INTRODUCTION

The tear film plays an important role in protecting and maintaining ocular surface health, and its composition is similar for humans and other mammalian species. However, some different aspects in anatomical, biochemical, physiological, and morphological characteristics are commonly found among species. Nevertheless, different species of primates have been used as experimental models for ocular surface assessments, due to the phylogenetic proximity with humans.

Capuchin monkeys (CM) are small neotropical primates that are adapted to anthropomorphic environments, are widely distributed on the American continent, and reproduce readily in captivity. This animal has several similarities to humans, including a high degree of binocular vision, similar fovea size, and similar cone density. CM has been already used as an experimental model for ophthalmic parameters, such as the Schirmer tear test and intraocular pressure; however, there is no report of its use...
in qualitative studies of tear film, which decreases the knowledge of CM tear film and reduces possibilities of using this species for comparative studies.

Considering the need for animal models to be used in the development of new methods, the objective of the present research was to evaluate tear production and its characteristics using meniscometry, tear osmolarity, and tear ferning test (TFT), and to determine the protein profile and biochemistry of tear fluid from CM and humans. Also, the biochemical composition of the two species tear films was assessed and compared.

2 | MATERIAL AND METHODS

2.1 | Humane care guidelines

Use of the animals was approved by the System of Authorization and Information on Biodiversity, Brazilian Ministry of Environment, and by the Ethics Committee on Animal Experimentation of the School of Veterinary Medicine and Zootecny of UFBA (Protocol number 72/2016). All procedures were conducted in accordance with the Association for Research in Vision and Ophthalmology’s Statement for the Use of Animals in Ophthalmic and Vision Research and NIH statement. Human participation was voluntary and adhered to the tenets of the Declaration of Helsinki. The research was approved by the Ethics Committee in Research and Information on Biodiversity, Brazilian Ministry of Environment, and by the Ethics Committee on Animal Experimentation of the School of Veterinary Medicine and Zootechnology of UFBA (Protocol number 2.388.777). Pain and distress were minimized during all the experiment.

2.2 | Species

A total of 11 healthy female CM, aged 5-15 years, were screened in this study. All animals were kept in the Center for Triage of Wild Animals (Salvador, Bahia, Brazil) and housed in an outdoor enclosure. A physical examination was performed by the authorized veterinary staff before the study, and animals that presented any clinical signs of systemic disease or gross abnormalities of the eye or periorcular region were excluded.

In order to validate our data, ten human volunteers (seven women and three men), aged 25-45 years, with normal tear function, were used. A case history and slit-lamp biomicroscopic examination of the ocular surface was performed to determine participant eligibility. Informed consent was obtained from all participants after all procedures had been explained.

Due to the monkey’s breeding characteristics and considering the fact that the animals were maintained in an outdoor enclosure, it was impossible to ensure that these animals could be in a total and complete previous fasting for the collect. In this way, it was considered that these collects were made in a postprandially way and, to ensure a proper comparison with the human’s results, the collect of blood and tear samples in humans was also made not considering a fasting period before collect.

2.3 | Procedures

Collection sequence was the right eye (RE) first and then the left eye (LE). All the CM were chemically restrained before the collect. Immediately after the onset of the anesthetic protocol, the animals were individually transported in wire cages to an experimental laboratory, with temperature and humidity ranging from 22.6 to 27.0°C and 58%-67%, respectively.

For the human volunteers, tests were performed without the use of anesthetic agents. Sampling was performed at a private practice, with controlled temperature and humidity ranging from 26 to 27.3°C and 45%-48%, respectively.

2.4 | Meniscometry tear test

The tip of an I Tear® Test (Imed, Dollard-des-Ormeaux, Canada) was carefully inserted for 5 seconds at the edge of the lower tear meniscus, without touching the eyelid or the ocular surface, and then immediately measured according to Dogru et al. After the procedure, the strip meniscometry test (SMT) value for that eye was determined.

2.5 | Osmolarity test

The tear osmolarity test consists of a single-use test card containing a microchannel held by a pen (i-Pen® Vet; Imed) designed to facilitate tear collection. The device was positioned at a 45° angle to the conjunctival surface of the lower eyelid with a portable reader unit (test card) in contact with the surface, according to the manufacturer’s instructions. Osmolarity results are presented in mOsm/L.

2.6 | TFT

The CM were anesthetized as previously described, and 5-μL tear samples was obtained with a micropipette (Labmate soft®—HTL Labmat, Rio de Janeiro, Brazil) from the lower conjunctival fornix of each eye and deposited in the center of a previously marked circle on a clean glass microscope slide. The tear sample was allowed to air-dry in the experimental laboratory.

Time and images of the crystallization were obtained using a polarized light microscope with 10 × magnification (Microscopio Zeiss, Scope A.1®, Oberkochen, Germany) and a coupled digital camera. Three evaluators with previous training in using the grading scales (veterinarian ophthalmologists from the Federal University of Bahia) classified the ferning images. Tear ferning patterns were classified according to the Rolando and Masmali grading scales.

2.7 | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile and biochemistry of tear film

The electrophoretic profile and biochemical tests were performed using pooled tear films from each species separately, obtained
through centrifugation of the Schirmer strips and kept at −20°C until further processing. The tear samples pool from both species were subjected to one-dimensional SDS-PAGE under denaturing conditions, according to the protocol described by Rebouças et al. Molecular masses were determined according to Kaleidoscope Prestained Standards® (Bio-Rad, Hercules, CA, USA). Protein bands were observed by Coomassie Brilliant Blue staining.

Blood sampling was performed postprandially by venous puncture at the selected sites. Serum was obtained after clotting by centrifugation for 10 minutes at 14 000 g and kept frozen at −20°C. A pooled aliquot per species was used for each quantification.

Quantification of total protein (Thermo Scientific®, Rockford, IL, USA), albumin, urea, glucose, and cholesterol in tears and serum was performed in duplicate using commercially available kits (LabTest®, Belo Horizonte, Brazil) according to the manufacturer’s recommendations. The results were expressed as means of the duplicates, and the values of each analyte concentration at the serum and tear film pooled samples were compared.

### TABLE 1  Median ± semi-interquartile range for SMT, tear osmolarity, and TFT for capuchin monkeys and humans

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CM/RE</th>
<th>CM/LE</th>
<th>Humans/RE</th>
<th>Humans/LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMT (mm/5 s)</td>
<td>7.5 ± 1.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5 ± 1.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5 ± 1.75&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Osmolarity (mOsm/L)</td>
<td>300 ± 9.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>304 ± 9.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>287 ± 8.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>293 ± 6.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFT, Rolando scale</td>
<td>3.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFT, Masmali scale</td>
<td>2.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LE, left eye; RE, right eye; SMT, strip meniscometry test; TFT, tear ferning test.

Different lowercase letters in a same line indicate significant difference between values (P < 0.05) (Mann-Whitney test).

### FIGURE 1  Examples of tear ferning patterns in capuchin monkeys and humans according to the Rolando grading scale.

A, Type I representation: dendritic fern growth is uniform, and there are no spaces between branches in humans. B, Type II in humans: small spaces begin to appear between the stems. C, Type II in capuchin monkeys: small spaces and thick ferns. D, Type III in capuchin monkeys: incomplete crystallization process; single and small formations, and rare or nonexistent branches.

![Examples of tear ferning patterns in capuchin monkeys and humans](image-url)
Statistical analysis was conducted using SPSS version 22.0 software® (IBM, Armonk, NY, USA), and the level of significance was set at 5% ($P < 0.05$). Shapiro-Wilk test was used to test data normality for SMT, osmolarity, and TFT values. Wilcoxon test was used for comparison of the same variables between eyes, and Mann-Whitney test was used to evaluate data between species. Spearman’s test was used to correlate SMT, osmolarity, and TFT grading.

3 | RESULTS

There were no significant differences between the RE and LE for SMT ($P = 0.797$), osmolarity ($P = 0.655$), TFT as defined by the Rolando scale ($P = 0.183$), or the Masmali scale ($P = 0.317$) (Table 1). The mean tear-drying time was $7.11 \pm 2.26$ minutes ($4.08$-$13.18$ minutes). The Spearman test showed no correlation between parameters evaluated.

The analysis of 22 tear images obtained from CM resulted in 13 eyes with Rolando grade type II (59.9%) and 9 eyes with type III (40.1%); 6 eyes were Masmali grade 1 (27.3%) and 16 eyes were grade 2 (72.7%) (Figures 1 and 2).

The SDS-PAGE analysis of the tear films was reproducible and presented bands ranging from 23-217 kDa, as it can be found in Figure 3. Six bands were found; however, some bands were more intense, mainly in the range of 37-55 kDa. Concentrations of total protein, albumin, urea, glucose, and relationship between tear film and blood concentrations of each analyte were detected when evaluating pooled serum and tear film samples, as it can be seen in Table 2.

**FIGURE 2** Examples of tear ferning patterns in capuchin monkeys and humans according to the Masmali grading scale. A, Grade 0 in humans: full crystallization without gaps between the ferns and branches. B, Grade 1 in humans: decreased branch density, and small spaces between them. C, Grade 2 in humans: small branches, sometimes thick and large, with clear gaps between the ferns. D, Grade 1 in capuchin monkeys: appearance of small spaces between ferns, with short and thick branches. E, Grade 2 in capuchin monkeys: spaces and gaps increase, and coarse crystals are formed.
The study of tear film and its components is important due to its ability to determine ocular surface health and to exhibit changes in response to several diseases. Nonhuman primates have been used as experimental models in ocular surface dynamic studies. This study characterized the tear film of a primate species that had not been previously evaluated for use as a possible experimental model for these tests.

Researchers have described the meniscometry method for humans, with a mean reference value for both eyes of $5.5 \pm 1.3 \text{ mm/s}$. Similar to the values found in the LE of human volunteers in this study. The significant difference observed between our volunteers’ RE and LE can be attributed to a tear reflex that occurs when the strip touches the surface of the first eye to be assessed and without the use of anesthetic drop. That situation can be confirmed by the values found for CM, where there was the use of systemic anesthesia before the collection and there was no statistical difference between the two eyes of the same recipient. However, the SMT values found for the CM were significant higher than those found for humans; as there are no reference values for primates nonhumans, it is difficult to perform any kind of assumption, as there is no previous report of SMT use in primates. Raposo et al. found that the use of ketamine and midazolam does not interfere with tear production in CM. Thus, considering the protocol adopted in the present study, the observed values can be considered as normal for the species.

Patients with dry eye have decreased SMT values and therefore, measuring the meniscus length and correlating it with osmolarity in these patients can yield parameters of great importance. In healthy CM, there was no significant correlation between SMT and osmolarity, in contrast to the results for humans, which might have been influenced by the significantly higher values of the LE. Larger studies on tear meniscometry are recommended.

Descriptions of tear osmolarity have previously been reported for dogs, cats, and horses, with differences attributed to microcomponents of the tear film. Osmolarity in the human volunteers represented normal values for healthy eyes. There is no previous report on osmolarity values in CM, and these were statistically

### TABLE 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CM/TF</th>
<th>CM/BS</th>
<th>TF/BS</th>
<th>Humans/TF</th>
<th>Humans/BS</th>
<th>TF/BS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/mL)</td>
<td>4.8</td>
<td>66.3</td>
<td>0.07</td>
<td>8.4</td>
<td>86.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>0.1</td>
<td>2.4</td>
<td>0.04</td>
<td>0.2</td>
<td>3.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>34.9</td>
<td>24.3</td>
<td>1.43</td>
<td>33.1</td>
<td>28.0</td>
<td>1.18</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>20.8</td>
<td>175.4</td>
<td>0.12</td>
<td>89.4</td>
<td>208.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>10.4</td>
<td>126.7</td>
<td>0.08</td>
<td>2.08</td>
<td>240.2</td>
<td>0.008</td>
</tr>
</tbody>
</table>

BS, blood serum; TF, tear film.

Blood sampling was performed postprandially for both species.

### FIGURE 3

SDS-PAGE profile of pooled tear film from humans and capuchin monkeys. Lanes: (1) molecular weight standards, (2) human tear film (50 μg), and (3) capuchin monkey tear film (50 μg). Staining with Coomassie Brilliant Blue. Numbers on the left indicate the molecular mass values for the protein standards.
similar to those of humans in our study, emphasizing the similarity of the two species’ tear films.

Tear ferning grading scales are based on previous studies with humans, and studies assessing TFTs in animals are scarce.2,38,39 To compensate the absence of a specific scale and reference values, two grading scales were taken into account, the Rolando and Masmali scales. In this study, the operators observed differences in human and CM’s tear film samples, as the crystal angulation and thickness, which could be attributed to variations in tear composition, sampling method, and temperature and environmental humidity.12,40,41

Rolando scale types I and II are described as suitable crystallization patterns; Masmali scale grades 0, 1 were obtained in most of the TFTs, particularly in humans. The type III found in CM can be attributed to peculiarities of the species, as healthy animals were used, and this score is normally found in human patients with dry eyes.25 These results are the first to show TFT applicability to CM. However, it is worth mentioning that eyes with normal tear production and with no changes in ophthalmic examination, such as those in the present study, should present crystallization patterns that are considered normal for the proposed scales. The 0.1-point increments among grades, primarily in the Masmali scale, may increase test sensitivity, as has been already proposed for humans.41

One of the biggest difficulties of the study on the biochemical profile of tear films is the small volume of sample that can be obtained in each collect, even more when considering the several measures to be taken in account to avoid contamination and discomfort.17 It must be cited that the collect of great volumes can result in exfoliative lesions of the eye surface and consequent contamination of the samples. As described by Posa et al.,42 the use of a pooled tear film sample can be an instrument for the biochemical characterization of tear films, as it has been doing for proteomic and glycomic analysis; in this way, we chose to use pooled samples for the SDS-PAGE and clinical biochemistry analysis of the CM and human’s serum and tear films.

Tear electrophoretic profile showed bands with similar molecular weight between humans and CM, and similar profiles can be found in other species.5,18,43,44 Bands ranging from 37 to 62 kDa—possibly lactoferrins, lysozyme, immunoglobulins, or prealbumin18,44—were found in higher quantities in humans. The protein profile of CM demonstrated a band of 217 kDa, which might be a high-molecular weight glycoprotein, such as mucin or immunoglobulin,5,18,43,44 that was present in higher quantities than in the human sample. These differences can be attributed to species-specific parameters and can result in different values found for other analysis, such as TFT.11

About 491 proteins have been described in human tears,45 and the total protein values cover a wide range—based on the sampling method—with values ranging from 4 to 9 mg/mL.42,46–48 Thus, the results found in this study for tears and blood serum are within the range of values described in the literature.49,50 Total proteins in the tear films of humans and chimpanzees have been quantified,45 with no difference in the observed values. This differs from our results, in which CM tears had decreased protein amounts.

It is well-known that the tear film is less complex than other body fluids, but the existence of a direct proportion between the concentration of components in the serum and in the tear film is yet unknown.17 The concentrations of the components herein evaluated in serum samples can be considered as normal results when comparing to the previously described reference values for postprandially collected samples of CM51,52 and humans,53–55 including the parameters described by biochemistry assay. The division of the values found in tear film by those found in serum could achieved a normalization of these results, and when this proportions in both species were compared, they showed a very marked similarity, with the exception of total cholesterol.

Albumin content in tears is an important marker of ophthalmic diseases.50,56 A prealbumin fraction that can be found in tears is absent in the blood serum.48,57 Albumin content of tears was lower for both species when compared to the blood serum concentration in this study; concentrations in human tear film in this study were similar to those previously described. However, the total protein and albumin contents can change with the sampling method, as occurs when measuring other proteins.50

The concentrations of urea in tear fluids and blood serum were similar for both species. This is due to the absence of a barrier to this substance in the lacrimal gland. In addition, as described for horses, the epithelial cell metabolism can also alter these values.48,58 Urea has been widely studied in patients with renal dysfunction, and this nitrogenous compound may influence tear osmolarity.59

Normal tear film has low concentrations of glucose.60 However, results are still controversial, due to possible inefficiency of the lacrimal gland barrier, as well as normal variations during circadian rhythm.60,61 In the present study, these results presented high glucose concentrations in the human blood serum and tears, and this situation may be related to the type of diet that the individuals that were included in this study have and the marked difference of human and monkey ingestion of carbohydrates.

Cholesterol is one of the main lipids in tear film and functions to maintain tear film stability. It can also influence other parameters, such as osmolarity and tear breakup time.57,62 However, the mechanism governing the synthesis of cholesterol and other lipids in the lacrimal gland has not been fully elucidated.63 As herein observed, there may be no exact correlation between cholesterol levels in the tear film and blood serum.

5 | CONCLUSIONS

In conclusion, CM possess tear film characteristics that are similar to those of humans and can be considered as potential experimental models for tear film assessment. The differences herein observed must be taken in account when using these animals as models and does not configure any problem for the use of CM in ophthalmic studies and the correlation of results with possible effects in humans. The results of meniscometry, osmolarity, TFT, SDS-PAGE, and tear biochemistry may serve as a reference baseline for CM.
ACKNOWLEDGMENTS

The authors are grateful to Nayone Lima Lantyer Cordeiro de Araújo, Maria Madalena Oliveira, Eunice Andrade, Ariane de Jesus Lacerda, and Thiago Assis Doria Barral from the Federal University of Bahia for technical assistance, and to Fernanda de Azevedo Libório and Josiano Cordeiro Torezani from the Triage Center for Wild Animals for providing health evaluations on the monkeys. A.C. Raposo had a scholarship from Coordination for the Improvement of Higher Level—or Education—Personnell.

ORCID

Ariane Pontes Oríd 
http://orcid.org/0000-0001-5093-6841

REFERENCES


