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## A Comparison of Phonation Threshold Pressure and Phonation Threshold Flow Between Pig and Rabbit Benchtop-Mounted Larynges

Amber Christeen Prigmore  
Brigham Young University

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A Comparison of Phonation Threshold Pressure and Phonation Threshold Flow Between  
Pig and Rabbit Benchtop-Mounted Larynges

Amber Christeen Prigmore

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

Kristine Tanner, Chair  
Christopher Dromey  
Scott L. Thomson

Department of Communication Disorders  
Brigham Young University

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## ABSTRACT

### A Comparison of Phonation Threshold Pressure and Phonation Threshold Flow Between Pig and Rabbit Benchtop-Mounted Larynges

Amber Christeen Prigmore  
Department of Communication Disorders, BYU  
Master of Science

Animal models are used extensively in voice research to study aspects of phonation, including physiology, kinematics, structure, and histology. Animals such as dog, cow, pig, sheep, deer, monkey, ferret, and rabbit have been used in voice research, with pig being one of the most common models. It is thought that the pig larynx is highly similar to the human larynx and one of the best models used in animal translational research. As with any model, however, the pig larynx does have some limitations. Perhaps a limitation most important to the rationale of this investigation is that pigs are difficult animals to study in vivo. Maintenance for a pig is challenging due to its large size and the variability of phonation use in the animal. Therefore, viable and practical alternatives are needed for in vivo voice research.

The current study collected preliminary normative data from an alternate animal model, the rabbit, which has been used more recently in studies to model human phonation. The rabbit model was chosen due to its histological similarities to humans, in vivo phonation patterns, size, and practicality. The rabbit represents a more practical model for some longitudinal designs, as well as ex vivo phonatory models with aerodynamic measures as the primary variables. The current study involved a comparison of two aerodynamic measures, specifically phonation threshold pressure (PTP) and phonation threshold flow (PTF) between two groups, pig and rabbit larynges. The purpose of this study was to determine normative aerodynamic values for rabbits and to compare these with normative values for pigs during excised larynx benchtop phonation. Each group consisted of 15 larynges that were finely dissected to reveal the true vocal folds. Each larynx was then connected to a pseudolung and humidified air was passed through it. Fifteen phonation trials were elicited and the results averaged for each larynx. The results indicated that PTP and PTF were significantly different between the two groups, with PTP and PTF being lower for the rabbit group. Additionally, PTP values for rabbits were closer than pigs to the typical human value; however, some methodological challenges to rabbit benchtop models, including size and structural integrity, also exist. But the results from this study indicate that rabbits should be considered a viable option for voice research that would be more feasible with a small animal option that translates well to humans than a large animal option.

Keywords: phonation threshold pressure, phonation threshold flow, pig, rabbit, excised larynx, benchtop model

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## DESCRIPTION OF THESIS STRUCTURE AND CONTENT

The current document, *A Comparison of Phonation Threshold Pressure and Phonation Threshold Flow Between Pig and Rabbit Benchtop-Mounted Larynges*, has been prepared using a hybrid journal-style thesis format. This work was funded by grants awarded to Dr. Kristine Tanner by the David O. McKay School of Education at Brigham Young University and the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (1R01DC016269-01A1). The research performed for this thesis was part of a larger multi-institutional, long-term project. The work was presented at the American Speech-Language-Hearing Association Annual Convention in Orlando, Florida on November 22, 2019. The data from this thesis will be combined with other work in Dr. Tanner's laboratory and ultimately submitted to a peer-reviewed journal for publication, with the thesis author serving as one of multiple coauthors. An annotated bibliography related to this hybrid thesis format is provided in Appendix A. Dissection and tissue preparation protocols are located in Appendix B. The data acquisition protocol used in this study is contained in Appendix C. Appendix D describes pressure and flow calibration. Appendix E provides information regarding pig and rabbit phonation trials. Details regarding LabChart™ installation and training are given in Appendix F. Finally, Appendix G provides details regarding the thesis timeline.

## Introduction

Excised larynx models have been used extensively in voice research. Jiang and Titze (1993) first developed an experimental setup to study vocal fold vibration independent of the vocal and respiratory tract in excised hemilarynges. Subsequently, this methodology was adapted to manipulate a variety of vibratory variables. This method for examining phonation outside the body, or *ex vivo*, was termed benchtop research; it has been used with a number of measurement tools and outcome variables, including those involving aerodynamics, acoustics, electroglottography and high-speed imaging, to name a few (Alipour & Jaiswal, 2008; Garrett, Coleman, & Reinisch, 2000; Hottinger, Tao, & Jiang, 2007; Jiang, Regner, Tao, & Pauls, 2008; Jiang, Verdolini, Ng, Aquino, & Hanson, 2000; Regner & Jiang, 2011; Regner, Robitaille, & Jiang, 2010; Regner, Tao, Zhuang, & Jiang, 2008; Smitheran & Hixon, 1981; Titze, 1992; Witt et al., 2009). These *ex vivo* benchtop mechanical models have contributed to the growing body of basic and translational voice research to inform clinical practice.

The *ex vivo* benchtop setup is a convenient, relatively inexpensive, and noninvasive method for studying certain characteristics of laryngeal structure and physiology, as well as characteristics of phonation. Parameters such as fundamental frequency ( $F_0$ ), sound pressure level, and other types of acoustic and aerodynamic measures help describe and quantify phonation both in human subjects as well as excised animal benchtop models. Fundamental frequency is the rate at which the vocal folds vibrate, quantified in Hertz (Hz). Aerodynamic measures of the voice, such as phonation threshold pressure (PTP) and phonation threshold flow (PTF), were the main outcome variables measured in the present study. In benchtop studies, PTP is the pressure observed at the onset of phonation (Titze, 1992); similarly, PTF is the flow observed at the onset of phonation (Jiang & Tao, 2007). Phonation threshold pressure has been

used extensively as a primary aerodynamic measure in phonatory research, but it can be highly variable in humans as well as animal models due to factors such as glottal and vocal tract configuration, mucosal wave velocity, vocal fold thickness, and tissue damping (Jiang et al., 2000; Smitheran & Hixon, 1981; Titze, 1992). It is crucial to understand this variance in both humans and various animal models. It has been suggested that PTF might be less variable than PTP (Hottinger et al., 2007), but it is a relatively new measure that requires further validation in benchtop research (Jiang et al., 2008; Regner et al., 2008; Witt et al., 2009).

Aerodynamic measures can be used to study human phonation in vivo and ex vivo (Mau, Muhlestein, Callahan, Weinheimer, & Chan, 2011; Plant, 2005). Laryngeal resistance as well as PTP and high-speed laryngeal imaging were employed to examine dysphonic females with posterior glottal chinks (Rammage, Peppard, & Bless, 1992). In another study, PTF values have been quantified during normal and disordered human phonation (Zhuang et al., 2009). Holmberg, Doyle, Perkell, Hammarberg, and Hillman (2003) used aerodynamic and acoustic measures to track patient progress in voice therapy. They found that aerodynamic measures were more sensitive to reflecting the presence of a vocal fold pathology than the acoustic parameters taken, suggesting that aerodynamic measures may be more appropriate in assessing voice disorders.

Although the ultimate goal of translational research is the application to humans, not all experimental designs are compatible with human subjects research. For example, many experimental studies require a control group and there are obvious ethical concerns about withholding treatment from a target human population. Furthermore, some studies require manipulation of the vocal folds (i.e., tissue or structural changes) to better understand human vocal fold pathologies. These studies are often more readily accomplished using animal models.

In addition, the animal benchtop model provides the flexibility and convenience of studying vocal fold structural, mechanical, and histological properties in an experimental design. Jiang et al. (2000) developed a benchtop apparatus to study the effects of dehydration on vocal fold vibration in dog larynges, analyzing specifically the PTP, glottal airflow, sound intensity of the acoustic signal, and vocal efficiency values of the larynges. The benchtop setup for the current work was heavily influenced by the setup in the Jiang et al. study, as well as the methodology described in Maytag et al. (2013). Benchtop models mechanically simulate in vivo phonation, providing a necessary step toward generalizing results to target populations.

Larynges from a variety of animals have been used in benchtop models to simulate phonation and report acoustic and aerodynamic measures. For example, dog larynges have been used to compare PTF onset and offset values (Regner et al., 2008). Dog and pig larynges have also been used to develop a reliable method for achieving phonation via neuromuscular activation (Howard, Mendelsohn, & Berke, 2015). Additionally, Alipour and Jaiswal (2008, 2009) used pig, sheep, and cow larynges to study glottal flow resistance as well as make a comparison of pig, sheep, and cow laryngeal phonatory characteristics to humans. While these various animal models are used extensively in the literature, there are certain advantages and drawbacks to each species.

### **Historical Review of Larynges Used in Benchtop Studies**

**Dog laryngeal model.** The excised dog laryngeal model is the most widely used model for phonatory and laryngeal research. Many studies have reported its effective comparisons to humans. When considering structure and mobility, the dog larynx is like the human larynx in size, vocal fold length and vibratory motion (Alipour & Scherer, 2007; Garrett et al., 2000; Regner et al., 2010). Garrett et al. (2000) found that the lamina propria of the dog vocal fold has

three layers—superficial, intermediate, and deep—which is the same structural organization as a human vocal fold. However, the dog vocal fold lacks a vocal ligament, which could interfere with studies sensitive to  $F_0$  control and change due to the tissue structure (Kim, Hunter, & Titze, 2004). Additionally, Kurita, Nagata, and Hirano (1983) found that the mucosa of the dog vocal fold is much wider than the human fold, comparing 3 mm width in dogs with an average of 1.1 mm width in humans. While a lack of vocal fold ligament and a thicker mucosa may be considered limitations for achieving  $F_0$  changes, Alipour, Finnegan, and Scherer (2009) recorded changes in  $F_0$  of dog larynges due to increases in airflow alone, despite the lack of a vocal ligament and while elongation and adduction remained constant. Still, the dog larynx is primarily used for barking, has a lower average  $F_0$  (i.e., 150 Hz) and has been reported to reach only as high as 450 Hz in chest or falsetto registers (Alipour et al., 2009). To compare, a human  $F_0$  may range from 75 Hz to over 1000 Hz for speaking and singing. Additionally, there are increasing objections to using domestic animal models in excised benchtop research designs, which leads to greater difficulty in obtaining dog ex vivo larynges for research purposes. These limitations fuel the search for other species that can provide the structural, histological, and vibratory model similar to a human larynx without the ethical concerns the use of dog larynges can have (Jiang, Raviv, & Hanson, 2001; Kim et al., 2004).

**Sheep, cow, and alternative animal laryngeal models.** While dog larynges are one of the most common animal species used in laryngeal research, other animal models such as sheep, cow, monkey, deer, and ferret have also been used and their strengths and limitations have been reported. For example, sheep seem to have histochemical similarities when compared to human laryngeal musculature (Happak, Zrunek, Pechmann, & Streinzer, 1989) and most of their laryngeal structure is similar to the human larynx, with some important differences (Zrunek,

Happak, Hermann, & Streinzer, 1988). Sheep vocal folds are similar in length to human vocal folds (Alipour & Jaiswal, 2008), but are soft and lack the stiffness found in human vocal folds. This softness makes differentiation between the false and true vocal folds difficult (Regner et al., 2010). They have large arytenoid cartilages, ranging anywhere from 20% to 70% larger than human cartilages in one study (Zrunek et al., 1988). Additionally, the size of their thyroid and cricoid cartilages has been found to be significantly larger than the human structures (Kim et al., 2004). Sheep may be a good physiological model of the vocal folds because of the histochemical similarities between sheep and humans, but their structural differences may limit their use in voice research.

Cows have been used in physiological research, but their use in voice research is limited (Alipour & Jaiswal, 2008; Kurita et al., 1983). Scherer, Cooper, Alipour-Haghighi, and Titze (1985) used cow larynges to measure contact pressure between the vocal folds. They have also been used to analyze glottal airflow resistance in excised animal larynx models (Alipour & Jaiswal, 2009). As mentioned earlier, Alipour and Jaiswal (2009) compared the phonatory characteristics of pig, sheep, and cow, and found that cow vocal folds are much longer and stiffer than the other species; additionally, their vocal folds oscillate in such a way as to produce large mucosal waves that travel in both vertical and horizontal directions. The authors also noted that the cow larynx differs structurally from the human larynx. Their vocal folds are longer, thicker, and pad-like. They lack a ventricle between the false and true vocal folds (Alipour & Jaiswal, 2008; Regner et al., 2010); however, Alipour and Jaiswal (2008) concluded that the cow larynx may be a good model to use when obtaining aerodynamic measures because of its low PTP and steady pitch. Alternatively, Regner et al. (2010) found that cow vibratory frequency and

amplitude differed significantly from human vibration, which may indicate some limitations in translating acoustic and aerodynamic measures of the cow larynx to the human larynx.

Other animal species such as deer, monkey, and ferret have been used sparsely in the voice literature with little support for their use in future studies. Deer larynges are generally available and their laryngeal dimensions appear to be within the normal size range of human larynges; however, they have limited range of motion at the cricothyroid joint, limiting the degree of vocal fold extension as compared to humans (Jiang et al., 2001). The monkey laryngeal model has also been used in a physiological study by Garrett et al. (2000). The monkey mucosa of the vocal fold was reported to be much thinner than the human mucosa and the histochemistry of the vocal fold was found to be significantly dissimilar to the human vocal fold. When comparing the histological properties of the lamina propria in various animal species, Hahn, Kobler, Starcher, Zeitels, and Langer (2006) and Hahn, Kobler, Zeitels and Langer (2006) found that the distribution of elastin fibers in the lamina propria of ferrets was significantly different from humans and that this may limit its use as a viable laryngeal model.

**Pig laryngeal model.** The pig model has been widely documented as one of the best laryngeal models regarding histology, physiology, and phonation. In a study comparing the histological properties of the lamina propria of human, dog, pig, and ferret, the pig lamina propria tissue was most similar to the human tissue (Hahn, Kobler, Starcher, et al., 2006; Hahn, Kobler, Zeitels, et al., 2006). In a study analyzing the pig larynx as a viable option for human laryngeal transplantation, it was found that the pig larynx anatomy and mucosal immunology was similar to the human larynx and that it provided an ideal preclinical model for transplantation (Gorti, Birchall, Haverson, Macchiarini, & Bailey, 1999). Furthermore, the origins and insertions of the intrinsic muscles of the pig larynx are similar to the human larynx

and the pathways and branching of the recurrent laryngeal nerve in the pig larynx closely match the innervations found in the human larynx, particularly in the posterior cricoarytenoid muscle (Knight, McDonald, & Birchall, 2005). This symmetry supports the use of the pig larynx as a model for human transplantation, as well as for physiological excised animal larynx studies. In addition to being physiologically compatible with the human larynx, the pig larynx also provides the ideal laryngeal model for phonatory research as well. Jiang et al. (2001) found that the mobility of the cricothyroid joint, the  $F_0$  ranges, and the structure of the vocal fold found in the pig larynx were like the values and structures recorded and observed in human larynges. The pig larynx has a modal frequency range of 100 to 450 Hz. The wide  $F_0$  range may be because the pig produces a wider range of noises, such as grunts and squeals (Alipour & Jaiswal, 2008; Jiang et al., 2001). In a high-speed video study, pig oscillation amplitudes were not found to be significantly different than those of humans and were most similar to the human oscillation amplitude range when compared to dogs, cows, and sheep (Regner et al., 2010). Additionally, the pig larynx is a relatively easy animal model to obtain from slaughterhouses where the pigs are slaughtered for food (Jiang et al., 2001). For these reasons, the pig larynx is widely used in excised benchtop research and its usefulness has been supported in numerous studies. While the versatility of the pig larynx has been widely documented, there are some limitations of the species. For example, it has been noted that pigs use their false vocal folds to phonate as well as their true vocal folds (Alipour & Jaiswal, 2008). This could influence the interpretation and transfer of research findings in certain studies to human subjects. Additionally, while laryngeal pig anatomy is like human anatomy, there may be some significant differences in the use of certain anatomical structures. For example, pigs have a large epiglottis and arytenoid cartilages with heightened walls to aid in swallowing while breathing. This structure forms a supraglottic



duct between the arytenoid cartilages and the thyroid wall. This duct can vibrate under certain conditions and could influence the phonatory data in certain studies (Alipour & Jaiswal, 2008; Harrison & Harrison, 1995). While a pig larynx has a clear defining line between the false vocal folds and the true vocal folds with a ventricle in between that aids in dissection, the folds slope in the larynx at a 40° angle, preventing airflow from oscillating the folds at a 90° angle as is the case in human larynges. This could limit the translation of acoustic and aerodynamic measures to humans. Lastly, while the pig provides an ideal excised animal model, it is a much greater challenge to study this animal in vivo. Pigs are big animals and the demands for care and upkeep of this type of animal are far greater than for a smaller animal. For any study with a control and experimental group in vivo, it is much easier to use a smaller and easily accessible animal. Unfortunately, few laryngeal studies have used animal models in vivo for any length of time. As this study is a part of a larger, five year experimentally controlled group study design, it was necessary to find another, smaller laryngeal animal model to maintain across the years beside the ones previously discussed. While selecting a smaller laryngeal model, it was crucial to maintain the reliability and transferability to humans that has been seen in dog and pig laryngeal animal models.

**Rabbit laryngeal model.** Rabbit models provide the necessary histological compatibility, in vivo phonation patterns, size, and practicality to be an ideal animal model in laryngeal research. The rabbit vocal folds have three layers of the lamina propria and the composition and histology of the layers is compatible with human vocal fold structure (Garrett et al., 2000; Hertegård, Dahlqvist, Laurent, Borzacchiello, & Ambrosio, 2003; Maytag et al., 2013). The extracellular matrix of the vocal folds in a rabbit is similar to the matrix of human vocal folds (Rousseau et al., 2008). For these reasons, the rabbit model has been used extensively in

histological studies, as well as studies regarding diseases and drug testing of the respiratory system (Keir & Page, 2008; Liang et al., 2008). Additionally, the rabbit is a relatively quiet animal, reducing vocal use variability between specimens, and is inexpensive and easy to care for in a laboratory setting, making it a convenient choice for long-term in vivo and ex vivo animal studies (Maytag et al., 2013; Rousseau et al., 2008). Phonation and vocal fold histology and healing characteristics have been studied in in vivo and ex vivo rabbit models (Awan, Novaleski, & Rousseau, 2014; Ge, French, Ohno, Zealear, & Rousseau, 2009; Maytag et al., 2013; Mills, Dodd, Ablavsky, Devine, & Jiang, 2017; Rousseau et al., 2008; Swanson et al., 2009). The rabbit model seems to be a reliable model for long-term studies requiring both in vivo and ex vivo experimental models (Branski, Verdolini, Rosen, & Hebda, 2005; Thibeault, Gray, Bless, Chan, & Ford, 2002). Rabbit larynges have been used extensively in histological studies but are only beginning to be used in ex vivo benchtop phonation studies. Maytag et al. (2013) established a reliable method for dissecting and mounting a rabbit larynx on a benchtop setup. They found that interlarynx variability between rabbit larynges was similar to interlarynx variability between dog larynges in the same study. Mills et al. (2017) found that values for PTP, subglottal pressure,  $F_0$ , and sound pressure level for rabbit larynges increased as elongation increased. In many ways, the combination of histologic compatibility with phonatory reliability in an ex vivo benchtop rabbit model, as well as the convenience and feasibility of using the rabbit in in vivo studies, makes the rabbit model an ideal animal model in laryngeal animal studies (Maytag et al., 2013; Rousseau et al., 2008; Thibeault et al., 2002).

### **Statement of Problem**

There is a lack of methodical correspondence between animal in vivo and ex vivo histological and excised experimental rabbit models in voice research. For example, there are

studies examining the healing characteristics of vocal fold scarring in rabbits ex vivo (Branski et al., 2005; Thibeault et al., 2002), but there are no excised benchtop models studying the phonatory characteristics of rabbit vocal folds after scarring. In another example, the rabbit larynx has been found to be a useful model for studying lung diseases such as asthma and rhinogenic chronic rhinosinusitis (Keir & Page, 2008; Liang et al., 2008), but there are not any ex vivo animal studies examining the phonatory characteristics of such diseases in the rabbit model. Furthermore, drug testing for certain lung diseases has been documented in the rabbit model, but there is no research studying the phonatory effects of such treatments in rabbits. While improvements in the diseases themselves have been seen with certain medications, the adverse effects on vocal fold tissue or vocal fold mechanical properties have not been analyzed. It is important to understand the adverse effects of disease and treatment in the rabbit model both in vivo and ex vivo to help inform human clinical research. This study attempts to close the gap between normal and disordered phonatory characteristics of the excised rabbit model, as well as contribute to the understanding of the relationship between in vivo and ex vivo normal and disordered rabbit histological properties and phonatory characteristics. This study quantifies normal rabbit aerodynamic measures of phonation with the goal of providing a baseline for future excised rabbit larynx research in disordered phonation.

### **Statement of Purpose**

For over two decades, benchtop mechanical models of phonation have been used to study different factors that affect phonation. Several types of animal larynges, particularly dog and pig larynges, have been used in these studies. Complementary synthetic models have also been used for scientific study when tissue experiments are insufficient. The expansion of excised larynx models to include other readily accessible, small animals would make an important contribution

to experimental methodologies available for voice research. Whereas most excised larynges are obtained from animals sacrificed for other research or nonresearch purposes, rabbits could be housed and included in prospective, longitudinal experimental designs. Large scale voice studies are also more feasible with small animals. This methodological advancement would improve benchtop research opportunities just as mice and rat models improved neural voice research (Basken, Connor, & Ciucci, 2012; Connor, Suzuki, Sewall, Lee, & Heisey, 2002; Inagi, Schultz, & Ford, 1998; Lenell, Newkirk, & Johnson, 2019). But it is essential to determine the benchtop phonatory characteristics of rabbit larynges as compared with other widely used animal models before translational rabbit diagnostic and treatment research can be pursued. The purpose of this thesis is to examine phonation in normal larynges from rabbits.

### **Research Questions**

The current thesis examined aerodynamic measures for pig and rabbit phonation using an excised larynx benchtop experimental setup. The purpose of this study was to determine normative data for the pig and the rabbit larynges. The research questions were: what are the normative PTP and PTF values for rabbit and pig phonation and is there a difference between the two species?

### **Method**

All research and data collection activities were completed in room 106 of the John Taylor Building Annex, as well as the Chemistry Store, room 126 of the Joseph K. Nicholes Building at Brigham Young University. Operational procedures were performed in compliance with Risk Management and Institutional Animal Care and Use Committee at Brigham Young University and the University of Utah. Food-grade excised pig larynges were obtained from a local butcher shop (Circle V Meats, Spanish Fork, UT); excised rabbit larynges were obtained from the

University of Utah. All larynges were obtained from animals sacrificed for purposes unrelated to this study.

### **Research Design**

This thesis was completed as part of a larger ongoing project being conducted by Kristine Tanner, Ph.D., associate professor in the Department of Communication Disorders at Brigham Young University. The current study used a between-subjects experimental design with group (i.e., rabbit versus pig) as the independent variable and PTP (cmH<sub>2</sub>O) and PTF (L/min) as the dependent variables. Rabbit larynges comprised one group and pig larynges formed the other group.

### **Larynx Preparation**

For the rabbit group, 15 larynges from white rabbits were excised immediately postmortem and submerged in either gallon sized plastic bags or cylindrical plastic containers (approximately 4 inches long and 1 inch wide) containing phosphate buffered saline (PBS) solution. These containers were placed in an isopropyl alcohol bath in order to reduce the formation of ice crystals and subsequently flash frozen using liquid nitrogen. Figure 1 depicts the flash freezing process. Larynges were stored in a research laboratory at the University of Utah and later transported in a foam cooler with ice to a -80 °C freezer at Brigham Young University, room 105 TLRB Annex. For the pig group, 15 larynges were obtained within 6 hours postmortem from adult pigs less than 2 years of age. Following rough dissection, larynges were flash frozen and stored at -80 °C in 105 TLRB Annex.



*Figure 1.* The flash freezing process. Pig larynges were submerged in PBS solution in either gallon plastic bags or plastic cylindrical containers and placed in liquid nitrogen. Rabbit larynges were also flash frozen according to protocols at the University of Utah.

Immediately prior to data collection, each larynx was thawed in a room temperature water bath and then finely dissected. The thyroid cartilage was removed except for the bottom portion below the thyroid notch; the epiglottis and false vocal folds were removed to reveal the true vocal folds and the trachea was cut below the cricoid cartilage. Disposable scalpels were used to transect and remove the thyroid cartilage using an anterior to posterior cut, the epiglottis using a “V” cut, and the lower trachea horizontally cut. As needed, metal hemostats were used to abduct the false vocal folds and aid in precise dissection of the false vocal folds without damaging the true vocal folds. A suture was added above the anterior commissure to aid in anterior attachment and overall larynx stability during phonatory trials. During dissection, each larynx was sprayed liberally with saline solution (0.9% Na<sup>+</sup>Cl<sup>-</sup>) to promote tissue hydration and

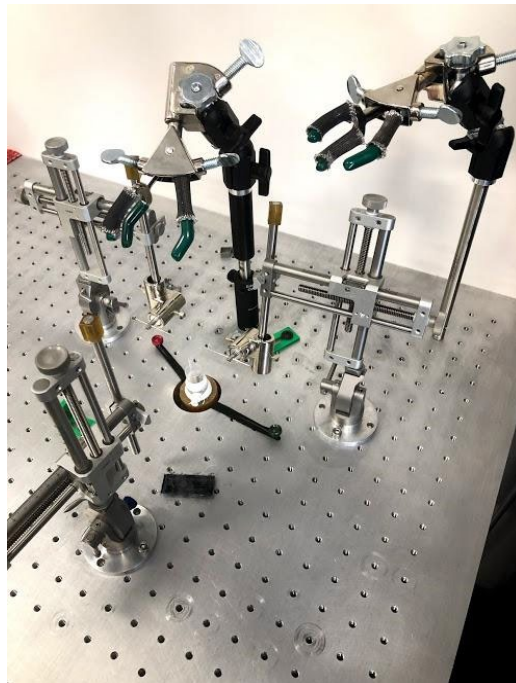
preservation. After dissection, each larynx was placed in a saline bath until mounted on the benchtop.

### **Benchtop Setup**

The benchtop model used for excised larynx comparison was based on the design by Jiang and Titze (1993) and modified by Maytag et al. (2013). Figure 2 contains a picture of the entire benchtop apparatus. A foam insulated custom pseudolung surrounded the plastic tubing below the breadboard benchtop (Thorlabs, Ann Arbor, MI). A PVC pipe was attached to the plastic tubing. Two silicone devices were designed and customized to taper the airflow, narrow the passageway from the PVC pipe to the trachea and fit the width of the tracheas of the pig and rabbit larynges. The trachea of each larynx was situated over the silicone piece and secured at the bottom using an adjustable metal hose clamp and Teflon™ tape. Micropositioners with one (or) three prongs were gently inserted into the arytenoid cartilages of the rabbit and pig larynges, respectively, to stabilize each larynx and adduct the vocal folds. These micropositioners were secured to the benchtop ¼-20 headless screws via custom bases. A third micropositioner was placed anteriorly. Figure 3 shows the types of micropositioners used. A string extending from the suture in the thyroid cartilage was attached to the anterior micropositioner. The micropositioner was then rotated to tighten the string slightly to stabilize the larynx but not lengthen the vocal folds.



*Figure 2.* The benchtop apparatus. Included in this picture are the humidifier, the signal acquisition system, the computer and monitor, the pseudolung, and the air tanks.

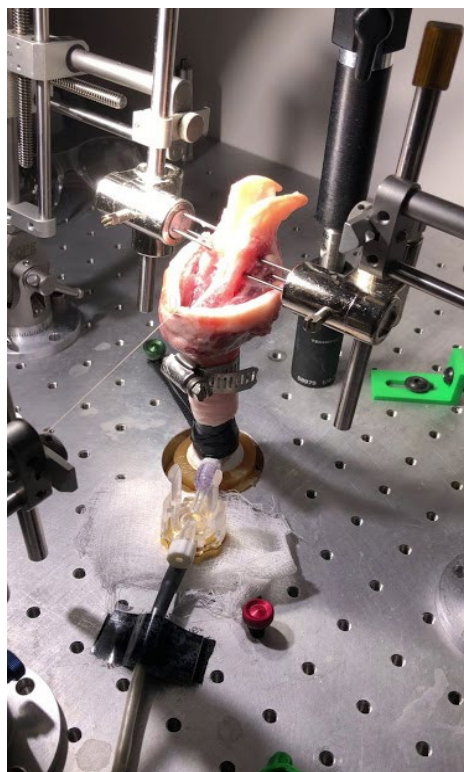


*Figure 3.* The micropositioners. Three-pronged micropositioners were inserted into the arytenoid cartilages to stabilize the larynx and bring vocal folds closer together. A suture sewed to the anterior thyroid cartilage was used for stability.



## Data Collection

**Voice onset.** Vocal fold vibration was elicited after each larynx was situated on the benchtop. Compressed air from an air tank was released and heated by a humidifier before being sent through the pseudolung and up to the larynx. Airflow administration from the tank was gradually increased by turning a knob on the air tank until phonation onset was achieved. After voice onset, the vocal folds vibrated for approximately 5 seconds and then the supply of air was turned off at the tank. Fifteen trials were performed for each larynx. Adduction and attachment remained constant between trials. This pattern continued for all trials of each larynx. Figures 4, 5, 6 and 7 depict the setup for both pig and rabbit larynges.



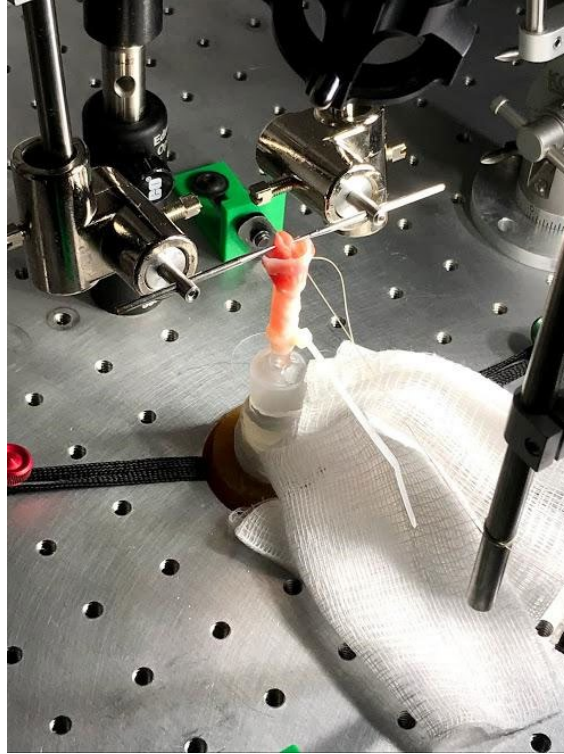
*Figure 4.* Adduction and attachment. Micropositioners were used to adduct arytenoid cartilages and bring the vocal folds together. Attachment via a suture to the anterior thyroid cartilage was used for stability.



*Figure 5.* Posterior view of micropositioners and larynx.



*Figure 6.* Single pronged micropositioners. These were used to adduct rabbit arytenoid cartilages and bring the vocal folds closer together. Cable ties were used to secure the trachea to the pseudolung.

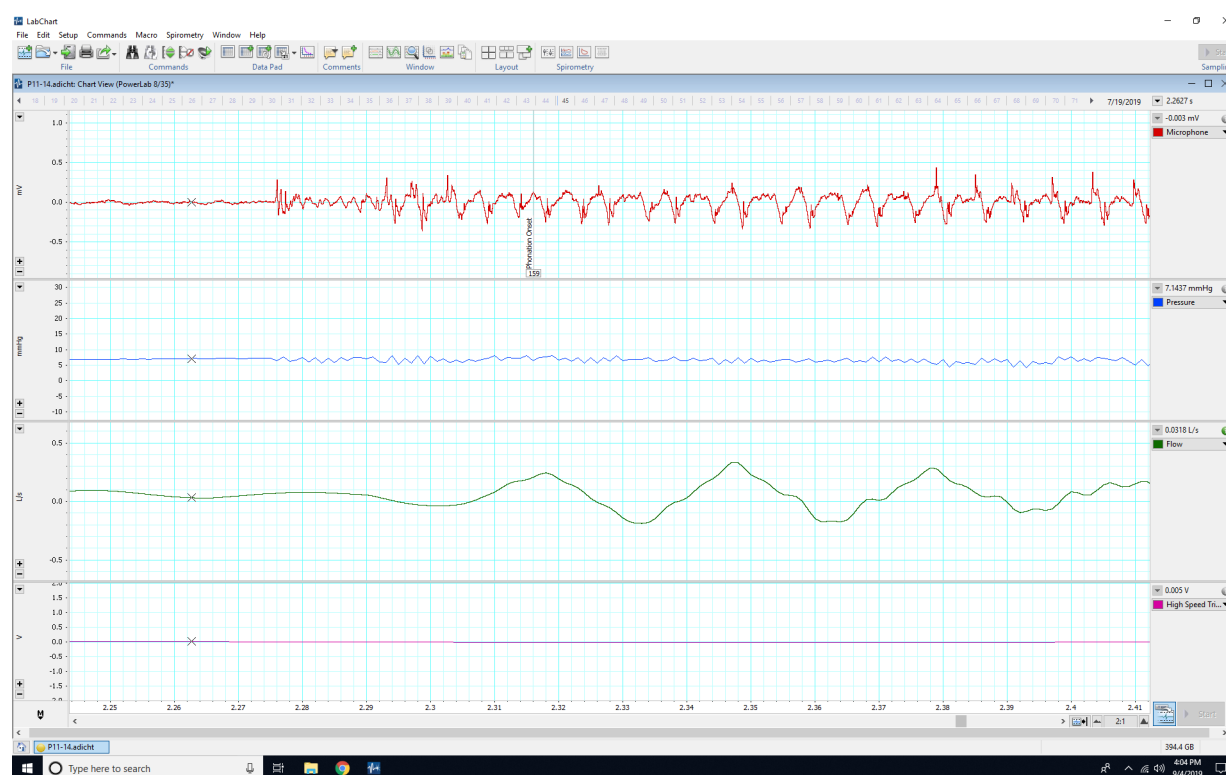


*Figure 7.* Mounted rabbit larynx. Measures were taken to ensure the researcher did not overly adduct the rabbit vocal folds.

**Signal acquisition.** The acoustic signal, airflow, and air pressure signals were obtained for each voice onset trial using LabChart™, version 8 (PowerLab™, ADInstruments, Colorado Springs, CO). A microphone was placed approximately 6 inches above the vocal folds and was used to acquire an acoustic signal. LabChart software was used to acquire signals at 20000 Hz per channel. A 100 L respiratory flow head was attached to a spirometer to sample airflow in L/min. A pressure transducer was calibrated using the PowerLab pressure calibration system at 40 mmHg and 120 mmHg and converted to cmH<sub>2</sub>O. A digital hygrometer was calibrated and then used to monitor environmental humidity levels in the room during the study.

**Data reduction and analysis.** Time-aligned acoustic and aerodynamic signals were segmented for each voice onset trial using LabChart™. A custom Matlab™ (MathWorks, Natick, MA) program was developed by thesis committee member Dr. Christopher Dromey,

specifically for the data analysis of the current work. Figure 8 shows what the raw signals looked like before analysis. Using previously established methodology (Stevens, 2017), onset pressure and flow were calculated by averaging pressure and flow values between 10 ms before and 10 ms after voice onset as identified from the acoustic vocal fold vibratory signal. The PTP and PTF values were exported to a spreadsheet for statistical analysis. Fifteen percent of the trials were identified using a random number generator, resegmented, and reanalyzed by the thesis author and a second examiner for purposes of intrajudge and interjudge reliability.



*Figure 8.* The data acquisition system. The red line is the acoustic signal. The blue line is the pressure signal. The green line is the flow signal.

**Statistical analysis.** The 15 PTP and PTF values from each trial were averaged for each of the 15 larynges in the pig and rabbit groups. Summary statistics were generated, including the mean, standard deviation, median, and range. The distributions of the data were examined using box plots. Independent-samples *t* tests were performed to compare PTP and PTF for pig and

rabbit groups. Intrajudge and interjudge reliability were examined using Pearson correlation coefficients.

## Results

### Pig and Rabbit Anatomical Measurements

As described in the method section, each larynx underwent fine dissection to reveal the true vocal folds and dissect away any tissue obstructing airflow to the true vocal folds.

Anatomical measurements were then taken from each larynx using a caliper. Measurements included thyroid cartilage width and height, larynx weight, vocal fold length and width, and trachea length. Tables 1, 2, and 3 detail the measurements in millimeters for both pig and rabbit larynges. These measurements reflect the relative sizes of each specimen in each species.

Table 1

*Vocal Folds Anatomical Size and Dimension*

Group	Session Date	Length (mm)	Width (mm)	Width from Vocal Folds to Thyroid Cartilage (mm)
<b>Pig</b>				
Pig 01	07/16/19	26.7	2.5	12.9
Pig 02	07/17/19	22.7	3.1	12.9
Pig 03	07/17/19	19.6	2.2	13.8
Pig 04	07/17/19	19.6	2.1	13.9
Pig 05	07/17/19	21.5	2.3	12.0
Pig 06	07/18/19	18.1	2.7	11.1
Pig 07	07/18/19	21.5	2.4	10.7
Pig 08	07/18/19	21.5	2.3	10.7
Pig 09	07/18/19	25.6	2.8	12.6
Pig 10	07/19/19	17.5	2.0	15.5
Pig 11	07/19/19	24.0	2.0	13.4
Pig 12	07/19/19	17.1	2.5	11.9
Pig 13	07/19/19	17.3	2.1	10.5
Pig 14	07/25/19	20.7	1.9	11.9
Pig 15	07/25/19	16.5	1.8	13.3
<b>Rabbit</b>				
Rabbit 01	07/26/19	5.5	0.9	2.8
Rabbit 02	07/29/19	7.5	1.4	3.9
Rabbit 03	07/29/19	4.3	1.0	2.2

Rabbit 04	07/29/19	4.6	2.0	3.0
Rabbit 05	07/31/19	7.1	1.9	4.0
Rabbit 06	07/31/19	5.6	1.9	4.6
Rabbit 07	07/31/19	6.1	2.0	4.0
Rabbit 08	07/31/19	4.8	1.8	3.5
Rabbit 09	08/01/19	5.4	2.5	4.0
Rabbit 10	08/01/19	5.8	2.7	4.7
Rabbit 11	08/01/19	5.8	2.4	4.6
Rabbit 12	08/01/19	6.2	1.9	3.6
Rabbit 13	08/01/19	4.2	1.2	3.8
Rabbit 14	08/01/19	4.4	1.8	3.5
Rabbit 15	08/01/19	6.7	2.3	4.4

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Table 2

*Thyroid Cartilage Anatomical Dimensions*

Group	Session Date	Height (protuberance to top; mm)	Height (protuberance to bottom; mm)	Width (mm)
<b>Pig</b>				
Pig 01	07/16/19	49.3	9.3	46.4
Pig 02	07/17/19	47.8	13.5	45.5
Pig 03	07/17/19	52.9	12.9	52.8
Pig 04	07/17/19	46.9	11.7	51.0
Pig 05	07/17/19	44.9	11.7	48.5
Pig 06	07/18/19	48.6	12.6	46.6
Pig 07	07/18/19	59.4	9.5	49.5
Pig 08	07/18/19	51.9	13.0	46.7
Pig 09	07/18/19	49.0	8.7	47.9
Pig 10	07/19/19	48.2	12.0	47.5
Pig 11	07/19/19	53.4	8.6	48.9
Pig 12	07/19/19	53.3	12.8	47.4
Pig 13	07/19/19	51.4	8.7	43.8
Pig 14	07/25/19	55.8	7.0	43.5
Pig 15	07/25/19	47.0	12.25	50.6
<b>Rabbit</b>				
Rabbit 01	07/26/19	5.9	2.0	14.2
Rabbit 02	07/29/19	6.9	3.2	12.7
Rabbit 03	07/29/19	6.5	3.1	8.8
Rabbit 04	07/29/19	6.5	4.8	11.2



Rabbit 05	07/31/19	5.4	5.1	14.9
Rabbit 06	07/31/19	5.8	3.8	14.2
Rabbit 07	07/31/19	6.1	3.7	13.1
Rabbit 08	07/31/19	3.9	3.6	10.6
Rabbit 09	08/01/19	6.0	4.6	14.0
Rabbit 10	08/01/19	5.6	6.0	14.5
Rabbit 11	08/01/19	6.1	4.0	11.6
Rabbit 12	08/01/19	4.9	4.3	12.6
Rabbit 13	08/01/19	6.8	3.7	10.6
Rabbit 14	08/01/19	6.4	2.5	11.0
Rabbit 15	08/01/19	6.5	3.3	14.4

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Table 3

*Trachea Anatomical Dimensions*

Group	Session date	Trachea length (mm)	Trachea width (mm)
<b>Pig</b>			
Pig 01	07/16/19	23.5	18.7
Pig 02	07/17/19	21.9	23.0
Pig 03	07/17/19	20.6	19.0
Pig 04	07/17/19	43.2	21.5
Pig 05	07/17/19	35.5	18.7
Pig 06	07/18/19	20.1	17.3
Pig 07	07/18/19	20.1	19.0
Pig 08	07/18/19	43.1	16.5
Pig 09	07/18/19	38.7	16.2
Pig 10	07/19/19	31.8	17.0
Pig 11	07/19/19	41.1	18.9
Pig 12	07/19/19	51.3	21.6
Pig 13	07/19/19	46.4	18.2
Pig 14	07/25/19	44.1	21.3
Pig 15	07/25/19	53.9	18.7
<b>Rabbit</b>			
Rabbit 01	07/26/19	23.1	5.7
Rabbit 02	07/29/19	22.3	5.5
Rabbit 03	07/29/19	17.5	3.5
Rabbit 04	07/29/19	21.4	4.8
Rabbit 05	07/31/19	30.8	6.7

Rabbit 06	07/31/19	21.3	5.7
Rabbit 07	07/31/19	20.3	5.4
Rabbit 08	07/31/19	26.2	4.5
Rabbit 09	08/01/19	28.5	5.6
Rabbit 10	08/01/19	28.1	5.2
Rabbit 11	08/01/19	22.2	5.6
Rabbit 12	08/01/19	22.1	5.9
Rabbit 13	08/01/19	21.4	3.7
Rabbit 14	08/01/19	19.0	3.8
Rabbit 15	08/01/19	25.9	4.9

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### Descriptive Statistics

The central tendency and variability of PTP and PTF values for pig and rabbit larynx groups were examined using SPSS, version 23 (IBM Corp., Armonk, NY). Mean PTP and PTF values were calculated based on the average of each of the 15 trials for each of the 15 pig and 15 rabbit larynges. The mean for PTP based on the pig larynx group was 19.98 (SD = 13.51), the mean for PTP based on the rabbit group was 8.7 (SD = 2.64). The PTF mean for the pig larynx was 0.27 (0.13); PTF mean for the rabbit larynx group was 0.08 (SD = 0.04). Significant differences were observed based on an independent-samples *t* tests for PTP,  $t = 3.173$ ,  $p < .006$ , degrees of freedom (df) = 15.07, and PTF,  $t = 5.665$ ,  $p < .001$ , df = 16.29. Differences between pig versus rabbit phonation for PTP and PTF are illustrated in Figure 9 and Figure 10, respectively. Descriptive statistics for pigs and rabbits in the complete data sets are displayed in Table 4, respectively.

Table 4

*Pig and Rabbit Groups Descriptive Statistics (N = 15 larynges)*

Statistic	Pig Onset Pressure (cmH <sub>2</sub> O)	Pig Onset Airflow (L/min)	Rabbit Onset Pressure (cmH <sub>2</sub> O)	Rabbit Onset Airflow (L/min)
Mean	19.98	0.27	8.70	0.08
SD	13.06	0.12	2.64	0.04
Median	17.84	0.30	8.52	0.07
Minimum	5.76	0.07	5.20	0.03
Maximum	60.59	0.43	15.16	0.14
Range	54.83	0.36	9.96	0.11

*Note.* *sd* = group standard deviation.

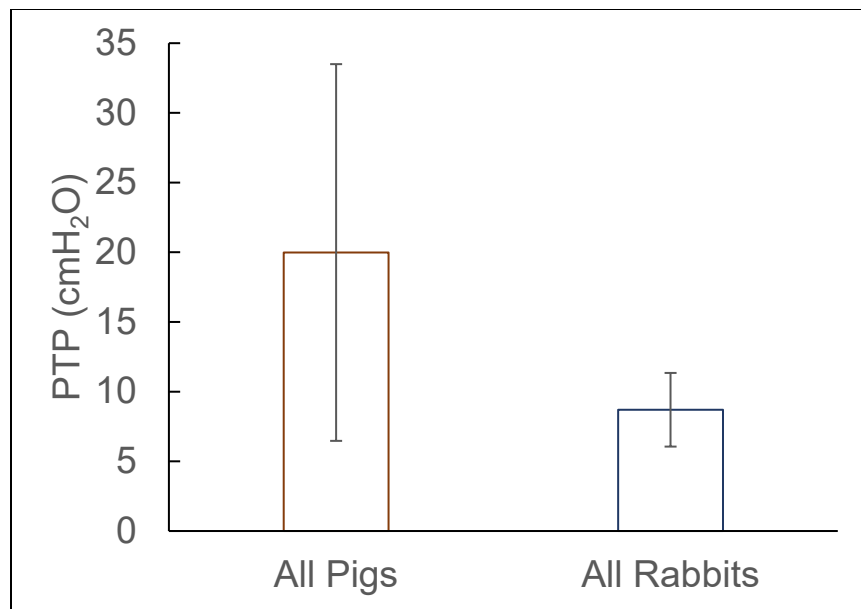


Figure 9. Average PTP values for 15 pig and 15 rabbit larynges, measured in cmH<sub>2</sub>O.

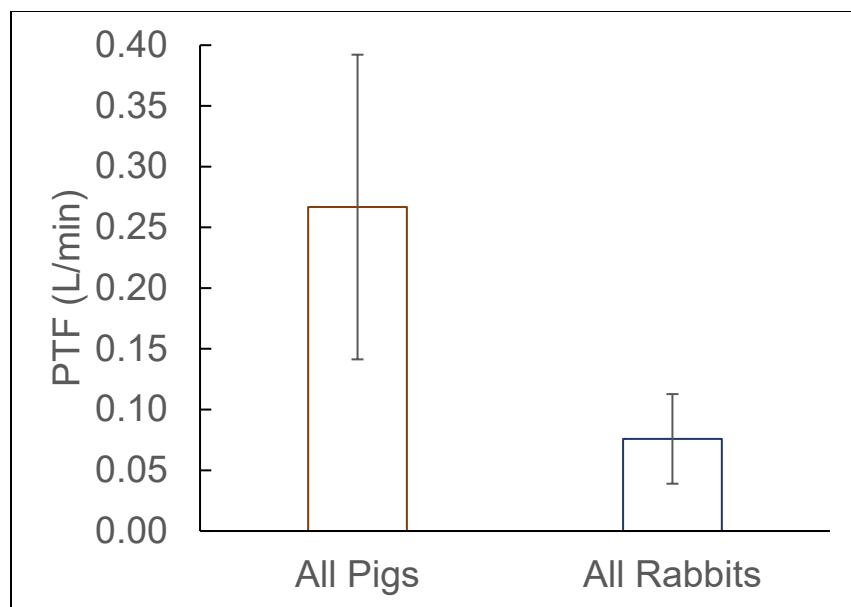


Figure 10. Average PTF values for 15 pig and 15 rabbit larynges, measured in L/min.

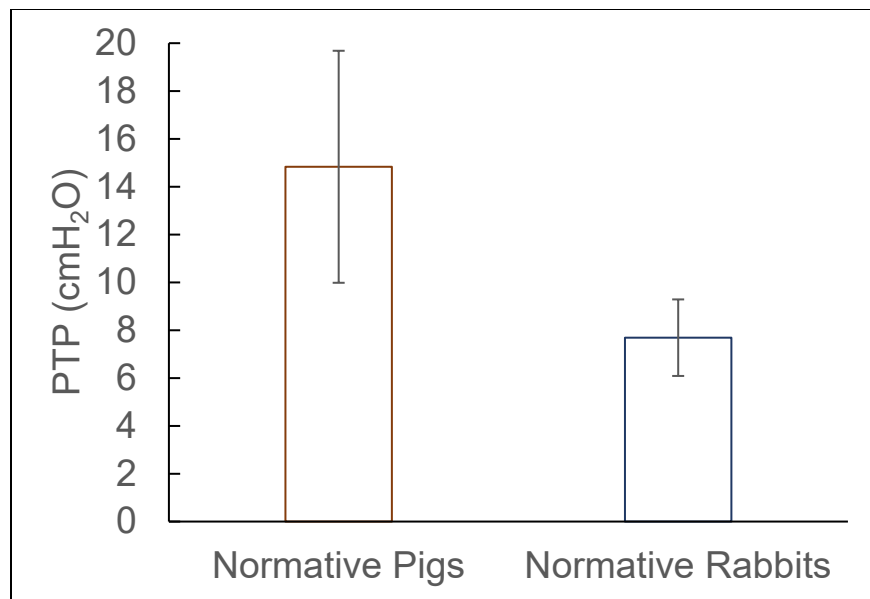
Based on the examination of central tendency and the presence of outliers in both the pig and rabbit groups, including Levene's test of equality of variances, it was determined that the 10 least variable larynges from each group would be selected for subsequent analysis for the primary purpose of establishing normative, comparative data sets. The average PTP value for the pig subset was 14.83 (sd = 4.85); the average PTP value for the rabbit subset was 7.69 (sd = 1.60). The average PTF value for the pig subset was 0.23 (sd = 0.14); the average PTF value for the rabbit subset was 0.06 (sd = 0.01). Significant differences were observed based on an independent-samples  $t$  tests for PTP,  $t = 4.426$ ,  $p < .001$ ,  $df = 10.93$ , and PTF,  $t = 3.886$ ,  $p < .003$ ,  $df = 9.34$ . Differences between normative pig versus normative rabbit phonation for PTP and PTF are illustrated in Figure 11 and Figure 12, respectively. Descriptive statistics for pigs and rabbits in the normative groups are displayed in Table 5, respectively.

Table 5

*Descriptive Statistics for Normative Pigs and Rabbits (N=10)*

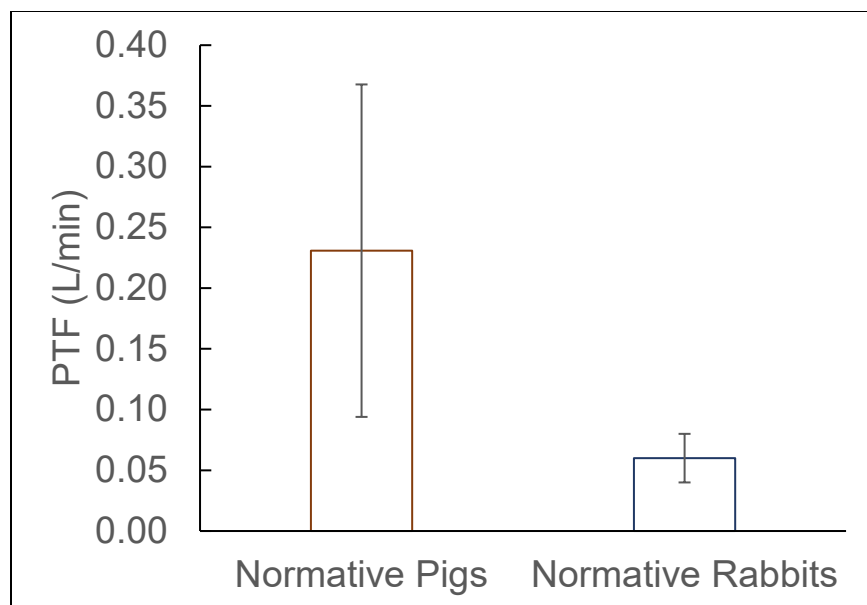
Statistic	Pig Onset Pressure (cmH <sub>2</sub> O)	Pig Onset Airflow (L/min)	Rabbit Onset Pressure (cmH <sub>2</sub> O)	Rabbit Onset Airflow (L/min)
Mean	14.83	0.23	7.69	0.06
SD	4.80	0.14	1.60	0.02
Median	16.13	0.23	7.64	0.06
Minimum	5.76	0.06	5.20	0.04
Maximum	21.46	0.43	10.37	0.09
Range	15.70	0.37	5.17	0.05

*Note.* *sd* = group standard deviation.



*Figure 11.* Average PTP values for 10 pig and 10 rabbit larynges, measured in cmH<sub>2</sub>O. This was the establishment of normative data.





*Figure 12.* Average PTF values for 10 pig and 10 rabbit larynges, measured in L/min. This was the establishment of normative data.

Interjudge and intrajudge reliability were calculated for 15% of repeated segmentation and analysis. Pearson product moment coefficients of .99 and .99 were obtained for intrajudge reliability for examiner one and examiner two for pig and rabbit samples, respectively.

Similarly, Pearson product moment coefficients of .99 and .99 were found for interjudge reliability between the two examiners for pig and rabbit larynges, respectively.

### Discussion

The present study examined PTP and PTF in pig and rabbit larynges. The purpose of the project was to establish normative PTP and PTF data for rabbit larynges and pig larynges. A traditional benchtop model setup (Witt et al., 2009) was employed for both groups, with corresponding tracheal fittings for each species. The operational procedures for larynx mounting and signal acquisition were the same for both species to minimize extraneous covariates for purposes of comparison. The results of this study indicated that there were significant differences in the pig and rabbit groups for PTP and PTF values. Significant differences existed

between the two groups for the complete data sets as well as the normative data set. Because the normative data sets demonstrated significant aerodynamic differences, the question regarding which species might be optimal for different voice research questions should be discussed.

### **Differences in Pig and Rabbit PTP and PTF Values**

There are several notable differences between pig and rabbit vocal folds which can help explain the significant differences found in PTP and PTF in the current study. Rabbit vocal folds are much smaller in length and width when compared to pig vocal fold length and width as seen in Table 1. Pig larynges have large structures just superior to the vocal folds that can influence phonation (Alipour & Jaiswal, 2008; Harrison & Harrison, 1995). Additionally, there are marked histological differences between the species. Pig lamina propria of the vocal folds consists of two indistinct layers (Garrett et al., 2000), while the rabbit vocal fold has three layers of lamina propria (Garrett et al., 2000; Hertegård et al., 2003; Maytag et al., 2013). The pig vocal folds have been found to be a less-than-ideal option for microflap surgery (Garrett et al., 2000). In contrast, healing properties in rabbit vocal fold tissue have been studied with the intention of further understanding vocal fold scarring mechanisms (Thibeault et al., 2002). Lastly, the rabbit is a relatively quiet animal, especially when compared with the pig species; as a result, there is less variability in phonation use before sacrifice. These differences may contribute to decreased intralarynx variability in the rabbit larynx as compared to the pig larynx (Hoggan, 2020).

### **Rabbit Translational Potential to Human Phonation**

In addition to several differences mentioned in the preceding paragraph, the rabbit larynx may provide a more optimal translational model when considering human phonation as compared to the pig model. For example, the tissue composition of the layering of the rabbit

vocal folds is like human vocal fold layering. In addition to the three-layered lamina propria found in both rabbits and humans, it was found that the extracellular matrix of the vocal folds in rabbits is similar to the matrix found in human vocal folds (Rousseau et al., 2008). Due to the similarities in tissue, rabbits have been used in tissue composition studies, and respiratory diseases and drug testing (Keir & Page, 2008; Liang et al., 2008). Not only is there histological compatibility between the two species, but PTP values for rabbit phonation are much closer to human PTP values than pig phonation values. The results of this study indicate that average PTP value for the normative data rabbits was 7.69 (sd = 1.60) as compared to 14.83 (sd = 4.85) for the normative pig data. To put these numbers into context, average human PTP values are reported to be 5.29 cmH<sub>2</sub>O during modal phonation (Mau et al., 2011). Since rabbit PTP values are closer to human values than pig PTP values, as well as other similarities such as histological properties, the rabbit model may be an appropriate model to use when conducting translational research or generalizing findings to the human species.

The translational potential of this study could also begin to reach future clinical applications in regard to voice measures. Many clinical voice measures are taken from sustained vowels or a combination of sustained vowels and connected speech. During these measures, the vocal folds are vibrating at a comfortable pitch and loudness, with minimal strain on the folds. Similarly, in the current work, the vocal folds of both the groups were vibrated at a presumed comfortable pitch and loudness. This comfortable pitch was achieved by not elongating the vocal folds. To contrast, many animal benchtop studies elongate the vocal folds to initiate and sustain phonation in the animal models (Jiang et al., 2008; Mills et al., 2017; Regner et al., 2008). The fact that this study did not elongate the vocal folds supports the conclusion that the folds were vibrating at a normal pitch and loudness, without unnecessary or atypical features

such as strain, which is also the aim of clinical voice sampling. This creates a more realistic comparison between rabbit phonation and human sustained phonation. Therefore, the current work has further translational potential for clinical application.

### **Decreased Distribution in Rabbit PTP and PTF values**

When considering the results of the current study, there is a marked difference between the *sd* between pig and rabbit groups regarding PTP values. Standard deviation accounts for the divergence away from the mean in a data set. The larger the *sd*, the greater the distribution of values away from the mean in the data set. The results of the current study indicated that for the normative data, the pig group had an *sd* of 4.85 with an average PTP value of 14.83, and the rabbit group had an *sd* of 1.60 with an average PTP value of 7.69. While PTP values for pigs were reported to be much higher than rabbit PTP values, this still does not account for the over 3 times difference in *sd* between the two groups. When the coefficient of variation is computed, which is the *sd*/mean and represents the dispersion of values around the mean, the pig values are about 57% higher than the rabbit values. These numbers show that while there is a larger deviation of pig values from the mean, there is a decreased distribution of rabbit values to create the normative data. In other words, there is a slightly stronger cluster of PTP values around the mean to create a strong normative data set in the rabbit group when compared to the pig group. This may indicate greater stability in the species itself. In contrast to the inherent variability of animal species, the rabbit group demonstrated a tighter distribution when compared to the pig group.

Additionally, a good range in values is also important to represent the diversity within “normal” for the species. In other words, the goal of normative data is to achieve both specificity and sensitivity within a group related to larynx responsiveness. The range for rabbit

PTP values was 5.17, while the range for pig values was 15.70. These ranges indicate a healthy range of normal for the rabbit group, but a large range for the pig group. Therefore, rabbit larynges may provide more reliable data. While this range in rabbits is important to consider when determining which animal option to use in benchtop studies, the author found that it was slightly more difficult to identify phonatory onset and locate PTP and PTF in auditory-visual perceptual analysis of the rabbit signal than the pig signal. Details regarding this difficulty and potential solutions will be addressed in the discussion section. Still, the sensitive and specific range in rabbits should be considered when determining which species to include in translational research using animal models.

### **Establishment of Normative Data**

A larger sample size of each specimen was used initially in this study ( $N = 15$ ), but only 10 specimens were considered when developing normative data statistics for several reasons. While a large sample size is always ideal in all research designs, outliers are inevitable. It is standard practice in many research designs to remove outliers with the objective of finding reliable and replicable averages. To support this reasoning, an analogy to listener experiments will be made. In listener experiments, listeners may be excluded from a study if they do not meet reliability criteria. Similarly, certain aspects of a listener's ratings may be excluded from statistical analysis if they do not meet intrajudge or interjudge reliability (Bergstrom, 2017). The rationale for this is that those listeners, or those specific ratings, are not reliable instruments when making conclusions about perceptual ratings. They differ too far from the average to be considered reliable listeners and useful rating instruments. In this way, they are considered outlier listeners who tend to produce less reliable ratings. A similar rationale was used to identify and exclude outliers from the current study. Due to the large sample size, outliers in the

data were anticipated. Certain variables such as vocal fold use before sacrifice, sex of the larynges, preexisting histological properties, and microstructural abnormalities could not be controlled. Larynges were considered an outlier based on the following criterion: if a larynx had PTP and PTF values farthest from the median, if acoustical analysis of the larynx revealed outlier characteristics (i.e., abnormal pitch, aphonia), and if a larynx had inconsistencies in PTP and PTF values between trials within itself. For these reasons, outliers in each data set were removed. Five outlier specimens from each group were removed to leave 10 larynges nearest the median to create the normative data set. Ten larynges from each group were chosen because this would provide the least amount of variability between the samples while also facilitating normative data statistical analysis. With such a large sample, the thesis author knew that they would capture several specimens with normal phonation. Normative data are necessary to establish a control group for future studies.

### **Limitations**

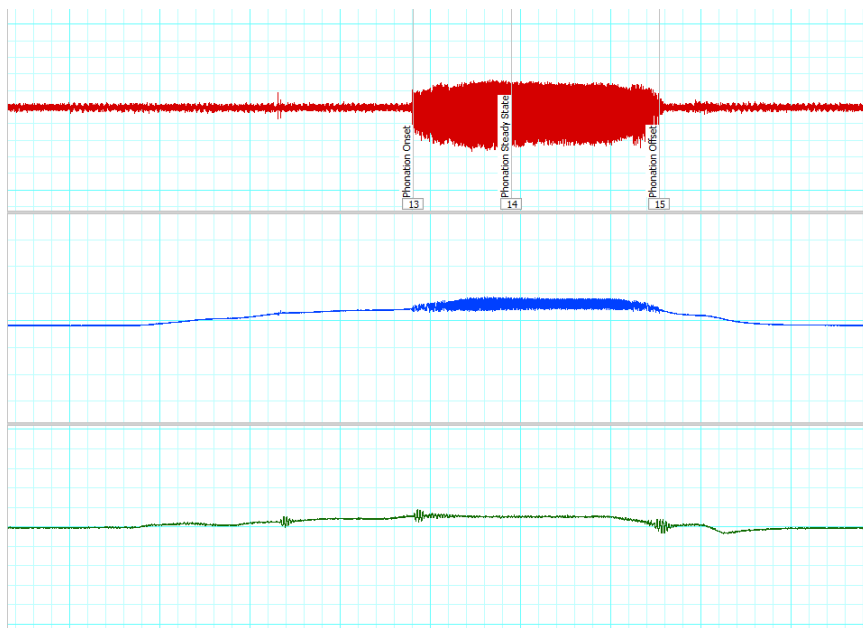
While measures were taken to ensure high quality research design, experimentation and analysis, there are several limitations in this study. First, the benchtop setup used in the current study was modified both to fit the small size of the rabbit larynges, as well as to accommodate the signal acquisition system, which could have potentially introduced new variables into the system. Significant structural modifications to the benchtop were made to allow mounting of such a small sized larynx as compared to the pig larynx. For example, the tubing from the pseudolung was tapered gradually by a series of smaller sized tubes until the rabbit larynx could fit over the smallest tube. This tapering introduced right angles into the airflow system, which could have contributed to back pressure as well as reverberation in the system and influenced the

PTP and PTF values. Additionally, much of the tubing from the air tank to the mounting apparatus was modified to accommodate new pieces of equipment.

Furthermore, there were some limitations with the analysis process. The author and another examiner used auditory-visual perception of the microphone signal to determine phonation onset, steady state, and offset; however, onset and periodicity were occasionally difficult to mark due to noise picked up from the microphone from air flow, beeping from the humidifier, fans from the freezer, and other ambient noise in the room. If the microphone had been set more posteriorly (i.e., out of the direct line of the air flow noise), if the author had used a more sensitive microphone, and if certain filters had been set in place to reduce noise amplification, it is expected that the audio signal would have been cleaner and the onset would have been easier to identify for some of the quieter larynges.

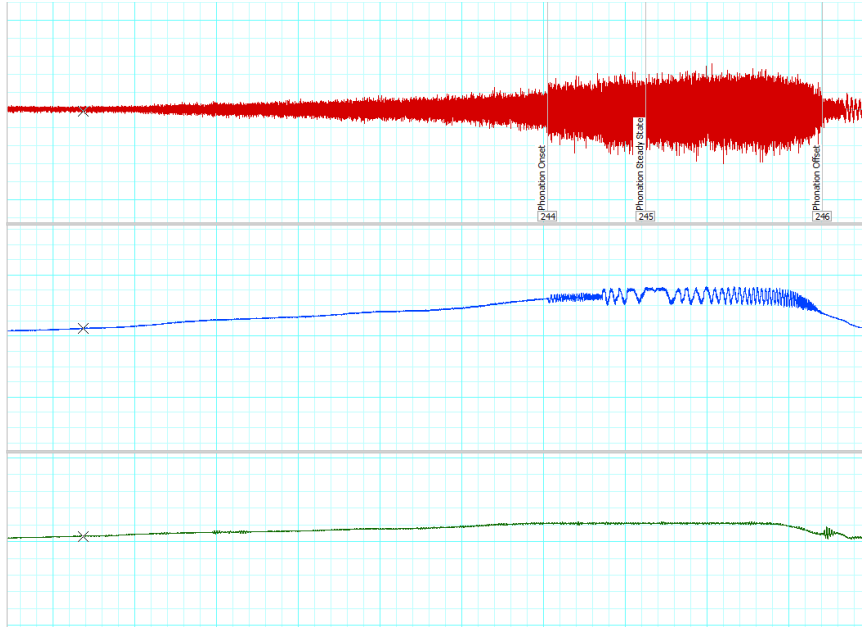
Ideally, onset would have been marked using both auditory and visual perception. Due to intermittent background noise, occasionally only auditory or visual perception could be used to mark onset through consideration of both the pressure and the microphone signals. In approximately 25% of the larynges in each species, auditory-visual perceptual analysis was not possible due to noise observed in the microphone signal. In order to achieve reliable values, other methods of analysis were developed to mark onset. In these cases, careful selection of onset was considered by looking at the pressure signal in conjunction with the audio signal. Onset was marked when a change in pressure was noted visually as well as a change in the audio signal auditorily. Figures 13, 14, and 15 show three different signals from three different rabbits analyzed in the current work. Figure 13 shows a typical signal where onset could easily be marked based on auditory and visual perception of the microphone signal (red line) alone. Figures 14 and 15 show two different rabbits in which auditory and visual perception of the

microphone signal alone was insufficient to mark onset. In these cases, visual perception of the pressure signal (blue line) in line with auditory perception of the microphone signal (red line) simultaneously was used to mark onset. Additionally, as has been stated previously, 10 ms before and after the onset marker were taken and averaged to achieve a true onset value. Having taken these precautions, the author is confident that the onset values reported in this study are reflective of the true average onset values for each species, respectively.

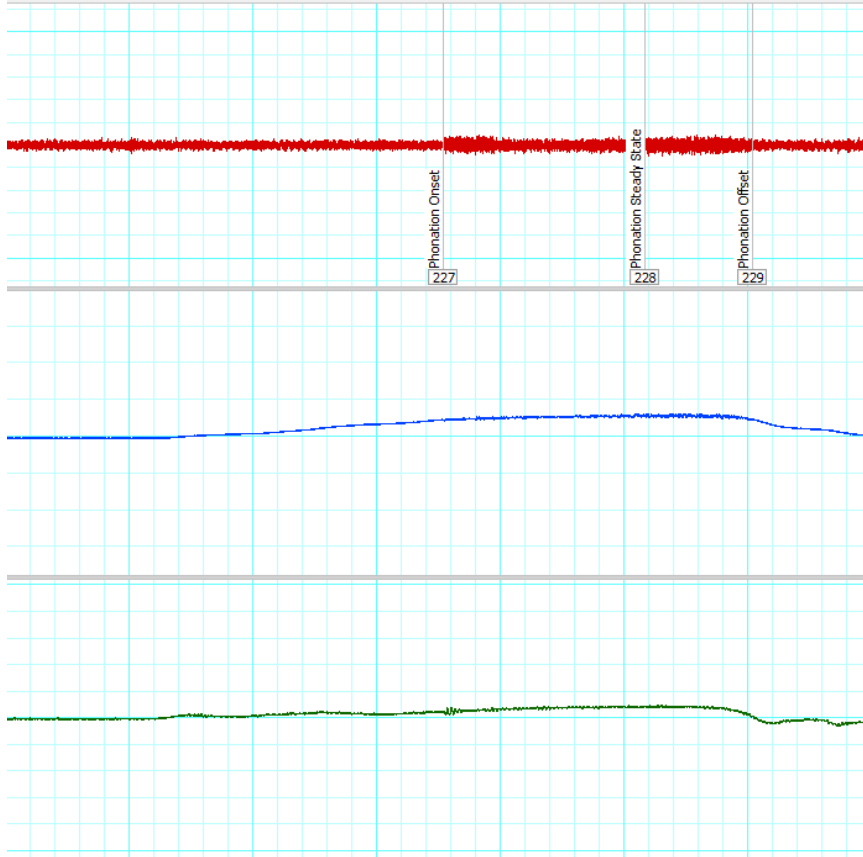


*Figure 13.* Data acquisition signal for a typical rabbit signal. In this signal, phonation onset could be marked through auditory and visual perception of the microphone signal (red line) alone.





*Figure 14.* Data acquisition signal for non-typical rabbit signal (R05). In this signal, phonation onset could not be marked through auditory and visual perception of the microphone signal (red line) alone. As a result, the pressure signal (blue line) was visually assessed to mark phonation onset in line with the microphone signal heard auditorily simultaneously.



*Figure 15.* Data acquisition signal for non-typical rabbit signal (R12). In this signal, phonation onset could not be marked through auditory and visual perception of the microphone signal (red line) alone. As a result, the pressure signal (blue line) was visually assessed to mark phonation onset in line with the microphone signal heard auditorily simultaneously.

Since this was an experimental comparison study between two groups, it would be ideal to reduce the number of variables between species. While efforts were made to decrease these variables, one variable that the author and researchers were unable to control for in both groups was the sex of each specimen. Since the pig larynges were food-grade and came from a butcher shop, control for the sex of the group was not feasible. The thesis author and two other examiners made a judgment via consensus as to the sex of each larynx based on the overall size of the larynx, the length of the vocal folds, and the angle of the vocal folds to the thyroid cartilage. It was also not possible to control for the sex and type of the rabbit specimen: some larynges were male, others female, and some rabbits were dwarf rabbits. Similarly, there are

other factors that could not be controlled for in these specimens. For example, vocal use prior to death was not quantified for the animal groups. While rabbits are traditionally quieter animals and this could be a relatively small variable to account for in that group, pigs are traditionally thought to be louder animals, and vocal use or abuse in pigs is much harder to quantify. This could explain the wide range of PTP and PTF values found in the pig group. These factors could have influenced the individual values for PTP and PTF found in each larynx, and for this reason, average values between the species were obtained to establish true normative values.

### **Implications for Future Research**

Future research should optimize the benchtop setup in this pilot study. Specifically, the tubing from the air tanks to the pseudolung and up through the larynx should be standardized as much as possible between species. Types of tubing, as well as length of tubing from air tank to larynx should be the same for all specimens. Also, tubing should be modified to eliminate possible back pressure or flow and reverberation that could influence aerodynamic measures. Additionally, an optimally quiet room with strategic microphone placement and mic sensitivity should be implemented to prevent auditory-visual discrepancies when determining onset, steady state, and offset. Future research should also examine sex-specific differences in PTP and PTF among both pig and rabbit larynges. Due to the specimen collection methods, sex could not be precisely determined for the pig and rabbit specimens. Performing a comparison study between sexes in both groups would provide helpful information in establishing and corroborating the normative data discovered in the current study. Lastly, replication of findings is crucial to establish the reliability of these data. While reliability within the study itself was achieved through interjudge and intrajudge reliability tests, the strength of the current work would be

increased through replication studies. The reliability of the current study when compared to other laboratories would be valuable information.

### **Conclusion**

The results from this study showed significant differences in PTP and PTF values for pig and rabbit groups. Significance was established both in the complete data set ( $N=15$ ) as well as the normative data set ( $N=10$ ). Additionally, other analysis revealed intralarynx variability in each species and within each specimen. Only three rabbit larynges were above 16% variability within trials for PTP and PTF (Hoggan, 2020). Phonation threshold pressure values of rabbit larynges were closer to human values and had less variability when compared to pig larynges. While methodological challenges to rabbit benchtop models exist when translating to humans, the results from this study indicate that rabbits should be considered a viable option for voice research. Specifically, when conducting *in vivo* studies and considering a small animal option that translates well to humans, the rabbit model should be considered an ideal candidate for translational phonatory research.

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## APPENDIX A

**Annotated Bibliography**

Alipour, F., Finnegan, E. M., & Scherer, R. C. (2009). Aerodynamic and acoustic effects of abrupt frequency changes in excised larynges. *Journal of Speech, Language, and Hearing Research*, 52, 465-481. doi: 10.1044/1092-4388

**Purpose of study.** The purpose of this study was to examine the differences in pressure and flow values in a dog model as vibration suddenly increased during vocal fold vibration obtained at chest and falsetto  $F_0$  ranges. The  $F_0$  can be defined as the lowest frequency in a complex sound and from which all harmonics are based from. Researchers hypothesized that rapid changes in pressure and flow (i.e., the pressure and flow sweep) might provoke a shift from chest to falsetto.

**Method.** Ten excised dog larynges were mounted onto a pseudotrachea which was attached to a pseudolung. Heated, humidified air was then pumped through the larynges and changes in vibration were measured as pressure and flow increased steadily.

**Results.** Changes in  $F_0$  and mode of vibration were observed after pressure and flow sweeps without any active manipulation of the larynx (i.e., elongation, purposeful adduction).

**Conclusion.** Pressure and flow sweep alone was sufficient to effect a change in vibration mode from chest to falsetto voicing. Muscle activation was not necessary to achieve changes in vibration modes. Subglottal pressure and flow may contribute more than previously thought to changes in vibration mode.

**Relevance to current work.** The current work focused on obtaining pressure and flow values in an excised animal model. While the animals are different, the basics of the setup, pseudolung and data collection are similar to this thesis. Understanding that changes in pressure and flow alone can cause changes in mode of vibration can also inform the current work's minimal pressure and flow values.

Alipour, F., & Jaiswal, S. (2008). Phonatory characteristics of excised pig, sheep, and cow larynges. *Journal of the Acoustical Society of America*, 123, 4572-4581. doi: 10.1121/1.2908289

**Purpose of study.** The purpose of this study was to examine the phonatory characteristics of pig, sheep, and cow excised larynges on a benchtop setup and determine which larynges are most useful when taking certain acoustic and aerodynamic measures.

**Method.** Eight pig, eight sheep and six cow larynges were obtained from a local butcher shop where the specimens were sacrificed for nonresearch purposes. Each larynx was cleaned and slow frozen and then later thawed and mounted to a tapered tube connected to a pseudolung. Electroglottographic signal, sound pressure level (SPL), subglottal pressure, mean flow rate and audio signal were recorded for each larynx. Sound pressure was defined as the deviation from atmospheric pressure that contributes to the sound. Sound pressure level was defined as the ratio between the deviation from atmospheric pressure and the normal hearing threshold. Mean flow rate was defined as the average rate at which the airflow was administered to the larynx. The  $F_0$  was acquired from the electroglottography signal and phonation threshold pressure (PTP) was acquired from the subglottal pressure values.

**Results.** The pig false vocal folds seemed to actively oscillate as seen from video-stroboscopic imaging. Pig larynges had large oscillation amplitudes and relatively loud SPLs and a higher  $dF/dP$  than the other animals. The cow larynx had a low PTP and a relatively steady pitch. Sheep vocal folds are known to be histologically similar to human vocal folds and the vocal fold length appeared to be similar to human folds as well as other laryngeal dimensions.

**Conclusion.** The pig larynx may be an ideal model to use when studying pitch control. The cow larynx may be useful when studying aerodynamic measures. The sheep larynx may be useful in physiological studies.

**Relevance to current work.** The current work was a comparison study between animal species similar to the species studied in this article. It was useful to compare pig pressure and flow signals reported in the study with the signals acquired in the current work. The different parameters measured and recorded in this article were considered for the current work and a few parameters, such as PTP, flow and the acoustic signal, were used in the current work.

Alipour, F., & Scherer, R. C. (2007). On pressure-frequency relations in the excised larynx. *Journal of the Acoustical Society of America*, 122, 2296-2305. doi: 10.1121/1.2772230

**Purpose of study.** The purpose of this study was to establish a relationship between a change in subglottal pressure causing a change in fundamental frequency ( $F_0$ ). Subglottal pressure was defined as the air pressure below the glottis, or the lung pressure. The  $F_0$  was defined as the lowest frequency in a complex sound and from which all harmonics are based. The rate of change in  $F_0$  was also studied and was represented as  $dF/dP$ . The  $dF/dP$  was defined as the rate of change in  $F_0$  as a function of change in subglottal pressure.

**Method.** Eight excised dog larynges were mounted to a pseudolung. Heated and humidified air was directed through the larynx. Pressure-flow sweeps were utilized to analyze the subglottal pressure needed for minimum and maximum phonation and to determine the change in  $F_0$  during various periods of phonation. A pressure-flow sweep was defined as a gradual increase in pressure and flow until the lowest level necessary for phonation was achieved, then up to the highest level where phonation could still be achieved, and the sweep was then finished with a gradual decrease in pressure and flow until the lowest level necessary to achieve phonation was met again. Airflow was sent through the pseudolung to the larynx and was gradually increased until maximum phonation was achieved and then gradually decreased until minimum phonation was achieved. Adduction and elongation were manipulated to change the tension of the muscles and simulate muscle contraction/relaxation.

**Results.** There was a nonlinear relationship between subglottal pressure and  $F_0$ . Adduction and elongation heavily impacted the subglottal pressure and  $F_0$  relationship. There seemed to be a reduction in  $F_0$  at greater adduction when subglottal pressure remained constant. Nonlinearity may be more pronounced with greater elongation and tension of the folds. Adduction and elongation seemed to play a more significant role in higher mode phonation than lower mode phonation.

**Conclusion.** The relationship between  $F_0$  and subglottal pressure may be helpful in changing speech and singing  $F_0$  control, independent from other methods of changing  $F_0$ .

**Relevance to current work.** The current work used a similar benchtop setup as the one described in this study. The effects of elongation and adduction to the relationship between  $F_0$  and subglottal pressure was important to consider when applying any adduction to the excised

larynges. Minimal adduction was utilized to prevent overtly influencing phonation and onset pressure and flow values.

Awan, S. N., Novaleski, C. K., & Rousseau, B. (2014). Nonlinear analyses of elicited modal, raised, and pressed rabbit phonation. *Journal of Voice*, 28, 538-547. doi: 10.1016/j.jvoice.2014.01.015

**Purpose of study.** The purpose of this study was to analyze different types of phonation (i.e., modal, raised, and pressed phonation) using acoustic measures that are nonlinear (i.e., phase space portraits, correlation dimensions, and descriptive spectrographic analyses).

**Method.** Seventeen white rabbits were used to evoke different types of phonation in an excised rabbit larynx model. Electrical stimulation was sent to the laryngeal musculature as well as subglottal air to initiate phonation. Different modes of phonation were achieved through changes in airflow rate and muscle stimulation current. Analysis of the phonation samples were taken from stable portions of the acoustic waveform signal. Sound spectrograms were also generated from the signal to make descriptive observations.

**Results.** Analysis of the acoustic signal revealed that phonation complexity increased as transglottal flow and electrical stimulation increased. Phonation increased from modal, to raised, to pressed as flow and electrical stimulation were increased. Increasing either flow or electrical stimulation led to nonlinear results, such as bifurcations in periodicity, as well as bifurcations of subharmonic content,  $F_0$ , and harmonic shifts.

**Conclusion.** Using transglottal airflow and electrical stimulation created different types of phonation in a rabbit model. The manipulation of these variables together was associated with the reliable study of nonlinear acoustic measures.

**Relevance to current work.** This study used a rabbit model to take acoustic measures of phonation, which is similar to the current work. While different acoustic measures and analyses were used, there are similarities between the principles of data collection for the rabbit phonation model. The current work also uses transglottal airflow to stimulate phonation.

Branski, R. C., Verdolini, K., Rosen, C. A., & Hebda, P. A. (2005). Acute vocal fold wound healing in a rabbit model. *Annals of Otology, Rhinology & Laryngology*, 114, 19-24. doi: 10.1177/000348940511400105

**Purpose of the study.** The purpose of this study was to examine vocal fold wound healing in the rabbit model during the first 3 weeks of tissue recovery. The long-term effects of wound and scarring on the vocal folds have been documented, but healing in the acute stages was largely unknown.

**Method.** Thirty two female New Zealand white rabbits were anesthetized and the right vocal fold of each specimen was cut through the epithelium and lamina propria along the entire fold. Four animals were then sacrificed at various stages of vocal fold healing, including 12 hours postinjury and then 1, 3, 5, 7, 10, 14, and 21 days postinjury. A total laryngectomy was performed on each rabbit and slides from the vocal fold epithelium and lamina propria were prepared and analyzed.

**Results.** Reepithelialization occurred relatively quickly after the vocal fold was severed. Collagen formation then follows, which eventually develops into a thick, fibrous tissue. By day 21, the right vocal fold epithelial tissue and lamina propria differed greatly from the uninjured

left vocal fold tissue. The scar tissue was significantly denser over the normal tissue as compared to the uninjured fold.

**Conclusion.** This study provided information on acute vocal fold healing and may influence future work on tissue regeneration and healing. Since reepithelialization of the vocal fold occurred relatively quickly after injury, this may inform clinicians in the future about the time necessary to keep patients on vocal rest while the vocal fold tissue recovers.

**Relevance to the current work.** This study contributed to the understanding of rabbit vocal fold histology and healing characteristics. This information was valuable to the current work when comparing the usefulness of the rabbit model to other animal models and when determining to use the rabbit model in combined phonatory and physiological studies.

Garrett, C. G., Coleman, J. R., & Reinisch, L. (2000). Comparative histology and vibration of the vocal folds: Implications for experimental studies in microlaryngeal surgery. *Laryngoscope*, 110, 814-824. doi: 10.1097/00005537-200005000-00011

**Purpose of study.** The purpose of this study was to find the most suitable animal model for vocal fold surgery and vibration. Histological comparisons as well as vibratory patterns between the vocal folds of dog, monkey, and pig were made.

**Method.** Three dog, pig, and monkey larynges each were compared to three excised human larynges. A standard microflap surgery was made on one vocal fold for each larynx, specifically raising the epithelium and the superior layer of the lamina propria. The histological slides were prepared for both the surgical vocal fold and the normal vocal fold in each specimen. After histological comparisons were made, the animals which appeared similar enough to human vocal folds were used in laryngeal video-stroboscopy in vivo. Vibratory patterns comparisons were made.

**Results.** It appeared that the dog vocal fold had more of a three-layered lamina propria than a two-layered lamina propria that was originally thought. The pig vocal fold had two layers of the lamina propria. The monkey vocal fold had indistinct layers and was not used further in the study. Video-stroboscopy images were assessed qualitatively by two skilled speech-language pathologists. The images of the pig vocal folds were much harder to record and assess than dog images due to the large size of the arytenoid cartilages. The vertical phase and mucosal wave differences were obtained from analyzations of the dog vocal fold vibrations.

**Conclusion.** The dog larynx seemed to be the most similar to the human larynx. The tissue structure and histology were similar, as well as the vibratory patterns of the dog vocal folds.

**Relevance to current work.** The current study made a comparison between two animals in the hopes of finding a more suitable model of human phonation, and the present study was also in part a comparison study to find the most suitable animal model for vocal fold surgery and vibratory patterns.

Ge, P. J., French, L. C., Ohno, T., Zealear, D. L., & Rousseau, B. (2009). Model of evoked rabbit phonation. *Annals of Otolaryngology, Rhinology, & Laryngology*, 118, 51-55. doi: 10.1177/000348940911800109

**Purpose of study.** The purpose of this study was to achieve reliable rabbit phonation in vivo through evoked muscle stimulation of the cricothyroid muscle with constant subglottal airflow. A second purpose was to describe its potential usefulness in small animal studies.

**Method.** Ten New Zealand white rabbits were used in this study. Each specimen was anesthetized before surgery and experimentation. For each specimen, electrodes were placed bilaterally into the cricothyroid muscle. A tracheotomy was performed on each specimen, controlled airflow was directed upward through the glottis, and electrical stimulation was induced every 5 seconds. Stimulation lasted for 2 seconds, and then the vocal folds were at rest for 3 seconds. Electrical activity of the cricothyroid muscle was recorded during every stimulation interval. The threshold of vocal fold motion as well as position of the vocal folds was observed through video-stroboscopy.

**Results.** Vocal fold motion threshold, operationally defined as the minimal stimulation necessary to achieve a twitch in the vocal folds, was seen at 1 mA. Increasing the stimulation current and frequency resulted in complete glottal closure between 50 to 60 Hz and 3 to 4 mA. Phonation intensity ranged from 69 dB SPL to 85 dB SPL. Airflow was delivered at a constant 143 cm<sup>3</sup>/s.

**Conclusion.** The study successfully described a method to achieve evoked rabbit phonation in vivo. Evoked phonation was achieved in the in vivo rabbits with bilateral electrode placement on the cricothyroid muscle with bipolar stimulation and constant airflow through the glottis. This work supports the use of smaller animals previously not used or underused in in vivo animal phonation research. The rabbit model may be a more convenient and reliable animal to use in small animal research than other animals, such as dogs.

**Relevance to current work.** The current work is a pilot study examining the feasibility and efficacy of using a smaller animal, the rabbit model in future studies as compared to larger animals, such as pigs. As this study was also a descriptive methodology study of the usefulness of the rabbit model in phonation research, it provided useful information about typical rabbit phonation that was valuable when preparing for expected rabbit threshold phonation airflow rates.

Gorti, G. K., Birchall, M. A., Haverson, K., Macchiarini, P., & Bailey, M. (1999). A preclinical model for laryngeal transplantation: Anatomy and mucosal immunology of the porcine larynx. *Transplantation*, 68, 1638-1642. doi: 10.1097/00007890-199912150-00006

**Purpose of study.** The purpose of this study was to investigate the viability of the pig larynx as a potential specimen to be used in preclinical laryngeal transplantation.

**Method.** Eight Minnesota mini pigs were dissected at the neck and anatomical structures were studied and vascular pathways were mapped. Tissue samples from the supraglottis, glottis, and subglottis were obtained and prepared for histology, immunohistochemistry, and immunofluorescence analysis.

**Results.** The pig laryngeal anatomy appeared to be relatively similar to the human laryngeal anatomy. The pig larynx had the same paired and unpaired cartilaginous structures as a human larynx. Most of the pig's blood supply to the larynx was through the caudal thyroid artery which branched off the brachiocephalic artery, with assistance from the cranial laryngeal artery, which branched off from the common carotid artery. The pig supraglottis was lined with stratified squamous epithelium in comparison with the human respiratory mucosal lining. The pig larynx contained a large amount of important immunological cells, and many which are similar to human cells.



**Conclusion.** The pig larynx is an ideal preclinical animal model for laryngeal transplantation because of its anatomical similarities to the human larynx as well as its immunological congruence with the human larynx.

**Relevance to current work.** This study contributed to the precedent of pig larynges being used in in vivo animal and ex vivo animal benchtop studies. It furthered the use of the pig larynx in laryngeal study. The current work supported the use of the pig larynx in phonation studies, while it also made a new comparison between the pig larynx and the rabbit larynx. The pig larynx has been used reliably and consistently in laryngeal and phonation research as seen in part from this study, but a newer, more convenient, and efficient animal model was now investigated in the current work.

Hahn, M. S., Kobler, J. B., Starcher, B. C., Zeitels, S. M., & Langer, R. (2006). Quantitative and comparative studies of the vocal fold extracellular matrix I: Elastic fibers and hyaluronic acid. *Annals of Otology, Rhinology, & Laryngology*, 115, 156-164. doi: 10.1177/000348940611500213

**Purpose of study.** The purpose of this study was to analyze the hyaluronic acid (HA) and elastic fiber content in human, dog, pig, and ferret vocal folds.

**Method.** The six human vocal folds were dissected within 24 hours postmortem and stained, prepared and frozen for tissue analysis. The six dog, pig, and ferret vocal folds were dissected within 6 hours postmortem and the tissue was stained and prepared in the same way as the human vocal fold tissue. Quantitative and qualitative analyses were taken.

**Results.** Compared to the human vocal folds, the animal vocal folds in each species had much higher levels of HA content. The HA content in all the animal species was 3 to 4 times higher than in the human folds. The elastic fiber distribution in the pig vocal folds was most similar to humans when compared with the other animals.

**Conclusion.** Of the animals studied, the pig larynx appeared to be the best model of HA and elastic fiber properties of the vocal folds when compared to human vocal folds.

**Relevance to current work.** The current work used the reliability of the pig laryngeal model to provide a comparison between a newer animal laryngeal model, the rabbits. This study contributed to the body of evidence supporting the use of pig larynges and instilled greater confidence in the researchers using the pig larynges as a stable comparison to a newer model.

Happak, W., Zrunek, M., Pechmann, U., & Streinzer, W. (1989). Comparative histochemistry of human and sheep laryngeal muscles. *Acta Oto-Laryngologica*, 107, 283-288. doi: 10.3109/00016488909127510

**Purpose of study.** The purpose of this study was to histologically compare type I and type II muscle fibers in the cricothyroid, transverse arytenoid, posterior cricoarytenoid, thyroarytenoid, and vocalis muscles in human and sheep larynges.

**Method.** The six human male and six female laryngeal muscles were compared with the six sheep laryngeal musculature. The muscles were extracted from the larynx within 6 days postmortem. In the sheep specimen, the musculature was extracted immediately following an acute lower limb surgery. The connective tissue and fascia were dissected away from the musculature. The tissue was then frozen and cut on a cryostat. The tissues were stained to differentiate between muscle fiber types.

**Results.** Significant differences in the two fiber types were observed in both human and sheep muscles. The individual fibers in the human muscles appeared to be much larger than in the sheep muscles with the exception of the cricothyroid muscle in the sheep. Additionally, all sheep musculature except for the cricothyroid muscle had significantly less type I muscle fibers than the human muscles. The sheep muscles also had less type II fibers in each muscle with exception to the cricothyroid muscle when compared to the human muscles.

**Conclusion.** Because of the increased amount of muscle fiber types in the cricoarytenoid muscle, the sheep larynx appeared to be a suitable animal model to use in sustained phonatory activities. The microscopic characteristics of human and sheep musculature are comparable and this may support the use of the sheep in functional laryngeal musculature research.

**Relevance to current work.** This was a comparative study between an animal species and the human. This work contributed to the body of evidence supporting the use of the animals in translational research to humans. Additionally, this study in part highlighted the usefulness of animal comparison studies to find ideal animal models to use in laryngeal research. The current work strove to identify a new animal model that could be considered in laryngeal research.

Hottinger, D. G., Tao, C., & Jiang, J. J. (2007). Comparing phonation threshold flow and pressure by abducting excised larynges. *Laryngoscope*, *117*, 1695-1699. doi: 10.1097/MLG.0b013e3180959e38

**Purpose of the Study.** The purpose of this study was to analyze PTP and PTF in dog larynges with varied glottal width. Phonation threshold flow was a new parameter not analyzed in previous studies. The study was designed to determine the reliability and usefulness of PTF to quantify certain physiologic changes to phonation.

**Method.** Ten dog larynges were excised and connected to a bench setup. Subglottal airflow and pressure were measured for each larynx as phonation began. Air was increasingly directed through the larynx until phonation was achieved; subsequently, pressure and flow values were determined.

**Results.** Analysis revealed that PTF increased as posterior glottal width increased; PTP demonstrated no change as posterior glottal width increased.

**Conclusion.** This study supported the use and reliability of PTF as an aerodynamic measure that is sensitive to posterior glottal width changes. It is possible that PTF might be more useful when researching voice problems that originate from abduction issue than PTP.

**Relevance to current work.** The current study also included PTF as its primary outcome measure. The benchtop setups were similar between these studies. Also, the collection of pressure and flow values was similar between studies. Both the current work and this thesis served as foundation for future clinical application studies involving PTF.

Howard, N. S., Mendelsohn, A. H., & Berke, G. S. (2015). Development of the ex vivo laryngeal model of phonation. *Laryngoscope*, *125*, 1414-1419. doi: 10.1002/lary.25149

**Purpose of study.** The purpose of this study was to describe a method to achieve phonation in an ex vivo dog larynx through neuromuscular innervation and blood supply in a simulated living excised animal model.

**Method.** The larynges of 19 dogs were surgically removed from the body. Care was taken to ensure the laryngeal nerves and major arterial blood supply lines were not damaged. Electrodes

were connected to the laryngeal nerves, and blood was administered through the arteries connected to the larynx to simulate in vivo laryngeal contraction and phonation. Different methods of electrical stimulation and blood supply were experimented with to create any phonatory response.

**Results.** Some methods for obtaining phonation were more successful than others. Autologous whole blood administered through a pulsatile pump rather than in a continuous flow through the organ produced the strongest and longest phonation.

**Conclusion.** The use of the dog larynx in a virtually living study with an ex vivo animal model appeared to be a repeatable and reliable method to achieve phonation. This study provided further insight into virtually living models in phonatory research as well as other organ transplant research.

**Relevance to current work.** This study supported the use of dogs in phonatory research. The dog animal model was considered for the current study. Following the comparisons between pig and dog larynges, as well as considering convenience and feasibility, pigs were chosen as the pig group in the current work.

Jiang, J. J., Raviv, J. R., & Hanson, D. G. (2001). Comparison of the phonation-related structures among pig, dog, white-tailed deer, and human larynges. *Annals of Otology, Rhinology, & Laryngology*, 110, 1120-1125. doi: 10.1177/000348940111001207

**Purpose of study.** The purpose of this study was to examine the attributes of certain animal models (i.e., pig, dog, and white-tailed deer) as compared to the human larynx and determine the best model for phonation.

**Method.** The larynges of two humans were used as a control comparison to three pig, three dog, and three white-tailed deer larynges. Vocal fold length, vocal fold stiffness, cricothyroid joint range of motion, and phonatory range were recorded for each species.

**Results.** The pig, dog, and human larynges all had similar ranges of motion in the cricothyroid joint, but the range of motion for the deer larynx was much smaller and more restricted. The deer and human vocal folds had greater lateral displacement and were less stiff when compared with dog and pig vocal folds. The pig vocal folds were slightly longer on average than the human vocal folds but there was greater variation in the pig specimen. Dog vocal fold length was the most similar to humans, and the deer vocal folds were less similar in length. The pig larynges had the greatest phonatory range, making noises from 100 to 450 Hz. The dog bark was approximately 150 Hz. The deer grunts were approximately 61 Hz.

**Conclusion.** The pig model is the most superior model in this study because of its variation in vocal fold length, making it a good phonatory model. Additionally, its vocal range is bigger than the other animals and can better simulate the human vocal range. The range of motion in the pig cricothyroid joint is also similar to that of humans, making vocal fold elongation simulation more similar to human elongation.

**Relevance to current work.** Similar to this study, the current work is also a comparative study between animal models. Similar to this study, the goal of the current work was to find a suitable animal model for phonation. This study established that the pig larynx is the best model for phonatory research, and the current work used the pig larynx as a group to compare with rabbit larynges.

Jiang, J. J., Regner, M. F., Tao, C., & Pauls, S. (2008). Phonation threshold flow in elongated excised larynges. *Annals of Otolaryngology, Rhinology, & Laryngology*, 117, 548-553. doi: 10.1177/000348940811700714

**Purpose of study.** The purpose of this study was to compare PTF in dog larynges that were elongated at various lengths.

**Method.** The 10 dog larynges used in this study were sacrificed for purposes other than this study. The larynges were dissected down to the vocal folds and mounted on a pseudolung and heated and humidified air was sent through the larynx. The acoustic signal, PTF, and PTP were sampled simultaneously using LabView™ software. Elongation effects on PTF and PTP were measured at + 0%, + 5%, + 10% and + 15% elongation. In this study, PTF and PTP were the dependent variables and percent elongation was the independent variable.

**Results.** The PTF increased with elongation increases. No significant correlation existed between larynx size and PTF values. Significant differences in PTF were observed between each elongation level. The PTP and PTF seemed to show some significant positive proportionality, specifically at the + 0% and + 5% elongation levels.

**Conclusion.** The PTF measure was able to independently show changes in mechanical manipulation of the vocal folds (i.e., elongation), which may indicate that this measure can be used to assess disordered phonation as well as typical phonation. Additionally, PTF can be used to establish normative data in a species.

**Relevance to current work.** The current work examined PTP as well as PTF in a new animal model, the rabbit. Part of the current work's aim was to provide normative phonation data for the rabbit species, which is similar to this study which established normative data for the dog species (i.e., + 0% elongation). Additionally, this study used PTF as a primary outcome measure, and the current work used PTF as well as PTP as primary outcome measures.

Jiang, J. J., & Tao, C. (2007). The minimum glottal airflow to initiate vocal fold oscillation. *Journal of the Acoustical Society of America*, 121, 2873-2881. doi: 10.1121/1.2710961

**Purpose of study.** The purpose of this study was to propose and exhibit the usefulness of a new aerodynamic measure, PTF, which is the minimal glottal airflow necessary to achieve phonation.

**Method.** The equations to establish PTF were on the body-cover model. Once the stability of this model was established, the critical conditions to produce phonation were stated. A stability analysis was then used to establish PTF. Clinical applications of PTF were then suggested.

**Results.** Mathematically, the body-cover theoretical model was established. A theoretical outline of the critical conditions necessary to produce phonation were then created from the body-cover model. After the critical conditions were set forth, the equation for PTF was produced. Preliminary studies on excised larynges showed promising results for the usefulness of PTF in benchtop work. Clinical application was also suggested, as PTF can be measured noninvasively through a flow meter attached to a mask covering the mouth and nose.

**Conclusion.** It was concluded that PTF is a useful new parameter to use when taking aerodynamic measures of phonation. Since measuring airflow is a noninvasive procedure, it may be more practical to use PTF clinically over PTP since measuring PTP is an invasive and uncomfortable procedure. Therefore, PTF has potential clinical application as well as its stated uses in phonatory research.

**Relevance to current work.** The current work used PTF as the primary outcome measure. This study was crucial to establishing the theoretical use of the measure in research, and provided the basis from which the current work could use the measure with confidence in its usefulness.

Jiang, J. J., Verdolini, K., Ng, J., Aquino, B., & Hanson, D. (2000). Effects of dehydration on phonation in excised canine larynges. *Annals of Otolaryngology, Rhinology, & Laryngology*, 109, 568-575. doi: 10.1177/000348940010900607

**Purpose of study.** The purpose of this study was to determine the effects of surface dehydration on PTP. Systematic dehydration has been observed to increase PTP in humans through the use of diuretics. However, direct dehydration directly on the epithelium of the vocal folds has not been studied.

**Method.** The 17 dog larynges used in this study were divided into two groups. The experimental group consisted of 15 larynges. These larynges were mounted and connected to a pseudolung, and nonhumidified air was administered to them. The control group consisted of two larynges and these larynges were administered humidified air and phonated for the same amount of time as the experimental group. All larynges were taken from dogs that were sacrificed for other purposes. The larynges were placed in saline solution and refrigerated for 24 to 48 hours until experimentation. The larynges were mounted to a benchtop apparatus very similar to the one used in the current work. Phonation trials lasted for 10 seconds each (with a rest period of 3 seconds in between trials) and the total drying period was about 5 minutes. The same phonation pattern was followed for the control group as well. Phonatory measures including PTP were recorded for each larynx.

**Results.** There were no significant changes in PTP or the other phonatory measures taken for the control group. There was, however, a significant increase in PTP for the larynges in the experimental group as dehydration increased. Since the only experimental factor was humidified or dry air, this change in PTP is considered to be in direct response to the nonhumidified air distributed to the experimental group.

**Conclusion.** Surface dehydration is one method to increase dryness in the surface epithelium of the vocal folds in an excised animal model. Surface dryness is somewhat more complicated in human vocal folds because of the lubrication from mucus glands below the false vocal folds. For this reason, this study does not have direct human application, but it does provide insight into the different methods of dryness that can increase PTP.

**Relevance to current work.** The benchtop apparatus used in the current work was modeled after the benchtop used in this study. The similarities between the setups was relied upon to achieve adequate phonation and interpret the results. Additionally, the phonatory measures used in this study, specifically PTP, was also used in a partner study with the current work.

Keir, S., & Page, C. (2008). The rabbit as a model to study asthma and other lung diseases. *Pulmonary Pharmacology & Therapeutics*, 21, 721-730. doi: 10.1016/j.pupt.2008.01.005

**Purpose of study.** The purpose of this article was to focus on the aspects of rabbits that make it a good animal model for studying various lung diseases.

**Method.** This article described the background of rabbit use in lung disease and lung histological research, specifically asthma. The paper progressed into discussing immunization similarities of the rabbit model to humans, as well as the pulmonary function in rabbits, the

airway hyperresponsiveness, the airway response to various antigens, the airway inflammation response, the nerves of the airway, the effects of drugs are various allergens introduced into the airway, airway wall remodeling, and airway smooth muscle in vitro.

**Results.** There were similarities between immunized rabbits and human asthma, which makes the rabbit a good model for studying the pathophysiology as well as various therapeutic treatments for asthma in humans. Overall, the airway inflammation response to asthma, allergens, histamines, and inhaled corticosteroids in the rabbit was similar to the responses seen in humans with asthma.

**Conclusion.** The rabbit histology and responses to allergens and therapeutic agents is so similar to humans that it seemed to be a good model for lung disease research. Additionally, the ability to introduce the allergen to the rabbits and then watch the response to the allergen as well as the responses to various therapeutic experiments in longitudinal studies made the rabbit an ideal candidate for lung disease research.

**Relevance to current work.** The current work is a part of a larger, five-year study looking at the effects of inhaled corticosteroids on rabbit vocal fold tissue and phonation. This article supports the use of rabbit in such a study because of the compatibility of rabbit tissue and responsiveness to allergens and therapeutic agents as compared to humans. The current work focused on obtaining pilot phonatory data for rabbits, but future studies will be using the rabbit model in a longitudinal study examining the effects of inhaled corticosteroids on phonation. This study highlighted the usefulness and practicality of the rabbit model in longitudinal studies.

Kim, M. J., Hunter, E. J., & Titze, I. R. (2004). Comparison of human, canine, and ovine laryngeal dimensions. *Annals of Otolology, Rhinology, & Laryngology*, 113, 60-68. doi: 10.1177/000348940411300114

**Purpose of study.** The purpose of this study was to compare laryngeal dimensions of dog, sheep, and human larynges with the goal of determining if the sheep larynx would be a good model for biomechanical studies of the larynx.

**Method.** The larynges of nine men, seven women, nine dogs, and 12 sheep were measured using 44 different parameters. Each larynx was mounted on a benchtop apparatus, and the 44 laryngeal dimension measures were taken.

**Results.** There appeared to be significant differences in the sheep larynx compared to the human larynx, but not a significant difference between the dog and human larynges. It was difficult to distinguish the false vocal folds from the true vocal folds in the sheep larynx because there was not ventricle separating the two. The laryngeal height in the sheep larynx was significantly larger than the human larynx. The space between the cricoid cartilage and the bottom of the thyroid cartilage was much smaller in the sheep larynges than the dog and human larynges, suggesting that it may have a smaller range of  $F_0$  control than the other species. The cricoid cartilage of the sheep larynges was much larger than those seen in the dog and human larynges as well.

**Conclusion.** The sheep larynx showed great differences in laryngeal dimensions when compared to the dog and human laryngeal dimensions. Since the structure is so different, this would affect the way the muscles in the sheep larynx move, and this could influence the biomechanics of the model. For these reasons, the sheep larynx may not be the most suitable model for phonatory research.

**Relevance to current work.** The current study is also a comparison study between pig and rabbits larynges with the purpose of finding a new animal model for phonatory research. Laryngeal dimensions similar to the ones recorded in this study were also taken in the current work, although they were not the primary focus of comparison in the current study.

Maytag, A. L., Robitaille, M. J., Rieves, A. L., Madsen, J., Smith, B. L., & Jiang, J. J. (2013). Use of the rabbit larynx in an excised larynx setup. *Journal of Voice*, 27, 24-28. doi: 10.1016/j.jvoice.2012.08.004

**Purpose of Study.** The purpose of this study was to determine the reliability and functionality of excised rabbit larynges for use with the excised larynx bench apparatus.

**Method.** Five rabbit larynges were dissected and mounted on a benchtop setup modified to study rabbit phonation. Heated and humidified air was passed through the larynx via a humidifier connected to a pseudolung. The base of the larynges were connected to the pseudolung through a Luer Lock™ and tightened by a cable tie. The true vocal folds were elongated by a string fastened to the thyroid cartilage and connected to a moveable positioner. Adduction was achieved through a micropositioner on each side of the arytenoid cartilages. Acoustic, aerodynamic, electroglottographic, and videokymographic measures were taken for each larynx. Setup for the dog larynges was similar and the same data was collected for the dog larynges for the purpose of comparison.

**Results.** Phonation was achieved for all rabbit larynges. Data for all outcome measures were reliable for both rabbit and dog larynges.

**Conclusions.** A method of collecting data for the rabbit model was established. The rabbit animal model was a reliable model for phonation experiments.

**Relevance to current work.** This study and the current thesis both involved the novel use of rabbit larynges to study phonation. Similar acoustic and aerodynamic measures, as well as similar methodologies and benchtop setups, were used.

Mills, R. D., Dodd, K., Ablavsky, A., Devine, E., & Jiang, J. J. (2017). Parameters from the complete phonatory range of an excised rabbit larynx. *Journal of Voice*, 31, e517-e519. doi: 10.1016/j.jvoice.2016.12.018

**Purpose of study.** The purpose of this study was to determine PTP, PTF, and other measures from the complete phonatory range of the excised rabbit larynx. The rabbit larynx is histologically similar to the human larynx, which makes it a preferable model to use in phonatory research. Phonation was manipulated by elongation of the vocal folds at 0%, 5%, 10%, 15% and 20% of the vocal fold length. It was thought that the phonatory range would decrease as elongation increased.

**Method.** The seven rabbit larynges used in this study were mounted on a benchtop apparatus similar to the one used in the Jiang, Regner, Tao, and Paul (2008) study. Each larynx was elongated at 0%, 5%, 10%, 15% and 20% of the vocal fold length. Percentages of elongation increase were determined by measuring the vocal fold length from the anterior point of the vocal fold to the end of the arytenoid cartilage.

**Results.** The results indicated that PTF did not differ significantly across elongation increases; however, PTP did increase significantly as elongation increased. The phonatory range decreased as elongation percentage increased.

**Conclusion.** The histological similarities between rabbit vocal folds and human vocal folds, combined with the aerodynamic results obtained from this study, support the conclusion that the rabbit larynx may be a more suitable choice for benchtop laryngeal research.

**Relevance to current work.** The current work also used PTF and PTP to make comparisons between pig and rabbit larynges. This study used these parameters in part to identify the usefulness of the rabbit larynx in phonatory research. Similarly, the current work used these parameters to compare rabbit larynges to pig larynges.

Regner, M. F., Robitaille, M. J., & Jiang, J. J. (2010). Interspecies comparison of mucosal wave properties using high-speed digital imaging. *Laryngoscope*, *120*, 1188-1194. doi: 10.1002/lary.20884

**Purpose of study.** The purpose of this study was to compare cow, sheep, dog, and pig vocal folds vibratory patterns to in vivo vocal fold human vibratory patterns.

**Method.** The 17 female and 18 male subjects were used as the control group in this experimental design study. The seven dog, seven pig, six cow, and five sheep larynges were used as the experimental groups. Each larynx was mounted on a benchtop apparatus used to simulate respiration. High-speed video was recorded using a high-speed video camera. Each high-speed video was 0.1919 seconds long at 4,000 frames per second. The vocal fold edge length was recorded for each specimen.

**Results.** Dog larynges had vocal fold oscillation frequencies most similar to humans, both male and female. Pig larynges also had vocal fold oscillation frequencies were high compared to human frequencies. Both sheep and cow vocal fold oscillation frequencies were low. Cow larynges had the largest oscillation amplitudes of the species studied. Sheep had the second largest oscillation amplitudes. Pig and dog larynges had similar oscillation amplitudes to humans but pig larynges were the most similar.

**Conclusion.** Dog and pig larynges appeared to be the most similar to humans of the species studied when considering vocal fold oscillation frequencies and amplitudes as observed in high-speed video. These two models seemed to be the most suitable models of the species studied for laryngeal phonatory research.

**Relevance to current work.** This study used pig larynges as a specimen similar to the current work, although the pig larynges were used as one of the experimental groups in this study and they were used as one of the groups in the current work. Additionally, the benchtop apparatus used in this study is similar to the benchtop used in the current work.

Regner, M. F., Tao, C., Zhuang, P., & Jiang, J. J. (2008). Onset and offset phonation threshold flow in excised canine larynges. *Laryngoscope*, *118*, 1313-1317. doi: 10.1097/MLG.0b013e31816e2ec7

**Purpose of study.** The purpose of this study was to determine if the onset PTF was higher than the offset PTF, and to show that the ratio of the two parameters falls between .707 and 1.0.

**Method.** The 10 dog larynges used in this study were sacrificed for other purposes. Each larynx was dissected and frozen in 0.9% saline solution until experimentation. After each larynx was thawed, it was mounted to a benchtop apparatus similar to the one used in the current work. Three trials were recorded at each elongation stage: 0%, 5%, 10% and 15% elongation.



**Results.** In all trials, the offset PTF was lower than the onset PTF, as hypothesized. Additionally, approximately 80% of the data collected fell in the boundaries described for the ratio between .707 and 1.0.

**Conclusion.** Onset PTF and offset PTF may be useful parameters to use clinically. Onset PTF has been shown to be useful in diagnosing laryngeal disorders, but offset PTF may also be useful clinically, especially in instances where the onset PTF cannot be established.

**Relevance to current work.** This study highlighted the relationship between PTP and PTF as a powerful diagnostic tool, and the current work also used PTP and PTF in conjunction with each other to support the comparison between the two animal species. This study's main parameter was PTF, which is a primary outcome measure of the current work as well.

Sjöblom, E., Höjer, J., Kulling, P. E., Stauffer, K., Suneson, A., & Ludwigs, U. (1999). A placebo-controlled experimental study of steroid inhalation therapy in ammonia-induced lung injury. *Journal of Toxicology: Clinical Toxicology*, 37, 59-67.

**Purpose of Study.** The purpose of this study was to determine if the use of corticosteroids were effective in treating lung injury in rabbits as compared to a saline placebo.

**Method.** Sixteen New Zealand white rabbits inhaled ammonia to induce lung injury and were randomly assigned to control and experimental groups. Lung pressure was measured at both 5 minutes and 15 minutes postinjury. The control group was a placebo group that received nebulized saline treatments and the experimental group received nebulized corticosteroids. Each treatment was administered 30 and 120 minutes after the initial injury to the experimental group.

**Results.** There was no difference between the effects of nebulized corticosteroids and saline on treatment for the induced lung injury. Corticosteroids did not improve the gas exchange or increase airway pressure levels as compared to the placebo.

**Conclusions.** Corticosteroids did not improve ammonia induced lung injury in rabbits.

**Relevance to current work.** This study compared the effects of nebulized inhaled corticosteroids and nebulized saline solution on lung injury, which is relevant to the current work because this thesis lays the foundation for future rabbit studies of respiratory disease.

Witt, R. E., Regner, M. F., Tao, C., Rieves, A. L., Zhuang, P., & Jiang, J. J. (2009). Effect of dehydration on phonation threshold flow in excised canine larynges. *Annals of Otology, Rhinology & Laryngology*, 118, 154-159. doi: 10.1177/000348940911800212

**Purpose of study.** The purpose of this study was to determine the effects of dehydration on PTF in excised dog larynges. Additionally, a cessation point of phonation due to dehydration was observed in certain larynges.

**Method.** The 11 dog larynges used in this study were divided into three groups. The control group consisted of two larynges. In the control group, the larynges were mounted on a benchtop apparatus similar to the setup in the current work. Heated and humidified air was sent through the larynx for a total of 10 seconds for each trial, followed by 3 seconds of rest. This continued for approximately 5 minutes. After each phonation trial, the vocal folds were sprayed with saline solution to aid in hydration. In the experimental group, eight larynges were mounted to the same benchtop apparatus as the control group; however, the humidifiers were taken out of the setup and presumably dry air was administered to the larynges. The pattern of phonatory trials, 10 seconds of phonation with 3 second break for approximately 5 minutes, was the same for the

experimental group. No saline solution was sprayed in between each trial. Additionally, one larynx which underwent the control trials and then the dehydration trials to rule out the variability of larynges as the possible reasoning for a dehydration effect seen in the results.

**Results.** There was a significant difference between the initial PTF values and the final PTF values of all dehydration larynges. To contrast, there was no significant difference in the initial and final PTF values in any of the control larynges. Additionally, PTF values showed a significant increase between trials for each experimental larynx. Lastly, there appears to be a critical point in dehydration when the vocal folds cannot initiate or sustain phonation any longer. For the one larynx that underwent both the control and experimental trials, there appeared to be a significant difference between the two sets of trials.

**Conclusion.** A positive correlation was observed between dehydration and PTF values; as dehydration increased, PTF values also increased. A large PTF value clinically may indicate laryngeal dysfunction, possibly due to dehydration.

**Relevance to current work.** The current work used a similar benchtop setup to the one described in this study used for the control group. The current work also used PTF as a primary outcome measure and this study did as well. In part from the results of this study and studies like it, the researcher of the current work was careful to hydrate the vocal folds after every three trials to prevent possible dehydration and affect the results of the study.

Zhuang, P., Sprecher, A. J., Hoffman, M. R., Zhang, Y., Fourakis, M., Jiang, J. J., & Wei, C. S. (2009). Phonation threshold flow measurements in normal and pathological phonation. *Laryngoscope*, 119, 811-815. doi: 10.1002/lary.20165

**Purpose of study.** The purpose of this study was to identify PTF values in normal and disordered in vivo human larynges and compare those values between genders, and between normal and disordered phonation.

**Method.** Forty normal speakers in the study (21 female and 19 male) were used as a control comparison to each other as well as to the disordered phonation types (21 patients with vocal fold nodules and 23 patients with vocal fold polyps). All groups were controlled for race and gender. The polyp group consisted of 12 female subjects and 11 male subjects. The nodule group consisted of only females because vocal fold nodules occur almost exclusively in female speakers. To sample PTF, patients were instructed to phonate softly and gradually decrease intensity for 3 to 5 seconds until no phonation was detected. This was repeated 10 times for each subject, with a break of 5 to 6 seconds in between each trial.

**Results.** The difference between male and female PTF values in normal speakers was statistically significant. When adjusted for gender differences, PTF differences among genders were statistically significant, and gender differences were included in the statistical analysis of the parameters. There was a significant difference between normal speakers and speakers with vocal fold polyps. A significant difference was not found between normal and vocal fold nodule groups or between disordered groups.

**Conclusion.** These findings indicate that PTF is a useful diagnostic tool when assessing voice disorders, particularly vocal fold polyps. The PTF may not have been significantly different among normal speakers and subjects with vocal fold nodules because the presence of nodules did not increase the prephonatory glottal width as seen in pretrial stroboscopy observations, which has been shown to increase PTF.

**Relevance to current work.** The current work used PTF as a primary outcome variable, which is similar to this study. The current work is also a comparison study between populations, as is this study. In part, this study aimed to find PTF values in normal male and female speakers, and the current work is also aiming to find PTF values in normal rabbit phonation.

Zrunek, M., Happak, W., Hermann, M., & Streinzer, W. (1988). Comparative anatomy of human and sheep laryngeal skeleton. *Acta Oto-Laryngologica*, 105, 155-162.

**Purpose of study.** The purpose of this study was to quantify the skeletal laryngeal dimensions of the sheep larynx and compare the dimensions to the human larynx to aid in possible laryngeal reinnervation research using the sheep laryngeal model.

**Method.** Eleven male and 10 female human larynges were used as the control comparison group for the 11 male and 11 female sheep larynges. Seventeen dimensions were described and recorded for each larynx. But one parameter, the length of the thyroid lamina, could not be obtained for sheep because of the unique shape of the sheep larynx. For each group and each parameter, mean and standard error of mean were calculated and significance differences were acquired.

**Results.** The differences in size between male and female laryngeal dimensions was statistically significant. The differences in size between male and female sheep larynges was much smaller, though, and only five of the 17 parameters taken were statistically significant between the sexes. Most of the laryngeal dimensions recorded for sheep were within the range of values for male and female human larynges, with some notable differences. The arytenoid cartilages of the sheep larynx were as much as 20% to 70% larger than the human arytenoid cartilages. The cricoid cartilage was more oval shaped in the sheep larynx as compared to the human larynx. Additionally, the thyroid height in the sheep larynx was different from the thyroid height seen in the human larynx.

**Conclusion.** The sheep larynx appeared to be within human ranges for most laryngeal dimensions, and as such appeared to be a suitable model for laryngeal research, particularly research studying the functionality of the larynx and possible surgical applications of this model.

**Relevance to current work.** This study was a comparison study between two groups, and the current work is also a comparison study between two groups. The current work used two animal species as comparison groups, and this study helped educate the researcher on possible animal laryngeal models that have been used in similar and supportive laryngeal research.

## APPENDIX B

**Dissection and Tissue Preparation****Materials for Dissection and Tissue Preparation: Pig and Rabbit Groups**

1. Stainless steel disposable scalpels (sizes 10, 11, and 15)
2. Disposable plastic aprons
3. Gloves (black nitrile powder free examination gloves)
4. Dissection mat
5. Gallon sized plastic bags
6. ThermoScientific Nalgene™ bottles (1000 mL, closure diameter 63 mm, low density poly-ethylene)
7. Hemostats (4)
8. Sutures (silk black braided 45 cm suture, 24 mm needle)
9. Protective goggles
10. Dissection table
11. Phosphate-Buffered Saline (PBS) solution
12. Red hazardous waste box (for scalpel and suture needle disposal)
13. Sani-Cloth™ germicidal disposable wipes
14. Lab sink
15. Room temperature water
16. Digital Caliper (UltraTECH™ no. 1433)
17. Digital scale (Ozeri Model ZK14-S™)
18. ThermoScientific™ Freezer
19. Food-grade Refrigerator
20. Styrofoam boxes
21. Permanent Marker
22. Cryogenic gloves
23. Liquid nitrogen (provided by BYU Chemistry Store)
24. Timer

**Dissection and Tissue Preparation Protocols: Pig Group***Gross Dissection Protocol*

1. Pick pig larynges up from Circle V Meat (609 Arrowhead Trail, Spanish Fork, Utah, 84660).
2. Deliver larynges to room 106 Taylor Building Annex (106 TLRA).
3. Put on protective goggles, a disposable plastic apron, and dissection gloves.
4. Lay two sheets of dissection mat on the dissection table.
5. Using a No. 10 stainless steel disposable scalpel, carefully remove all extralaryngeal tissue and structures, including the esophagus, extrinsic laryngeal muscles, tendons, lungs, vascularization, innervation, glands, and fat. Leave approximately 60 mm of trachea, if possible.

6. Check the larynx for any damage to the superior 30 mm of the trachea, or thyroid, cricoid, and arytenoid cartilages.
7. Discard any larynx that appeared to have superior damage to the trachea or perforations in the thyroid, cricoid or arytenoid cartilages.
8. Briefly rinse the larynges under room temperature water to remove excess blood clots or tissue.
9. Place each larynx in its own gallon sized plastic bag, or Nalgene™ bottle (if the larynx width is smaller than 63 mm).
10. Pour PBS solution in the plastic bag or Nalgene™ bottle until the entire larynx is immersed.
11. Dispose of the scalpels by rinsing them and placing them in the red hazardous waste box.
12. Using a permanent marker, write the date of dissection on the plastic bag or Nalgene™ bottle.
13. Wipe down all surfaces in the lab with disinfectant wipes.
14. Transport the larynges over to the BYU Chemistry Store to undergo flash freezing process with liquid nitrogen.

#### *Flash Freezing Protocol*

1. Immediately following gross dissection, transport the larynges in two Styrofoam boxes, timer, and extra plastic bags to the BYU Chemistry Store.
2. At the BYU Chemistry Store, ask an employee fill both Styrofoam boxes with liquid nitrogen.
3. Place cryogenic gloves over hands and lower arms.
4. While wearing the cryogenic gloves, carefully place the closed Nalgene bottles into one of the Styrofoam boxes and begin a 10 minute timer.
5. In the other Styrofoam box, carefully place the plastic bags in the liquid nitrogen, and begin a 10 minute timer. The plastic bags should be open and suspended in the liquid nitrogen. If the plastic bags break, place the ripped bag in a new bag and continue to freeze.
6. When the timer ends, check each of the bottles and plastic bags for remaining PBS solution that has not frozen completely. If there is still liquid, submerge the bags for another minute. Continue to check each minute until the larynx and solution are completely frozen. Do not leave the bottles in the liquid nitrogen for longer than necessary as the plastic has been known to explode.
7. Pay for liquid nitrogen using McKay School of Education grant.
8. Once all of the larynges are frozen, ask a BYU Chemistry Store employee to discard the remaining liquid nitrogen. Place the larynges in the Styrofoam boxes, and transport them back to the lab.
9. Place all of the larynges in the ThermoScientific™ Freezer at -80 °C until they are to be used for data collection. Larynges should be frozen for at least 24 hours before data collection.

*Note:* Cable ties were used in the bagging process to decrease the amount of PBS solution needed to fill the bag and fully submerge the larynx. The bag was folded around the larynx, and a cable tie was placed around the bag. It was then tightened until the bag was securely folded around the larynx. Excess solution was then poured from the bag. After being cable tied, the larynx was then put into a separate bag to facilitate suspension in liquid nitrogen during the flash freezing process. This method was only used once and abandoned shortly thereafter due to the difficulty in

tying the bags, as well as tissue damage occasionally induced from the cable ties being too tight. When this method failed, the author decided to use temperature-resistant plastic Nalgene bottles to immerse the larynges in PBS solution and freeze them. The author also used plastic bags to immerse the larynx in saline, seal the bag, put it in another bag, and freeze the larynx this way. However, this way was not as efficient. Lab assistants would have to suspend the larynx in the liquid nitrogen by holding the bag, and although they used cryogenic gloves to protect their skin from being freezer burned, the process was uncomfortable and inefficient.

### *Fine Dissection Protocol*

1. Remove frozen larynges from the ThermoScientific™ freezer and place in a room temperature water bath in the lab sink.
2. Leave the larynges in the water until fully thawed (approximately 60 minutes when in the plastic bags and 90 minutes in the Nalgene™ bottles).
3. Lay two sheets of dissection mat on the dissection table.
4. Put on protective goggles, a disposable plastic apron, and dissection gloves.
5. Using a No.10 stainless steel disposable scalpel, remove the superior portion of the epiglottis.
6. Using the same scalpel, make an incision approximately .5 cm above the thyroid prominence into the thyroid cartilage.
7. Continue to cut the thyroid cartilage bilaterally at a slight upward angle posteriorly.
8. Carefully cut away the muscles and fascia that connect the thyroid cartilage to the ventricular folds.
9. Leaving the arytenoid cartilage and true vocal folds intact, dissect away the ventricular folds using a hemostat and No. 11 stainless steel scalpel.
10. Carefully separate the fascia on the superior surface of the thyroarytenoid muscle from the true vocal folds and dissect away the fascia.
11. Remove any leftover tissue superior and posterior to the vocal folds to prevent vibration of tissue outside of the vocal folds.
12. Trim the inferior end of the trachea, leaving at least 30 mm of length for ease in mounting the larynx to the benchtop.
13. Tie a large knot to the end of the suture thread. Secure the suture needle with a hemostat and while providing digital pressure to the thyroid cartilage, insert the suture needle just above the anterior commissure of the thyroid cartilage.
14. Repeat the suture four times to secure attachment: the suture should not be able to rip through the cartilage.
15. Using the scale and the measuring device, measure the following of each larynx: weight (in ounces) before and after fine dissection, length and width of trachea, width of the thyroid cartilage, length of the thyroid cartilage from the prominence to the top and from the prominence to the bottom, length and width of the vocal folds, and the width of the thyroarytenoid muscle (width of the vocal fold to the inner surface of the thyroid cartilage). The sex of the pig or rabbit should also be noted if possible.
16. Immerse the larynx in a plastic bag filled with fresh PBS solution, and place in the refrigerator until mounted on benchtop. The larynx number should be written on the bag with a permanent marker.

## Dissection and Tissue Preparation Protocols: Rabbit Group

### *Gross Dissection Protocol*

1. Researchers at the University of Utah harvested the rabbit larynges used in this study from rabbits that were sacrificed for other research purposes.

### *Flash Freezing Protocol*

1. Researchers at the University of Utah flash froze the rabbit larynges in PBS solution at the University of Utah facility.
2. The researchers then transported the larynges in a Styrofoam container with ice from the University of Utah to the Brigham Young University laboratory.
3. Researchers at Brigham Young University placed frozen larynges in ThermoScientific™ Freezer at -80 °C.

### *Fine Dissection*

1. Remove frozen larynges from the ThermoScientific™ freezer and place in a room temperature water bath in the lab sink.
2. Leave the larynges in the water until fully thawed (approximately 30 minutes).
3. Using No. 15 stainless steel scalpel, remove upper thyroid cartilage to just above the false vocal folds.
4. Attach a hemostat to each of the two facia of false vocal folds. Spread the false folds apart using the hemostats.
5. Using No. 15 stainless steel scalpel, remove bilateral fat pads superior from false vocal folds.
6. Using a No. 15 stainless steel scalpel, make an incision at the anterior commissure of the false vocal folds.
7. Follow cut posteriorly toward arytenoid cartilages, cutting false fold from the facia of the true vocal folds.
8. Carefully cut away facia along the thyroarytenoid muscle and the vocal ligament.
9. Create an attachment mechanism through the thyroid cartilage using a surgical suture. Tie a knot at the end of the string, use a hemostat to guide the needle through the cartilage, and sew four loops through the cartilage.
10. Cut the needle off the end of the string, and place in red hazardous waste basket.
11. Place larynx in small cylindrical container and immerse in fresh PBS solution. Store in refrigerator until mounted on benchtop. The larynx number should be written on the container.

## APPENDIX C

**Data Acquisition****Materials for Acquisition:**

1. Dell computer
2. Dell computer monitor
3. PowerLab™ data acquisition hardware (ADInstruments)
4. LabChart™ data acquisition software (ADInstruments)
5. Custom benchtop apparatus
  - a. Tubing (Vinyl: 1½” outer diameter (OD), 1” inner diameter (ID); Clear Vinyl: 1⅛” OD, ⅞” ID; 1” OD, ¾” ID; ¾” OD, ½” ID; ⅞” OD, ⅝” ID; ⅝” OD, ½” ID; ½” OD, ⅜” ID; ⅜” OD, ¼” ID; 5/16” OD, 3/16” ID; 3/16” OD, ⅛” ID)
  - b. Metal hose clamps (22 mm diameter, 25 mm diameter, 30 mm diameter)
  - c. Screwdriver for tightening hose clamps
  - d. Micropositioners, three (Model 1460, Kopf Industries)
  - e. Micropositioner prong attachments, single prong and three prong (Kopf Industries)
  - f. 20 cm aluminum foam insulated custom pseudolung
  - g. TheraHeat™ humidifier (Model RC70000, Smiths Medical)
  - h. Distilled water
  - i. Medical-grade air tanks (2) containing compressed, low-humidity air (30 psi, <1% relative humidity)
  - j. Flow head meters (Model MLT300L, AD Instruments)
6. Physiological pressure transducer (Model MLT844, AD Instruments)
7. Sphygmomanometer (AD Instruments)
8. Syringe (25 cc/ml)
9. Pressure calibration block
10. Gauze (decreasing reverberation signals under the pressure transducer)
11. Teflon™ tape for sealing air leaks
12. Cable ties
13. Velcro™ for securing transducers during calibration and data collection
14. Custom tray for securing airflow transducer during data collection
15. Pneumotach Calibration Unit (MCU-4, Glottal Enterprises)
16. Audio Output Extension
17. Dynamic microphone (Model SM-48, Shure)
18. Bose™ Amplifier
19. Pulse transducer (AD Instruments)
20. Polygrip™ (sealing up air leaks in the supraglottis)
21. Sani-Cloth™ germicidal disposable wipes
22. Permanent Marker
23. AcuRite™ Hygrometer (Model 01083M)



### *LabChart™ Signal Acquisition Protocol*

1. Power on the computer, (Dell™, bottom shelf of black storage unit), the monitor (Dell™), and PowerLab™ unit.
2. Open LabChart™ 8 Application; open the template entitled “Official Pig Template” or “Official Rabbit Template” depending on which species is participating in data collection.
3. Allow the application to run for 15 minutes before calibrating or collecting data.
4. Confirm settings under “Channel Settings.” Three channels should be used: 1) Microphone, 2) Pressure, 3) Flow.
  - a. For pig data, the microphone should be set at a sampling rate of 20k/s, with a range of 10mV. The mains filter should be activated. Pressure should have a sampling rate of 1k/s and a range of 50mV, and should be measured in millimeters of Mercury (mmHg). Flow should have a sampling rate of 1k/s and a range of 200mV with the unit of measurement being liters per minute (L/min).
  - b. For rabbit data, the microphone should be set at a sampling rate of 20k/s, with a range of 10mV. The mains filter should be activated. Pressure should have a sampling rate of 1k/s and a range of 20mV, and should be measured in millimeters of Mercury (mmHg). Flow should have a sampling rate of 1k/s and a range of 200mV with the unit of measurement being liters per minute (L/min).
5. Add a comment noting that channel settings have been set.
6. To begin data acquisition, press “start.” At the onset of phonation, select the predetermined key for phonation onset. Once phonation has ceased, select “stop.” Continue this pattern for all 15 trials of each larynx.

#### **Microphone Signal:**

- SHURE™ SM-48 microphone is placed posteriorly and superiorly to the larynx and is secured approximately 15 cm away from the glottis.
- The microphone signal can be played back if the signal in channel one is selected, the “Setup” drop down bar is clicked, “Audio Output” is clicked, and the source is identified as channel one. The “Play Selection” button may then be pressed and an audio signal is produced from the Bose™ amplifier.

#### **Measuring Humidity:**

- Record percentage of humidity at the beginning and end of the 15 trial period for each larynx.
- Hygrometer is located 50 cm above each larynx.
- AcuRite™ Hygrometer will show degree of accurate calibration.

#### **Humidifier:**

- Thera-Heat™ Heated Humidifier-Portex, by Smiths Medical™ sits below the benchtop apparatus.
- The level of distilled water within the humidifier should fall within the set maximum and minimum range.
- The humidifier should be powered on at least 15 minutes before data acquisition. This allows it to warm up the water.

- Extra noise from the humidifier can be muted by pressing the red mute button in the upper left-hand corner.

## APPENDIX D

### Pressure and Flow Calibration

#### Pressure Calibration

1. With the transducer being securely attached to the pressure calibration block and sphygmomanometer, unscrew the white stop cock (allowing the transducer to be open to atmospheric air pressure) and run data acquisition software for 5 seconds.
2. Stop the acquisition. Select the pressure signal; under the “Pressure” drop down box, select “Bridge Amp”; zero the signal; add a comment “Zero.”
3. Pull the plunger to the 25 cc/mL limit; attach the syringe to the open input on the sphygmomanometer’s stop cock.
4. Start the acquisition. While watching the sphygmomanometer dial, push the plunger into the syringe until the dial has reached 40 mmHg; hold this for 5 seconds.
5. Select the wavelength from the “zero” plateau up to halfway through the 40 mmHg plateau; under the “Pressure” drop down box, select “Units Conversion.”
6. For “Point 1,” click on a point of data that resembles zero pressure; press the right arrow. Manually insert “0” in the following text box and click the mmHg unit option.
7. For “Point 2,” click on a point of data that resembles 40 mmHg of pressure; press the right arrow. Manually insert “40” in the following text box. Click “OK;” add a comment “40 mmHg.”
8. Attach pressure transducer to the plug on the trachea mount on benchtop apparatus; make sure there is an airtight seal.
9. System is ready to record pressure data. Press “start” and “stop” to begin and pause data collection.

#### Flow Calibration

1. Remove tubes from either side of the flow head meter. While maintaining its position on the flow calibration tray, start the acquisition and run for 5 seconds.
2. Stop the acquisition. Select the flow signal; under the “Flow” drop down box, select “Spirometer”; zero the signal; add a comment “Zero.”
3. Power on the pneumotach calibration unit; attach the flow head meter to the input on top of the unit via the blue seal piece.
4. Make sure that the flow rate is switched to “½” and the liter volume is at “1.” Start the acquisition on LabChart™. Flip up the “Start” switch on the pneumotach unit while data is being collected on LabChart™. Allow pneumotach unit to run airflow for several seconds. It will automatically stop when complete.
5. Stop the acquisition when pneumotach turns itself off. Select the middle “exhalation” signal (up); click the “Flow” drop down box, select “Spirometry Flow”.
6. Next to “Flow Head”, find the MLT 300 L option; select “Calibrate”; insert 1 L as the injected volume; click “OK”; add a comment “1L.”
7. Attach tubing from pressurized air tank to the right side of the flow head with the arrow pointing toward the humidifier, and then attach the tubing that runs to the humidifier on the left side of the flow head.

8. System is ready to record flow data. Press “start” and “stop” to begin and pause data collection.

## APPENDIX E

### **Pig and Rabbit Phonation Trial and Error**

Various methods were taken to achieve phonation. Both pig and rabbit phonation were best achieved with larynges whose vocal folds were adducted at rest, and whose vocal folds were on a level plane. A clean dissection with few punctures to the thyroarytenoid (TA) muscle also seemed to facilitate vibration in the pig larynx; however, this method was abandoned due to its invasive nature. Micropositioners were used to approximate the arytenoid cartilages and help with vocal fold adduction in both species. When adduction was maximized but air leakage through the posterior glottis (between the arytenoid cartilages) persisted, dental cream (Poly-grip™) was used to seal the posterior glottis off, prevent air leakage posteriorly, and facilitate directed airflow through the vocal folds exclusively. Stabilization of the larynx was in part achieved via a suture string tied to a positioner which moved posteriorly.

Air leakage at other areas of the setup proved to be a common problem for each larynx. In addition to air leakage out of the posterior glottis, air could potentially leak out the bottom of the trachea, a hole in the trachea, or a loose tube in the airflow pathway. Air leakage at the base of the trachea was reduced by using a hose clamp to secure the edge of the trachea to the benchtop. If air leakage persisted, then Teflon™ tape was wrapped around the base of the trachea to the air flow tube. Some pig larynges had cuts in the trachea due to prior excision. These were either taped shut or sutured to provide an adequate seal for airflow.

Additionally, if a larynx was not phonating properly, all the tubes through the airflow pathway were checked for possible air leaks.

## APPENDIX F

### **LabChart™ Installation and Training**

LabChart™ with LabView™, a state-of-the-art data viewing and acquisition system was used in the current study. Since this system was new to the Brigham Young University Voice Research Laboratory and few voice research labs around the country used the system, regular and intensive training and troubleshooting of the equipment setup and software was required. An application and technical representative of the company was sent to the lab to provide a brief introduction to the system and demonstrate the many uses of LabChart™. However, increased training and education were necessary to properly understand the system and adapt it to the benchtop setup. Each member of the research team read and understood the detailed user's manual, and it was referred to frequently when adapting the system to the unique needs of the lab. Additionally, a PhD student at Purdue University who used a version of LabChart™ with similar equipment was also consulted when setting up the system for the benchtop. The research team frequently held video conferences with a software engineer of ADInstruments to ask questions about equipment purposes, parameters, the meaning and interpretation of signals seen in LabView™, as well as data analysis features in the system. The entire training, education, implementation, and completion of LabChart™ and the benchtop setup took approximately one year to finish.

## APPENDIX G

**Thesis Timeline**

- 8/18: supervise construction and renovation of laboratory
  - Oversee shelf and cabinet carpentry, construction, and placement
    - Worked with Brigham Young University (BYU) Carpentry to design/assemble
  - Move lab equipment
    - Worked with BYU Moving to transport equipment to the lab
    - High-speed camera and computer, stroboscopy equipment
    - Experimental benchtop: assemble pseudolung, humidifier, flow head, high-speed camera connection, pressure transducer connection
    - Move and set up data collection computer
    - Move dissection table
      - Scalpels, sharps container, sanitation aprons, sutures, scale, calipers, dissection mats, gloves, hemostats, plastic bags
    - Move craftsmen storage container
      - Tools: drills, drill bits, wrenches, screwdrivers, protective eyewear, Teflon™ tape, duct tape, vinyl, PVC, and plastic tubing, scissors, nebulizers, cable ties, superglue
    - Move other lab materials
      - Saline solution, nebulizers, dissection mats, tubing, pressure transducer
- 9/18 Oversee delivery and installation of freezer
  - Coordinate delivery of ThermoScientific™ freezer
  - Set up freezer with alarm backups in place to notify of significant temperature drops, movement of freezer, history of temperature changes, door openings
- 10/18 Install LabChart™ Data Acquisition Hardware and Software
  - Bridge amp, spirometer, PowerLab™ connections
  - Conference calls with software and technical engineers of ADInstruments to understand data acquisition signals, calibration procedures, options for various flow heads
  - Adaptation of benchtop setup for ADInstruments equipment
    - Adapt air flow tubing to secure pressure transducer to the unit
      - Collaborate with mechanical engineering professor to develop sound setup without air leaks
      - Custom build pieces from materials in lab and at hardware stores
    - Develop custom silicone pieces for pig and rabbit attachment to the air flow system
      - 3D printing
    - Adapt flow head to attach to spirometer, humidifier, air tank, and pseudolung
    - Order new flow heads for pig, rabbit, and human flow values
  - Attach microphone to PowerLab™
    - Develop XLR-BNC cord connection

- Learn how to read signals in LabChart™ software
  - Conference calls with software engineers to understand signals coming in
- 1/19
  - Oversee installation of computers from Education Computing Services (ECS)
    - Crashplan™ installation in four computers; set up external backups
    - Computer cleanup (deletion of old files and freeing up space on computers)
- 2/19
  - further consultation with technical engineers of ADInstruments to understand signal acquisition, troubleshoot pressure transducer, adapt system to record airflow instead of fluid flow as the default
- 4/19
  - make plans for data collection
  - obtain pig larynges from slaughterhouse and prepare them for fine dissection and flash freezing
  - obtain rabbit larynges used for further study of the larynx itself and prepare for fine dissection of the specimen
- 5/19
  - continued consultation with technical engineers of ADInstruments to answer questions, establish firm understanding of system
  - read user manual to understand fine details of signal acquisition system
- 7/19
  - complete data collection for both pig and rabbit larynges
- 8/19
  - consultation with thesis committee member Dr. Christopher Dromey for audio signal understanding backflow, discussion of sinusoidal wave form found in audio signal, filtering of the audio signal to decrease noise effect, and overall understanding of signal to aid in future analysis
- 9/19
  - complete Prospectus meeting in which optimal benchtop setup as well as air flow system was discussed.
  - Segment data, clean data, and prepare all data for future analysis
- 10/19
  - collaborate with thesis committee member Dr. Christopher Dromey to develop MatLab™ (Mathworks, Natick, MA) program to analyze data
    - develop three different modifications to the program due to trial and error of data analysis (including introducing another analyzation system, Audacity™)
  - began data analysis
- 11/19
  - complete data analysis of PTP and PTF using modified MatLab™ and Audacity™ programs; use Praat™ to find true  $F_0$  in each pig and rabbit trial
- 12/19
  - submit all data analysis for statistical analysis



- 1/20
  - complete first written draft of thesis
- 2/20
  - schedule and complete oral defense