



Histomorphological evaluation of thermal injury following palatoplasty performed with CO₂ laser or LigaSure device in dogs with brachycephalic obstructive airway syndrome

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ABSTRACT

The elongated soft palate is an abnormality that characterizes most brachycephalic dogs and contributes to the brachycephalic obstructive airway syndrome (BOAS). Palatoplasty is routinely performed in brachycephalic dogs; several surgical techniques exist. The use of surgical instruments such as monopolar electrocoagulation, CO₂ or diode laser, bipolar vessel sealing device and harmonic shears has become routine to reduce the operating time, the intraoperative risk of bleeding and the postoperative oedema. This prospective study aimed to compare the histomorphological effect of a CO₂ laser and LigaSure device in palates of dogs undergoing palatoplasty. Twenty owned brachycephalic dogs were included, 10 palatoplasties were performed using CO₂ laser and 10 using LigaSure™ device. The dogs were positioned in sternal recumbency. A transoral approach was performed: the elongated soft palate was grasped with Allis forceps and brought rostrally, the palatoplasty was performed using the tonsillar crypts as anatomical landmarks. Surgical specimens were routinely fixed in 10 % formalin. Two sections perpendicular to the surgical margins were trimmed from each sample, paraffin-embedded and stained with hematoxylin and eosin (H&E). Tissue damage induced by the two types of surgical devices was graded (1–4, from minimal to severe) and the depth of thermal injury measured in µm on captured images (using an image analysis program - ImageJ). Mean values and standard deviations (SD) were calculated based on six measurements for each sample.

The tissue damage was graded 3.7 ± 0.48 in group LigaSure™ and 2.8 ± 1 in group Laser. The mean depth of thermal injury was 874.94 ± 184.92 µm in the LigaSure™ group and 451.76 ± 137.86 µm in the Laser group. The comparison between the two groups showed significant lower grade and extension of thermal injury in the palate samples obtained with CO₂ laser ($p < 0.05$).

Additionally, there is a lack of literature that correlates the histological changes with the clinical outcomes of the different palatoplasty methods in brachycephalic dogs. By comparing histological changes and clinical outcomes, we aim to provide valuable insights for optimizing the surgical approach for palatoplasty in brachycephalic dogs, ultimately improving postoperative outcomes for these patients.

Introduction

Brachycephalic Obstructive Airway Syndrome (BOAS) is a complex and debilitating respiratory disorder primarily affecting certain canine

and feline breeds characterized by brachycephalic conformation (Dupré and Heidenreich, 2016; Riecks et al., 2007; Torrez and Hunt*, 2006). This syndrome encompasses a multitude of anatomical abnormalities, including stenotic nares, elongated and thickened soft palate, aberrant

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nasopharyngeal turbinates, enlarged or everted tonsils, macroglossia, hypoplastic trachea, everted laryngeal saccules and, laryngeal collapse collectively resulting in chronic upper airway obstruction (Dupré and Heidenreich, 2016; Packer and Tivers, 2015; Tamburro et al., 2019; Torrez and Hunt*, 2006). Affected animals exhibit clinical signs of respiratory distress, exercise and, heat intolerance, compromising their overall quality of life (Lilja-Maula et al., 2017; Riggs et al., 2019). BOAS represents a critical concern in veterinary medicine, necessitating multidisciplinary clinical management, including surgical corrective procedures, to enhance the quality of life and well-being of affected animals.

The surgical correction of BOAS focuses on addressing the primary anatomical abnormalities responsible for upper airway obstruction, thereby enhancing respiratory function and improving the affected animal's overall quality of life. Stenotic nares are one of the hallmarks of BOAS (Dickerson et al., 2020). Surgical correction involves the resection of a portion of the alar fold, thereby widening the nostrils. This procedure, known as rhinoplasty, aims to reduce airflow resistance during inspiration (Franklin et al., 2024). Elongated soft palates play a crucial role in causing airway obstruction in BOAS. Palatoplasty involves partial resection of the soft palate, thereby preventing its interference with the airflow during breathing (Dickerson et al., 2020). The traditional surgical approach to palatoplasty involves scissor cutting and joining the oropharyngeal and nasopharyngeal mucosa by sutures. Over the past decade, much interest has been focused on the use of electrosurgical tools for palatoplasty. Various methods, including monopolar electrocoagulation, CO₂ and diode laser, bipolar vessel sealing devices, and air plasma-mediated bipolar radiofrequency ablation, Harmonic technology, have been explored (Brdecka et al., 2007; Conte et al., 2022; Davidson et al., 2001; Tamburro et al., 2019). Histological evaluations have been previously conducted to assess soft palate damage resulting from different electrosurgical techniques, to assess tissue damage and compare the effectiveness of the different techniques (Conte et al., 2022; Tamburro et al., 2019). A CO₂ laser instrument and a LigaSure™ device were compared in an experimental model in 2007 and they found a similar depth of thermal damage 96 hours after surgery. However, no study comparing histological changes during palatoplasty procedures in brachycephalic patients has ever been reported with the instruments mentioned above. LigaSure™ technology combines bipolar radiofrequency (RF) energy with pressure to effectively seal blood vessels and soft tissues, minimizing bleeding and promoting efficient tissue fusion (Driessen et al., 2023). Carbon dioxide (CO₂) surgical lasers emit a focused beam of infrared light at a wavelength of 10,600 nanometers. The CO₂ laser works based on the principle of tissues absorbing infrared light. The energy from the laser beam is absorbed by water in the tissues, leading to tissue separation by vaporisation allowing precise tissue removal or ablation (Paczuska et al., 2014). The techniques and instruments used for palatoplasty were compared in previous studies but the results have not yet demonstrated the ideal instrument for performing palatoplasty (Conte et al., 2022; Davidson et al., 2001; Dunié-Mérigot et al., 2010). To the authors' knowledge, there are no studies comparing the use of LigaSure™ and CO₂ laser in brachycephalic breeds. The aim of the study was to compare the histomorphological characteristics of palate specimens excised by the aid of either CO₂ laser or LigaSure™.

Materials and methods

Animals

The study protocol was approved by the University of Teramo Ethic Committee (Approval number, 22374; Approval date, 19/07/2023). Twenty dogs were included as reported in a previous study (Tamburro et al., 2019). A written informed consent was obtained from all dog owners. Brachycephalic dogs presented to the Small Animal Teaching Hospital of the University of Teramo with the diagnosis of BOAS, and for

endoscopic examination, rhinoplasty and caudal palatoplasty. Prior to surgery, all dogs underwent a complete upper airway and digestive endoscopic examination to evaluate congenital and acquired anomalies of the upper airway and stomach and duodenum. Thoracic radiography was performed to evaluate tracheal hypoplasia and intrathoracic structures. Dogs were randomly assigned into two equally numbered groups: in one, group A, palatoplasty was performed with a CO₂ laser model, ML020-CA (Shanghai Wonderful Opto-Electrics Tech. Co., LTD, Shanghai, China) and in the other (group B), a LigaSure™ Curved Jaw Open Sealer/Divider (Medtronic, Dublin, Ireland) device was employed.

Surgical technique

The dogs were sedated with methadone (0.2 mg/kg, IV) and dexmedetomidine (0.001 mg/kg, IV) and anesthesia was induced with propofol (4 mg/kg, IV). A single dose of maropitant (1 mg/kg, IV) was administered before starting complete upper airway examinations. Following this, the dogs were intubated to perform gastro-duodenoscopy.

Thereafter, the dogs were transferred to the operating room, dogs were positioned in sternal recumbency with the mouth opened. The oral cavity was aseptically prepared with a 0.05 % chlorhexidine diacetate solution. An intraoral approach was used to perform palatoplasty. The mid portion of the soft palate was grasped with Allis tissue forceps and retracted rostrally. The caudal border of the tonsillar crypt was used as reference point for the extent of palatoplasty. In group A (laser group), wet surgical swabs, as previously recommended (Davidson et al., 2001), were positioned behind the soft palate and surrounding the endotracheal tube. This aimed to absorb laser energy, preventing unintentional laser exposure to tissues in the posterior oropharynx, and to mitigate the risk of combustion. For the resection procedure, the laser is configured to operate at 12–15 watts in the continuous wave, Ultra Pulse mode. In group B, the LigaSure™ was used, the jaws of the instrument were positioned to fully encompass the soft palate. The procedure involved advancing and activating the device on setting 2 as needed to excise the planned elongated portion of the soft palate. Laryngeal sacculectomy and tonsillectomy were performed in patients if deemed necessary by the surgeon.

In both groups, after the surgical procedure, 0.1 ml of dexamethasone (2 mg/ml) was injected into the center of the soft palate cut surface to reduce postoperative oedema and pain (Abdel-Aziz et al., 2012; Kerekhanjanarong et al., 2001; Liu and Su, 1996); the oral cavity was observed for few minutes to ensure absence of bleeding. Resected soft palate tissue samples were fixed in 10 % formalin solution.

All dogs underwent correction of stenotic nostrils by vertical wedge rhinoplasty. Times to complete palatoplasty were recorded (from the palate grasp to completion of soft palate surgery), as well as any intra/post-operative complications.

Information about complications was collected during surgery and in the 24-hour post-surgical period. Major complication required surgical revision and included mild/severe bleeding, severe oedema (causing dyspnea) and insufficient palate removal. Minor complication did not require surgical revision and included self-limiting hemorrhage or mild oedema (without respiratory effort).

Postoperative management

All dogs were admitted to the intensive care unit after surgery. Specifically, all dogs received methadone (0.2 mg/kg, IM), an injection of dexamethasone (0.1 mg/kg IV), and lactated Ringer's solution intravenously.

During hospitalization, vital parameters were monitored, focusing on respiratory pattern, frequency, and effort. Dogs without signs of regurgitation/vomiting, respiratory distress, or those that did not require administration of sedatives to reduce respiratory effort, were fed with small meals and soft meat balls six hours after surgery.

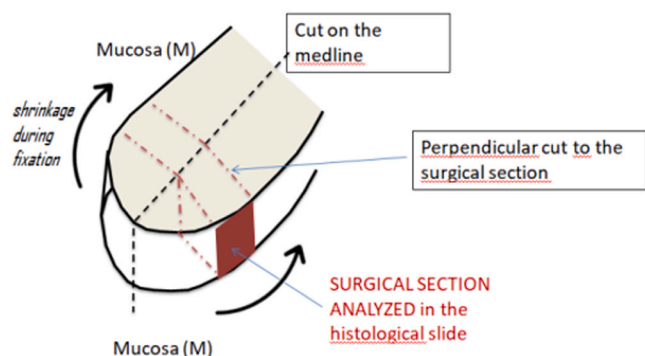


Fig. 1. Representative illustration of sample preparation for histological examination.

Prednisolone 1 mg/kg once daily for 2–3 days and omeprazole 1 mg/kg once daily for 7 days were administered. Dogs were discharged 24/48 hours after surgery.

Histological assessment

Surgical specimens were fixed in 10 % formalin, only cases that shown minimal shrinkage after fixation were included in the present work. From each fixed sample, two sections perpendicular to the surgical margins were trimmed following the scheme indicated in the drawing (Fig. 1). All the samples were processed at the same time to reduce differences in the processing procedure among samples. Paraffin-embedded; 4µm sections were stained with hematoxylin and eosin (H&E). Tissue damage on the cut border, induced by the two types of surgical devices, was evaluated by two board-certified pathologists (L. B., L.D.S.), blinded as to the method of surgery, and graded based on the

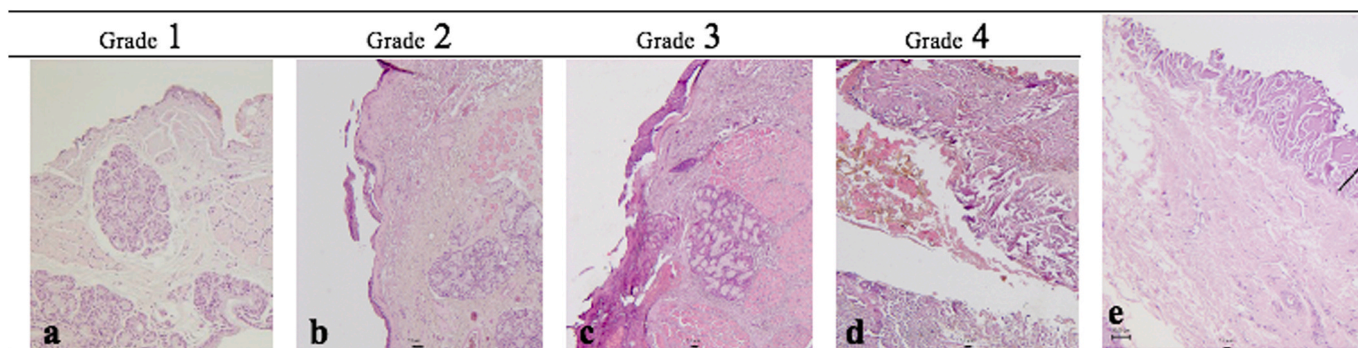


Fig. 2. a-d: Representative pictures for the histological grading applied; severity, extension and depth of the thermal injury was considered in both the tissue sections on the slides for each case, considering the area of the tissue covered by lesions, using four ranges of percentage: a) Grade 1: minimal extent and depth of the lesions (<1 %); b) grade 2: mild extent and depth of the lesions (1–5 %); c) grade 3: moderate extent and depth of the lesions (6–10 %); d) grade 4: severe extent and depth of the lesions (11–15 %). e: representative pictures of method applied for the measurement of the damaged tissue on the incision margins.

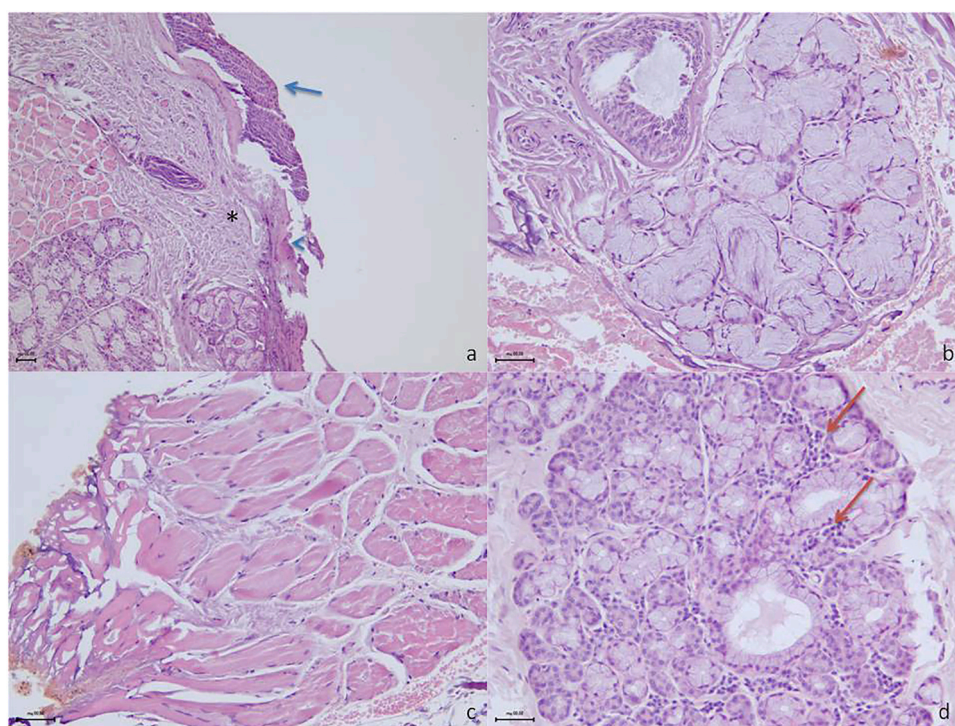


Fig. 3. a) Separation (arrow) and coagulative necrosis (arrowhead) of the oral epithelium and collagen denaturation of the connective tissue at the incision margins (asterisk). Degeneration and necrosis of the striated muscle (b) and glands along (c) the margins. d) Background lesions, characterized by mild, multifocal chronic inflammatory infiltrate, with lymphocytes and plasma cells in the glandular interstitial tissue (arrows).

Table 1
Demographic and surgical data of the two groups.

	Dog	Age (years)	Body weight (kg)	Breed	Sex	Surgical time (minutes)	Complications
Laser	1	4	9	French bulldog	NF	27	no
	2	5	26	English bulldog	NM	22	no
	3	1	12	French bulldog	F	25	no
	4	11	8	Pug	M	32	no
	5	1	10	French bulldog	M	33	no
	6	1	10,5	French bulldog	NF	35	no
	7	2	11	French bulldog	NF	28	no
	8	1	9	French bulldog	F	26	no
	9	4	30	American Bulldog	NM	28	no
	10	6	25	Boxer	NM	30	no
Ligasure	1	2	12	French bulldog	F	33	no
	2	3	23	English bulldog	M	34	no
	3	2	25	English bulldog	NF	30	no
	4	3	10	French bulldog	M	28	no
	5	1	11	French bulldog	NF	32	no
	6	1	11	French bulldog	NF	32	no
	7	2	9	Pug	M	28	no
	8	4	13	French bulldog	NM	28	no
	9	3	29	English bulldog	NM	36	no
	10	3	12	French bulldog	NF	35	no

evaluation of the severity, depth and extent (defined as the area of the tissue sample analysed covered by the lesions and expressed as a range of percentage) of the thermal injury (including detachment of the epithelium from the basal membrane, epithelial coagulative necrosis and collagen denaturation); the following grading was applied: Grade 1: minimal extent and depth of the lesions (<1 %); Grade 2: mild extent and depth of the lesions (1–5 %); Grade 3: moderate extent and depth of the lesions (6–10 %); Grade 4: severe extent and depth of the lesions (11–15 %) (Fig. 2, a,b,c,d). For each slide, three digital images from distinct fields (10x magnification) were captured. Using an image analysis program (ImageJ), the depth of thermal injury induced by laser or LigaSure™ surgical devices was measured in µm (Fig. 2, e) according to previously published methods (Tamburro et al., 2019). Mean values and standard deviations of the mean (SD) were calculated based on six measurements for each sample.

Statistical analysis

All data were tested for normality using commercial software (Jamovi, version 2.3) with the Shapiro-Wilk W test. Normally distributed data were statistically analyzed by Student’s t-test and considered statistically significant for values of P < 0.05.

Results

Twenty brachycephalic dogs were included in the study: twelve were French bulldogs, four English bulldogs, two Pugs, one American Bulldog, and one Boxer.

Of them, 5 were entire males, 5 neutered males, 3 were entire females and 7 were spayed. All dogs underwent elongated soft palate correction and rhinoplasty divided in two groups as described above.

Mean body weight was 15,05± 8,4 kg in group A and 15,5± 7.25 kg in group B; mean age was 3.6± 3,2 years in group A and 2.4 ± 1 years in group B; mean surgical time was 28,6±3.95 minutes in group A and 31,6 ± 3 minutes in group B. No intraoperative or postoperative complications occurred in either group.

No statistical difference in demographic data was present between the two groups.

Histological evaluation

In all soft palate samples, indications of thermal injury were noted along the surgical incision margins induced by both devices. As previously described, various epithelial modifications were detected at the

boundaries of the surgical incisions, ranging from individual cell detachment to complete separation of the epithelium from the basal membrane, culminating in epithelial coagulative necrosis (Fig. 3, a). The connective tissue at the incision margins displayed a thick and dense eosinophilic layer in H&E stained slides, characterized by collagen denaturation (Fig. 3, a), interstitial edema, and coagulative necrosis affecting both striated muscle (Fig. 3, b) and glands along the margins (Fig. 3, c). Muscle damage was characterized by fragmentation and hypereosinophilia of the sarcoplasm. Table 1

The thermal tissue damage was graded as 2.8±1 in group A and 3.7 ±0.48 in group B. The mean depth of tissue damage was 451,76±137,86 µm in group A and 874.94±184.92 µm in group B (Table 2). The comparison between the two groups showed significant lower grade, less extension and less depth of thermal injury in the palate samples obtained by CO2 laser transection compared to the samples obtained by Liga-Sure™ (p<0.05).

Background lesions, referring to chronic inflammation, were also reported, as multifocal small lympho-plasmacytic aggregates in the interstitial tissue of nasal and oral glands (Fig. 3, d).

Discussion

Palatoplasty holds significant importance in mitigating respiratory challenges associated with elongated soft palates, a common anatomical anomaly in brachycephalic dogs. The microscopic structure of the soft palate in brachycephalic dogs consists of an increased degeneration and muscular necrosis; there is a decreased proportion of muscle fibers and an augmented presence of stromal collagen and glandular tissue compared to mesaticephalic dogs, leading to an increase in thickness (Arrighi et al., 2011; Crosse et al., 2015; Pichetto et al., 2011).

Palatoplasty aims to alleviate airway obstruction by excising the elongated portion of the soft palate, the procedure improves breathing efficiency, enhances overall respiratory function, reduces frequency of regurgitation and vomiting (Seneviratne et al., 2020). The importance of palatoplasty is underscored by its potential to alleviate symptoms of obstructive sleep apnea, reduce snoring, and enhance the quality of life for affected dogs. In the realm of surgical options, various techniques and tools are employed, ranging from traditional methods involving scissors and suturing to more advanced approaches utilizing laser technology, such as Diode or CO2 lasers or bipolar or ultrasonic devices (Conte et al., 2022; Gilman et al., 2023; Holloway et al., 2022; Kirsch et al., 2019; Miller et al., 2024). Each option presents unique benefits and considerations, and the choice of the surgical method depends on factors such as the severity of the condition, the dog’s overall health, and

Table 2

Legend: average length of the 6 measurements of the depth of thermal injury for each case; Grade of the thermal tissue injury (extent and depth, 1: minimal; 2: mild; 3: moderate; 4: severe).

Lenght of thermal lesions	Average length per case (µm)	GRADE of the thermal lesions (1–4)
Laser		
603,664	511,424	4
366,059		
827,625		
483,958		
381,108		
406,131		
221,046	370,189	4
469,814		
419,073		
311,945		
358,602		
440,656		
380,049	362,847	3
172,441		
298,45		
228,803		
515,227		
582,112		
1085,299	609,403	4
742,741		
453,85		
400,373		
586,522		
387,633		
157,13	348,523	1–2
154,976		
241,796		
416,03		
840,416		
280,792		
281,125	338,918	1–2
492,655		
515,615		
300,771		
69,43		
373,911		
258,48	504,239	2
252,275		
1023,622		
482,577		
635,214		
373,263		
487,917	706,938	3
391,398		
519,724		
811,611		
983,47		
1047,509		
758,539	499,305	2
290,174		
383,131		
634,382		
499,3		
430,301		
254,55	266,835	3
349,777		
332,178		
213,55		
217,576		
233,378		
Ligasure		
508,994	707,047	3
859,833		
632,746		
842,972		
713,823		
683,913		
337,621	738,712	4
557,707		
428,571		

Table 2 (continued)

Lenght of thermal lesions	Average length per case (µm)	GRADE of the thermal lesions (1–4)
1039,606		
1033,876		
1034,888		
453,678	644,124	3
1031,489		
216,195		
268,866		
968,547		
925,967		
1099,517	755,09	3
1172,79		
779,691		
574,052		
548,738		
355,751		
620,06	921,281	4
1174,547		
902,813		
880,506		
936,34		
1013,418		
338,466	703,33	4
439,94		
833,72		
953,265		
618,248		
1036,338		
801,02	1018,477	4
961,24		
1145,557		
963,431		
1426,99		
812,623		
618,552	1028,794	4
562,708		
1295,851		
1338,713		
1138,27		
1218,667		
1247,567	1138,729	4
996,274		
1018,622		
1057,217		
1274,401		
1238,291		
1307,494	1093,808	4
1126,228		
1156,540		
1012,977		
1003,875		
955,736		

the surgeon’s preference and expertise (Jones and Kennedy, 2024). The introduction of new techniques to replace scissors and suturing, results in better hemostasis, reduced oedema and considerably less surgical time (Davidson et al., 2001; Dunié-Mérigot et al., 2010; Michelsen, 2011).

The choice of surgical technique, particularly between conventional methods and advanced technologies, presents advantages but merits considerations: traditional palatoplasty with a scissor may pose challenges in achieving precise tissue removal and hemostasis (Conte et al., 2022). On the other hand, the precision of laser cutting technology facilitates fine incisions, promoting more efficient hemostasis but creates more damage to the surrounding tissue due to thermal spread (Tamburro et al., 2019). Additionally, the reduced risk of bleeding and potential for decreased postoperative discomfort stand out as significant benefits (Jones and Kennedy, 2024; Kirsch et al., 2019). However, it is essential to acknowledge that laser technology demands specialized training and equipment, and the associated costs may need to be considered (Bhattani et al., 2019). Furthermore, comparative studies exploring outcomes, complication rates, and long-term efficacy between

traditional scissor and cutting palatoplasty and advanced technological approaches are crucial to inform evidence-based decision-making in veterinary practice.

The first report of histological evaluation of excised soft palates dates from 2007 on experimental models, palate samples of normal dogs were obtained during surgery and 48 or 96 hours after procedure using LigaSure™ device and CO₂ laser (Brdecka et al., 2007). The LigaSure™ group required less surgical time compared to the Laser group. The average depths of maximum tissue injury at 48 hours were 4.7 ± 1.2 mm for the LigaSure™ and 5.3 ± 1.5 mm for the CO₂ laser. The results of this study showed a reduced thermal damage with LigaSure™; however, the zone of thermal injury was similar between the two groups. This could be the result of technological evolution of the instruments employed in our study. Additionally, in the cited study, at 96 hours post-surgery, palates resected by both methods exhibited comparable histopathological changes and intraoperative and postoperative complications were not reported.

In 2019, samples of palates obtained from palatoplasty performed with Airplasma® device and a diode laser were analyzed and no significant differences were observed in the mean depth of thermal injury or surgical time but two dogs from air plasma group presented a major complication (Tamburro et al., 2019). In the current study no significant difference in surgical time was observed nor in complications rate, but depth and grade of thermal injury in excised palate samples was greater in the LigaSure™ group. In the laser group, two samples were graded “1–2” because they showed a degree of lesions in between samples graded as 1 and 2. Pathologist decided to not create 4 grading groups in order to not change the grading system already applied by Tamburro et al., 2019 and be consistent.

A recent study compared clinical effects and histological changes of palates obtained with Harmonic shears scalpel (HSS), laser diode (DLS) and scalpel and scissors (SIS) (Conte et al., 2022). The HSS was the faster technique, SIS was associated with bleeding and required more time to complete surgery. Lateral spread of tissue damage was significantly higher in HSS group and DLS compared with SIS. HSS caused more structural damage to connective tissue, muscle and salivary gland tissue but less oedema. Comparing these results with our study, the Harmonic technology appears superior to both Laser and LigaSure™ but a comparison between Laser and Harmonic technology using the same sample preparation and histological evaluation system should be done to facilitate surgical decision making. The use of better performing tools than LigaSure™ could lead to a further reduction in thermal damage.

The use of CO₂ laser and LigaSure™ device had superimposable clinical effects in the present study similar to data reported previously. No differences in intra- or post- operative complication rate, anesthesia or surgical time were noted (Kirsch et al., 2019).

The risk of tissue carbonization may compromise the integrity of tissue samples, leading to altered cellular structures and obscuring pathological features. Instruments known for their thermal impact, such as certain cautery devices or lasers, may inadvertently induce tissue carbonization, introducing artifacts that can impede accurate histopathological interpretation (Fornaini et al., 2016). The impact of the surgical Plasma or Lasers results in four distinct tissue zones: the first being charred, followed by a region of fused tissue, then altered, and finally normal (Ruidiaz et al., 2011). Histological examination of specimens obtained with LigaSure™ exhibited three distinct zones: a crush zone, representing the tissue within the jaws of the device; a transition zone, characterized by thermally damaged parenchyma; and beyond that, a viable zone of normal parenchyma (Mayhew et al., 2012).

The tissue effects obtained with the two different methods could account for the significant difference observed in the results of the present study.

Moreover, it is plausible to assume that the residual tissue avoids thermal damage identical to that observed in the excised tissue, given that it is vital tissue with adequate vascularization. Consequently, the histological changes experienced may be less severe and potentially

reversible.

The study exhibits several limitations, among them is the relatively small sample size. Additionally, it is conceivable that sample preparation and fixation could be enhanced by employing suitable supports to prevent retraction of excision margins. Furthermore, the extent of carbonized tissue cannot be quantified.

In a clinical study performed on owned dogs, it would not be ethically acceptable to remove palate samples following a surgical procedure and, consequentially, it was not feasible to conduct a histological comparison between the excised specimens and the remaining vital tissues.

The thickness of the palates was not assessed in the study and the number of instrument applications necessary to perform the palatoplasty was not evaluated. This is a limitation of the study because there can be considerable differences in thickness in brachycephalic dogs which makes necessary repeated instrument application to perform palatoplasty. Different application time of the instrument may influence the histological grading and depth of damage. It would be interesting to evaluate this aspect in future studies.

Conclusions

In this study we found that histomorphologically partial soft palate ablation by a CO₂ laser induced less thermal tissue damage than an excision using a LigaSure™ device, they produced clinically the same effect and a similar scarcity of post-operative complications; however, the long-term clinical significance of this observation remains to be elucidated.

CRediT authorship contribution statement

Francesco Collivignarelli: Data curation. **Leonardo Della Salda:** Writing – review & editing, Investigation, Conceptualization. **Jorge Llinás Ceballos:** Methodology, Investigation. **Gert W. Niebauer:** Writing – review & editing, Conceptualization. **Amanda Bianchi:** Writing – original draft, Investigation. **Roberto Tamburro:** Writing – original draft, Conceptualization. **Laura Bongiovanni:** Writing – review & editing, Methodology, Data curation. **Andrea Paolini:** Investigation. **Melissa Teofani:** Investigation. **Roberto Bussadori:** Validation, Methodology, Conceptualization.

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper

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