
16 test (TFBUT) [18], which might represent a limitation. The TFBUT has not been standardized to date
17
18 in foals and values range from 2-3 seconds [20] to 24.9 seconds [21].
19
20

21
22 A lower tear production in foals than in adult horses could be a predisposing factor for the
23
24 development of corneal ulcers in foals as already reported in a previous study [5]. An additional
25
26 predisposing factor for the development of corneal ulcers in newborn foals might also be tear film
27
28 instability, however further studies are needed to confirm this correlation.
29
30

31
32 The menace response (MR) is a learned protective response after a sudden movement towards the
33
34 eye resulting in closure of the eyelids and retraction of the globe and/or an avoidance movement of
35
36 the head [8]. Foals learn with experience how to react to the menace [3,7]. The time needed to
37
38 develop MR is not well defined and with wide intervals ranging from 4 days to three weeks of life
39
40 [9-11,22]. None of the foals enrolled in our study showed the MR in the first four weeks of life,
41
42 which seems to be longer than previously found [9,10]. This difference might be due to the type of
43
44 management of the mares and foals during the study. In particular, foals were housed in a box with
45
46 the mare throughout the first month of life. It is possible that, as foals may not have been exposed to
47
48 external stimuli, the MR may have been delayed because it took them a longer time to learn it [7,11].
49
50

51
52 The housing system used in the present study might have influenced our results and thus represents a
53
54 limitation for an objective evaluation of the MR. However, our controlled indoor environment might
55
56
57
58
59
60
61
62
63
64
65

1 actually have helped to make the external stimuli uniform and to standardize the results obtained,
2 both for the STT I and MR evaluation.
3
4
5
6

7 **5. CONCLUSIONS**

8
9 In conclusion, to the best of our knowledge this is the first study to assess STT I values and MR
10 during the first month of life in a population of healthy Standardbred foals. The results showed that
11 lacrimal secretion in newborn foals is lower than in adult horses, and no menace response was
12 evident in the first four weeks of life. These features might be predisposing factors for the
13 development of corneal ulcers. Further studies are needed to determine the stability of the pre-
14 corneal tear film in foals.
15
16
17
18
19
20
21
22
23
24
25

26 **FIGURE CAPTIONS**

27 **Figure 1**

28
29 Mean±SD of STT I values over time obtained for 31 foals. Statistical differences were observed
30 between T0 and T2 (p=0.0355), T0 and T3 (p=0.0004) and T0 and T4 (p=0.0139) and between T1
31 and T3 (p=0.0131). The different letters above the bar charts denote a significant difference over the
32 observational time (a≠b,c,d; a,c≠b,d: p < 0.05).
33
34
35
36
37
38
39
40
41
42
43

44 **ACKNOWLEDGMENTS**

45
46 The authors would like to thank La Piaggia srl.
47
48
49
50

51 **FUNDING SOURCES**

52
53 This research did not receive any specific grant from funding agencies in the public, commercial, or
54 not-for-profit sectors.
55
56
57
58
59
60
61
62
63
64
65

DATA ACCESSIBILITY STATEMENT, INCLUDING A LINK TO THE REPOSITORY

USED

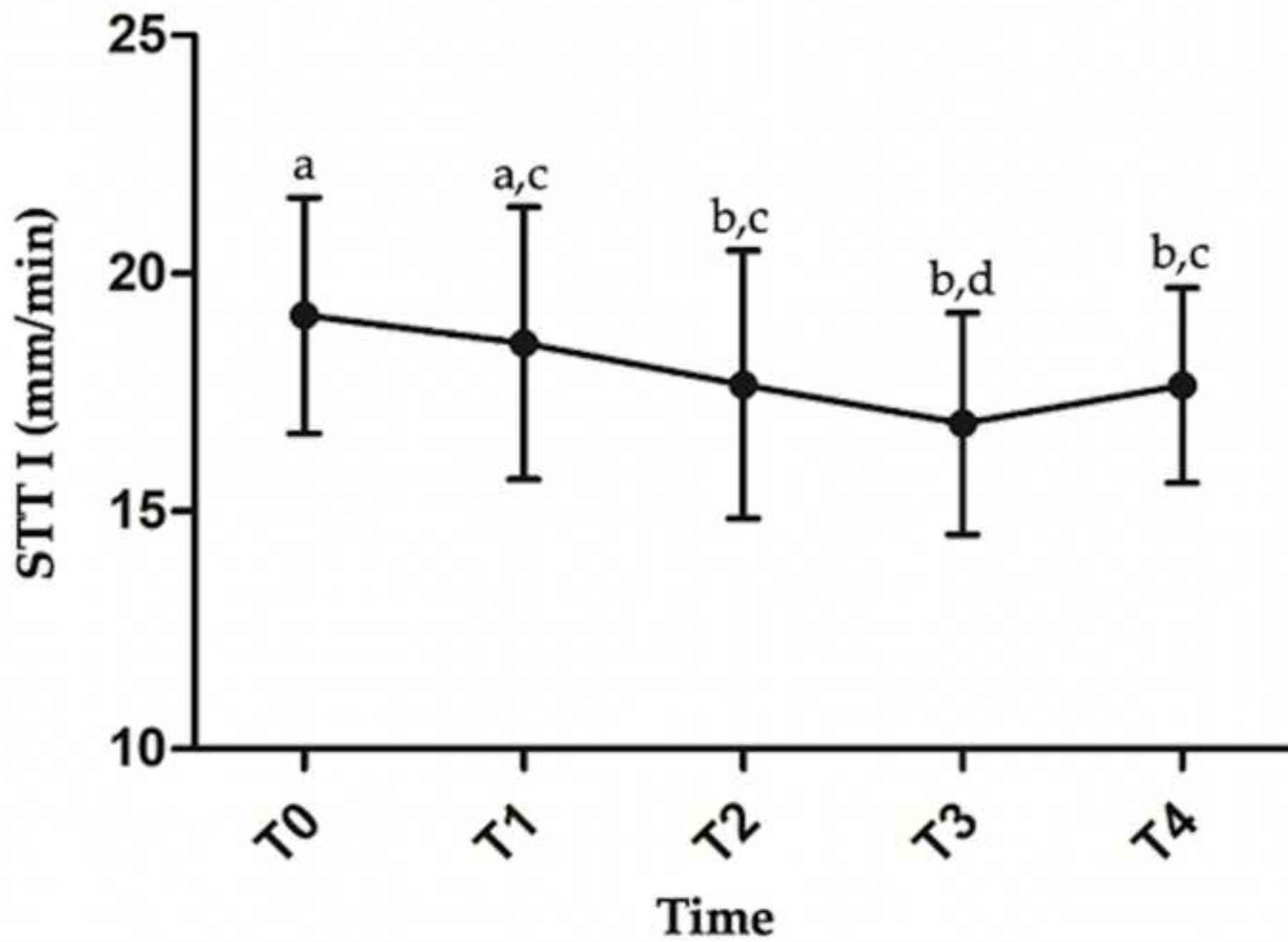
The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Samuelson DA. Ophthalmic Anatomy. In: Gelatt KN, Gilger BC and Kern TJ, editors. *Veterinary Ophthalmology*, 5th ed., Ames, IA: Wiley-Blackwell; 2013, p. 58-60.
2. Miller PE. Lacrimal System. In: Maggs D, Miller PE and Ofri R, editors. *Slatter's Fundamentals of Veterinary Ophthalmology*, 4th ed., St. Louis, MO: Elsevier; 2008, p. 157-174.
3. Featherstone HJ, Heinrich CL. Ophthalmic Examination and Diagnostics, Part 1: The Eye Examination and Diagnostic Procedures. In: Gelatt KN, Gilger BC and Kern TJ, editors. *Veterinary Ophthalmology*, 5th ed., Ames, IA: Wiley-Blackwell; 2013, p. 533-613.
4. Trbolova A, Ghaffari MS. Results of the Schirmer tear test performed with open and closed eyes in clinically normal horses. *Acta Vet. Scand* 2017;59(1):35.
5. Brooks DE, Clark CK, Lester GD. Cochet-Bonnet aesthesiometer-determined corneal sensitivity in neonatal foals and adult horses. *Vet Ophthalmol* 2000;3:133-137.
6. Sgorbini M, Barsotti G, Nardoni S, Mancianti F, Rossi S, Corazza M. Fungal flora of normal eyes in healthy newborn foals living in the same stud farm in Italy. *J Equine Vet Sci* 2008;28:540-543.
7. Hendrix DV. Eye examination techniques in horses. *Clin Techn Equine Pract* 2005;4:2-10.
8. Gilger BC, Reeves KA, Salmon JH. Ocular parameters related to drug delivery in the canine and equine eye: aqueous and vitreous humour volume and scleral surface area and thickness. *Vet Ophthalmol* 2005;8:265-269.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
9. Latimer CA, Wyman M. Neonatal ophthalmology. *Vet Clin North Am: Equine Pract* 1985;1:235-259.
10. Enzerink E. The menace response and pupillary light reflex in neonatal foals. *Equine Vet J* 1998;30:546-548.
11. Leiva M, Peña T, Monreal L. Ocular findings in healthy newborn foals according to age. *Equine Vet Educ* 2011;23(1):40-45.
12. Ray WA, O'day DM. Statistical analysis of multi-eye data in ophthalmic research. *Invest Ophth Vis Sci* 1985;26:1186-1188.
13. Beech J, Zappala RA, Smith G, Lindborg S. Schirmer tear test results in normal horses and ponies: effect of age, season, environment, sex, time of day and placement of strips. *Vet Ophthalmol* 2003;6:251-254.
14. Wieser B, Tichy A, Nell B. Correlation between corneal sensitivity and quantity of reflex tearing in cows, horses, goats, sheep, dogs, cats, rabbits, and guinea pigs. *Vet Ophthalmol* 2013;16(4):251-262.
15. Knickelbein KE, Scherrer NM, Lassaline M. Corneal sensitivity and tear production in 108 horses with ocular disease. *Vet Ophthalmol* 2018;21:76-81.
16. Piccione G, Giannetto C, Fazio F, Giudice E. Daily rhythm of tear production in normal horse. *Vet ophthalmol* 2008,11:57-60.
17. Labelle AL, Hamor RE, Townsend WM, Mitchell MA, Zarfoss MK, Breaux CB, Thomasy SM, Hall T. Ophthalmic lesions in neonatal foals evaluated for nonophthalmic disease at referral hospitals. *J Am Vet Med Assoc* 2011;239(4):486-492.
18. Monclin SJ, Farnir F, Grauwels M. Determination of tear break- up time reference values and ocular tolerance of tetracaine hydrochloride eyedrops in healthy horses. *Equine Vet J* 2011;43(1):74-77.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
19. Hazlett L, Dudzik D, Harries B. Development of ocular mucin: scanning EM analysis.
Ophthalmic Res 1986;18:28-33.
 20. Plattner U, Nell B. Measurements of eye blink frequency, corneal sensitivity, Schirmer's tear test I and II, tear film break-up time, and intraocular pressure in newborn foals and calves.
40th Annual Meeting of the American College of Veterinary Ophthalmologists, Chicago, IL, USA, November 4-7; 2009;12(56):386-389.
 21. Nunnery CM, Brooks DE, Plummer CE, Barrie KP. Equine tear film break-up time. 39th Annual Meeting of the American College of Veterinary Ophthalmologists, Boston, MA, USA, October 15-18; 2008;11:413-429.
 22. Turner AG. Ocular conditions of neonatal foals. Vet clin North Am: Equine Pract, 2004;20(2):429-40.



1 DECLARATIONS OF INTEREST

1
2 2 Declarations of interest: none.
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

ETHICAL ANIMAL RESEARCH

The present study was authorized by the Institutional Animal Care and Use Committee of the University of Pisa (D. Lgs. 116/1992).

OWNER INFORMED CONSENT

Owners gave consent for their animals' inclusion in the study.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 **AUTHOR CONTRIBUTIONS**

2 MS and SC conceived, designed and supervised the project.

3 IN, BA and LD executed the experiment. IN, BA and LG analysed the data.

4 GC executed the formal analysis.

5 All the authors interpreted the data, wrote and critically revised the manuscript for intellectual

6 content and approved the final version.

7 IN and MS had full access to all the data in the study and take responsibility for the integrity of the

8 data and the accuracy of the data analysis.



Click here to access/download

e-Component

E4AC manuscript declaration[1].pdf

