Evaluation of tear production with modified Schirmer tear test-1 during the neonatal period in cats

Luciano Fernandes da Conceição, Alexandre Pinto Ribeiro, Maria Lucia Marcucci Torres, Maria Lucia Gomes Lourenço and José Luiz Laus


The study aimed to evaluate tear production by means of modified Schirmer tear test-1 (mSTT-1) in neonate cats. Likewise, correlation between mSTT-1 and STT-1 was assessed in vitro. Standard SST strips were cut in half and after eye lid opening, tear production of neonates (n=15) was daily measured in both eyes (mSTT-1), until the 7th day, and at day 14, 21, and 28. Animals were daily weighted until 28 days of age. Results were compared statistically (P<0.05). During the first 7 days, the overall mSTT-1 mean was 0.76 wetting/minute. Significant differences between right and left eyes were not observed at any time point (P=1.00). Tear secretion increased significantly, from the 14th to 28th day, in comparison with 7 first days (P<0.05). Positive correlation between maturity parameters and tear secretion was observed (P<0.0001). Distance between slopes of each strip changed significantly (P<0.0001). It was concluded that tear secretion in the neonatal period of cats is very below the reference values described for young and adults of the same species. It is not possible to extrapolate results obtained with mSTT-1 to standard STT-1.

INDEX TERMS: Ophthalmic semiotechnique, standardization, tear production, Schirmer tear test, cats.

INTRODUCTION

The preocular tear film is composed of three layers: one outermost consisted of lipids produced by the tarsal glands...
In cats, no study regarding tear production during the neonatal period has been carried out. Thus, the objectives of the present article were to quantify tear production by means of a modified Schirmer tear test-1 (with standard strips cut in half) in newborn kittens, from the eyelid opening till the end of the neonatal period, and to determine in vitro whether the uptake of saline solution of the cut STT strips correlates with the uptake of the standard STT strips.

**MATERIALS AND METHODS**

This study was approved by the Ethics Committee on Animal Experimentation of the University Center of the Octávio Bastos Foundation Teaching, São João da Boa Vista/SP, Brazil, and followed the ethical guidelines of the Association for Research in Vision and Ophthalmology (ARVO; National Institutes of Health, Publications no.85-23. revised 1985).

Three female (average age of 2 years and mean weight of 3.47±0.44 kg) and one male (average age of 3 years and weighing 4.2 kg) Domestic Short Hair cat, bred in a shelter at the University Center of the Octávio Bastos Foundation Teaching, without history of ocular or systemic illness confirmed after clinical, ophthalmic and hematological evaluation, were enrolled in the study. Once selected, females were kept in individual cages, one close to each other, and received commercial dry food and water *ad libitum*. All of the three females synchronized the oestrus and mating occurred in a natural fashion. After 65 days of pregnancy, two queens delivered kittens within a 12 hour interval; while the third queen delivered 28 hours later (67 days of pregnancy). Fifteen newborn kittens (6 males and 9 females), weighing on birth about 280g were used in the study.

After eyelid opening, basal tear production was assessed in both eyes by means of modified Schirmer tear test-1, with a commercial 5.0mm strip cut in half with a nº 15 scalpel blade, over a clean metal table. Tear production and the weight of kittens were daily measured for 7 days, and at day 14, 21, and 28. The time taken to open the eyelids and its impact over tear secretion was also evaluated. Values of mSST-1 below 1 mm/minute were calculated with a rule, by subtracting the 1 mm standard mark from the moistened part of the strip. All measurements occurred at the same time (5:00pm) and environment, and were performed by the same investigator.

The wetting capacity per minute of the 5.0mm standard strip (n=4) and the 2.5mm cut strip (n=4) were *in vitro* assessed, in order to evaluate the reliability of the modified STT-1 used in our study.

Five different volumes of 0.9% saline solution (3, 5, 7, 9, and 10μL) were applied to the notched end of each strip, and mean values obtained for each tested volume were plotted for statistical analysis.

Data were evaluated by nonparametric or parametric repeated measures, followed by Dunn’s or Tukey multiple comparison post-hoc test, in accordance with normality distribution. Person’s test was used to assess correlation between tear production and maturity parameters. Linear regression models were used to compare the wetting capacity of 5.0 and 2.5mm strips. Significance was set at a value of *P*<0.05 for all analyses.

---

4 Teste de Schirmer, Ophthalmos, São Paulo, SP.
RESULTS

Results are expressed as mean and standard deviation (±SD). Throughout the period of evaluation, the lowest and the highest mean ±SD of the modified STT-1 (mSST-1) were, respectively, 0.60±0.26 wetting/minute and 2.14±1.20 wetting/minute. Means between right and left eyes of each individual did not change significantly, as well as between males and females evaluated at the same time point (>0.05) (Table 1). From day 14 to day 28, average mSTT-1 increased significantly (P<0.001) (Fig.1); however, average mSST-1 among this period did not change significantly (P>0.05).

Mean ±SD eyelid opening time was of 13.73±1.79 days. Tear production did not vary significantly among kittens in which palpebral fissure opened at day 12 (n=7), 14 (n=2), 15 (n=2), and 16 (n=4) (P=1.00) (Fig.2). During the period of evaluation, both age (r²=0.98; P<0.0001) and weight (r²=0.96; P<0.0001) of neonates showed strong positive correlation with mSTT-1 values (Fig.3).

Average wetting capacity per minute between standard (5.0mm) and cut strips (2.5mm) differed significantly with volumes of 5-10μL (P<0.001). Although the wetting capacity per minute of the 2.5mm strip have reached values 44%

Table 1. Descriptive statistics of the modified Schirmer tear test-1 (wetting/minute) between right and left eyes in neonates of cats, in each time point evaluated

<table>
<thead>
<tr>
<th>Days of evaluation</th>
<th>Right eye*</th>
<th>Confident interval</th>
<th>Left eye*</th>
<th>Confident interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.67±0.18</td>
<td>0.10</td>
<td>0.69±0.21</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>0.65±0.22</td>
<td>0.12</td>
<td>0.65±0.18</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>0.60±0.26</td>
<td>0.14</td>
<td>0.70±0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>4</td>
<td>0.74±0.27</td>
<td>0.15</td>
<td>0.70±0.26</td>
<td>0.14</td>
</tr>
<tr>
<td>5</td>
<td>0.78±0.30</td>
<td>0.16</td>
<td>0.78±0.19</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>0.84±0.18</td>
<td>0.10</td>
<td>0.82±0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>7</td>
<td>0.88±0.16</td>
<td>0.08</td>
<td>0.96±0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>14</td>
<td>1.30±0.46</td>
<td>0.25</td>
<td>1.24±0.37</td>
<td>0.20</td>
</tr>
<tr>
<td>21</td>
<td>1.44±0.50</td>
<td>0.28</td>
<td>1.60±0.63</td>
<td>0.35</td>
</tr>
<tr>
<td>28</td>
<td>2.08±1.00</td>
<td>0.55</td>
<td>2.14±1.20</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Dunn’s test (P>0.05).

Fig.1. Mean (±SD) of modified Schirmer tear test-1 (STT-1) in neonates of cats in all time points evaluated (days). **Significant in comparison to other time points (Dunn’s test P<0.001).

Fig.2. Mean* values of the modified Schirmer tear test-1 (SST-1) in neonate cats in regard of eye lid opening day. *Dunn’s test (P>0.05).

Fig.3. (A) Linear regression curves indicating positive correlation between tear secretion (wetting/minute) and age (days), and (B) between tear secretion and weight (grams) in neonate cats.

*Person’s correlation test (r²= 0.98; P<0.0001)a, (r²= 0.96; P<0.0001)b.
The present study showed that in neonate cats, tear production increases significantly after the 14th day of life (P<0.001). However, values of the modified Schirmer tear test-1 (mSTT-1) observed in our study are very below reference values described for the standard Schirmer tear test (SST) in healthy young and adult cats (Cullen et al. 2005a, Lim & Cullen 2005, Lim et al. 2009).

In neonate dogs with ages ranging from 22 to 28 days, average SST-1 and STT-2 values are 13.6±3.07 wetting/min., and 3.63±1.77 wetting/min., respectively (Silva & Galera 2004). In the present study, average mSTT-1 value at day 28 (2.11±0.82 wetting/min.) was very below the SST-1 and STT-2 values observed in dogs of the same age (Silva & Galera 2004). In human beings, only preterms infants secret lower amount of tears in comparison to term infants (Tokter et al. 2002, Akar et al. 2004, Dogru et al. 2004). Considering that at day 15, the average weight of the kittens was of 469.33±138.84 grams (reference values in cats with same age: 240±10g), and that the three queens used in the study labored in periods considered normal for cats (66.00±1.47 days of pregnancy), is reasonable to admit that newborn kittens were not preterms (Johnston et al. 2001, Grundy 2006).

In this research, average time for eyelid opening was of 13.73±1.79 days, which can be assumed as physiological for this species (Grundy 2006). Although at day 28, animals that opened their eyelids at day 14 have shown decreased STT-1 values (1.10±0.28 wetting/min), in comparison to other kittens, in which the eyelid opening occurred at day 12 (2.27±0.78 wetting/min.), 15 (2.35±1.13 wetting/min), and 16 (2.21±0.834 wetting/min.), average values of modified SST-1 did not change significantly throughout the study (P=1.00).

The positive correlation observed between tear production and parameters related to maturity, such as age (r²=0.98; P<0.0001) and weight (r²=0.96; P<0.0001), suggests that anatomical components responsible for tear secretion are not completely developed in newborn kittens. In human beings, two different studies reported that tear production correlates positively with the weight of the babies, but not with the post-conceptional ages of the full term neonates (Tokter et al. 2002, Rohatgi et al. 2005), whereas in another study, the opposite was noted (Akar et al. 2004). The amount of light that reaches the retina and the status of corneal innervation are variables that also contribute to tear secretion (Cullen et al. 2005b). Therefore, the lower corneal sensitivity threshold, in addition to the underdeveloped retina of neonates, may also be associated with the low STT-1 values observed in this study (Lavely 2006, Bedford 2009).

Harley et al. (2005) showed that STT-1 values of dogs increase in the amount of 0.7mm at the afternoon (16:00p.m.), in comparison with values collected in the morning (10:00a.m.). Hence, one may considerate that time of the day did not play an important role over the STT measurements taken in this research, once all the exams were performed at the same time of day (5:00p.m.). Differences between gender were not observed herein (P=0.84), which corroborates with findings of one study where dogs were used (Harley et al. 2006). Regarding morphometric analysis of lacrimal glands, bitches present smaller glands than male dogs (Cabral et al. 2005). However, it is believed that such variable did not interfere in the modified SST-1 values in present study, due to underdeveloped gonades of cats during the neonatal period (Johnston et al. 2001).

Due to small palpebral fissure of neonates, it was decided to cut in half the 5.0mm standard strips to perform

### Table 2. Descriptive statistics of the In vitro wetting capacity per minute between the cut (2.5mm) and the standard strips (5.0mm) after instillation of different volumes (µL) of 0.9% saline solution

<table>
<thead>
<tr>
<th>Saline solution (µL)</th>
<th>Coefficient of variation(%)</th>
<th>0.25mm</th>
<th>Coefficient of variation(%)</th>
<th>5.0mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4.50±0.57*</td>
<td>12.83</td>
<td>2.57±0.43*</td>
<td>18.89</td>
</tr>
<tr>
<td>5</td>
<td>12.50±1.00*</td>
<td>8.00</td>
<td>5.12±0.25*</td>
<td>4.88</td>
</tr>
<tr>
<td>7</td>
<td>18.25±0.95*</td>
<td>5.25</td>
<td>8.47±0.55*</td>
<td>6.49</td>
</tr>
<tr>
<td>9</td>
<td>23.75±0.95*</td>
<td>4.03</td>
<td>11.25±0.86*</td>
<td>7.70</td>
</tr>
<tr>
<td>10</td>
<td>25.50±0.64*</td>
<td>5.06</td>
<td>12.00±0.81*</td>
<td>6.80</td>
</tr>
</tbody>
</table>

*Tukey test (P<0.001), *(P>0.05).
the modified STT-1. From the in vitro study, it can be assumed that the smaller area of the 2.5mm cut strips allowed increased values of STT-1, compared with the 5.0 mm standard strips. For all tested volumes, values regarding the wetting absorbance of the standard 5.0mm strip observed in this study, were a little higher than previous results described in another in vitro study (Williams 2005). Yet, from our in vitro results, it may be speculated that after eyelid separation, the amount of tears accumulated in the conjunctival fornix during the neonatal period of cats is less than 3μL. However, during the experiment with neonates, one cannot be sure what level of tears was lost through the nasolacrimal duct and by tear evaporation.

Anesthetic procedure (Cullen et al. 2005) and instillation of tropicamide (Margadant et al. 2003) deteriorate quantitatively tear secretion in cats. In addition, corneal sequestra, feline herpes virus type 1 (FHV-1), Chlamydophila and Mycoplasma infection may produce qualitative tear film abnormalities, principally because the impaired secretion of mucin, which may be attributed to conjunctival scamous metaplasia, inflammation and a decreased amount of conjunctival goblet cells (Cullen et al. 1999, Lim & Cullen 2005, Lim et al. 2009). In one study, despite the impaired quality of the tear film, assessed by means of tear film break up time and conjunctival biopsies, Lim et al. (2009) showed that STT-1 values increased significantly in young cats inoculated experimentally with FHV-1. Considering our results, further studies evaluating possible correlations between SST-1 and -2 and the stability of the tear film by means of evaluation of tear film break up time in neonate cats are warranted.

In summary, it is concluded that baseline tear production in neonates cats, evaluated by means of the modified Schirmer tear test-1, are really diminished in comparison of values reported in the current literature for adult and young subjects of the same species. Likewise, tear secretion increases in parallel with age and weight, notably 14 days after the eyelid opening. It is not possible to extrapolate results obtained with mSTT-1 to standard STT-1.

REFERENCES