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Hydrodissection in the Construction of Conjunctival Flaps in Dogs

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ABSTRACT

***Background*:** Hydrodissection is a minimally invasive procedure that consists of injecting fluid into an anatomical space to facilitate dissection during surgery. Although this procedure is employed in several areas of veterinary medicine, including ophthalmology, there are no reports of the use of this maneuver in conjunctival procedures in dogs. The use of this technique can facilitate the construction of conjunctival pedicle flaps, thereby improving the results. The purpose of this work was to evaluate the use of hydrodissection in the construction of conjunctival pedicle flaps in dogs.

***Materials, Methods & Results*:** The sample consisted of 20 eyes from 10 healthy dogs that had been subjected to elective surgical procedures of ovariohysterectomy and orchiectomy. The dogs were divided into 2 groups; the 1st group of 10 eyes underwent hydrodissection and the 2nd group of 10 eyes did not. All the patients underwent a complete ophthalmic examination and assessment of their systemic conditions. The patients were then anesthetized and the procedures were performed under a surgical microscope. In the group subjected to hydrodissection, the conjunctival flap was prepared by means of a previous subconjunctival injection of 0.7 mL of 0.9% sodium chloride, followed by preparation of the flap. In the group without hydrodissection, the flap was prepared by means of conventional divulsion using iris scissors. After producing the conjunctival flaps, a conjunctival fragment was collected from both groups for histological analysis and evaluation of the presence of the Tenon capsule. The operating time, degree of hemorrhage and ease of handling the conjunctiva in the intraoperative period were evaluated. Postoperative evaluations were performed at 1, 7 and 14 days after surgery and included: blepharospasm, conjunctival hyperemia and edema, which were classified as absent, mild, moderate or severe; tear production was evaluated using the Schirmer test, and the appearance of the conjunctival scar was assessed based on photographs taken in the postoperative period, and by a visual analogue scale, with healing classified as fair, good or excellent. The 2 groups showed no statistical difference in terms of operating time, bleeding, ease of handling and conjunctival divulsion. A volume of 0.48 ± 0.12 mL of 0.9% sodium chloride was administered to the conjunctiva. Postoperative assessments of hyperemia, blepharospasm, conjunctival edema, and tear production also did not differ statistically. Conjunctival scarring was considered optimal until the 14th postoperative day, with no statistically significant difference between the 2 groups. These results demonstrate that both maneuvers are effective in creating conjunctival flaps. The Tenon capsule could not be identified in histological stains.

***Discussion*:** The literature offers numerous descriptions of the use of hydrodissection in surgical procedures in humans in order to facilitate dissection and reduce surgical duration and handling, thereby improving the clinical recovery of patients. Conversely, this technique has not been described frequently in veterinary medicine, notably with respect to conjunctival procedures. In this study, we demonstrated that conjunctival hydrodissection was perfectly feasible, contributing to the divulsion and preparation of conjunctival flaps, thus proving to be a viable option for this type of procedure. The absence of the Tenon capsule in the evaluated samples demonstrates that, in both groups, the techniques were effective in separating them from the conjunctiva. It was therefore concluded that the hydrodissection technique is a feasible maneuver in the construction of conjunctival flaps, providing a new option for surgeons, especially for novice ophthalmologists.

Keywords: ophthalmology, corneal ulcer, divulsion, pedicle flaps, conjunctival hydrodissection.

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INTRODUCTION

Hydrodissection is a minimally invasive procedure for injecting fluid into an anatomical space to facilitate dissection during surgery [2,17,19,20]. In veterinary medicine, the hydrodissection technique is applied in ophthalmology, phacoemulsification surgery [1], enucleation [16], removal of a 360-degree conjunctival flap attached to the cornea [9], general surgery, nephrectomy, ear canal [16] and in oncological mastectomy [5].

The conjunctival flap is a surgical technique used for the treatment of deep, chronic, progressive corneal ulcers and descemetoceles [21]. The flap is taken from the bulbar conjunctiva and during its preparation the conjunctiva must be carefully dissected from the Tenon capsule. The inclusion of the capsule in the flap may contribute to surgical failure due to increased tension on the conjunctival graft [11].

Procedures that require additional care in dissection benefit from this maneuver and its use in the construction of conjunctival flaps can be advantageous to facilitate the separation of the conjunctiva from the Tenon capsule. The purpose of this study was to evaluate the use of hydrodissection in the construction of conjunctival flaps in dogs.

MATERIALS AND METHODS

Patients

This study involved the eyes of 10 healthy dogs (20 eyes), male or female, 8 months to 8 years old, which had undergone elective surgical procedures of orchiectomy or ovariohysterectomy at the Veterinary Hospital (HVU) of the State University of Maranhão (UEMA). Inclusion criteria were normal ophthalmic examination, which included the reflex test, Schirmer tear test[1], slit-lamp biomicroscopy[2], indirect binocular[3] and direct monocular ophthalmoscopy[4], applanation tonometry[5], sodium fluorescein staining test[1] and eye fundus examination[3]. After evaluating the tests and confirming their normality, the patients were selected and divided into 2 groups. The systemic conditions of the patients were evaluated based on a physical examination, blood count, study of liver and kidney functions and blood glucose levels.

Groups

The 10 dogs were divided into 2 groups: the right eye (n =10) underwent conjunctival flap construction without hydrodissection (NH), by means of conventional divulsion using iris scissors, while the left eye (n =10) was subjected to conjunctival flap construction by the hydrodissection technique (WH). All the procedures were performed by the same surgeon.

Conjunctival pedicle flap

The patients underwent the routine anesthetic protocol and were placed in the supine position. A commercial aqueous solution of iodopyrrolidone6 was used on the eyelids, and the same solution diluted 1:50 in lactated Ringer's solution[7] was applied to the ocular surface for antisepsis. After antisepsis, anesthetic eye drops containing 1% tetracaine hydrochloride and 0.1% phenylephrine hydrochloride[8] were instilled, after which the surgical field was prepared and the eyelids were held apart with a blepharostat. The procedures were performed under the magnification of an illuminated surgical microscope[9] equipped with microfocus function, and the ocular surface was positioned parallel to the lens. In the group without hydrodissection, the conjunctival incision was made immediately behind the limbus using iris scissors and blunt dissection, forming a pedicle to separate the Tenon capsule and release the flap in a dorsal position. This step in the hydrodissection group was performed by means of a previous subconjunctival injection of 0.7 mL (average) of 0.9% sodium chloride[8] (a sufficient volume to allow the flap to be made), using a 26 G needle and 1 mL syringe, immediately behind the limbus, followed by dissection using iris scissors to prepare the pedicle (Figure 1). At the end of the procedures, a fragment of the pedicle was collected from each group for histopathological analysis. Diclofenac sodium anti-inflammatory eye drops[8] were instilled in both eyes at 6h intervals for 5 days.

Intraoperative evaluation

The operating time of the procedure in each eye was recorded using a stopwatch, starting the count from the moment of hydrodissection in the 1st group and incision in the 2nd group until the flap was made and the conjunctival fragment was collected. The amount of bleeding was assessed by counting sterile swabs used for tamponade in the operative field during the procedure. The ease of handling the conjunctiva was also evaluated, considering the degree of difficulty in conjunctival divulsion and flap preparation.

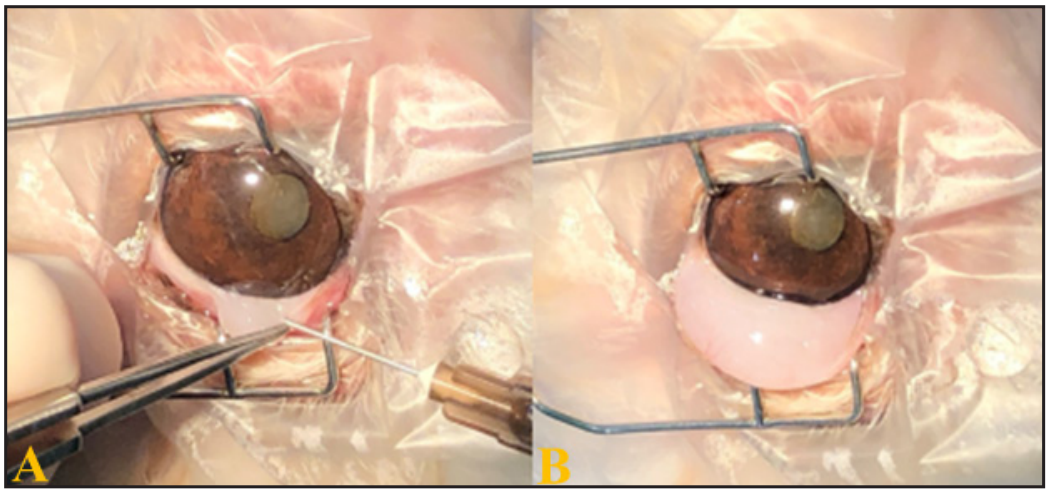

Figure 1. Canine patient, left eye. A- Conjunctival hydrodissection. B- Conjunctival distension after hydrodissection.

Postoperative evaluation

Clinical evaluations began before the surgical procedure (time zero: T0), followed by 24 h (T1), 7 days (T7) and 14 days (T14) after surgery. Blepharospasm, hyperemia and conjunctival edema were evaluated [1], and were classified as absent, mild, moderate or severe. Scarring of the conjunctiva was subjected to a blind assessment by a surgeon and monitored by means of photographs taken postoperatively and based on a visual analogue scale from 1 to 3 (1=excellent, 2=good and 3=fair). The evaluation parameters were: return of the conjunctival epithelium to the limbus region and the formation, or not, of granulation tissue. The Schirmer test was also evaluated at all the times.

Histopathological analysis

During the surgical procedure to create the conjunctival flaps, conjunctival samples were collected for histopathological analysis to ascertain the presence or absence of the Tenon capsule. The samples were fixed in 10% formalin and sent to University HVU pathology lab for processing and embedment in paraffin, according to established protocols. Sections of 5 µm were stained with Gomori trichrome[10], hematoxylin and eosin (H&E)[10] and Sirius Red[10] and analyzed under a light microscope.

Statistical analysis

The parametric data were tabulated and arranged in a 2x4 factorial design of treatments and times and tested for the assumptions of normality of errors and homoscedasticity. Having made these assumptions, the data were subjected to analysis of variance (ANOVA) and the means analyzed by Tukey's test. Qualitative variables were compared using the Wilcoxon test (between treatments) and the Kruskall-Wallis test over time. A statistical significance (*P*-value) of 95% ($P < 0.05$) was considered in all the tests.

RESULTS

No difference was found between the 2 groups in terms of operating time, as indicated in Table 1.

Regarding the bleeding, the median number of swabs used for hemostasis was 0; hence, there was no statistical difference (Table 2) between the groups concerning the number of swabs used to stem bleeding during the creation of the flap.

Postoperative evaluations

The production of tears was assessed based on Schirmer's test, which indicated that this function remained stable throughout the evaluated periods, based on preoperative values. There was no statistical difference between the 2 groups, as indicated in Table 3.

Blepharospasm was absent from the eyes throughout the evaluated period. Both groups showed mild hyperemia on the 1st and 7th postoperative days, which was significantly higher than in the preoperative period. Conjunctival edema was observed in the group without hydrodissection in the immediate postoperative period but was absent from subsequent evaluations. No statistical differences were observed between the 2 groups with respect to these parameters (Table 4).

As for conjunctival scarring, there was no statistical difference between the 2 groups, as indicated in Table 5. The conjunctival epithelium was restored up to the limbus

region during the first 7 postoperative days in each evaluated group, with absence of hyperemia and healing considered optimal up to the 14th postoperative day (Figures 2 & 3). In Phase I, 7 eyes showed the presence of granulation tissue 7 days after the procedure, but they had resolved by the 14th postoperative day without complications. Of the 7 eyes showing the presence of granulation tissue, 5 belonged to the group without hydrodissection.

Histopathological analysis

A total of 20 conjunctival fragments were evaluated: 10 from the NH group and 10 from the WH group. In the histological analysis, the Tenon capsule was not identified in both groups.

These conjunctival fragments, which were taken from healthy patients, and showed a normal histological architecture [13]; therefore, the analysis of the fragments indicated no difference between the WH and NH groups. The conjunctival tissues were composed of stratified squamous epithelium of varying thickness in different areas of the fragment, supported by lamina propria composed of fibroblasts, abundant disorganized extracellular matrix, and blood vessels of varying calibers (Figure 4).

Table 1. Operating time in the 2 evaluated groups.

Group	Variable	Mean	Standard deviation	Coefficient of variation (%)
NH	Operating time	4.67^{A}	1.5	32.1
WH		6^{A}	1.22	20.4

Means followed by the same letters do not differ from each other by Tukey's test at $P > 0.05$. Data with normal distribution of errors by the Cramer-Von Mises test (W=0.05, $P > 0.25$). NH: No hydrodissection; WH: With hydrodissection.

Table 2. Median number of swabs used for hemostasis in the 2 evaluated groups.

Group	Variable	Median
NH	Bleeding	0^{A}
WH		0^{A}

Medians followed by the same letters do not differ from each other by the Wilcoxon at $P = 0.14$. NH: No Hydrodissection; WH: With Hydrodissection.

Table 3. Mean and standard deviation of Schirmer's test values in the 2 groups and at the times evaluated.

Group	Variable	Times (days)				Mean ± sd
		0	1	7	14	
NH	Schirmer test	$20.8^{aA} \pm 3.4$	$17.3^{aA} \pm 3.9$	$20.6^{aA} \pm 2.2$	$17.4^{aA} \pm 2.7$	$19.1^{A} \pm 3.5$
WH		$20.8^{aA} \pm 3$	$18.4^{aA} \pm 4.9$	$19.3^{aA} \pm 2.8$	$19.6^{aA} \pm 2.2$	$19.6^{A} \pm 3.4$

Means followed by the same letters, lowercase on the line and uppercase in the column, do not differ from each other by Tukey's test at $P > 0.05$. Data with normal distribution of errors by the Cramer-Von Mises test (W=0.09, $P = 0.11$). NH: No Hydrodissection; WH: With Hydrodissection.

Table 4. Median values of blepharospasm, hyperemia and conjunctival edema in each group at different evaluation times.

Group	Variable	Times (days)				*P* value
		0	1	7	14	
NH	Blepharospasm	0	0	0	0	1
WH		0	0	0	0	1
NH	Hyperemia	0	1*	1*	0	0.001
WH		0	1*	1*	0	0.001
NH	Conjunctival edema	0	1*	0	0	0.004
WH		0	0	0	0	0.1

*Different medians relative to the initial term (0) by the Kruskal-Wallis Test. There are no differences between treatments according to the Wilcoxon test at $P > 0.05$. NH: No Hydrodissection; WH: With Hydrodissection.

Table 5. Scar appearance of the conjunctiva in the 2 groups and at the evaluated times.

Group	Variable	Times			*P* value
		1	7	14	
NH	Appearance Scarring	2	2	1*	0.002
WH		2	2	1*	0.01

*Different medians relative to the initial term (1) by the Kruskal-Wallis Test. There are no differences between treatments according to the Wilcoxon test at $P > 0.05$. NH: No Hydrodissection; WH: With Hydrodissection.

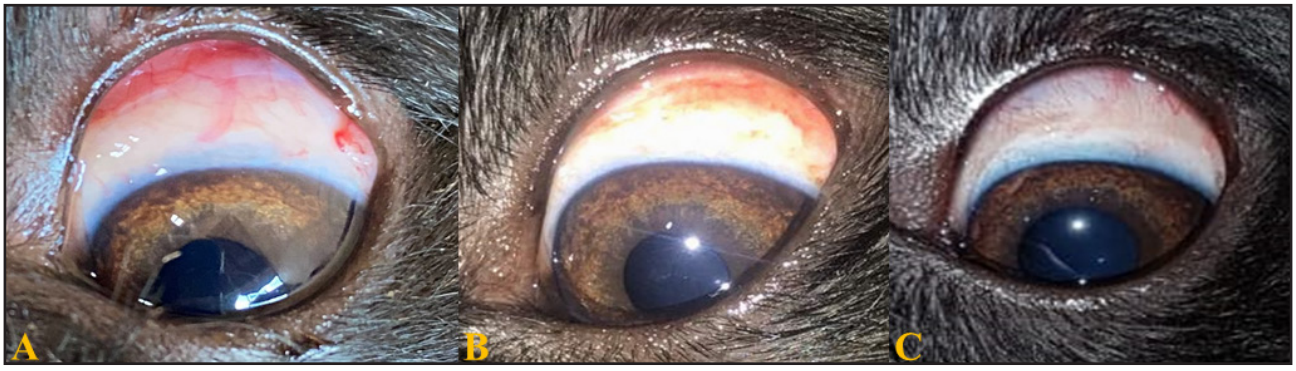

Figure 2. Canine patient, right eye. Conjunctival scarring in a patient in the group without hydrodissection, Phase I. A- 1 day after surgery. B- 7 days after surgery. C- 14 days after surgery.

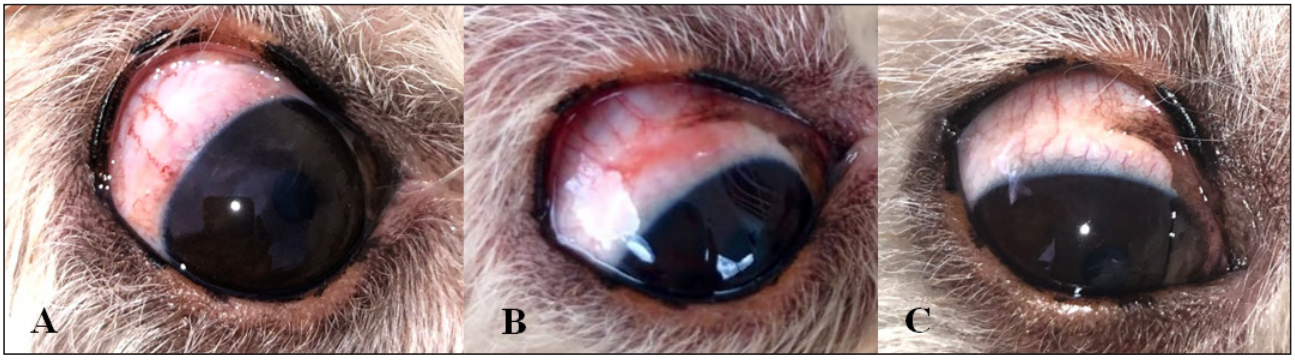

Figure 3. Canine patient, left eye. Conjunctival scarring in a patient in the group with hydrodissection, Phase I. A- 1 day after surgery. B- 7 days after surgery. C- 14 days after surgery.

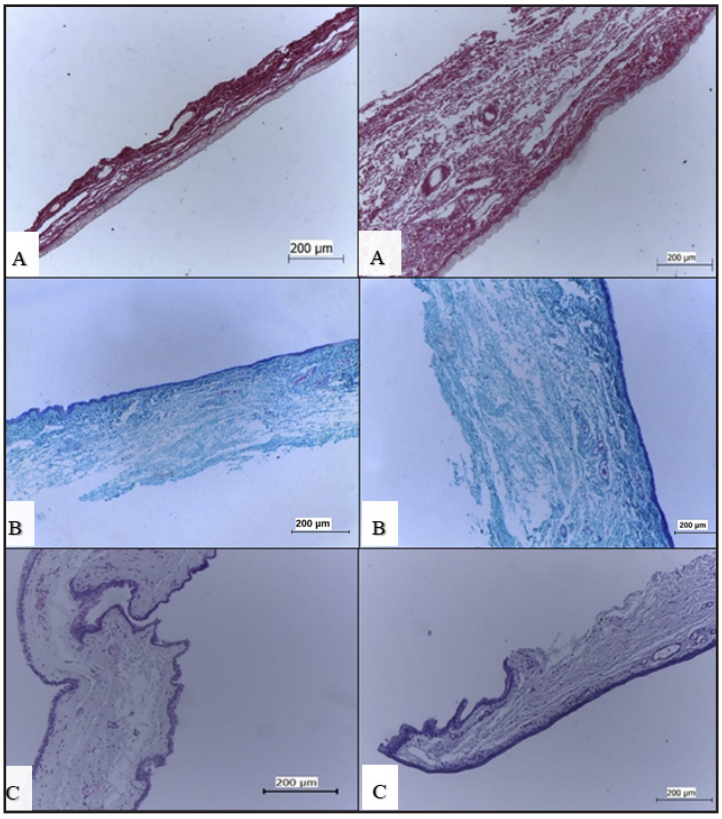

Figure 4. Photomicrograph of the bulbar conjunctiva of healthy dogs evaluated based on different histological stains. Note the presence of 3 to 5 layers of stratified squamous epithelium, followed by loose connective tissue and blood vessels. A- Sirius Red. B- Gomori Trichrome. C- Hematoxylin and Eosin. Left side: NH, and Right side: WH.

DISCUSSION

This study demonstrated that the use of conjunctival hydrodissection was easily feasible, contributing to the divulsion and preparation of the conjunctival flap, and that it is a practicable alternative for this type of procedure. The use of hydrodissection in surgical procedures on humans facilitates dissection, reducing the operating time, involving less handling, and improving the clinical outcome of patients [2,17,19,20]. However, the application of this maneuver in veterinary medicine is rarely described, especially with respect to conjunctival procedures. Hydrodissection was used to remove a conjunctival cyst from a human patient and the authors reported that the technique is useful to dissect the conjunctiva [12].

As for operating time, there was no difference between the groups, demonstrating that the two procedures are effective and did not affect this duration. Operating time is an important parameter to be analyzed in surgical procedures. A systematic review revealed that longer operating times contribute significantly to the risk of complications, particularly infection [3]. However, the use of hydrodissection to make a conjunctival flap did not change the operating time in the two phases. Some factors may contribute to extend operating times, such as preoperative planning, surgeon experience, fatigue, and others [6]. Nevertheless, it should be noted that the procedures were performed by the same surgeon, who is a graduate of the Brazilian College of Veterinary Ophthalmology, whose learning curve for this technique has stabilized, thus minimizing this type of influence on the results of this research.

On the other hand, other procedures have benefited from the use of hydrodissection with respect to time. The effectiveness of hydrodissection was evaluated in performing face lipoplasty in humans and a significant difference was observed in operating time ($P < 0.01$), with a time of 8:18 ± 0:47 min in the treated group and of 14:08 ± 2:28 min in the control group [20]. The operating time in women undergoing mastectomy using hydrodissection was also lower compared to that of standard mastectomy [18]. In longer or more complex procedures, hydrodissection can facilitate dieresis maneuvers, contributing to reduce the final operating time, although when used for simpler and faster procedures such as the conjunctival flap, it does not offer any additional benefit for this parameter.

Intraoperative bleeding was not significant and there was no statistical difference between the groups. This finding indicates that hydrodissection did not reduce bleeding when compared to the traditional conjunctival flap preparation technique. Bleeding is a parameter commonly evaluated in research involving hydrodissection. Intraoperative bleeding in humans undergoing face lipoplasty was also not significantly different between groups, as all patients had less than 5 mL, equivalent to 1 gauze, of intraoperative bleeding [20].

From the surgeon's point of view, creating the conjunctival flap was easy in all the evaluated groups, indicating that both maneuvers are effective for handling the conjunctiva.

The volume of liquid used in hydrodissection varies considerably. The literature reports different volumes depending on the medical specialty. For example, face lipoplasty for buccal fat pad reduction was facilitated by using 15 mL of a solution composed of 250 mL of saline with epinephrine and lidocaine [20]. An average volume of 410 mL of saline was used to facilitate the dissection of hepatic tumors of the inferior vena cava in humans [10]. The volume used in this study was much smaller than those described in the literature, since it involved the anatomy of the conjunctiva, so the tissue received a maximum of 0.7 mL. Subconjunctival drug delivery is similar to conjunctival hydrodissection, with volumes similar to those described for this route of drug administration. A volume of 0.15 mL of liposomes containing Rapamycin was used subconjunctivally in dogs with keratoconjunctivitis sicca [8]. Dexamethasone and Gentamicin (0.3 mL of each drug) were administered subconjunctivally to dogs with chronic glaucoma [4]. A dose of 0.2 mL of autologous platelet-rich plasma was used to treat refractory corneal ulcer [22]. The volumes cited in the described studies were similar to those used in this work.

Based on the evaluated data, tear production showed no statistical difference between the groups, demonstrating that hydrodissection did not affect this function in the patients of this research. The flap is produced from a band of conjunctiva, so tear production could be affected. Therefore, the Schirmer test was performed to assess the variation in tear production in the patients. Tear production is influenced by the conjunctiva because its internal structure contains goblet cells, which are responsible for producing the mucous portion of the precorneal tear film [7,15].

Conjunctival hydrodissection did not affect ocular comfort and the clinical parameters of inflammation evaluated here, such as blepharospasm, hyperemia and conjunctival edema, remained similar and self-limiting, with no statistical difference between the evaluated groups. This indicates that neither of the maneuvers caused important clinical consequences.

The healing aspect of the conjunctiva was considered excellent in the patients of this study, demonstrating that despite the injury generated in the conjunctiva by producing the flap, healing was satisfactory and no complications were observed. Because the conjunctival tissue has a high capacity for wound repair, conjunctival injuries heal naturally [15], as was observed in this study. Granulation tissue was identified in 7 eyes 7 days after the procedure, which may be attributed to an individual immunological reaction of these patients. These patients were not given additional care to treat the granulation tissue, and by postoperative day 14, all had been resolved.

In dogs, the Tenon capsule is connective tissue located on the external aspect of the sclera. It is separated from the sclera by a narrow space filled with loose connective tissue called Tenon's space. This capsule, which is attached to the sclera near the corneoscleral junction and becomes continuous with the fascia that surrounds the extraocular muscles, consists of small compact bundles of collagen that lie parallel to the surface of the episclera [14].

This structure plays an important role in conjunctival flap surgery, as it must be separated by conjunctival dissection in order to avoid scar retraction, which can lead to rupture or loosening of the flap [21]. In dogs, the bulbar conjunctiva, the Tenon capsule and the sclera are firmly joined approximately 3 mm from the limbus [14]. Therefore, careful dissection is essential to separate the Tenon capsule from the conjunctiva. To ensure adequate thickness of the pedicle, the blades of the scissors must remain visible under the thin released bulbar conjunctiva [21].

The conjunctival fragments evaluated in this work did not show the presence of the Tenon capsule, indicating that both the techniques used here are effective in separating it from the conjunctiva and that they provided adequate divulsion of this structure. We were unable to find any studies in the literature specifically characterizing the Tenon capsule in dogs, and to the best of our knowledge, this is the first study aimed at identifying this structure and describing its importance in conjunctival flap construction. Furthermore, there are no reports of pathological processes associated with the Tenon capsule in dogs [11].

CONCLUSIONS

We conclude that hydrodissection is an easy procedure that can optimize conjunctival flap construction, providing surgeons with a new option, especially for novice ophthalmologists. Clinical phase 2 trials are needed to assess its effects and complement the study.

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***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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