Flexible and Rigid Endoscopic Evaluation of In Vivo Rabbit Larynges: A Pilot Study

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Flexible and Rigid Endoscopic Evaluation of In Vivo Rabbit Larynges: A Pilot Study

Shauntel Mei Li Anderson

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

Christopher Dromey, Chair
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ABSTRACT

Flexible and Rigid Endoscopic Evaluation of In Vivo Rabbit Larynges: A Pilot Study

Shauntel Mei Li Anderson
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Master of Science

The purpose of this investigation was to determine the comparability of flexible endoscopy to rigid endoscopy in the examination of rabbit vocal folds in vivo. This work was conducted within a larger parent project that has investigated the adverse effects of combination inhaled corticosteroids (ICs) on voice function in animal models and humans. In this study, both flexible and rigid vocal fold imaging was performed on eight rabbits; four having received ICs and four that were from control conditions. Original recordings were examined frame-by-frame to identify similar views, zoomed in and zoomed out, for flexible and rigid samples. Images were extracted, cropped, and placed in a slide presentation for purposes of visual-perceptual ratings. Two board-certified laryngologists from the parent project performed ratings that included the following: (a) superiority or equivalence (> , <, or =) of randomized rigid and flexible images; (b) erythema severity using a visual analog scale (VAS) for all randomized images; and (c) edema severity using VAS for all randomized images. Each rating was performed twice, in random order, for purposes of computing interjudge and intrajudge reliability. The results indicated that images from rigid endoscopy were of superior quality than those from flexible endoscopy. Additionally, the magnitude of severity ratings was greater for flexible versus rigid images. These findings offer confirmatory evidence that rigid endoscopy remains the preferred method for endoscopic analysis; however, flexible endoscopy is a novel, viable alternative that permits reliable visual-perceptual analysis during in vivo small animal voice research.

Keywords: asthma, inhalers, endoscopy, visual-perceptual, steroids
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DESCRIPTION OF THESIS STRUCTURE AND CONTENT

This thesis, *Flexible and Endoscopic Evaluation of Rabbit Larynges: A Pilot Study*, was funded by the National Institute of Deafness and Other Communication Disorders and National Institutes of Health (1R01DC0162901A1). Dr. Kristine Tanner, the primary investigator, obtained funding for this longitudinal research project in collaboration with labs at Brigham Young University and the University of Utah.

The work presented in this thesis was submitted for presentation at the 2024 annual convention of the American Speech-Language-Hearing Association in Seattle, Washington. It is written in a hybrid format that follows both traditional thesis requirements and journal formats required for publication submission.

This thesis format contains two reference lists. The references cited throughout the main body of the text are just below the conclusion. The second list includes all citations used in Appendix A, entitled “Annotated Bibliography.” Appendix B contains rating instructions provided for the raters.
Introduction

Asthma is a chronic and heterogeneous disease of the lungs. This condition affects all ages around the globe, regardless of ethnicity, sex, and living environment. In a Centers for Disease Control (CDC) report (2022), 8.7% of adults and 6.2% of children currently live with asthma. Asthma presents with variable degrees of dyspnea due to airway stenosis, increased airway sensitivity, and mucous secretions. Efficient vocal fold vibration and generation of the voice is dependent on adequate airflow. Individuals with asthma experience significant resistance and obstruction to airflow, especially during exhalation. To overcome for this excess resistance in the airway, increased muscle contraction is required to expel sufficient air out of the lungs. These compensatory respiration changes can affect the ideal posture for healthy voice production, thus resulting in unhealthy voicing behaviors (Dogan et al., 2007). Airway inflammation may cause elevated secretion of mucus, resulting in increased coughing and throat clearing, which can also be harmful to laryngeal tissue and voicing.

Inhaled Corticosteroids

Inhaled corticosteroids (ICs), also known as glucocorticosteroids, are commonly prescribed as a treatment for individuals with asthma and have been extensively researched. They are now considered the first line of therapy for all ages and severity levels of asthma and can achieve treatment effects in low doses in most patients (Barnes, 2010). IC treatments target several respiratory symptoms by combining a corticosteroid and a long-acting beta agonist (LABA) into a single treatment (Erickson & Sivasankar, 2010). Similar to ICs, LABA is a bronchodilator; however, it has properties that act on a different aspect of the pathophysiology of asthma. LABA suppresses the release of mast cells, a type of white blood cell, and reduces
activation of sensory nerves. Barnes (2002) claimed that neither ICs nor LABA can treat asthma on their own; both drugs are essential in effective asthma management.

As with other medications, potential side effects are unavoidable. While ICs are the standard treatment for asthma, their local and systemic side effects must be considered. According to the current literature, one of the most frequently associated local effects of IC therapy is dysphonia, which has an incidence as high as 5% to 58% in patients with asthma (Barnes, 2010; Galván & Guarderas, 2012; Naunheim et al., 2023). Dysphonia is a disordered vocal quality resulting in altered vocal pitch, range, loudness, and increased effort. This can significantly reduce quality of life, affecting an individual socially, emotionally, physically, and in their vocational lives. According to Galván and Guarderas (2012), about 7.2% of the population misses work at least 1 day per year due to voice difficulties, resulting in about two and a half billion dollars in annual work productivity lost in the United States. Therefore, while ICs target and help manage the symptoms of asthma, there is a tradeoff due to the interference with typical voice production. Other local side effects include pneumonia in patients with chronic obstructive pulmonary disease (COPD), oropharyngeal candidiasis, and cough (Barnes, 2010). As the efficacy of ICs has been investigated, there have been concerns regarding the systemic side effects, especially in pediatric populations and with usage over long periods of time. Whether ICs cause systemic side effects is largely dependent on the amount of drug absorption into the systemic circulation; nearly 80% of the IC dosage is swallowed and then absorbed in the gastrointestinal tract. The systemic side effects that have been recognized include adrenal and growth suppression, bruising, osteoporosis, cataracts, glaucoma, metabolic effects, and although rare, psychological disturbances (Barnes, 2010).
Barnes (2010) suggested that when ICs are prescribed in larger doses, patients should rinse with mouthwash immediately and use a spacer device, which is a holding chamber that can gradually deliver the treatment, to prevent delivering the dosage all at once. However, it should be noted that rinsing with mouthwash and using a spacer does not address the adverse effects of dysphonia at and below the level of the vocal folds. Although more research needs to be done on effective prevention of IC side effects, withholding asthma treatments from those who need them would be a significant limitation to human studies. To mediate this problem, alternative research models have been used to study the effects of ICs on the voice, including both in vivo and ex vivo animal models.

**Animal Models**

Animal models are routinely used in voice and airway research. Translational in vivo and ex vivo animal studies are critical to the advancement of voice assessment and asthma management. In vivo models can be useful because researchers can study aspects of voice that could otherwise not be accomplished in humans, such as injury, scarring, and wound healing. Using in vivo animal models can also be beneficial because phonation and laryngeal structures can be observed in real-time without the potential confounding variables of laryngeal excision, vocal fold fine dissection, and external airflow manipulation (Novaleski et al., 2016). There have been promising and viable findings using various animal models throughout current voice and airway literature, including tigers, sheep, lions, dogs, pigs, rabbits, deer, and cows (Alipour & Jaiswal, 2008; Jiang et al., 2001; Klemuk et al., 2011; Mills et al., 2017). Animal models avoid the difficulties associated with living human subjects. Limitations of conducting research with the human populations include participant recruitment and blinding and ethical considerations in withholding treatment from a control group (Erickson & Sivasankar, 2010). However, there are
also limitations in using larger animals, such as dogs and pigs, in clinical voice research. For example, larger animals are more challenging to keep and care for in longitudinal animal research. Additionally, it is also difficult to control vocal use and, therefore, possible phonotrauma in these animals.

While the vocal folds and laryngeal structures from other species have similarities to humans, the rabbit model is ideal for research investigating the effects of in vivo administration of combination ICs (Tanner et al., 2023). Furthermore, they state that the rabbit model is advantageous because it is a small animal, rabbits have similar vocal fold epithelium and vocal fold histology to humans, and rabbits have been previously used in voice and asthma research. Because rabbits are quiet by nature and do not typically use their voice, the potential effects of phonotrauma or other harmful vocal behaviors on the vocal folds are not a concern. However, rabbits’ reduced phonation may be a limitation due to reduced carry over or translation to humans who phonate routinely and much more extensively.

Unlike larger animals such as dogs, rabbits are more convenient, easier, and cost effective to house and care for in longitudinal animal studies. While canine models are similar in gross size and structure to a human larynx, such as similar vocal fold length and size of the cricothyroid muscle, the vocal fold layers between the two are significantly different (Jiang et al., 2001). Both dogs and humans have a lamina propria with three layers; however, dogs have thin layers of collagen and elastin that lies superior to the loose ground substance. Additionally, dogs have a poorly defined vocal ligament (Maytag et al., 2013). These differences in tissue composition are not ideal for translational human voice research. However, because rabbits possess similar lamina propria histology, evidence has shown that rabbits are reliable animal
models in research for comparisons with humans, especially studies investigating vocal fold tissue change, such as the effects of ICs (Maytag et al., 2013; Tanner et al., 2023).

**Endoscopy**

The word endoscopy derives from the Greek prefix, “endon,” meaning inside and the verb “scope’ın” which means to watch carefully (Antoniou et al., 2012). Endoscopy is a diagnostic and therapeutic technique used in medicine to interiorly visualize organs or cavities within the body via a rigid or flexible tube. Endoscopy is particularly beneficial due to its minimally invasive nature. Common vendors of endoscopes used both in medicine and research include STORZ, Olympus, and Kay Pentax. Components of an endoscope include control, insertion, and connector sections, including a video system center that uses an LCD monitor which then displays live imaging (Olympus, n.d.). The control section allows the tip or distal end of the endoscope to be maneuvered horizontally or vertically for insertion and a 360-degree view of a given body cavity. The insertion section includes the wide-angle lens, image sensor, and light guide fiber bundles that help to conduct light from the external light source. Through a universal cord, the connector section connects the endoscope to the video system center. While endoscopy is used to examine functional and structural changes within the body, it is also a tool used to perform surgical procedures such as biopsy of tissues and nerves via channel scopes (Moore, 2003). These channel scopes have an additional component called the instrument channel outlet to aid in such procedures. Endoscopy is often used to examine the respiratory system, gastrointestinal system, thoracic cavity, abdomen, urinary tract, and joints (Weil, 2009).

The technology of endoscopes has progressively improved and changed over many decades. For instance, flexible fiberoptic laryngoscopy was first introduced in 1975 (Williams et al., 1975). At the time, this scope was commercially available and used due to its affordability
and effectiveness in intubation; however, it proved to be unsatisfactory due to its wide diameter, limited optics, and poor ability to fully meet the needs of otolaryngologists (Silberman et al., 1976). Endoscopy for the voice has improved with access to digital distal-chip endoscopes. While digital flexible endoscopes provide significant improvements in image quality, they are also typically larger in diameter, particularly at the tip of the endoscope. Depending on types of cameras and light sources, image quality can be variable across endoscopes. Furthermore, as technology has advanced with time, endoscopes have been improved and modified to have digital image processing via a distal chip. This has proven to be advantageous in laryngeal imaging because it eliminates the use of optical fibers and the associated image quality degradation, thus producing clearer, high-quality images (Eller et al., 2008; Plaat et al., 2014). A variety of other options are available in clinical voice practice, including pediatric sizing and continuous and stroboscopic light sources.

Endoscopy of the nose, throat, and upper airway have now become the gold standard for instrumental voice assessment (American Speech-Language-Hearing Association, n.d.; Patel et al., 2018). Various sizes of rigid and flexible scopes can be used to visualize structures of the upper and lower airway. Otolaryngologists use this technology routinely in clinical practice. Speech-language pathologists perform endoscopic examination of the velopharynx and larynx, including visualizing the function of the oropharynx and nasopharynx in the assessment of voice and swallowing, such as for respiration, upper airway obstruction, and paradoxical vocal cord dysfunction. Given the advances in image quality and greater access to a variety of assessment options, voice and airway researchers can employ state-of-the-art imaging techniques to examine voice and airway in research participants. Although less accessible than traditional endoscopy and stroboscopy, high-speed video (HSV) imaging is another tool that is used in voice research.
Both HSV and videostroboscopy techniques allow clinical observation of the interaction of laryngeal structures, aerodynamic forces, tissue mechanics, and muscle activation patterns (Patel et al., 2018; Poburka et al., 2017). In recent years, the use of HSV has increased across voice clinics to overcome the limitations to using videostroboscopy, such as poor temporal resolution, pitch detection, and tracking of severely impaired voices. HSV traditionally uses a rigid endoscope and a very bright constant light source to sample vocal fold vibration and offers improved temporal resolution for short intervals at up to 8,000 frames per second (fps) versus stroboscopy at 30 fps, allowing enhanced measurement of vocal fold vibration in patients with severely disordered voices (Poburka et al., 2017). Several researchers have also developed custom experimental set-ups that include light sources bright enough to support flexible endoscopy (Mehta et al., 2015; Popolo, 2018; Woo & Baxter, 2017).

Two methods that are used for the purposes of this thesis and that are consistently used in clinical voice practice and research include rigid and flexible endoscopy. Laryngeal imaging is critical in the diagnosis of patients presenting with disordered voice symptoms and complaints. Depending on clinician preference, availability, and the needs of the patient, rigid or flexible scopes may be chosen. Additionally, rigid and flexible laryngoscopy both have their own advantages and disadvantages. Flexible scopes enter through the patient’s nostril to bypass the nasal cavity and nasopharynx and through the oropharynx to get to the hypopharynx. Rigid endoscopic exams consist of the scope entering through the oral cavity with the patient’s tongue gently pulled forward. In some patients, this can be problematic due to a sensitive pharyngeal reflex, commonly known as the gag reflex, and low tolerance for the scope (Hassen & Abo Hasseba, 2016). The insertion sites for both rigid and flexible scopes lead to several benefits and drawbacks. For instance, the flexible scope allows the patient to participate in a series of
assessment tasks, such as breathing, swallowing, coughing, and voicing. Additionally, flexible endoscopy allows for connected and more naturalistic speech tasks, while rigid endoscopy does not. During a rigid scope examination, the scope can provide a clear, superior view of laryngeal structures and patients can perform sustained vowels (Milstein et al., 2005). Because the patient can only phonate a vowel with the rigid scope in their mouth, they cannot articulate any sounds. This consequently gives clinicians a limited context in which to view vocal fold activity.

Current Problem and Purpose

Imaging is a necessary component to many animal studies. Generally, rigid endoscopy is used for surgical procedures and in studies using high speed imaging (Novaleski et al., 2016). Deciding which endoscopy method to use is determined by the purpose of the imaging, to either view structures (e.g., as needed for surgery) or to observe function. As discussed previously, many animals have been used in voice research. Flexible endoscopy in small animals can be a significant challenge due to size; however, more recent advances in smaller endoscopes have made imaging in animals possible (Jekl & Knotek, 2007). The literature has already reported useful applications for flexible endoscopy. For example, Durkes and Sivasankar (2016) investigated the effects of simulated reflux in an in vivo porcine animal model with each animal undergoing repeated endoscopy three times a week for 4 weeks. This demonstrated that flexible endoscopy can be a viable approach for imaging animals, especially for long-term studies requiring regularly occurring scoping procedures.

When determining which endoscopy technique is best in animal research, many factors must be considered, including image quality, longevity of the animal, recovery post procedure, and size of the animal. While rigid endoscopy is currently considered the gold standard for image quality and its ability to capture structural changes in the larynx, it is not ideal for long term
studies due to its more invasive nature, higher associated mortality rates, and the animal requiring deep anesthesia (Boles et al., 2023; Milstein et al., 2005). Most laryngeal function evaluations in animals are performed using a form of anesthesia (Moore, 2003; Weil, 2009). The deeper the sedation, the greater the likelihood of arytenoid cartilage movement being diminished; however, the ideal technique of using anesthesia in animals for laryngeal endoscopy has yet to be established (Weil, 2009). Radlinsky et al. (2004) used a transnasal flexible scope on dogs to diagnose laryngeal paralysis. Each dog underwent intramuscular sedation and topical anesthesia, which demonstrated that traditional laryngoscopy could be successful in dogs without the use of general anesthesia, thus avoiding any potential associated complications.

This thesis is an extension of an existing large-scale project examining the adverse effects of combination ICs. The parent study includes animal experiments using flexible endoscopy in rabbits. The purpose of the present study was to determine whether flexible endoscopy is a viable alternative to rigid endoscopy for in vivo rabbit research. Similarities and differences in endoscopy for laryngeal imaging in rabbits were explored to evaluate the utility of images acquired with both flexible and rigid endoscopes. While many studies in the current literature have performed a comparison between types of endoscopy techniques, they have primarily been within human models (Boles et al., 2023; Eller et al., 2008; Milstein et al., 2005; Plaat et al., 2014; Poburka et al., 2017). This thesis provides the first endoscopy comparison study in rabbits, providing the foundation for future research in endoscopy in small animal models.

**Research Questions**

Are flexible endoscopy images comparable to rigid for purposes of examining structural changes in rabbit larynges? Specifically:

a. Are flexible endoscopy images better, worse, or equivalent to rigid endoscopy images?
b. How do severity ratings for erythema compare between flexible and rigid endoscopy images?

c. How do severity ratings for edema compare between flexible and rigid endoscopy images?

Method

This thesis was part of a larger ongoing study funded by the National Institutes of Health with Kristine Tanner, Ph.D., as the principal investigator. The grant that funded portions of this research was provided by the National Institute on Deafness and other Communication Disorders (1R01DC016269). This thesis study was conducted in her laboratory at Brigham Young University, Provo, Utah and the University of Utah Comparative Medicine Center (CMC), Salt Lake City, Utah. The rabbits for this work were all obtained from the Charles River Laboratories and maintained at the University of Utah under the animal research protocol 21-0308. All animal procedures complied with National Institutes of Health and related guidelines. The parent study of this thesis investigated the threshold and reversibility of combined ICs and visual perceptual changes in laryngeal tissue in rabbits. This work included a pilot study on laryngeal visualization comparing rigid and flexible endoscopy in a rabbit model.

This work involved two primary methodologies. The first included a pilot study performing rigid and flexible endoscopy in rabbits to compare imaging techniques. The second methodology consisted of visual-perceptual ratings of the images acquired from the rigid and flexible endoscopy procedures. A between-groups case-control experimental research design was used. The independent variables were the group a rabbit was randomly assigned to (baseline control group or experimental group) and the scoping method (rigid or flexible endoscopy). The dependent variables were the visual-perceptual ratings of true vocal folds for edema and
erythema. In addition to ratings for severity in changes in laryngeal tissue, a comparison between rigid and flexible endoscopy image quality was completed.

**Endoscopy Procedure**

The pilot study used eight New Zealand White rabbits, ages 7 to 8 months and weighing between 3.1–4.8 kg. Four rabbits were treated with ICs to induce tissue changes, and four were paired controls who received saline. As part of the parent study, rabbits were randomly assigned to be in the experimental group or control group. Experimental rabbits received IC salmeterol fluticasone propionate twice daily which was administered via a metered dose inhaler (MDI) and using a facemask and spacer where rabbits inhaled through their nares for 18 breaths. Control group rabbits received nebulized isotonic saline (0.9% NaCl) twice daily via a facemask for 18 breaths.

**Parent Study**

As part of the larger study’s protocol, all rabbits, including the animals used for this pilot study, underwent transnasal flexible videoendoscopy at baseline and every 2 weeks thereafter. In other words, a single rabbit would receive repeated endoscopy. Once endoscopy procedures revealed tissue changes to the vocal folds from baseline, animals were humanely sacrificed with an injection of 1 mL/10lb of sodium pentobarbital via IV injection (1mL/4.5 kg) through the ear vein. If a rabbit was not scheduled to be euthanized that day, post-endoscopy monitoring was performed by CMC personnel. Vital signs were observed until the rabbit was perceptually judged to be completely recovered from the anesthesia, which was about an hour and a half for each animal.

**Pilot Study**

The scoping sessions completed in the pilot study for this thesis were the final endoscopy
session for all eight animals. Because rigid endoscopy procedures were not initially documented
and approved for in the original animal protocol 21-03008, an amendment was proposed to the
Institutional Animal Care and Use Committee (IACUC). Once the IACUC approved this
amendment, the eight rabbits included in this thesis underwent routine flexible endoscopy first
and then rigid endoscopy immediately after, for a total of two procedures for each rabbit. Each
rabbit’s timeline from the baseline flexible endoscopy procedure, completed as part of the parent
study’s protocol, until later euthanasia are as follows: rabbits 23-006 and 23-007 had a timeline
of 10 weeks, rabbits 23-013 and 23-014 had a timeline of 6 weeks, and rabbits 23-009, 23-10,
23-011, and 23-012 had a timeline of 8 weeks.

For flexible endoscopy procedures, a 2.9 mm diameter, 30 cm working length Slim-Line
video rhino-laryngoscope with a light source was used. Settings for the flexible endoscopy
procedures included white balancing prior to all video recordings and the use of chroma setting
to provide more definition on vascularity. Additionally, this scope had an attached digital video
acquisition system, a Karl Storz C-MAC monitor. For the rigid scope, a Karl Storz Hopkins
10005 AA 0° 4 mm diameter, 30 cm working length telescope with a fiber optic light
transmission was used. Similar to the flexible scope, this rigid scope was connected to the Karl
Storz Tele Pack x LED TP 100 video unit. Both the rigid and the flexible endoscopes had similar
camera resolutions. Figure 1 shows the rigid and flexible endoscopy cart set up, including the
digital video systems for each. Video playback and live image capturing was possible, with the
highest brightness settings used for both endoscopic techniques. Figure 2 demonstrates the
capturing of a live image during a rigid endoscopy procedure. All rabbits were in a supine
position for rigid and flexible endoscopy procedures in an animal operating room at the CMC. At
least two trained CMC staff assisted during each procedure to sedate the rabbits, monitor their
vitals, and for later euthanasia. Additionally, during each procedure, a trained lab member would perform the endoscopy, while a second individual assisted to help position the rabbit and administer oxygen. Each animal was given the same dosages of anesthetics for both the rigid and flexible procedures. They were given 5–10 mg of Xylazine/ketamine via intramuscular (IM) injection. Isoflurane was administered with titrate dosage via inhalation if the animal would benefit from increased relaxation. The level or depth of anesthesia was monitored by observing the animal’s heart rate, respiratory rate, oxygen saturation, and the response to stimulus.

**Figure 1**

*Rigid Endoscope Karl Storz Video Monitor on the Left and Flexible Endoscope C-MAC Video Monitor on the Right*
Before each flexible scoping examination, each rabbit was administered nasal decongestant through the nares. Medical grade alcohol wipes were used to clean the tip of the scope prior to insertion. Lubricant jelly was then applied to about 10 cm of the scope’s tip to aid in smoother insertion. The scope was inserted in either the left or right nares, depending on which passageway appeared to be more open and less narrow. Figure 3 shows a flexible endoscopy procedure with insertion through the rabbit’s right nares. Once the true vocal folds, arytenoids, and laryngeal vestibule had been visualized, the live recording of the procedure
ended, and the scope was withdrawn. As seen in Figure 4, the scope was placed in Cidex to sanitize for approximately 12 minutes in between each procedure using a timer. Once 12 minutes had passed, water was passed through the scope using a syringe to clear any debris. As seen in Figure 5, for the rigid endoscopy procedures, the scope was inserted into the rabbit’s oral cavity, with the tongue gently pulled aside. Once the target laryngeal structures were visualized and the video recording ceased, the scope was withdrawn. As was the case for the flexible procedures, the rigid endoscope was then sanitized in Cidex for 12 minutes. Immediately after each procedure had been completed, either rigid or flexible endoscopy, the video recording was played back, saved to a thumb drive that was inserted to the video monitor, and then transferred to a PC laptop to be uploaded to the lab’s Box (www.box.com) account.

Figure 3

*Flexible Endoscopy with Insertion Through the Rabbit’s Right Nares; Oxygen Administered to the Left Nares*
Figure 4

Setup for Sanitization of the Flexible Endoscope in Cidex for 12 Minutes

Figure 5

Rigid Endoscopy with Insertion Through the Rabbit’s Oral Cavity
With each of the eight rabbits receiving one flexible endoscopy procedure, seven out of the eight flexible scoping procedures took approximately 2 to 7 minutes. The flexible examination for one out of the eight rabbits took about 27 minutes due to a narrow nasal passageway, minor internal and external nasal bleeding, and repeated insertions of the scope. With each of the eight rabbits receiving one rigid endoscopy procedure, these procedures took approximately 3 to 11 minutes across all rabbits, with one rabbit needing to be scoped a second time due to visualization difficulties. These endoscopy methods are common and are not expected to cause the animal any harm. The complications observed during these procedures were minimal signs of distress, especially while the scope was in the process of being passed. Additionally, minor internal and or external nasal bleeding occurred in a few rabbits. Once each animal received its last sessions of both methods of endoscopy, it was similarly euthanized with the same dosage of sodium pentobarbital and method as previously discussed for all rabbits as part of the larger study’s protocol.

**Data Acquisition**

Once all endoscopy procedures were completed, all 16 endoscopy recordings, including eight flexible and eight rigid endoscopy procedures, were carefully analyzed to determine the two best images for each rabbit’s rigid and flexible endoscopy procedure. More specifically, this included one image of the true vocal folds for flexible, one image of the supraglottis/laryngeal vestibule for flexible, one image of the true vocal folds for rigid, and one image of the supraglottis/laryngeal vestibule for rigid. The best image quality was perceptually determined by the highest level of brightness, sharpness, and visualization of the target laryngeal structures. Using OpenShot version 3.1.1, a video editing software program, exact image acquisition was completed by proceeding frame-by-frame. The original file size for each image approximately
ranged from 4.5 to 7.9 MB. To preserve image quality, each image was taken directly from the original files and then exported as .png files to avoid file compression.

Visual-Perceptual Analysis

Based on subjective clinical judgement, the perceptual rating of vocal quality is a routine practice during voice evaluation (Kempster et al., 2009; Kreiman & Gerratt, 1998). Two commonly used rating scales used in voice research and clinical practice are the Likert scale and the visual analog scale (VAS). While Likert scales provide ordinal data, with each level being assigned a numeric value, they are limited in response sensitivity, especially in between two samples. Furthermore, these ordinal judgements can significantly limit its application to research design and statistical analysis (Kempster et al., 2009). Visual analog scales are typically presented as a 10 centimeter horizontal line, with defined anchors on the far left and right of the scale. The Consensus Auditory Perceptual Evaluation of Voice (CAPE-V) is a clinical and research assessment tool used to measure auditory perceptual judgements of vocal quality (Kempster et al., 2009). The CAPE-V is an example of a VAS that is routinely used due to its use of open-ended right and left anchor points as a way to prevent end effects of the scale. Furthermore, Kempster et al. (2009) argue that the VAS is user friendly for raters and in the past 2 decades, has recently become a common rating method in voice research. For example, Otto and et al. (2006) used a VAS to evaluate the effects of high definition and magnification on the perceived image quality in laryngeal imaging. In addition to the previously discussed rationale, a VAS was used in this thesis because of its advantage to detect subtle tissue changes, such as erythema and edema.

In the current study, visual perceptual ratings were performed using a VAS and a better, worse, and equal rating scale. After each endoscopy image was standardized for position, crop,
and lighting using Adobe photoshop version 25, all laryngeal images were de-identified and randomly compiled into a PowerPoint, version 16, slideshow presentation. The VAS was used to rate erythema and edema in the true vocal folds; no erythema and edema was located on the far left of the scale, while severe erythema and edema was located on the far right of the scale. For the VAS, raters were presented with two endoscopy images per slide, for a total of 64 images, representing each of the eight rabbits and their rigid and flexible endoscopic images. One image was a zoomed in view to visualize the true vocal folds, while the second image was a wide view to visualize the laryngeal vestibule/supraglottis. The two images on each slide were within the same rabbit. Raters were asked to place a red tick mark on the horizontal scale to indicate the severity of tissue change. Figures 6 and 7 are screenshots of the PowerPoint rating slides demonstrating the VAS measuring the severity of edema and erythema for flexible and rigid endoscopy. VAS ratings were then scored as follows after the PowerPoint slides were saved as jpg images. The total number of pixels of the VAS line was determined and then the number of pixels from the far left to where the rater placed the red tick mark was measured in pixels. The number of pixels from the far left to the red tick mark was then divided by the total number of pixels of the VAS line and then multiplied by 100. Thus, the VAS scores ranged between 0 and 100.
Figure 6

Example Rating PowerPoint Slide Used to Compare Severity of Erythema in Flexible Endoscopy Images

Figure 7

Example Rating PowerPoint Slide Used to Compare Severity of Edema in Rigid Endoscopy Images
In addition to the VAS, the better–worse rating scale was used for comparison purposes between rigid and flexible endoscopy images. For the better–worse rating scale, raters were presented with four images per slide, representing two rigid and two flexible endoscopic images within the same rabbit. One rigid endoscopy image was a zoomed in view to visualize the true vocal folds, while the second rigid endoscopy image was a wide view to visualize the laryngeal vestibule/supraglottis; these image views were also acquired for flexible endoscopy images. Raters were asked to place a greater than, less than, or equal sign in the provided table on each slide to compare the two side by side images. Figure 8 shows a screenshot of the PowerPoint rating slide for the better–worse scale to compare image quality between the two endoscopy techniques. All ratings were completed in one sitting by two trained laryngologist physicians, each familiar with endoscopic imaging and rating laryngeal anatomy.

**Figure 8**

*Example Rating PowerPoint Slide Used to Compare Image Quality Between Rigid and Flexible Endoscopy*
The first few slides in the PowerPoint included orientation to the VAS, better–worse rating scale, and definitions of anatomical locations. Images were randomly presented in three sections within the PowerPoint slideshow. Furthermore, for each of the three sections, two of the same images were randomly presented for a total of 80 images. Raters rated each image within each section twice for the purposes of intra and inter-rater reliability. The first section of images the raters judged were flexible versus rigid endoscopy images to compare the two imaging techniques using the better, worse, and equal rating scale. The second section of images were all rigid and flexible endoscopy images to be judged for edema and the third section of images were all the rigid and flexible endoscopy images to be judged for erythema. Ratings were organized in three sections to enhance the raters’ understanding of how they were rating the given images within each section. Additionally, presenting the better–worse scale in the first section of ratings allowed the raters to be oriented to both flexible and rigid endoscopy images side by side, especially because the next two rating sections included both flexible and rigid endoscopy images randomly mixed in.

**Results**

The current study compared image quality between flexible and rigid endoscopy images and examined how severity ratings for erythema and edema compared between flexible and rigid endoscopy images. Intra-rater reliability and inter-rater agreement were first computed to ensure that the data were valid to address the research question.

**Rater Reliability**

A Pearson correlation was computed between the first and second ratings of the same randomly sequenced images to determine intra-rater reliability for each rater for each parameter. Rater 1 had a correlation of .666 for erythema and .810 for edema. Rater 2 had a correlation of
.835 for erythema and .867 for edema. Taken together, these correlation values indicate acceptably strong intra-rater reliability. The intraclass correlation coefficient (ICC) was used to determine inter-rater agreement for raters 1 and 2. For erythema their average measures ICC was .786, \( p < .001 \), and for edema it was .681, \( p = .001 \), indicating acceptably good agreement between the raters.

**Image Quality Ratings**

Both raters were asked to rate whether flexible or rigid endoscopy images were better or equal in image quality. Table 1 shows the number of ratings where both raters judged flexible to be higher in image quality, rigid to be higher in image quality, or flexible and rigid images to be equal in image quality. Table 1 includes both the original ratings and the repeated ratings. For Rater 1, 7/8 repeated ratings were in agreement with the initial rating; one rabbit’s image was initially judged as flexible being worse than rigid. The repeated rating for the same rabbit image was subsequently judged as being equal in quality. Rater 2 similarly had 7/8 repeated ratings that were in agreement with the initial rating; one rabbit was initially judged as flexible being worse than rigid. The repeated rating for the same image was subsequently judged as flexible being better than rigid.

**Table 1**

*Totaled Comparison Ratings for Rater 1 and Rater 2*

<table>
<thead>
<tr>
<th></th>
<th>Rater 1</th>
<th>Rater 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexible Better (&gt;)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Equal (=)</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Rigid Better (&lt;)</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>
Severity Ratings

The reader is referred to Table 2 for descriptive statistics of the flexible and rigid endoscopy ratings of erythema and edema, pooled across the two raters. Table 3 reports the results of the paired samples $t$-tests comparing the means of flexible and rigid endoscopy ratings. Ratings of erythema and edema were significantly higher for flexible endoscopy than for rigid endoscopy. Figure 9 shows the mean severity rating for erythema across raters for flexible and rigid endoscopy and the standard error of measurement. Similarly, Figure 10 shows the results of the mean severity rating for edema across raters and the standard error of measurement.

Table 2

*Mean and Standard Deviation of Flexible and Rigid Endoscopy Ratings of Erythema and Edema*

<table>
<thead>
<tr>
<th></th>
<th>Flexible</th>
<th>Rigid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>63.0</td>
<td>28.1</td>
</tr>
<tr>
<td>Edema</td>
<td>51.1</td>
<td>25.6</td>
</tr>
</tbody>
</table>

Table 3

*Paired Sample T-Tests Comparing Flexible and Rigid Ratings of Erythema and Edema*

<table>
<thead>
<tr>
<th></th>
<th>$t$</th>
<th>$df$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td>7.306</td>
<td>15</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Edema</td>
<td>5.877</td>
<td>15</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
Figure 9

Rating of Erythema Severity for Flexible and Rigid Endoscopy

Figure 10

Rating of Edema Severity for Flexible and Rigid Endoscopy
Discussion

During the past 2 decades, in vivo animal models have expanded to include rabbits for purposes of addressing experimental questions related to voice disorders. Their use in surgical studies and vocal fold wound healing has rapidly increased in recent years (Maldonado et al., 2014; Novaleski et al., 2016). Rabbits have many advantages in voice research, including comparability of the vocal fold layered structure and the ability to house and examine them easily, reducing facility burden and cost. At the same time, rabbits are fairly fragile animals that have been known to exhibit higher mortality rates than some other animals, particularly with repeated surgical procedures involving endoscopy (Steehler et al., 2011; Wistermayer et al., 2020). One of the contributors to experimental rabbit mortality is general anesthesia; therefore, it is possible that alternative laryngeal imaging techniques involving less anesthesia might be advantageous in voice research, particularly longitudinal studies.

The overall objective of this thesis was to determine whether flexible endoscopy images are comparable to rigid endoscopy images for examining structural changes in rabbit larynges. Specifically, this thesis examined the equivalence of rigid versus flexible endoscopic images of eight rabbits that were part of a larger parent project studying the effects of combination ICs on voice function. Laryngologists with experience interpreting inflammatory changes in rabbit vocal folds performed visual-perceptual ratings of flexible and rigid still images from a small cohort of rabbits enrolled in the parent project. Ratings included judgements of equivalence (i.e., better, worse, or equal), as well as the severity of erythema and edema, if present, between both scoping techniques. It is important to note that experimental and control groups were equally represented in this study; that is, four rabbits had received twice-daily combination IC administration and four were from a sham nebulized isotonic saline control group. Due to the small sample size and
known challenges associated with visual-perceptual laryngeal judgements (Poburka & Patel, 2021; Poburka et al., 2017), all judgements were repeated by both raters for reliability purposes. The results confirmed our experimental hypothesis that rigid endoscopy resulted in better image quality, which was observed for the majority of flexible versus rigid comparisons. The visual-perceptual ratings also indicated significant differences in the magnitude of change for erythema and edema between flexible and rigid endoscopy. An interpretation of these findings within the context of the experimental questions and extant literature is provided below.

**Image Quality Ratings**

In this study, rigid endoscopy resulted in better image quality when compared to flexible endoscopy in nearly all visual-perceptual image samples. These results are consistent with the experimental hypothesis of this thesis and provide additional confirmatory evidence to the literature; specifically, this study adds important translational evidence regarding animal imaging as compared to humans. The subject of imaging methods, including flexible versus rigid modalities, but also type of endoscope, camera, recording and compression effects continue to be a topic of interest in clinical settings (Boles et al., 2023). Although the judgement of image quality is subject to clinician interpretation, brightness, sharpness, and adequate visualization of the target structures—as defined in this study—represent central components to the quality of images for endoscopy interpretation.

In a similar vein, it is important to consider that experience level of the rater has been shown to influence visual-perceptual judgements when viewing and interpreting vocal fold images (Poburka et al., 2017). For example, studies have shown that visual-perceptual judgements and interpretation of videolaryngostroboscopy are notoriously challenging from
reliability and agreement perspectives. Even very experienced clinicians demonstrate variability and bias when interpreting specific features of laryngeal imaging, including anticipated versus observed findings based on diagnosis (Ellerston et al., 2023; Sauder et al., 2023). In the current study, it became apparent that experience level could have influenced the ratings. For example, the more experienced laryngologist found that 30/32 rigid endoscopy images were superior to flexible endoscopy images from the same rabbit. For the newer laryngologist, quality was judged to be equivalent for 22/32 images and rigid endoscopy superior for 10/32 images. While both laryngologists were fellowship-trained with experience in the interpretation of in vivo rabbit laryngeal imaging, the findings regarding experience level are consistent with other studies involving perceptual judgements and voice (Boles et al., 2023; Cammarota et al., 2006; Ellerston et al., 2023). It is important that ratings from each reviewer be considered as accurate and clinically meaningful when interpreting results from the current study. Perhaps with experience comes greater expectations for image quality, whereas images may be deemed adequate for a newer rater. It is also essential to note that both raters were involved in the interpretation of flexible endoscopy rabbit images from the 2-year parent project and judged all images as acceptable for purposes of visual-perceptual ratings. So, it is also possible that raters differed on the level of acceptability versus superiority in this thesis dataset. Regardless, the overarching results from this study demonstrate that flexible endoscopy is a viable method for in vivo small animal research, with particular advantages for longitudinal studies; however, rigid endoscopy still yields the highest overall image quality.
Severity Ratings

Although the primary aim of this study was to determine the comparability of flexible versus rigid endoscopy in the rabbit animal model, a secondary purpose was to compare visual-perceptual severity ratings of vocal fold erythema and edema. The examination of these secondary questions was undertaken with the goal of providing additional details and potential contributing factors that might influence flexible versus rigid endoscopy interpretation. Parameters of vocal fold erythema and edema were chosen because of the larger parent project that documented adverse voice function changes associated with visual-perceptual ratings of erythema and edema. Specifically, increases in onset pressure and flow have been documented in the experimental group rabbits from this study, which were euthanized, and larynges extracted for benchtop aerodynamic assessment once visual-perceptual detection of erythema or edema was completed (Bullock, 2024). This is a particularly important point as applied to the current study because the vocal fold abnormalities identified in this study were corroborated in a parallel study of aerodynamic voice function changes in the same larynges. Therefore, for purposes of this thesis, it is known that the ratings of vocal fold abnormality in this study were indeed correct with respect to the presence or absence of edema and/or erythema. Furthermore, the inter-judge and intra-judge reliability of severity ratings in this study were fairly strong, certainly within acceptable limits for a smaller study such as this.

As discussed above, the results from this study indicated that rabbits from the experimental group had some degree of abnormality related to vocal fold erythema and edema. Both raters identified these changes. The VAS rating approach in this study was a departure from that of the parent project, which relied on presence or absence ratings and subsequent consensus discussion, if needed, to determine when rabbits should change phases of the experiment. This
study required severity scaling versus just presence or absence. Severity scaling was included in this study to attempt to elucidate why certain vocal fold images might be better or more useful than others in animal research. The results of the current study showed that flexible versus rigid ratings were significantly different in severity for both severity parameters. The results are best characterized in Figure 9 and Figure 10, wherein the differences in magnitude for flexible versus rigid endoscopy images are depicted. Clearly, images from flexible endoscopy resulted in markedly greater severity ratings as compared to rigid. Put another way, based on the rigid scope images, this sample of larynges were either at baseline or at the threshold of demonstrating abnormal changes to the true vocal folds. Therefore, one would expect the VAS ratings to be smaller in magnitude, reflecting this minimal change. It could be that the magnitude of change seen in rigid endoscopy procedures was a more valid reflection of the presence or absence of erythema and/or edema, whereas the flexible endoscopy images may have exaggerated changes in the laryngeal tissue. However, it could also be suggested that flexible endoscopy may have been more sensitive to those changes than rigid.

The laryngology raters in this thesis had experience rating in excess of 1,000 flexible endoscopy rabbit images from the parent project, but fewer from rigid. So, it is possible that familiarity with flexible endoscopy images resulted in the observed scaling differences. It is also important to consider the flexible camera used, which supported vascularity enhancement and might have also contributed to scaling; nonetheless, this enhancement does not introduce abnormality and yielded accurate ratings for randomized baseline image ratings in the parent project. Taken together, the findings from this study support previous work on the overall superiority of rigid versus flexible endoscopy (Boles et al., 2023; Eller et al., 2008; Milstein et
al., 2005); however, it also demonstrates the utility and sensitivity of flexible endoscopy as a tool for in vivo animal studies of voice.

**Limitations**

There are several limitations to the current study that warrant attention. It is important to acknowledge that this study included small sample sizes for both rabbits and raters. If this thesis had included more raters, this would have likely resulted in increased reliability and assisted in the determination of whether a given rater was reliable for the study. Manipulation of images for color cast was purposely avoided so that the ratings were as close to clinical ratings as possible. Physicians are used to seeing images with different color cast for different cameras; however, due to the specific settings that were used during flexible endoscopy procedures, it is likely that this could have influenced the raters’ perceived erythema changes due to the enhanced vascularity from scope settings. Another limitation to this study includes the level of expertise, experience, and training with endoscopy equipment of the team members involved in this study; individuals with less experience and training could have impacted image quality for both flexible and rigid endoscopy procedures (Patel, 2007).

Another limitation is that the changes related to IC use can influence the entire larynx and not just the vocal folds. This can be challenging for both human and animal subjects due to the difficult nature of quantifying the level of erythema and edema when the entire larynx is affected. One of the advantages of performing flexible endoscopy in humans is that it allows for more representative posture of the larynx; however, when performing rigid endoscopy in rabbits, it is difficult to avoid disturbing the rabbit’s resting respiration posture (Tran et al., 2001). Because of this, a limitation of the current study was that it was occasionally challenging to see
the entirety of the arytenoid cartilage and true vocal folds close up using rigid endoscopy in a single image; this was easier to do using flexible endoscopy.

**Implications for Future Research**

Based on the findings of this thesis, there are significant implications for future animal research into tissue changes in the larynx. Researchers will need to consider using the same endoscopy method throughout a given study, especially studies that are primarily measuring changes over time. While both raters in this study have extensive experience viewing rabbit and human larynges, including rating about 100 rabbits, Rater 2 has more overall experience. For future research, it is recommended that the level of training for raters be consistent. Similarly, there should be standardization of training among individuals who perform endoscopy procedures. Additionally, researchers may want to consider color calibration of the scope system and evaluate potential bias in the system they use (Krantz, 2008).

**Conclusions**

The preliminary data found in this study indicate that rigid endoscopy is better than flexible endoscopy regarding image quality; this is consistent with other literature. However, different levels of rater experience in the current work had some impact on the interpretation of whether rigid and flexible were comparable. Both methods demonstrated sensitivity to erythema and edema changes, even though the flexible endoscopy procedures might have exaggerated the magnitude of those changes. This thesis aligns with other work on the potential applications of flexible endoscopy as a method for small animal laryngeal imaging. As imaging technology advances, including the development of smaller flexible endoscopes, we anticipate flexible endoscopy to approach the quality of rigid endoscopy as it is in the clinical setting.
References


APPENDIX A

Annotated Bibliography

This annotated bibliography contains a literature review of research articles used in the formation of the research questions and experimental design of this thesis, including use of animal models in voice and airway research, endoscopy methods, and visual-perceptual data. Each article contains a description of the purpose, method, results, conclusion, and the article’s relevance to the current work, as well as its reference.


**Purpose of the study:** The objective of this study was to investigate the phonation of excised pig, sheep, and cow larynges to determine the best model of human phonation. Because many animal species have been employed in the current literature, the authors of this study set out to explore both similarities and differences between humans and other species that may account for differences in the phonatory mechanism and productions.

**Method:** Acquired from a local butcher shop, this study used eight pigs, eight sheep, and six excised cow larynges, each undergoing fine and gross dissection. Each larynx was mounted over a tapered tube on an ex-vivo benchtop mount that produced pressurized, heated, and humidified air. Using sutures to mimic vocal fold adduction, phonation trials were performed for each larynx to measure pressure and flow. During each trial, the subglottal pressure, electroglottograph, flow rate, acoustic signal, and sound pressure level were recorded.
**Results:** This study found that pig, sheep, and cow larynges each demonstrated different phonatory ranges and pressure-frequency behaviors. Results showed that all three species shared similar 2-4 cm length supraglottic ducts. The pig had the highest fundamental frequency, while the cow had the lowest. Additionally, the pig had the largest oscillatory range out all three models.

**Conclusion:** The findings of this study suggested that pig, sheep, and cow excised larynges are suitable models for phonation and aerodynamic measures, especially when larynx size and phonatory range need to be considered.

**Relevance to the current study:** The current study is using a rabbit model as a translational study to the effects of ICs on the human voice. This thesis explores how and why a rabbit model is more suitable for the current work although research has demonstrated success with other animal species in voice research.


**Purpose of the study:** The purpose of this study was to provide a comprehensive description of the surgical anatomy and approach to the nasal vault and septum in rabbits.

**Method:** The method of this study was an ex vivo design. Rabbit head specimens were used to gain familiarity with relevant anatomical structures and to gain practice with various approaches. This study used four live Pasteurella free New Zealand white rabbits, between 9 and 12 months. To gain entrance to the nasal vault, a three-osteotomy approach was used, which allowed for the nasal cavity to be exposed
bilaterally. This also helped to maintain the solidity of the mucoperichondrial, post septal cartilage harvest. All rabbits received daily observation for 4 weeks within the postoperative period to watch for signs of infection, pain, and complications.

**Results:** The results of this study found that post-operation, all rabbits could be studied for 4 weeks and had no complications. There were no abnormalities or septal perforations observed. Results also showed that entrance to the nasal septum bilaterally was successful in all the rabbit specimens. Additionally, after extubation, all procedural sites looked like the preoperative nasal dorsum, with no tissue lesions. While edema was observable in all rabbits post procedure, this had no effect on the animals’ ability to breathe nasally. After 4 weeks, there were no signs of the incision site and upper respiratory tract infection. Additionally, all rabbit snouts maintained their original shape and appearance.

**Conclusion:** The authors of this study concluded that submucosal resection in the septal cartilage of a rabbit, using a three-ostomy dorsal approach is successful. They deem that the technique demonstrated in this study was safe and resulted in minimal distortion and no complications during and after the operation. Overall, this study’s technique and method preserved the integrity of the rabbit nasal airway, anatomy, and dynamics.

**Relevance to the current work:** This study provided a significant overview of rabbit nasal anatomy, its structures, and their locations. The current study is exploring the feasibility of endoscopy in rabbits.

Purpose of the study: The focus of this work was to determine the validity and reliability of the Reflux Finding Score (RFS), an 8-item clinical severity scale.

Method: Forty patients with a clinical diagnosis of LPR were employed in this study. Before treatment and 2, 4, and 6 months after treatment, all patients were evaluated with proton pump inhibitors. Daily, each patient was treated with either 20 mg omeprazole, 30 mg lansoprazole, or 20 mg rabeprazole. Each patient underwent flexible fiberoptic endoscopy with laryngeal photo documentation and RFS rating each visit. Final scores on this scale can range from zero to 26, with zero representing no pathology, 11 and higher indicating LPR, and 26 representing a severe pathology. For the purposes of validity, the pretreatment RFS was compared to the three posttreatment RFS. For test-retest intra-rater reliability, the RFS for the photo documented larynges were rated by one blinded laryngologist on 2 separate days, less than 24 hours apart. To assess inter-rater reliability, two different laryngologists who were blinded provided an RFS for the photo documented larynges.

Results: The results of this study found that pre-treatment RFS ratings for patient’s diagnosed with LPR had a mean score of 11.5. This score improved to 9.3 at the 2-month post treatment mark, 7.3 at 4 months, and 6.1 at 6 months. Participants in the control group had a mean RFS of 5.2. Both the inter and intra rater reliability assessments resulted in correlation coefficients all greater that 0.90, demonstrating excellent reliability measures.

Conclusion: The authors concluded that while the RFS is subjective, it can reliably and accurately document improvement with treatment targeting reflux.
Additionally, this article’s findings suggest determining improvement in patients with LPR is dependent on clinicians’ ability to document and record abnormal laryngeal findings.

Relevance to the current work: Like this study, the current work is examining laryngeal erythema and edema. This thesis is comparing endoscopy techniques with visual perceptual ratings of erythema and edema in experimental and control rabbit larynges undergoing IC treatment.


Purpose of the study: The purpose of the study was to study the effects of inhaled corticosteroids in adults to examine potential laryngeal and pharyngeal symptoms.

Method: The methodology of the study included a pilot respiratory questionnaire distributed to 20 participants who were confirmed to have pharyngolaryngitis due to the use of inhaled corticosteroids. Based off this pilot questionnaire, a new one was created to add additional questions based on symptoms. Overall, 190 patients were included in this study based on current inhaled corticosteroid use and asthma. Participants were labeled as mild, moderate, or severe asthmatics using the British Thoracic Society guidelines. Data including smoking history inhaler use, including duration, strength, type, and dosing regimen were recorded. Side effects such as hoarseness, voice weakness, voice loss, sore throat, throat irritation, and persistent cough were
investigated. Using a 10-point visual analogue scale, inhaler induced throat discomfort was assessed.

**Results:** The majority of the study’s participants were classified as mild or moderate asthmatics. Results showed that the median and mean discomfort scores on the analogue scale were 4.8 and 4.26, with zero indicating no discomfort and 10 indicating the highest level of discomfort caused by the inhaled corticosteroid. Most of the participants reported using their inhaled corticosteroid for between 1 and 5 years. The most observed side effects of inhaled corticosteroid usage were hoarseness, throat irritation, sore throat, and cough. Results showed that there was a positive correlation between worsening asthma severity and frequency of aphonia. Additionally, inhaled corticosteroid use was positively correlated to hoarseness. Hoarseness and persistent cough were present in individuals with more severe asthma. Overall, pharyngeal and laryngeal symptoms were more prevalent as the asthma severity in an individual worsened.

**Conclusion:** Further research is warranted to investigate how and why inhaled corticosteroids cause local pharyngeal and laryngeal symptoms. It is critical that this area of research be performed in the collaboration of pulmonologists, voice care specialists, and laryngologists who specifically treat asthmatic patients.

**Relevance to the current work:** The current study is part of a larger parent studying the effects of inhaled corticosteroid use on vocal function. This study investigates pharyngeal and laryngeal symptoms, including throat irritation. This thesis will include visual perceptual ratings of endoscopic images of healthy control and experimental rabbits undergoing inhaled corticosteroid use.
Purpose of the study: The primary objective of this study was to do a comparison between flexible distal chip laryngoscopy (FDL) and rigid telescopic laryngoscopy (RTL) to investigate their image quality and diagnostic accuracy.

Method: The subjects in this study included 18 healthy adults who underwent both FDL and RTL. RTL exams used a rigid 70-degree scope with a 10 mm outer diameter and FDL exams used a 130-degree flexible scope with a 3.5 mm diameter. Participants were asked to produce sustained vowels with varying pitch during both the FDL and RTL exams. A survey was then administered to three trained laryngologists to blindly rate the image quality and diagnostic ability of both FDL and RTL exam video footage. Additionally, blind raters were asked to report and describe any abnormalities they observed in either of the imaging videos.

Results: The results of the study found that RTL was rated higher in color fidelity, resolution, and tissue vascularity when compared with FDL. While inter-rater reliability demonstrated slight agreement for color fidelity, resolution, and vascularity in RTL, there was poor agreement between raters for illumination. Abnormalities were detected with both visualization techniques; however, an abnormality was observed six times with RTL, but was not detected using FDL.

Conclusion: This study showed that rigid laryngoscopy is significantly better at producing higher quality images and providing diagnostic accuracy. This demonstrates
consistent findings with prior research where RTL has been determined to be the gold standard for superior image quality.

**Relevance to the current study:** The current study is performing a similar comparison between rigid and flexible endoscopy to determine the most advantageous technique for visualization of rabbit larynges. The current study will also include blind raters for a visual perceptual analysis of rigid and flexible scope images.


**Purpose of the study:** The purpose of this work was to perform a comparison between flexible distal chip laryngoscope (FDL) and rigid telescopic laryngoscope (RTL) to examine patient satisfaction, comfort, and experience with both imaging techniques.

**Method:** This study used a randomized crossover design. Twenty-three adults participated in the study and underwent both FDL and RTL in a randomized order. Anesthetics were applied surrounding the participants’ nares for the FDL procedure but were not used for the RTL procedure. Post procedure, for both exams, participants and clinicians were asked to fill out a 7-point Likert scale questionnaire regarding worry, discomfort, pain, satisfaction, and willingness to have the procedure again. Clinicians had an additional rating on their questionnaire asking them to report the difficulty of each procedure. Both participants’ and subjects’ perceptions were then analyzed.

**Results:** The results of the study showed that 10 out of the 23 subjects reported preference over undergoing FDL again, while 13 subjects reported preference over
undergoing RTL again. Participants rated FDL to be more uncomfortable and painful than RTL. Additionally, clinicians reported similar ratings regarding patient worry, discomfort, and satisfaction for both exams, and stated that both FDL and RTL procedures were similar in difficulty.

**Conclusion:** The authors of this study concluded that flexible laryngoscopy was perceived by patients to be more uncomfortable and painful than rigid laryngoscopy. However, both methods are associated with high levels of satisfaction.

**Relevance to the current work:** The current study is performing a similar comparison of rigid and flexible endoscopy to determine best visualization of laryngeal structures within a small animal model.


**Purpose of the study:** The purpose of the study was to examine the epithelium effects of induced reflux in an in-vivo porcine model.

**Method:** This study included eight domestic pigs that were randomly assigned to a reflux and control group. While receiving 100% oxygen, each animal underwent sedation using a combination of Telazol and xylazine hydrochloride via intramuscular injection, with inhaled isoflurane to maintain sedation. Animals were scoped using a QIF 160 Olympus endoscope to visualize the larynx and an endoscopic aspiration catheter was used to administer either saline or acidified pepsin directly onto the membranous portion of the vocal folds. This procedure was repeated three times a week across 4 weeks. Each animal was then immediately euthanized.
**Results:** The results of the study indicated no significant differences or changes in vocal fold epithelium, lamina propria, and the thyroarytenoid muscle between the experimental and control groups.

**Conclusion:** Based on the results of this study, the authors suggest that healthy porcine vocal folds can effectively fight and protect against acidified pepsin. This study contributed to laying the foundation for future research in understanding the pathophysiology of laryngopharyngeal reflux.

**Relevance to the current work:** This study’s methodology employed repeated use of flexible endoscopy under sedation and anesthesia in an animal model. The current work is exploring visualization techniques within a rabbit model using anesthesia and comparing rigid and flexible endoscopes.


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**Purpose of the study:** The objective of this study was to compare distal chip (DC) endoscopes and high quality fiberoptic (FO) endoscopes to determine which provided more diagnostic information. Additionally, this study sought out to explore whether DC scopes provide sufficient image quality to replace traditional rigid endoscopy for routine videostroboscopy assessments.

**Method:** Using the KayPENTAX strobe database, this study used 34 total patients, with 17 in the FO group and 17 in the DC group. Flexible and rigid stroboscopy footage were grouped by type and set in a random order. For the purposes of intra-rater
reliability, three FO patients and three DC patients were chosen at random to be presented twice in each data set group. All stroboscopy segments were rated by experienced and trained individuals in the field of otolaryngology. Raters were asked to provide a diagnosis if possible and to describe the vocal fold mucosal wave. Both types of flexible endoscopes were compared to determine the closest similarity to a rigid endoscopy procedure.

**Results:** The results of this study showed that the raters diagnosed more lesions with the rigid endoscopy segments than with the flexible exams. Furthermore, for vocal fold mass diagnoses alone, the FO endoscope identified 86.3% of the lesions within the same patient using a rigid endoscope. Similarly, the DC endoscope identified 87.3% of masses, indicating an insignificant difference between the two. Both types of flexible scopes identified more pathologies within the mucosal wave than with the rigid scope.

**Conclusion:** This study concluded that DC endoscopes do not provide any advantages over traditional FO endoscopes when identifying vocal pathologies, indicating that either technologies can provide high quality examination of the voice and larynx. However, the findings of this study included limitations, including varying range of rater experience matching the demographics of those who regularly use these types of endoscopes could have produced confounding variables.

**Relevance to the current work:** The current thesis is similarly performing a comparison study between two types of scoping techniques: flexible and rigid endoscopy. Additionally, this thesis has a visual perceptual component like this study with experienced raters; however, the rating measures for the current work are for erythema and edema in laryngeal structures.
Purpose of the study: The purpose of this study was to examine the adverse effects of ICs on phonatory measures, including PTP and perceived phonation effort (PPE). A second aim of this study was to investigate the relationship between PTP and PPE in individuals that received the experimental treatment of ICs.

Method: The method of this study employed nine women and five men, all in good general health with healthy vocal ability. To qualify, all participants completed the Voice Handicap Index (VHI) and underwent routine rigid videostroboscopy. In counterbalanced order, subjects received either the IC or sham treatment in two experimental sessions at the same time on consecutive days. The dependent variables of this study, PTP and PPE, were measured prior to the experimental and sham treatments to get baseline measures, immediately after receiving the treatments, 1 hour post treatment, and 2 hours post treatment. PPE was collected with each participant using a vented pneumotachograph where they were instructed and trained to produce a five syllable /pi/ string. For PTP, the pneumotachograph mask was fitted to low and wide bandwidth pressure transducers that were coupled to a Glottal Enterprises MSIF-2 system to collect additionally collect airflow measures with the pressure measures. For PPE, participants used a visual analog scale to rate perceived vocal effort.

Results: The findings of this study showed that IC treatment had an acute adverse effect in increasing PTP, while the sham treatment did not. It was observed that the increase in PTP lasted about 2 hours immediately following administration of the IC
treatment. The results of the participants’ ratings indicated that PTP was not significantly related to PTP.

**Conclusion:** This study concluded that IC treatments can have adverse effects in phonation in a population of healthy adult individuals. Additionally, the data in this study showed that the effects on vocal ability can occur in a brief amount of time.

**Relevance to the current work:** The current thesis is part of a parent study investigating the adverse effects of ICs on vocal health, similar to this study. Additionally, the current work is employing a similarly structured visual analog scale to rate the severity of erythema and edema in rabbit larynges that were exposed to IC treatment.


**Purpose of the study:** The focus of this study was to investigate the effects of ICs in asthmatic patients. Specifically, this study sought to determine vocal and laryngeal changes.

**Method:** This study included 15 male and 15 female participants, each diagnosed with bronchial asthma and undergoing current IC treatment, during a minimum duration of 4 months. Each patient underwent a voice evaluation protocol that included a comprehensive case history and a routine ENT assessment, including rigid endoscopy; although, a small number of participants underwent flexible nasolaryngoscopy due to low tolerance with the rigid scope. Images were then acquired and systemically analyzed from each endoscopy procedure by two expert phoniaticians. Ratings were provided on a
4-point scale, observing vocal fold edema, erythema, bowing, atrophy, irregular vocal fold edges, interarytenoid thickening and supraglottic hyperfunction. All study subjects underwent acoustic analysis of their voice using a multidimensional voice program software.

**Results:** Results of this study found that 53% of participants had dysphonia, with 36.7% with mild, 16.7% with moderate, and 47% with difficult or abnormal voice symptoms. Vocal fold erythema was observed in 56.7% of the patients, interarytenoid thickening was present in 56.7%, bowing was present in 5.3%, and vocal fold atrophy was present in 5.5%. About 36.7% of patients presented with LPR symptoms. This study also found that vocal fold atrophy and bowing was significantly related to the duration of IC treatment.

**Conclusion:** This study concluded that ICs demonstrate adverse effects on laryngeal structure and function in humans.

**Relevance to the current work:** The current thesis is part of a larger study investigating the adverse effects of ICs. This study is relevant because the current work is investigating the comparison between rigid and flexible endoscopy procedures in rabbits that underwent IC treatment. Similarly, the current working is using visual perceptual ratings to observe vocal fold erythema and edema.


**Purpose of the study:** The objective of this study was to evaluate the oral cavity of different mammals using a pediatric laryngoscope and rigid endoscope.
**Method:** This study used 170 small mammals, including 65 rabbits, 35 chinchillas, 38 guinea pigs, 19 degus, and 13 prairie dogs. Each animal underwent a clinical examination to determine health and observe anatomical structures. Animals then received laryngoscopy without sedation. If any observable pathological changes were seen, animals would then undergo rigid endoscopy for further evaluation. For the rigid endoscope procedures, a xenon light source was used for clearer visualization and animals were sedated and anesthetized. To collect tissue samples during rigid endoscopy, forceps were used.

**Results:** Laryngoscope procedures in all animals to evaluate the oral cavity was safe. In five procedures, minor tongue bruising was observed; however, there were no other observable injuries from the laryngoscope during examination. There was a total of 305 procedures, with each animal undergoing between one to four examinations.

**Conclusion:** Endoscopy was concluded to be the most effective in gaining detailed visualization of pathologies within the oral cavity and oropharynx in small mammals.

**Relevance to the current work:** The current study includes a pilot comparison between rigid and flexible laryngoscopy in New Zealand White rabbits to determine best image quality.

Purpose of the study: The objective of this work was to conduct a pilot study using pigs to determine the effectiveness of flexible endoscopic thyroidectomy, utilizing a single operating channel.

Method: Six pigs were utilized for this study. First, each pig underwent endotracheal intubation in supine position with general anesthesia. After an incision was made, forceps were used to expose a channel that led to the thyroid gland, and a guidewire was inserted to expand the channel via a 1.5 cm diameter balloon. Next, a flexible endoscope with one channel was introduced to reach the site of the thyroid. Using an IT knife, the thyroid gland was dissected and underwent histopathological analysis. This procedure was performed and repeated five additional times, consecutively after the first pig.

Results: All six procedures using a flexible endoscope with a single channel to perform a thyroidectomy was successful in a porcine model. Both before and after the procedure, neither bleeding or adverse effects were observed.

Conclusion: This study concluded that flexible endoscopy techniques can be modified from a rigid endoscopy approach and be successful in a porcine animal model to identify and preserve the recurrent laryngeal nerve and parathyroid gland.

Relevance to the current study: This pilot study performed transoral flexible endoscopy to perform thyroidectomy on pigs under anesthesia. The current study similarly involves a pilot study using a rabbit model to perform flexible nasoendoscopy to visualize laryngeal tissues under anesthesia.

**Purpose of the study:** The purpose of this study was to adjust and accommodate the excised larynx benchtop model to make it suitable with rabbit larynges.

**Method:** This study included the use of five excised larynges from adult New Zealand white rabbits. The larynges were mounted on a custom created phonatory benchtop apparatus. The arytenoid cartilages were forced in adducted position by rods and humidified air used to initiate vocal fold oscillation. For each rabbit larynx, acoustic, aerodynamic, electroglottographic, and video kymographic data were collected and recorded. The same data collection was acquired for five canine larynges to do a comparison between the dog and rabbit larynges.

**Results:** The results of this study showed consistent phonation in each larynx. The following data is reported for each rabbit larynx: acoustic fundamental frequency (F0), percentage of shimmer, signal to noise ratio, pressure and flow for phonation onset and offset, F0, closed and speed quotient, jitter and shimmer, contact quotient, mucosal wave amplitude, and phase difference.

**Conclusion:** The authors of this study concluded that when data from the leporine and canine larynges were compared, intra-larynx variability was found to be similar between both sets of larynges. They also concluded that their study was successful in developing a method for recording acoustic, aerodynamic, video kymographic, and electroglottographic data for rabbit larynges.
Relevance to the current work: This study included a comparison of human and rabbit laryngeal histology and methodology of high-speed video. A challenge and remedy to the use of high-speed imaging is briefly discussed. The current work is studying best practices for imaging rabbits using endoscopy.


Purpose of the study: The purpose of the study was to investigate phonatory parameters, including phonation threshold pressure (PTP) and phonation instability pressure (PIP) using a benchtop apparatus in an excised larynx rabbit model.

Method: Seven excised rabbit larynges were harvested and mounted to a benchtop apparatus. For each larynx, aerodynamic acoustic measures and high-speed video were recorded. Airflow was manually controlled until PIP was reached. To prevent the effects of dehydration, larynges were misted with 0.9% saline between phonation trails. Trials were repeated and implemented various elongation conditions.

Results: The findings of this study showed that once PTP was reached, the sound pressure level, subglottal pressure, and fundamental frequency increased as elongation increased. While there appeared to be no effect on elongation variability on PTF, phonation instability flow (PIF) and phonation flow range (PFR) significantly decreased as elongation increased. Additionally, at PTP and PIP vibratory amplitude decreased as elongation increased.

Conclusion: As previous findings in the current literature have supported, this study demonstrated the reliability of using a rabbit model to explore phonatory
parameters. The authors of this study also reported that due to the similarities in vocal fold histology between rabbits and humans, their findings increase the validity of using a rabbit model for tissue inflammation studies.

**Relevance to the current study:** This study presents findings that support the excised rabbit model in phonatory and inflammation experiments. The current study is utilizing rabbits undergoing inhaled corticosteroid treatment and endoscopy to visualize adverse effects, including erythema and edema.


**Purpose of the study:** The objective of this study was to identify the prevalence of Ear Nose Throat (ENT) findings in the typical, healthy adult population, compare flexible and rigid endoscopy examinations to explore the diagnosis specificity of reflux in endoscopic laryngeal procedures.

**Method:** The method of this study employed 24 male and 28 female vocally healthy volunteers. Qualified study participants were determined they filled out a self-administered questionnaire. Randomly assigned, qualified subjects underwent both flexible and rigid stroboscopy evaluations consecutively using a Kay Elemetrics Digital videostroboscopy system. A comprehensive profile of laryngeal and pharyngeal findings visualized during endoscopy was created by practicing ENT physicians and voice clinicians. Video recordings of each procedure were then evaluated by three examiners
for the presence of laryngeal and pharyngeal findings, with a yes or no scoring system. All examiners were blinded to the study participants’ questionnaires.

**Results:** Results indicated that laryngeal pathology signs are more likely to be detected in a population of healthy voice users with a flexible endoscope than a rigid endoscope. Furthermore, flexible laryngoscopy was significantly more likely than rigid to identify posterior pharyngeal wall, interarytenoid mucosa, pseudosulcus, interarytenoid mucosa, and arytenoid complex abnormalities. Overall, it was found that inter-rater agreement was relatively low.

**Conclusion:** This study suggested that laryngeal pathology identification is dependent on intra and inter-rater reliability but is also significantly dependent on the tools that are used. This study demonstrated that laryngeal examination via the traditional flexible endoscope has higher sensitivity but lower specificity in identifying potential tissue changes related to LPR.

**Relevance to the current work:** The current study is investigating an ideal imaging technique to visualize in-vivo rabbit larynges. Similar to this study, this thesis sought out to determine advantages and disadvantages between rigid and flexible endoscopy techniques. Additionally, the current work is identifying the potential adverse effects on laryngeal tissues from ICs.

**Purpose of the study:** The purpose of this study was to investigate the extent of associated effects of inhaled corticosteroid (ICS) use, such as dysphonia, within the adult population.

**Method:** Using the institution’s patient data registry, study participants diagnosed with dysphonia by an ear, nose, and throat physician were determined. Each patient was then matched with a paired control, resulting in a study of 6,551 participants. For each paired group of subjects, the following were evaluated: exposure to ICS, the time between dysphonia diagnosis and date of prescribed ICS. Using Fischer’s exact test, both pulmonary diagnoses and exposure of ICS use were compared between subjects.

**Results:** The results of the study demonstrated that inhaled corticosteroid use is significantly associated with dysphonia. The prevalence of ICS use and subjects diagnosed with dysphonia was 9.7%, while the paired control groups had a 2.1% prevalence. Findings also indicated that MDI and DPI steroids were both associated with dysphonic changes in the voice. No significant differences were found between inhaler particle types or the specific active medications in the inhalers, such as fluticasone.

**Conclusion:** This study supports previous research findings investigating the adverse effects of ICS. The higher the dosage of ICS, the more risk a patient is at for developing a voice disorder. The findings in this study demonstrate a critical need for physicians to consider ICS usage, including dosage, type, and administration when assessing patients with dysphonia.

**Relevance to the current study:** The current study is part of a parent study that is investigating the adverse effects of ICS on New Zealand White rabbits. This thesis will
provide data on a pilot study examining image quality between rigid and flexible laryngoscopy.


**Purpose of the study:** The objective of this study was to explore a nonstimulated rabbit phonation model using an Isshiki type IV thyroplasty surgery and a constant source of humidified airflow.

**Method:** This study used six male New Zealand white rabbits, each weighing 3 to 4 kg. All rabbits were anesthetized with ketamine, xylazine, and acepromazine via intramuscular injection. Vitals were consistently monitored to assess the animal’s well-being and state of sedation. Rabbits then underwent surgery including an Isshiki type IV thyroplasty. Using high speed video imaging, phonation parameters were examined, and an acoustic and aerodynamic analysis was performed, including F0 (Hz), vocal intensity (dB), subglottal pressure (cm H20), and airflow (mL/s). After, animals were humanely scarified, and their larynges were excised. Magnetic resonance imaging (MRI) was completed for the purposes of validation of glottal configuration and previous laryngeal models used in other research simulations.

**Results:** The use of high-speed laryngeal video imaging demonstrated sustained vocal fold phonation throughout the experimental thyroplasty procedure. Analysis of acoustic signals indicated a mean vocal intensity of 61 dB and an F0 of 590 Hz. Additionally, aerodynamic measures resulted in a mean airflow rate of 85.91 mL/s and a
mean subglottal pressure of 9 cmH20. MRI procedures revealed that glottal configuration was maintained.

**Conclusion:** This study concluded that an in-vivo nonstimulated rabbit model with sustained glottal airflow was successful. The authors of this study suggest that this finding and methodology can produce sustained phonation and phonatory measures that are consistent with other research findings that used neuromuscular stimulation.

**Relevance to the current work:** Like this study, the current thesis is employing an in vivo rabbit model, rather than an ex-vivo model, to complete a novel methodology, which is a comparison between endoscopy techniques.


**Purpose of the study:** The purpose of the study was to examine the changes in sustained subglottal air pressure and airflow during phonation in experimental excised rabbit larynges receiving inhaled corticosteroids. Additionally, this study included visual perceptual ratings to compare edema and erythema in both experimental and control rabbit larynges.

**Method:** The method of this study included a benchtop model using excised and finely dissected larynges to collect data on aerodynamic measurements of phonation. As each larynx underwent 15 trials, airflow, pressure, and acoustic signals were collected using LabChart. Still images of taken of each larynx post mounting and before phonation trials for a visual perceptual analysis. Six blinded raters rated each laryngeal image of the
vocal folds for the severity of edema and erythema on the arytenoid and vocal fold tissues.

**Results:** The results of this thesis showed that larynges that received inhaled corticosteroid drugs had significantly higher levels of sustained pressure and airflow when compared to the control group, who received nebulized isotonic saline. The visual perceptual analysis showed higher ratings of edema and erythema of the vocal folds in experimental larynges versus the control larynges.

**Conclusion:** The author of this thesis concluded that inhaled corticosteroid treatment has negative effects on vocal function and laryngeal tissues.

**Relevance to the current study:** The current study is part of a larger study that is researching the effects of inhaled corticosteroid use on the voice. This thesis will also include a pilot study that will have visual perceptual ratings of endoscopic images of healthy control and experimental rabbits undergoing inhaled corticosteroid use.


**Purpose of the study:** The focus of this study was to provide recommended protocols for instrumental voice assessment, including laryngoscopic imaging and acoustic and aerodynamic analyses.

**Method:** To develop the recommendations for this work, an expert panel reviewed voice assessment protocols from various sources, including textbooks, peer-
reviewed and non-peer reviewed articles, and materials acquired from protocol requests from the voice special interest group from the American Speech Language Hearing Association (ASHA). When there was a lack of evidence, the panel came to a consensus through multiple discussions. The protocol was revised and edited three times with 10 consensus meetings before being presented at the 2015 ASHA annual convention.

**Results:** The results of this work provided a complete Instrumental Voice Assessment Protocol that included recommendations for laryngeal endoscopy, data acquisition for acoustic and aerodynamic measures, voice and speech tasks, and data analysis to reach a uniform method to evaluate vocal function in clinical and research settings.

**Conclusion:** The authors of this work concluded that the recommended protocols for vocal function assessment are valid and reliable measures that be employed and compared across various populations of clients and facilities.

**Relevance to the current work:** With the product of this article and the determination that endoscopy is the gold standard in voice assessment, the aim of the current thesis is to determine whether flexible endoscopy is a viable alternative method to imaging an in-vivo rabbit model to observe adverse laryngeal effects from IC treatment.


**Purpose of the study:** The aim of this study was to introduce a novel rating and training protocol that includes visual perceptual ratings for videostroboscopy and HSV imaging.
This study sough to evaluate the inter and intra judge reliability of raters using the proposed rating form, the Voice-Vibratory Assessment with Laryngeal Imaging (VALI).

**Method:** This study used video samples from 10 adult male, 19 adult female, and one child participant with dysphonia. Using the grade, roughness, breathiness, asthenia, and strain scale (GRBAS), auditory perceptual ratings of each participants’ voice quality was performed. Each subject underwent stroboscopy and HSV video imaging, using a rigid 70-degree endoscope. Videostroboscopy was captured at 30 fps with a 640x480 spatial resolution, whereas the black and white footage with HSV were captured at 4,000 fps with a spatial resolution of 256x512 pixels. Both endoscopy procedures were captured within the same visit using standardized instructions, identical physical position, and endoscope. Nine experienced speech-language pathologists were trained to use the VALI form to rate 66 de-identified and randomized samples from dysphonic individuals. Inter and intra rater reliability was completed.

**Results:** Inter-rater reliability ranged from 0.57 to 0.96 for video stroboscopy and from 0.81 to 0.94 for HSV imaging. Both HSV and stroboscopy parameters showed strong correlation coefficients, indicating strong agreement between raters.

**Conclusion:** The VALI form is a newly developed rating form for stroboscopy and HSV techniques that can be used to make reliable visual perceptual judgements when combined with sufficient training. The authors of the study conclude that further revisions and refinement to training and the form could increase its usefulness and higher reliability measures.

**Relevance to the current work:** The current thesis includes visual perceptual ratings as part of the work’s methodology to compare rigid and flexible endoscopy
techniques in an in-vivo rabbit model. Additionally, like this study, the current work sought to determine inter and intra rater reliability measures among raters.


**Purpose of the study:** The purpose of this study was to investigate various techniques using flexible high speed video laryngoscopy to determine the best approach to enhance the imaging brightness.

**Method:** The method of this study included a systematic system, implementing various scope manipulations, including the camera frame rate, the lens coupler focal length, camera software parameters, using the scope's outer diameter, using a supplemental light fiber bundle and light source, and the distance from the distal end of the scope to the glottis.

**Results:** The results of the study showed that to gain consistently bright colored images KayPENTAX Model 9710 and high-speed video laryngoscopy can be used. Authors found that using the High Gain camera setting, short focal length (20 mm) lens coupler, and proximity of the distal end of the scope to the glottis resulted in the best image brightness. Outcomes also indicated that using additional gain in the sensor resulted in poorer image quality, as evidenced by observed image fuzziness. Small to moderate outer diameter flexible scopes were found to be usable when obtaining color imaging of the vocal folds, using frame rates of 1000 to 2000 frames per second (fps).

**Conclusion:** The conclusions of this study indicate the need for analysis of laryngeal structures and observation of phonation through high-speed color images. Authors concluded that a large outer diameter scope can be used at 2000 or 3000 fps from
a higher position in the laryno-pharyngeal space, this can result in improved imaging brightness. More research and adjustments need to be made to optimize visualization techniques.

**Relevance to the current work:** The current study involves a literature review on laryngeal imaging using flexible and rigid endoscopy. This article explores various visualization techniques in humans using a high-speed video laryngoscope, which will aid in the current investigation to determine methodology for imaging rabbit larynges.


**Purpose of the study:** The purpose of this study was to use a rabbit model to examine nasal inflammation and ulceration that is secondary to damage that is produced by an intranasal device.

**Method:** This study employed 48 New Zealand white rabbits of both sexes. Animals were assigned to either a 4- and 8-week experimental drug group or the control group. Rabbits in the 4-week group received treatment once a day, while rabbits in the 8-week group received treatment twice a day. One male and female rabbit was set aside for recovery phases. Four animals, two female and two male, did not receive the control or drug treatment, and did not come in contact with the intranasal delivery device.

**Results:** The outcomes of this study found that all rabbits from the drug and control groups, and that were treated for both 4 and 8 weeks, had observable histopathological changes in parts and full sections of the nose. Histopathological changes were also seen in the eyes of some rabbits that were either treated with the drug
or control treatment for 8 weeks. Nasal inflammation was noted in both control and drug-treated rabbits, which was ultimately due to the trauma created by the repeated use of the device, rather than the experimental drug itself.

**Conclusion:** The authors of this study concluded that the histopathological changes indicated in this study is critical for future safety studies, especially in rabbits. Due to rabbits’ nasal anatomy, they are more prone to obstruction in the nasal passageway and infection, which could lead to eye infection.

**Relevance to the current work:** This study presents valuable information on the potentially harmful effects of intranasal device administration. The current study is interested in the feasibility of endoscopy in rabbits via the nasal passageway. This study provides knowledge and the risks that can be associated when dealing with the rabbit’s nasal anatomical structures.


**Purpose of the study:** The purpose of the study was to examine the effects of ICs on healthy adult voice users. The primary questions of the study were whether repeated short-term use of ICS affects acoustic characteristics of voice and if sex influenced the side effects of ICS on vocal function.

**Method:** Fifteen females and 15 males were included in the study. All participants had no history of communication disorders and were healthy voice users. Inhaled corticosteroids were administered, and audio recordings of each participants’ voice were collected over 6 days consecutively. A baseline voice recording was taken
prior to the administration of a 500-microgram dose of ICS for each participant. All participants were instructed to avoid participating in any vocally abusive behaviors. Acoustic measurements, including fundamental frequency, formant frequency, formant bandwidth, long time spectral analysis (LTAS), first spectral peak, and spectral tilt were all taken.

**Results:** The fundamental frequency analysis indicated that ICS use did not significantly change vocal F0 in the male or female participants. Females were found to have a higher first formant frequency (F1) than males, while no significant effects of ICS were found in the male and female participants for second formant frequency (F2). Similarly, there were no observed effects in the first and second formant frequency bandwidths. Women were found to have higher first spectral peaks than males and was found to be variable across the administration of ICS. Spectral tilt was also found to be significantly lower post ICS use on the first day of treatment; however, it was noted that changes in spectral tilt and first spectral peak reversed within 24 hours post-ICS.

**Conclusion:** This study confirmed previous findings that inhaled corticosteroid use can have adverse effects on voice production, especially on voices that were previously healthy. Further research is warranted to determine the effects of ICS based on dosage.

**Relevance to the current study:** The current study is part of a larger study that is investigating the adverse effects of inhaled corticosteroid use on vocal function. This study had similar objectives and purposes. This thesis will include visual perceptual ratings of endoscopic images of healthy control and experimental rabbits undergoing inhaled corticosteroid use.

**Purpose of the study:** The objective of this study was to investigate aerodynamic effects, PTP and PTF, of combination inhaled corticosteroids in a rabbit model.

**Method:** This study included 22 male white New Zealand, aged 7 to 8 months old. A double-blind randomized experimental and matched control group design was employed. Rabbits in the experimental group received Advair HFA IC doses twice daily while rabbits in the control group received aerosolized isotonic saline doses twice daily. After 8 weeks, all rabbits were euthanized, and their larynges excised to be immersed in phosphate buffered saline and stored at -80 degrees Celsius. Each larynx was then mounted on an ex vivo benchtop model procedure to measure PTP and PTF, with each larynx undergoing 15 phonation trials.

**Results:** The results of this study indicated significant differences between the experimental and control groups. Rabbits that received ICs had higher mean PTP and PTF (worse) values than the matched control rabbits.

**Conclusion:** Rabbits that received the experimental treatment of Advair HFA required greater amounts of air pressure and flow to initiate phonation, equating to vocal effort. The authors of this study report that these findings may suggest that long-acting beta2-adrenergic agonist (LABA) ICs may put patients at risk for voice disorders within a short period of time.
**Relevance to the current study:** This thesis is part of a parent study investigating the adverse effects of ICs on rabbits. This article provides a rationale behind why the rabbit model was chosen over other commonly used animal models in voice and airway research. The current study is performing a pilot study on a rabbit model partly due to the rationale and evidence described in this article.


**Purpose of the study:** The purpose of this study was to provide an overview of current upper airway assessment and endoscopic techniques to understand the comparison between rigid and flexible endoscope methods.

**Method:** The method of this study included two parts: structural/anatomic and physiological/dynamic. Obtaining patient history, including data from various activity states, such as at rest, during exercise, and sleep is the first part of evaluation. Other techniques that are useful in airway evaluation are radiographic studies, computed tomography (CT), and magnetic resonance imaging (MRI). However, the gold standard for upper airway evaluation is endoscopy. This paper provides a review on rigid versus flexible endoscopy and their combined use together in evaluation of the upper airway.

**Results:** This study found specific advantages and disadvantages each scoping method has. Benefits of using a flexible scope include easy sterilization between uses, increased visualization of the upper airway, specifically the anterior commissure, immediate availability, and cost effective. Limitations to the flexible scope are difficulty in viewing the subglottic space, posterior commissure, proximal part of the cervical trachea, and can be a traumatic experience for the patient. Advantages to using the rigid
scope include high-resolution images, admits simultaneous use of other instruments while still under direct visual control, and allows visualization of abnormalities that would not be seen using flexible scope. Disadvantages to the rigid scope are that it is difficulty to use on children, more expensive, more difficult to visualize the nasopharyngeal airway, and it requires the mouth to be opened, the neck extended, and the tongue base lifted in order to see the larynx; this introduces anatomic and airway distortion. Results also showed that when rigid and flexible endoscopy are used together, in addition with sedation/anesthesia, the most precise and beneficial information will result.

**Conclusion:** The authors of this study concluded that the most effective method to evaluate upper airway includes a combination of rigid and flexible endoscopy. Using both techniques will produce detailed, defined visualization of anatomical structures and will yield the most accurate diagnostic information of the upper airway.

**Relevance to the current work:** This article outlined the advantages and limitations to rigid and flexible endoscopy. The current study is investigating the use of flexible endoscopy in rabbits to understand the best approach in imaging their laryngeal anatomy.
APPENDIX B

Instructions for PowerPoint Ratings

Introductory Instructions
Please complete this rating task in one sitting. There are 80 total rating slides (including repeats for reliability), requiring no more than one hour to complete.

This slide deck contains still images of rabbit vocal folds taken *in vivo* at various points in our inhaler study (from baseline to eight weeks). Ratings from our inhaler study were based on flexible endoscopy. This slide deck includes additional images for eight rabbits using rigid endoscopy. For these eight rabbits, flexible and rigid images were taken at the same timepoint and day. In this study, we want to compare the flexible and rigid images on the following parameters:

- General image quality
- Ratings of true vocal fold erythema
- Ratings of true vocal fold edema

For general image quality, you will use better than or worse than symbols (> <) or the equal (=) symbol if you believe the image qualities are equivalent. For erythema and edema ratings, we will use a visual analog scale, with the extreme left of the scale indicating no erythema or no edema, and the extreme right indicating severe erythema or edema. For both rigid and flexible endoscopy, there are zoomed in and zoomed out views. Please use the same criteria for abnormality (erythema/edema) as you did for the inhaler study.

You will see images repeated for purposes of reliability, similar to our inhaler study. Severity (zero to eight weeks) and flex/rigid image presentation will be randomized. Please complete ratings in the sequence presented and do not go back to change previous ratings. Reference photos for the true vocal folds at baseline and eight weeks are provided on the next slide. Instructions will be repeated for the visual analog scale ratings immediately prior to those sections.
Instructions for Erythema Ratings
For erythema and edema ratings, we will use a visual analog scale, with the extreme left of the scale indicating no erythema or no edema, and the extreme right indicating severe erythema or edema.

For visual analog scale ratings, please “drag and drop” the vertical line indicating the magnitude of severity. You can use the entire scale or not depending on your judgements. Please only place one vertical line on each scale.

For both rigid and flexible endoscopy, there are zoomed in and zoomed out views. There is only one rabbit and one type of endoscopy on each slide. Please use the same criteria for abnormality (erythema/edema) as you did for the inhaler study.

You will see images repeated for purposes of reliability, similar to our inhaler study. Severity (zero to eight weeks) and flex/rigid image presentation will be randomized. Please complete ratings in the sequence presented and do not go back to change previous ratings. Reference photos for the true vocal folds at baseline and 8 weeks are provided on the next slide.

Instructions for Edema Ratings
For edema ratings, we will use a visual analog scale, with the extreme left of the scale indicating no edema, and the extreme right indicating severe edema. For visual analog scale ratings, please “drag and drop” the vertical line indicating the magnitude of severity. You can use the entire scale or not depending on your judgements. Please only place one vertical line on each scale.
For both rigid and flexible endoscopy, there are zoomed in and zoomed out views. There is only one rabbit and one type of endoscopy on each slide. Please use the same criteria for abnormality (edema) as you did for the inhaler study.

You will see images repeated for purposes of reliability, similar to our inhaler study. Severity (zero to eight weeks) and flex/rigid image presentation will be randomized. Please complete ratings in the sequence presented and do not go back to change previous ratings. Reference photos for the true vocal folds at baseline and 8 weeks are provided on the next slide.