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CLINICAL RESEARCH

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The effect of repeated freezing and thawing on the suture pull-out strength in equine arytenoid and cricoid cartilages

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Abstract

Objective: To assess the effect of repeated freezing and thawing on the suture - pull-out strength in arytenoid and cricoid cartilages subjected to the laryngoplasty (LP) procedure.

Study design: Ex vivo experimental study.

Sample population: Ten grossly normal equine cadaveric larynges.

Methods: Bilateral LP constructs were created using a standard LP technique. One hemilarynx was randomly allocated to the single freeze and thaw group and the other allocated to the repeated freeze and thaw (3 complete cycles) group. The suture ends of each LP construct were attached to a load frame and subjected to monotonic loading until construct failure. Mean load (N) and displacement (mm) at LP construct failure were compared between groups.

Results: All LP constructs failed by suture pull through the arytenoid cartilage. The mean load at failure was similar between groups (118.9 ± 25.5 N in the single freeze and thaw group and 113.4 ± 20.5 N in the repeated freeze and thaw group, $P = .62$). The mean displacement at failure was similar between groups (54.4 ± 15.1 mm in the single freeze and thaw group and 54.4 ± 15.4 mm in the repeated freeze and thaw group, $P = .99$).

Conclusion: Repeated freezing and thawing did not affect the suture pullout strength of the arytenoid and cricoid cartilages.

Clinical significance: Laryngeal specimens that have been subjected to repeated freezing and thawing can be utilized in the experimental evaluation of LP procedures because there is no alteration of the suture pull-out strength of the relevant cartilages.

1 | INTRODUCTION

Cadaveric larynges are commonly used ex vivo to evaluate modifications to the laryngoplasty (LP) technique that are designed to enhance its biomechanical performance.[1–4] Some studies have utilized fresh cadaveric specimens for ex vivo testing;[2,5,6] however, this approach may be impractical for multiple reasons including logistic difficulties in obtaining fresh cadaveric tissues at the testing location, collection and testing of cadaveric specimens before postmortem changes affect the physical properties of the tissue, the need to test a large number of specimens at once to minimize testing variability, securing age- or breed-matched specimens, transportation between dissection and testing locations, or availability of testing or laboratory equipment. Storing and preserving cadaveric larynges is therefore common practice until ex vivo testing is performed.

Most studies have used 1 of 2 methods for storing laryngeal specimens before testing. One method involves preserving specimens in a 2% solution of 2-phenoxyethanol at 0-4 °C and then allowing the specimens to reach 20 °C before use.[1,7,8] The second method preserves specimens by wrapping them in gauze soaked with 0.9% sodium chloride and storing them at −20 °C. The specimens are then thawed at room temperature (20 °C) for 12 to 24 h before use.[9] This method is widely used,[3,10–15] but it is unknown if freezing and thawing cadaveric larynges multiple times adversely affects the mechanical properties of the cartilages.

Previous investigations on the effect of freezing and thawing on tendons and ligaments found the tensile strength to be similar between fresh specimens and those that underwent a single freeze-and-thaw cycle.[16–18] However, the results of studies on the effect of freezing and thawing on articular cartilage have been inconsistent. While some studies have found no changes in biomechanical properties following a single freeze and thaw cycle,[19–21] other studies have reported a decrease in cartilage stiffness and in peak strain of samples that had been frozen and then thawed.[22–24] In some cases, statistically significant changes were only noted when the effect of how rapidly the articular cartilage was frozen was examined.[25]

The objective of this study was to assess the effect of repeated freezing and thawing on the suture pull-out strength in arytenoid and cricoid cartilages subjected to an LP procedure. We hypothesized that there would be no difference in the maximal load and displacement at failure of LP constructs in specimens frozen and thawed once compared to those specimens frozen and thawed several times.

2 | MATERIALS AND METHODS

2.1 | Sample collection and storage

Grossly normal larynges were obtained with owner consent from 10 horses after humane euthanasia for reasons unrelated to the study and unrelated to respiratory tract disease. Horses were of the same age (17 years old; 6 mares, and 4 geldings) and different breeds (2 Quarter horses, 2 Standardbreds, 2 American Paint horses, 2 Morgans, 1 Arabian, and 1 Percheron). Larynges were collected within 5 h of euthanasia, wrapped in gauze soaked in saline (0.9% NaCl) solution, and stored at −20 °C until the time of the study. Specimens were thawed for 12 h at 20 °C before the start of the study.[3,15]

2.2 | Construct preparation

Each specimen had both cricoarytenoideus dorsalis muscles removed before LP construction to facilitate consistent suture material placement. In addition, the dorsal aspect of each cricoarytenoid joint was incised to mimic the clinical scenario in which arthrotomy of the cricoarytenoid joint is performed to induce ankylosis and thereby reduce postoperative loss of arytenoid abduction.[26] Laryngoplasties were then performed bilaterally using a standard technique[27] utilizing a single strand of #5 polyester coated polyethylene suture (Fiberwire; Arthrex Inc., Naples, Florida). Briefly, the suture material was passed through the cricoid cartilage approximately 2 cm rostral to the caudal border and 1 cm abaxial to its dorsal ridge. The suture was then passed through the muscular process of each arytenoid in a caudomedial to rostrolateral direction, ensuring the spine of the muscular process was engaged.

One hemilarynx of each specimen was randomly allocated (by flipping a coin) into the single freeze and thaw group, and the contralateral hemilarynx was allocated into the repeated freezing and thawing group. The LP constructs allocated into the single freeze and thaw group were subjected to monotonic loading to construct failure within 2 h of LP placement. In contrast, the LP constructs allocated to the repeated freezing and thawing group were subjected to 3 freeze and thaw cycles before monotonic loading to construct failure. The repeated freezing and thawing protocol was as follows: following monotonic loading to construct failure on the hemilarynx in the single freeze and thaw group, specimens were placed in a Styrofoam container with ice for 6 h, moved to a −20 °C freezer for 24 h, thawed at 20 °C for 12 h, placed in a Styrofoam container with ice for 8 h, moved to a −20 °C freezer for 24 h, and thawed at 20 °C for 12 h. The remaining hemilarynges then underwent monotonic loading to construct failure (Figure S1 in the supplementary material). This protocol was specifically chosen to mimic the experimental design for another study.[28]

2.3 | Mechanical testing

For monotonic loading in all specimens, the LP suture was secured using a single surgeon's throw and the suture ends secured to the stationary crosshead/load cell and a servohydraulic actuator of a load frame (Instron 880; Instron Co., Norwood, Massachusetts). The LP sutures were loaded at 100 mm/minute until construct failure.[15] The load (N) and displacement (mm) at time of construct failure, as well as the mode of failure (suture pull through or fracture of the muscular process, suture pull through or fracture of the cricoid cartilage, or suture breakage) were recorded.

2.4 | Statistical analysis

The Shapiro-Wilk and Anderson-Darling tests were used to verify that the continuous data obtained during monotonic tensile testing of the LP constructs were normally distributed. Differences in maximal load and maximal displacement (mean ± SD) at the failure of the LP constructs were compared between both groups using paired *t*-tests. The significance level was $P \leq .05$. All statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

3 | RESULTS

Twenty LP constructs (10 in the single freeze and thaw group and 10 in the repeated freeze and thaw group) underwent monotonic loading until construct failure. All LP constructs failed by suture pull through at the muscular process of the arytenoid cartilages. The mean maximal load at failure was similar between the single freeze and thaw and the repeated freeze and thaw LP constructs (118.9 ± 25.5 N and 113.4 ± 20.5 N respectively, $P = .62$) (Figure S2). The mean maximal displacement at failure was similar between groups (54.4 ± 15.1 mm in the single freeze and thaw group and 54.4 ± 15.4 mm in the repeated freeze and thaw group, $P = .99$) (Figure S3).

4 | DISCUSSION

The main finding of our study was that the mean maximal load and displacement at the time of construct failure were similar between groups, which suggests that repeated freezing and thawing of the larynges did not affect the suture pull-out strength in arytenoid and cricoid cartilages under monotonic loading. Although the applied loads to the LP constructs tested were beyond those needed to achieve appropriate arytenoid abduction clinically,[29] the force at which all LP constructs failed in this study was similar to values previously reported for specimens subjected to single freezing and thawing cyles'[15,30] and specimens refrigerated at 5 °C before testing.[5] We therefore suggest that cadaveric larynges subjected to repeated freezing and thawing can be utilized for ex vivo experiments without concerns regarding alteration of the suture pull-out strength of the cartilages.

We did not evaluate the biomechanical properties of LP constructs in fresh cadaveric specimens because obtaining suitable cadaveric specimens and having the testing equipment available to complete the study in a timely fashion was not feasible. However, this comparison was addressed in a recent study, and the biomechanical properties of fresh and single cycle frozen/thawed equine laryngeal cartilages were very similar.[31] Subjectively, our experience is that tissue handling of fresh specimens and those frozen and thawed once is also similar. Collectively, these outcomes demonstrate that the biomechanical properties of fresh laryngeal specimens and specimens subjected to 1 or multiple freeze-thaw cycles are comparable.

The LP constructs were only subjected to single-cycle-to-failure rather than being subjected to cyclic loading. Viscoelastic deformation of the cartilages and possibly slowly progressive tissue tearing may influence suture retention or pull-out strength. It could also be argued that cyclic loading would be more representative of a clinical situation, where repeated stress on constructs during strenuous breathing and swallowing occurs. Future studies should evaluate the effect of freezing and thawing on LP constructs under cyclic loading to address this knowledge gap.

Donor horses used in this study were much older than those prone to recurrent laryngeal neuropathy. Although age has not been shown to influence LP prosthesis retention in cadaveric larynges harvested from horses 3 to 5 years of age,[32] it is possible that age-related arytenoid cartilage mineralization might affect LP prosthesis retention in older horses. However, as all of the horses used in our study were of the same age, this effect should have been uniform across our study population and therefore is unlikely to have affected our conclusions.

We chose to emulate testing that represents the use of the cadaver cartilage in ex vivo laryngeal testing rather than performing the more common cartilage compression or frictional properties evaluation.[33] We recognize that this choice means that the biomechanical strength was not measured directly but was instead extrapolated from construct failure. However, this testing approach makes our results more directly applicable to investigation of equine LP constructs.

There were several limitations to the study. Only 1 model of repeated freeze-thaw cycles was used, and care should therefore be taken when extrapolating these outcomes to different freeze-thaw protocols or additional freeze-thaw cycles. The method of evaluating the biomechanical strength could be viewed as a limitation; however, as explained above, we chose to use maximal load and displacement at construct failure to measure suture pull-out strength as this approach mimics how the specimens are used in ex vivo testing.

In conclusion, 3 repeated freeze and thaw cycles did not change the biomechanical properties of equine laryngeal cartilage as assessed by the maximal load, displacement, or method of cartilage failure. Cadaveric larynges subjected to 3 cycles of repeated freezing and thawing can therefore be utilized in ex vivo studies to evaluate different LP techniques because there is no alteration of the suture pull-out strength in arytenoid and cricoid cartilages.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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