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ORIGINAL RESEARCH

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Acoustic, aerodynamic, and vibrational effects of ventricular folds adduction in an ex vivo experiment

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Abstract

Objectives: The excessive adduction of ventricular folds has been observed in patients with dysphonia and professional singers. Whether these changes in the ventricular folds are the cause or just a result of disease progression remains unclear, and their potential pathological and physiological implications are yet to be determined. This study aimed to examine the impact of different degrees of ventricular adduction on acoustics, aerodynamics, and vocal fold vibration.

Methods: The excised models of mild and severe ventricular adduction were established. We recorded the vibration pattern of vocal folds and ventricular folds and measured acoustic metrics, including fundamental frequency (F0), Jitter, Shimmer, harmonic-to-noise ratio (HNR), and sound pressure level (SPL). Furthermore, we evaluated the aerodynamics index through phonation threshold pressure (PTP), phonation instability pressure (PIP), mean flow rate (MFR), phonation threshold flow (PTF), and phonation instability flow (PIF).

Results: Irregular vibrations of the ventricular fold were observed during ventricular adduction. Notably, mild and severe ventricular adduction conditions showed a significant increase in PTP, Shimmer, and Jitter, whereas MFR, PIF, and HNR decreased compared with the control condition.

Conclusions: Ventricular adduction leads to the deterioration of acoustic and aerodynamic parameters. The aperiodic and irregular vibration of the ventricular folds may be responsible for this phenomenon, although further experiments are warranted. Understanding the functioning of ventricular folds can be beneficial in directing the treatment of muscle tension dysphonia and improving voice training techniques.

Level of evidence: level 4.

KEYWORDS

acoustics, aerodynamics, excised larynx, ventricular folds, ventricular vibration

Zhixue Xiao and Jing Kang contributed equally and were regarded as co-first authors.

1 | INTRODUCTION

Ventricular folds, also known as false vocal folds (FVFs), are a pair of thick wedge-shaped mucosal folds adjacent to the ventricle and above the true vocal folds (TVFs). Studies have confirmed that the ventricular folds' secretion is crucial for vocal fold lubrication and mucosal repair.[1,2] Additionally, the ventricular folds play a role in chest pressure and help the process of swallowing function.[3]

However, the impact of ventricular folds on phonation has yet to be understood. In clinical situations, excessive adduction of the ventricular folds is often observed in conjunction with pathological phonations in diseases such as muscle tension dysphonia (MTD)[4] and spasmodic dysphonia (SD).[5,6] Surprisingly, ventricular fold adduction can also occur in healthy individuals during physiological or artistic states.[7,8] Research into the acoustic interactions of vocal source and vocal tract theory has determined that a narrow epilarynx tube consisting of the epiglottis, ventricular folds, and aryepiglottic folds is crucial for producing a desired singer's formant.[9] Moreover, narrowing the epilarynx tube also enhances the efficiency and economy of the voice.[10,11] The question of whether alterations in the ventricular folds, which are also closely tied to the structure of the epilarynx tube, play a role in phonation that is either physiologically normal or pathologically problematic remains unclear.

Studies using computer simulations and animal models have demonstrated that the presence of ventricular folds plays a crucial role in enhancing the acoustic signal and reducing the scattering of the glottal flow.[9,12–14] Despite these findings, there has been limited research on how ventricular adduction affects the phonatory process and vocal fold vibration.

Kucinschi et al.[15] reported that a narrow ventricular gap resulted in a straighter glottal jet compared to a model with a wide ventricular gap. Li et al.[16] conducted a study on the effect of ventricular adduction using static larynx models and discovered that the glottal flow resistance was lowest when the ratio of the ventricular gap to the glottal gap was between 1.5 and 2. Farahani et al.,[9] using computationally rigid models, revealed that in cases of small false vocal fold gaps, rebound vortices could create complex vorticity patterns, leading to significant pressure fluctuations on the surface of the larynx. Computational models may be limited by the lack of pliable and moving ventricular folds during the vibration period, which may lead to the oversight of their impact on the vocal folds under certain states. A few studies have investigated the impact of ventricular adduction using a canine larynx model and found that wide ventricular gaps were associated with increased fundamental frequency and decreased glottal resistance.[13,17,18] Nevertheless, specific significant acoustic and aerodynamic changes such as jitter, shimmer, and phonation threshold pressure and flow have yet to be observed. These changes are essential to explore and enhance our understanding of voice production during ventricular adduction. Furthermore, the clinical query of whether ventricular adduction is beneficial or harmful remains unsettled.

Therefore, we used the ex vivo canine model to investigate how different degrees of ventricular fold adduction affect aerodynamics and acoustics. Additionally, high-speed laryngeal imaging was utilized to assess the mucosal wave in both vocal and ventricular folds during the vibration.

2 | MATERIALS AND METHODS

2.1 | Larynx preparation

All the larynges were obtained postmortem from canines killed for purposes unrelated to this study. The study was conducted on the certified platform of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), the Guangzhou General Pharmaceutical Research Institute, which complied with the Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee of Guangzhou Huateng Biomedical Technology Co., Ltd. (Guangdong Province, China) reviewed, approved, and monitored this study's protocol (B202309-4). The animal experiment and surgery were carried out by qualified operators. The study used fifteen larynges of canines that were previously sacrificed for non-research purposes. Fifteen larynges were harvested from canines sacrificed for non-research purposes. All larynges were maintained at a temperature of -80°C in the freezer. Prior to experimentation, each larynx was thawed in a 37°C bath to reintroduce pliability. A thorough and careful examination of each larynx ensured that no irregularities or trauma to the vocal folds were present. Canine larynges were dissected based on the method Jiang and Titze described while preserving the ventricular folds.[19] During the experiment, each larynx was placed on a bench and hydrated by regularly applying 0.9% saline solution. The outer mucosa of the ventricular folds was sutured with the inner side of the thyroid cartilage to expose the ventricular folds and maintain their normal tension (Figure 1A). A 3–0 silk suture passed through the thyroid cartilage of the anterior commissure to create a forward tension and maintain the natural length of the vocal folds.

2.2 | Apparatus

During the experiment, each larynx was placed on a bench, and a metal hose clamp was used to secure the trachea to a pipe. A bilateral three-pronged micrometer stabilizes and precisely controls arytenoid adduction to ensure complete vocal fold closure.

The air pump (Wenling Xiaba Machinery Co. Ltd., Zhejiang Province, China) simulated the continuous stable airflow of the lungs, which was passed through a humidifier (SH300, Wuxi Jike Electronics Co., Ltd., Jiangsu Province, China) to humidify and warm the air. The airflow meter (LZM-6 T, Nanjing Shunlaida Measurement and Control Equipment Co. Ltd., Nanjing Province, China) controlled the airflow by manually adjusting the knob. The digital pressure meter (Az Instrument Corp, Taiwan Province, China) displayed real-time subglottic pressure. A microphone (Xion GmbH, Germany) was placed about 10 cm from the larynx. Subglottal pressure, airflow, and

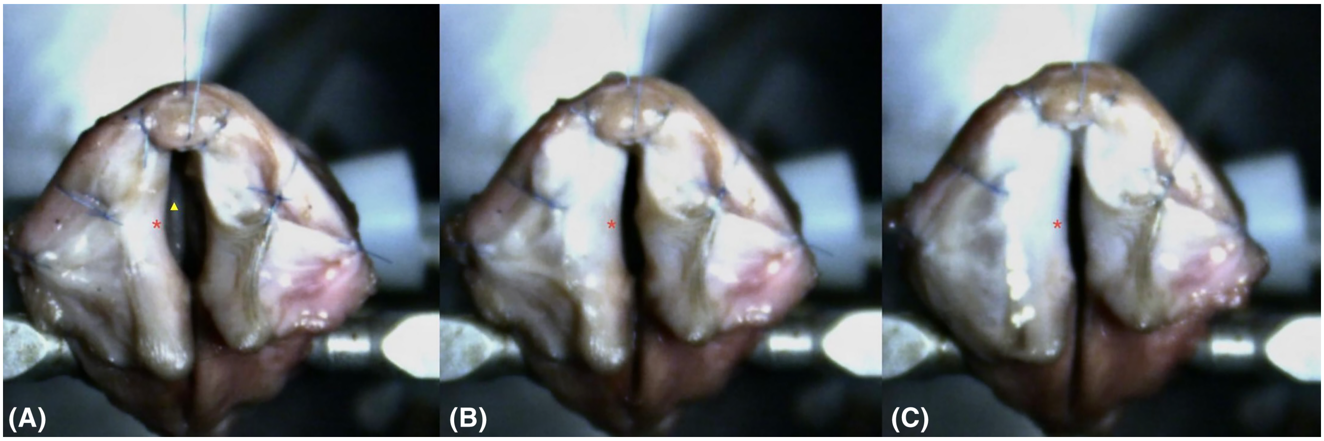

FIGURE 1 The setup of the canine larynx: (A) under normal conditions, (B) exhibiting mild ventricular adduction where the vocal folds are partly occluded, and (C) displaying severe ventricular adduction where the vocal folds are almost occluded. (* denoted ventricular folds, ▲ denoted vocal folds.)

acoustical signal were recorded simultaneously using the BL-420S biological function experimental system. A high-speed camera (NPX-GS6500UM; Dongguan Qingcheng Electronic Technology Co. Ltd., Guangdong Province, China) was placed about 40 cm above the excised larynx to capture the vibration metric of the ventricular and vocal folds. The apparatus was housed in a sound-attenuated room to reduce background noise and stabilize humidity levels and temperatures.

2.3 | Protocol

To examine the effects of varying levels of ventricular adduction on laryngeal function, all larynges were utilized across three conditions: control, mild ventricular adduction, and severe ventricular adduction. Mild ventricular adduction was conducted, referring to Jiao Y et al.'s model,[18] and involved the injection of 0.1 mL of fructose solution (12.5 g fructose solution for intravenous injection dissolved in 50 mL of normal saline) into the superficial layer of the bilateral ventricular folds, as depicted in Figure 1B. This resulted in a mild degree of ventricular fold adduction, which partially obstructed the vocal folds. To simulate severe ventricular adduction (Figure 1C), the mild adduction model was then injected with 0.15 mL fructose solution (0.25 mL total), resulting in near-complete obstruction of the vocal folds (Figure 1C). It is worth noting that the vocal folds were fully adducted in all three conditions.

2.4 | Data collection

We observed the vibrations of TVFs and FVFs and gathered acoustic and aerodynamic metrics for each condition through three trials in all fifteen larynges. The mean value was used for statistical analysis.

The acoustic parameters of Jitter, Shimmer, F0, and SPL were calculated using Praat software (version 5.3) when subglottal airflow gradually increased to 25 cmH_2O during stable vibration of vocal folds. Meanwhile, the high-speed camera recorded the vibration of the vocal and ventricular folds at a rate of 2255 frames per second. A 1-ml syringe needle was used as a marker in a photographic field to compare the regularity and symmetry during the vibration period.

During each trial, phonation threshold pressure (PTP), phonation threshold flow (PTF), mean flow rate (MFR), phonation instability pressure (PIP), and phonation instability flow (PIF) were determined manually using traces of pressure or flow over time. The airflow was gradually increased until phonation began, and the resulting pressure and flow were recorded as PTP and PTF, respectively. The airflow was then increased until the subglottal pressure reached 25 cmH_2O during stable vibration; at this point, the flow rate was recorded as MFR. As the airflow continued to increase, chaotic and irregular oscillations were observed, and the resulting pressure and flow were recorded as PIP and PIF.

2.5 | Statistical analysis

Statistical analysis of the data was carried out using SPSS 26.0. Acoustic and aerodynamic parameters were compared across conditions using a one-way analysis of variance (ANOVA) with repeated measures. The vibrational frequency and amplitude of ventricular and vocal folds were compared using an independent samples *t*-test. To determine pairwise comparisons between different levels of ventricular adduction, multiple LSD (Least Significant Difference) trials were used as post hoc analysis in all measurements.

3 | RESULTS

3.1 | Vibration metrics

Ventricular vibration was observed in 14 (14/15, 93.3%) canine larynges in both ventricular adduction conditions, while no observable vibration was found in the control condition. Moreover, ventricular vibration was unstable, with a significant irregularity in amplitude.

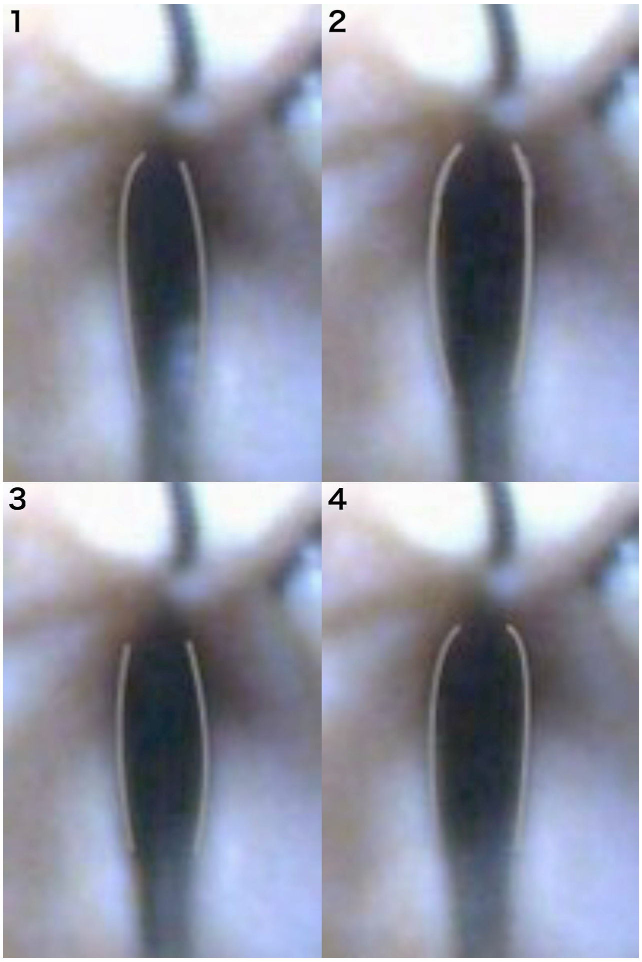

FIGURE 2 The mucosal wave of ventricular folds when mild adduction occurs during one vibration period.

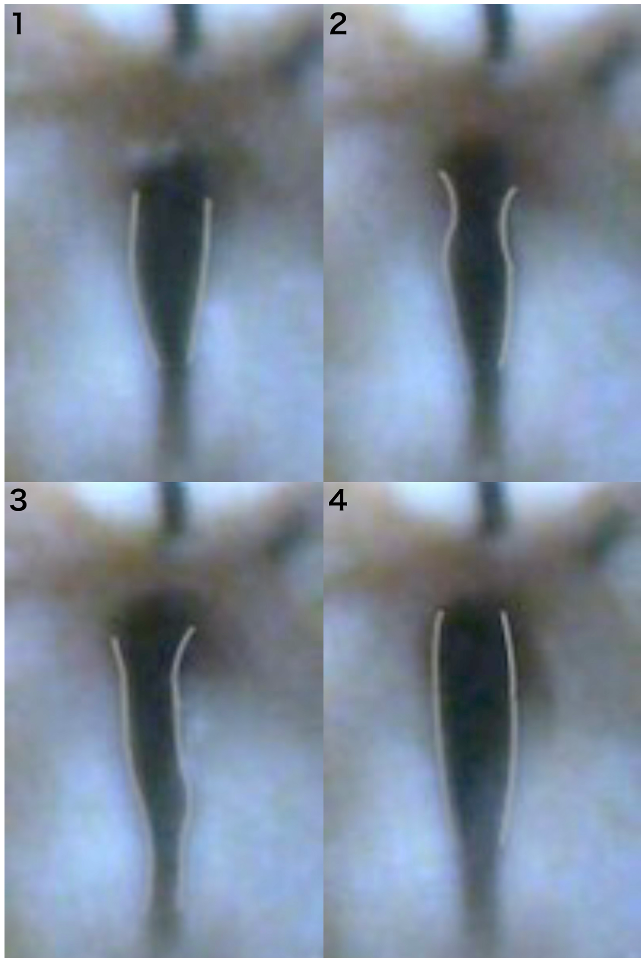

FIGURE 3 The mucosal wave of ventricular folds when severe adduction occurs during one vibration period.

Figure 2 outlines the vibrational edges of the ventricular folds during a single vibrational phase, demonstrating the fluctuation and irregularity in the gap between the ventricular folds. This observation is more pronounced in Figure 3, where severe ventricular adduction occurred. The typical vibration high-speed videos under different conditions were provided on the website (Videos S1–S3). We slowed down the video to one-fourth of the original speed to better observe the vibration of the ventricular folds.

3.2 | Acoustic analysis

Regarding Shimmer and Jitter, significant differences were detected between the three conditions ($p < .001$, Table 1). The results from within-group comparisons showed that Shimmer and Jitter were obtained in the severe adduction condition compared with the control and mild adduction condition, as shown in Figure 4.

No significant change was detected for F0 and SPL (Table 1). In contrast, significant decreases were observed in mild and severe adduction compared to control conditions for HNR ($p < .001$, Figure 4).

3.3 | Aerodynamic analysis

Considering PTP, significant differences were found in the main effects of the condition ($p = .002$, Table 2). Results from between-group comparisons demonstrated that PTP significantly increased in the experimental conditions (mild and severe adduction) compared with the control ($p = .049$, $p = .001$, respectively, Figure 5). However, no significant changes were detected in PTF.

No significant difference was observed in PIP, whereas there were substantial changes in PIF between the three conditions ($p = .006$, Table 2). Significant decreased PIF were obtained in mild and severe adduction compared to control conditions ($p = .040$ and $p = .002$, Figure 5).

For MFR, a declining trend was found in MFR when ventricular fold adduction ($p = .026$, Table 2). The results from between-group comparisons demonstrated that MFR reduced significantly in the severe adduction condition compared with the control condition ($p = .008$, Figure 5).

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TABLE 1 Results from two-way ANOVA with repeated measures for acoustic metrics.

Variables	Control	Mild adduction	Severe adduction	p
Shimmer (%)	12.27 ± 3.17	15.70 ± 2.69	18.59 ± 3.62	<.001*
Jitter (%)	0.57 ± 0.33	1.13 ± 0.52	1.54 ± 0.80	<.001*
F0 (Hz)	251.61 ± 74.87	250.86 ± 92.17	241.79 ± 91.42	.942
SPL (dB)	43.21 ± 3.23	42.14 ± 4.45	41.67 ± 4.83	.594
HNR (dB)	4.30 ± 2.26	1.77 ± 0.98	0.87 ± 0.75	<.001*

Note: * indicates statistical significance $P < 0.05$.

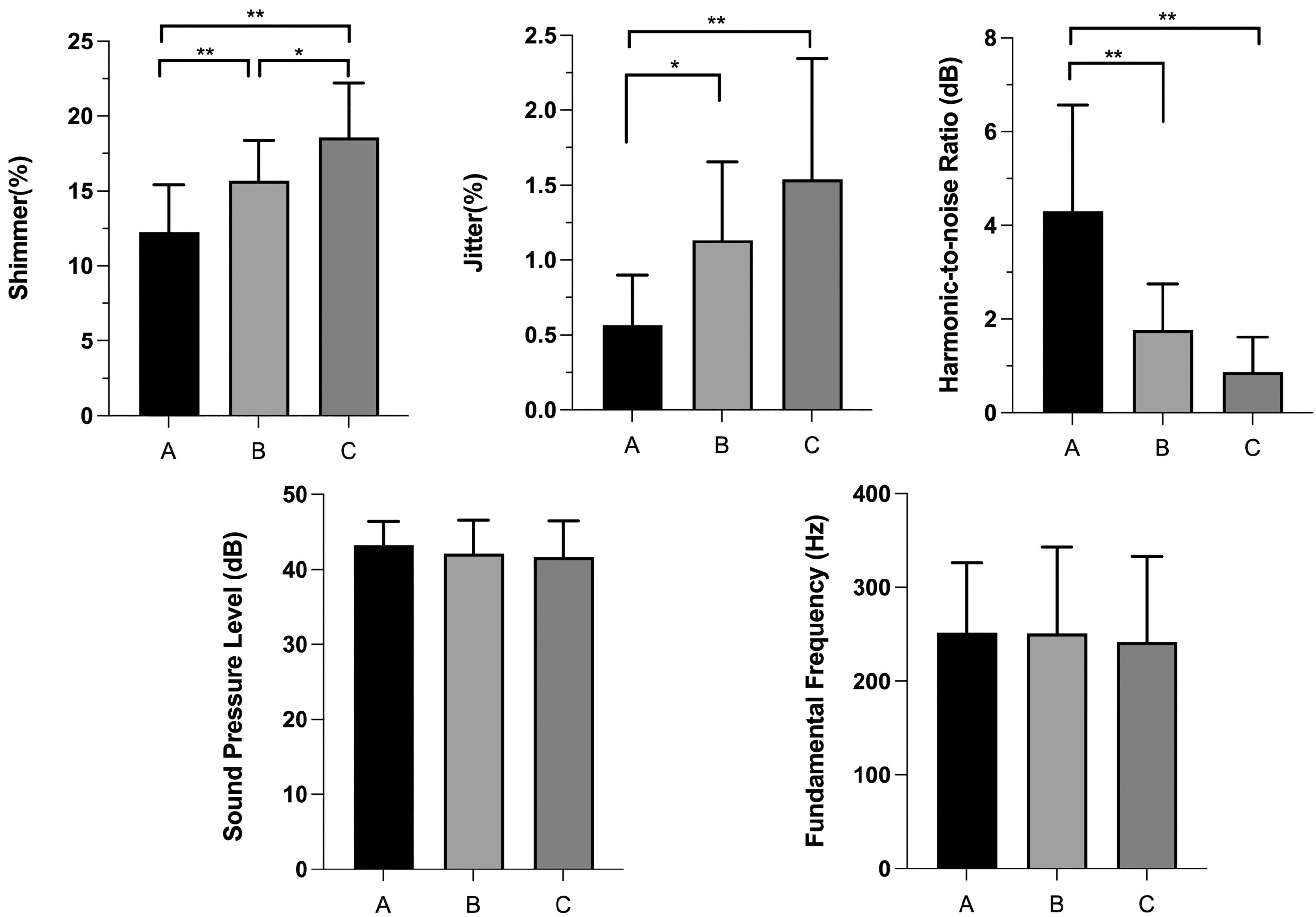

FIGURE 4 Comparison of acoustic variables among control condition, mild adduction, and severe adduction of ventricular folds.

TABLE 2 Results from two-way ANOVA with repeated measures for aerodynamics index.

Variables	Control	Mild adduction	Severe adduction	p
PTP (cmH_2O)	11.89 ± 4.37	16.09 ± 5.49	19.61 ± 6.89	.002*
PTF (L/min)	16.05 ± 2.63	16.95 ± 2.71	18.10 ± 3.41	.173
MFR (L/min)	27.46 ± 7.97	23.09 ± 6.25	20.94 ± 4.65	.026*
PIP (cmH_2O)	32.66 ± 8.76	28.17 ± 9.45	26.61 ± 9.39	.187
PIF (L/min)	38.03 ± 12.18	29.99 ± 11.00	25.39 ± 7.32	.006*

Note: * indicates statistical significance $P < 0.05$.

4 | DISCUSSION

No significant ventricular fold vibration was observed in the control condition; however, the vibration was detected in both mild and severe adduction models. The results of high-speed camera analysis revealed that in mild and severe adduction, vocal folds and ventricular folds can co-oscillate, presenting a dual source of vocal vibration. The combined vibration of the vocal folds and the ventricular folds has been observed

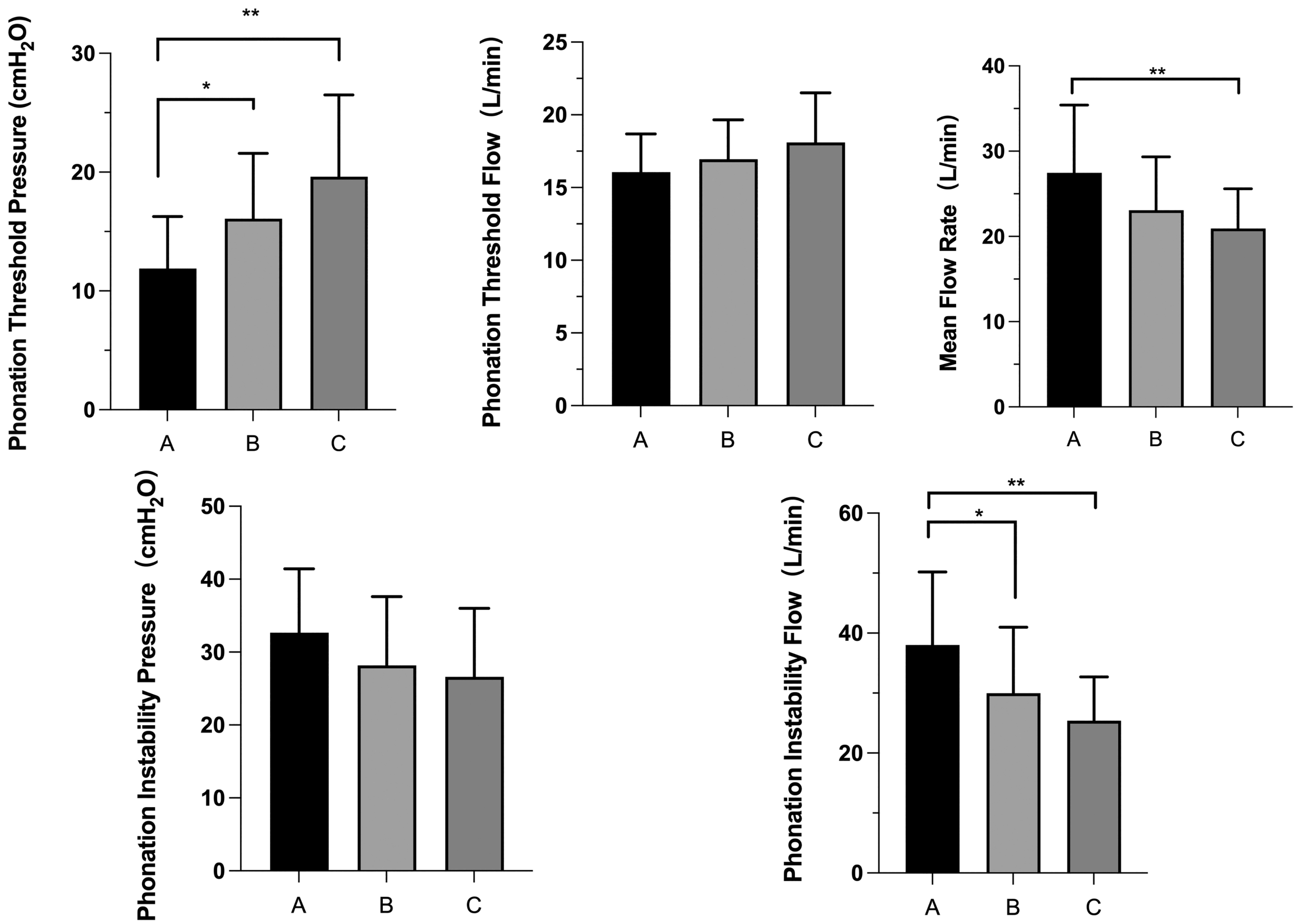

FIGURE 5 Comparison of aerodynamic variables among control condition, mild adduction, and severe adduction of ventricular folds.

during artistic timbre production, such as Asian throat singing and Mediterranean traditional polyphony.[20–22] To produce a low-pitched bass-type sound, the ventricular fold vibratory movement is periodic, occurring every two glottal cycles, in antiphase with the glottal vibration.[23]

However, it was noted that there was a significant irregularity in amplitude, and the vibration was unstable during our observation of ventricular vibration. The ventricular folds have a higher elastic shear modulus (G´) and dynamic viscosity (ŋ´) in comparison to the vocal folds.[24] This property difference could be attributed to the denser cellular and glandular structures and loose submucosal tissue of the ventricular folds.[25] To produce an artistic vocal timbre, singers must practice and regulate the strain of their laryngeal muscles to maintain a co-vibration mode of the ventricular and vocal folds. However, maintaining a periodic and regular vibration of the vocal-ventricular phonation mode can be challenging for individuals without professional vocal training due to the differing viscoelastic properties of the ventricular and vocal folds.[26] As a result, patients who experience voice complaints during ventricular adduction may exhibit aperiodic and irregular vibration of the ventricular folds, similar to our ex vivo model.

A higher shimmer parameter indicates greater sound wave amplitude instability. The Jitter parameter evaluates the perturbation of the fundamental frequency by comparing one cycle with another. The HNR parameter estimates the relationship between the percentage of harmonics and the percentage of chaotic noise present.[27] These parameters are related to irregular vocal fold vibrations. There exist two potential causes for the perceptible variations in acoustics. The first factor is attributed to heightened turbulence in the glottal airflow, resulting from irregular vibration of ventricular folds,[9,28] which has a detrimental effect on the periodic vibration of the vocal folds. The second factor is that when the ventricular folds oscillate in conjunction with the vocal folds, it amplifies the amount of noise in the sound produced.

In this experiment, ventricular fold adduction results in elevated PTP and PTF, demonstrating that more subglottal pressure and airflow were required to initial the vibration. However, these results diverged from previous acoustic interactions of the voice source and the vocal tract theory,[29] positing that a narrow epilarynx tube would facilitate vocal fold vibrations by lowering the oscillation threshold pressure. In Kucinschi's symmetric static scale model of the human larynx, it was reported that the glottal jet was straighter in a narrow ventricular gap compared to a wide ventricular gap. This suggests that a narrow ventricular gap suppresses jet deflection and facilitates vocal fold vibration.[15] These conflicting results in various research may be attributed to the co-vibration of ventricular folds, which has been overlooked in computer simulation experiments.

We hypothesized when considering the ventricular folds as a static model, their convergence leads to a narrow epilarynx tube. This, in turn, suppresses jet deflection and facilitates vocal fold vibration. However, when the ventricular folds begin to vibrate, the glottal airflow fluctuates, resulting in aerodynamic variation. According to the results of high-speed photography, we have identified that the ventricular vibration exhibited irregular and unstable patterns. An implication of the elevated PTP and PTF is the possibility that aperiodic ventricular vibration aggravates glottal resistance and glottal airflow turbulence, leading to difficulty in vocal fold vibration. Additionally, a higher PTP of severe adduction was acquired compared to mild adduction, indicating a more significant effect in severe adduction.

The irregular vibration of ventricular folds could also be the reason for the significantly increased PIF in adduction conditions. When subglottal pressure or airflow is sufficiently high, vocal fold vibrations become irregular, and the pressure and airflow at which the vocal folds change from regular vibration to irregular vibration are defined as PIP and PIF.[19,30] Ventricular fold vibration results in unstable glottal airflow, making it challenging for the vocal folds to maintain regular vibration, leading to decreased PIP and PIF.

Our results showed that MFR significantly decreased during ventricular adduction, which was consistent with the results in Finnegan and Alipour 's excised laryngeal experiment.[13] They observed that when the epiglottis was elevated to an upright position, the epilarynx tube took on the shape of a more elongated open tube, and the gap in the false vocal fold increased. This resulted in a decrease in subglottal pressure and an increase in flow rate, indicating decreased laryngeal resistance. As a result, ventricular adduction leads to an increase in glottal resistance, likely contributing to the decreased MFR.

In conclusion, this study confirmed that ventricular adduction has the potential to adversely affect both acoustic and aerodynamic indicators. These outcomes have significant implications for identifying and managing vocal diseases such as MTD. MTD typically exhibits supraglottic constriction upon laryngoscope examination.[4] One common type of MTD involves the adduction of the ventricular folds towards the midline.[31] Consequently, ventricular adduction is deemed an unfavorable pronunciation pattern that necessitates appropriate voice training. Our study has corroborated this finding, emphasizing that the improper vocal tract structure during ventricular adduction can alter the glottis vibration mode, leading to a decline in vocal efficiency and acoustic output.

Paradoxically, ventricular adduction is seen in professionally trained singers and ordinary people. Petekkaya E et al. found that opera singers who had received voice training exhibited lower Shimmer and HNR values, and two sopranos displayed varying types of ventricular fold compression.[7] In addition, Behrman et al.[8] and Stager et al.[32] also pointed out that ventricular adduction is common in normal individuals, which raised questions about its classification as a functional articulation disorder. According to this experiment, we predicted that ventricular fold co-vibration determines whether ventricular fold adduction would result in a positive or negative outcome. Professionally trained singers could potentially sustain the laryngeal muscle tension to prevent ventricular vibration when maintaining the adduction of ventricular folds under various levels of supraglottal airflow. This professional phonation mode composes an ideal narrow epilarynx tube according to the research into the acoustic interactions of vocal source and vocal tract theory mentioned before. However, individuals with MTD who lack vocal training have difficulty preventing irregular ventricular vibration during severe ventricular adduction. This erratic movement of the ventricular folds impacts the vibration of the vocal folds, leading to noticeable changes in acoustics and aerodynamics. Further research is needed to validate this hypothesis.

5 | CONCLUSION

The present study examined the impact of ventricular fold adduction on aerodynamics and acoustics, as well as the vibration pattern of ventricular and vocal folds in excised canine larynges. Ventricular fold adduction led to a significant increase in PTP, Shimmer, and Jitter whereas decreasing MFR, PIF, and HNR. Furthermore, the study identified the presence of aperiodic co-vibration of ventricular folds during mild and severe adduction. We hypothesized that ventricular vibration interfered with the mucosal wave motion of the vocal folds, making it easier to shift from periodic vibration to chaos, resulting in detectable acoustic and aerodynamic changes. Further research is needed to validate this hypothesis. A comprehensive understanding of the ventricular fold is imperative in providing effective clinical guidance for managing MTD and enhancing the outcomes of voice training interventions.

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CONFLICT OF INTEREST STATEMENT

None.

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SUPPORTING INFORMATION

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

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