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Permanent Tracheostomy for Long-Term Respiratory Studies in Canines

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Abstract

Introduction—We describe a modified surgical technique for permanent, anterior tracheal-wall stoma for chronic, repeated respiratory studies in trained, conscious dogs. These cannula-free tracheostomies require minimal daily maintenance, permit repeated intubation with endotracheal tubes modified for airflow respiratory measurement, and facilitate up to 6 hour, continuous administration of aerosol agents during long-term, repeated respiratory studies.

Methods—In 20 dogs, during a 30-40 minute procedure, portions of tracheal rings 2-4 were removed to create an oval stoma, approximately 2×1 cm. The dermis was secured to the transected cartilage and tracheal mucosa in such a manner that skin covered the sternohyoid muscles and grew-in flush with the tracheal mucosa at the stomal opening. Stomas were cleaned daily, and fur was clipped weekly around the stomal site. No other maintenance procedures or environmental modifications were needed. Animals breathed through both the stoma and the upper airway and barked normally.

Results—Stomas remained viable in long-term animals (n=4) ongoing for 70.3±32.2 months (Mean ±SEM), with an ongoing maximum of 126 months. Post-mortem examinations were performed on relatively short-term animals (n=16) sacrificed at 16.7±7.3 months. Thirteen showed no appreciable tracheal stenosis and three showed <10% stenosis at the level of the stoma. Histopathological examination of the stomal opening and surrounding tissue revealed minimal chronic inflammation and no evidence of necrosis or infection.

Conclusions—During long-term respiratory studies, this practical and dependable tracheal stoma provides a means for examining acute and chronic effects of environmental and pathophysiological influences on the respiratory system of conscious dogs.

Keywords

Tracheotomy; Permanent Tracheostomy; Chronic Respiratory Studies

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Introduction

Tracheotomy is a valuable clinical procedure for emergency or elective airway management. It has become standard in our laboratory for environmental exposure studies with limited material for aerosol inhalation and for chronic, repeated respiratory measurement in laboratory animals. The continued use of tracheostomy in clinical medicine and respiratory physiology research is testament to its medical value. However, tracheotomy and tracheal stoma intubation present noteworthy hazards and complications. Some of the more common complications include tracheal stenosis secondary to cuffed endotracheal tube inflation [2-4,20-22], pneumonia [10,14], tracheobronchitis [13,14], tracheitis [11], hemorrhage from innominate artery dissection [9,24], laryngeal trauma including laryngeal nerve damage [14], stoma occlusion [14], stoma closure [12-14], and tracheo-esophageal fistula [23,24].

Surgical procedures in canines have been described for permanent tracheostomy [13-17]. The literature present different surgical approaches with much contention concerning direction and type of incision, location of stoma placement, and merit of cartilage excision [5,6,8,16,17]. However, most studies were designed to look at short-term changes in tracheal structure over a period of weeks or months and do not consider longer-term stoma viability.

We describe a modified surgical technique for long-term, permanent anterior tracheal wall stoma for repeated intubation, collection of respiratory measurements, and continuous administration of aerosol agents. This study contains a description of the surgical techniques, post-operative care, and ongoing maintenance of the tracheal stomas. Recommendations are made to prevent common complications of tracheotomy surgery and for ongoing tracheal stoma maintenance. Using this methodology, stomas were maintained for more than 10 years in individual animals.

Materials and Methods

Animals and Anesthesia

All animals received humane care and were handled in accordance with National Institute of Health and Harvard University animal care committee guidelines. Experimental procedures followed approved animal studies protocols at The Harvard School of Public Health in Boston, MA.

Twenty adult female mongrel dogs, weighing an average of 15.4 kg (range: 12-19.3 kg), were used for this study. These were all research purpose-bred animals obtained from either Butler Farms (USDA # 21-A-003, Clyde, NY) or from Marshall Laboratories. All surgeries were performed aseptically while animals were maintained under a surgical level of anesthesia. In the operating room, the animals were pre-anesthetized using ketamine (10 mg/kg bolus), xylazine (1.5 mg/kg bolus), and atropine (0.04 mg/kg bolus). Animals were placed in the supine position on the operating table. A trans-oral endotracheal tube was inserted to initiate mechanical ventilation with pure oxygen and 1.5% isofluorane for anesthesia.

Surgical Procedure

The surgical site, centered on the ventral midline of the neck, was prepared for surgery. A 6 cm anterior neck incision was made parallel to the trachea from just below the thyroid cartilage to the 6^{th} tracheal ring (Figure 1A). The subcutaneous fascia was dissected. The sternohyoid muscles were exposed and divided down the midline. Three 3-0 Prolene stitches were used to fix the divided sternohyoid muscle to the lateral trachea in a hexagonal configuration (Figure 1B). This decreased tension on the skin-to-mucosa anastomosis and thereby decreased the tendency for dehiscence. Note that the central suture is more lateral than the top and bottom sutures.

Tracheal rings 2, 3, and 4 were identified and marked with cautery (Figure 1C). A # 11 blade was used to open the trachea at the 2nd intra-ring membrane with a vertical incision. Care was taken not to puncture the endotracheal tube used for ventilation and anesthesia. Anterior portions of the 2nd, 3rd, and 4th rings were removed to create a rectangular opening, 2 × 1 cm in the anterior wall of the trachea (Figure 1D). Minimal intra-ring membrane bleeding was cauterized as needed.

The skin flaps were elevated approximately 3 cm caudal to the cricoid cartilage and a 1-1.5 cm ellipse of skin was removed from either side of the tracheal opening (Dotted Blue Line, Figure 1E). The result is a four-leafed clover shaped incision (Figure 1E) which was closed above and below the stoma and secured to the tracheal mucosa at the edges of the stoma (Figure 1F). Non-absorbable sutures were used to secure the skin in direct apposition to the incised tracheal cartilage so that the sternohyoid muscles and exposed tracheal cartilage were covered with anterior neck skin that grew-in to form an anastomosis with the tracheal mucosa at the stomal opening. The small openings of skin above and below the stoma were loosely closed with a continuous 3-0 Vicryl suture. The final tracheal stoma is shown in Figure 2.

Post-Operative Care

During the first 48 hours post-operatively, the animals received intra-muscular doses of buprenorphine (0.005 mg/kg bolus) every 8-12 hours. There was no significant post-operative morbidity, and sutures were removed 10-12 days post-operatively. Care was taken to avoid inhalation of suture material into the respiratory tract. The animals were typically used in studies beginning 3 weeks post-operatively.

Post-Operative Stomal Maintenance

Stomas were cleaned of tracheobronchial secretions daily. The skin and stomal area were lightly scrubbed with a 10% Chlorahexadine solution. Once per week, or as needed, fur was clipped around the stomal site. Care was taken to avoid inhalation of clipped hair into the respiratory tract.

Intubation And Data Acquisition

Stomas were lubricated with sterile surgical lubricant and intubated with a # 5-8 inflatable cuff tracheostomy tube (Portex, Keene, NH). During the collection of continuous respiratory data, tracheostomy tubes were fitted with a minimal flow restrictor across which the pressure differential was recorded by pressure transducers [26]. Thus, each breath throughout the five or six hours of experimentation was available for analysis [25-29]. A complete, automated hardware and software system (BUXCO Electronics, Troy, NY) collected and processed the signals generated by the transducers (Figure 3).

Histopathology

At sacrifice, the trachea and its surrounding structures were removed. Gross samples were photographed and sections at the level of the stoma and at the level of unaltered trachea (ring # 17) were taken. Sections were fixed in 10% buffered Formalin (Fischer Scientific, Pittsburg, PA), processed routinely for paraffin histology, sectioned at 4 μm thickness, stained with hematoxylin and eosin (H&E), and examined by light microscopy by a pathologist to asses the presence and extent of chronic inflammation and changes to tracheal and surrounding structures (Figure 4). In several instances, large whole-mounts were made of the trachea, surrounding muscles, and other tissues in the area. These sections were processed with longer time periods for each step of the histological process to assure penetration of the larger specimen. After paraffin embedding, sections were cut at 10 μm thickness, mounted on plate glass, and stained with H&E.

Results

With this technique, tracheostomies were performed in 20 healthy dogs. Acute complications associated with tracheotomy surgery did not occur during the course of this study. In all animals, stridor was either absent or only demonstrable during exertion.

During surgery, the dermis was secured to the tracheal mucosa in such a manner that anterior neck skin covered the sternohyoid muscles and grew-in flush with the tracheal mucosa at the stomal opening. Granulation tissue sealed the skin to the tracheal cartilage lining the stomal opening. Excised cartilage did not regenerate. Stomas healed and were ready for intubation within three weeks of surgery.

In two animals, scratching caused the muco-cutaneous sutures to pull out. Swelling subsided within 10-14 days and repeat surgery was successfully performed.

In one animal, skin surrounding the stoma grew-in and narrowed the stomal opening. During experimentation with this animal, we uneventfully employed a smaller endotracheal tube for respiratory measurement.

Long Term Stoma Viability

In animals maintained for long-term studies (n=4), stomas remained viable, ongoing for an average of 70.3 ± 32.2 months (Mean± SEM), with an ongoing maximum of 126 months, during which time these animals have undergone repeated respiratory studies [27-29]. Figure 3 shows an example of continuous respiratory data simultaneously collected from two dogs—the top dog shows a resting breathing pattern while the bottom dog is panting. After experimentation, extubation has been accomplished without difficulty and none of the animals have developed clinical evidence of airway obstruction.

Post-Mortem Gross Anatomical Findings

In animals sacrificed during the course of our studies, gross anatomic and histopathologic examination (n=16) of the tracheas and surrounding tissues were carried out. These examinations were done at 16.7 ± 7.3 post-operative months. The neck skin attached contiguously with the luminal tracheal mucosa and completely covered the underlying sternohyoid muscles. The skin surrounding the stoma was removed and no gross deformities to the larynx or sternohyoid muscles surrounding the stoma were noted. Subcutaneous fascia secured the skin directly to the tracheal cartilage immediately surrounding the stoma. This tissue appeared healthy and well perfused. The sternohyoid muscles grew into an oval configuration surrounding the tracheal stoma as designed during surgery.

In 13 of the 16 animals sacrificed, after the sternohyoid muscles had been removed, no appreciable tracheal stenosis was noted. In the other 3 animals, minor stenosis was noted at the level of stomal opening. The defect occurred in the lateral wall of the trachea and accounted for a less than 10% stenosis. In the animal with the greatest stenosis, above and below the stoma, the tracheal diameter was 21 mm. At the level of stenosis, the tracheal diameter was 19 mm. This small indentation in the tracheal passage accounted for a less than 10% reduction in tracheal diameter. It should be noted that this was also the animal in which the skin surrounding the stoma grew-in and narrowed the stomal opening.

Histopathology Findings

In histological sections throughout the trachea and at the stomal site (Figure 4), minimal chronic inflammation was noted in the lamina propria of some sections, but most were without significant pathologic changes. In the tracheal mucosa, areas of squamous metaplasia were noted near the stoma and at the cuff site, but these were mild as illustrated below. No other pathologic changes were found. In Figure 4A,B, the typical gross appearance of the cross-section at the stoma is illustrated. Figure 4A is a gross section photograph of the cross-section including surrounding muscles after fixation. Figure 4B is a whole-mount section of the trachea with adjacent structures. Boxes illustrate the areas of sections shown at higher magnification in 4C-F. In Figure 4C, the junction of the skin and the tracheal mucosa illustrate the transition from squamous to respiratory mucosa. Figure 4D-F illustrate the respiratory mucosa at higher magnification and show minimal chronic inflammation. Figures 4D, F show that the mucosa has squamous metaplasia and no chronic inflammation. Figure 4E shows respiratory mucosa without metaplasia and minimal chronic inflammation at the lamina propria.

Discussion

Stomal Complications

Acute and chronic complications associated with tracheostomy did not occur during this study. Complications of tracheostomy have received extensive review in the medical literature, and tracheal stenosis within 6 weeks of tracheotomy surgery [7] has been especially emphasized [2-4,20,21,23]. Two factors can be considered: the endotracheal tube cuff pressure and the tracheal incision. Cuff pressure is an important etiologic factor in tracheal stenosis secondary to intubation with cuffed inflatable tracheostomy tubes. Studies have shown a progressive inflammatory response at the areas of maximum irritation from the tracheostomy tube and/or inflatable cuff [3,4,23]. Strictures developing at the level of the cuff are the result of circumferential mucosal ulceration of tracheal-mucosal membrane, which result from local ischemic change due to pressure exerted between the outside of the cuff and the tracheal wall [3,4]. The mucosa at these sites becomes hypoxic and ulcerates to expose the cartilaginous rings [3,4]. Destruction and absorption of the exposed underlying cartilage and subsequent healing by concentric fibrotic contracture narrow the tracheal passage [23]. As cited in previous literature [3,23], to avoid complications of stenosis, the use of high compliance-low pressure cuffs is recommended. These were used in our study. Furthermore, in previous studies with serious complications, the endotracheal tubes did remain in the stomas permanently [3,4,13, 14]. During our studies, intubations were performed for a maximum of six hours per day, twice per week. Likely, our choice of cuff and intermittent rather than chronic intubation prevented the development of more severe inflammation and subsequent major tracheal stenosis.

In previous studies, the tracheal incision has received much scrutiny and has been a technical point of contention. Several types of tracheostomy incisions in dogs, including transverse and vertical, U-shaped flap through two tracheal rings, and excision of 1 cm segments of 1 and 2 rings have been compared [5,6,8,16,17,22]. Vertical tracheostomy shows the most consistent healing [5,6]. However, vertical incisions have been associated with higher incidence of stenosis [22]. In these studies, a stoma was fashioned around a single vertical slit made through the intra-ring membrane and held open with sutures fixed to the surrounding muscle. It is possible that this approach causes undue pressure-strain to the tracheal wall resulting in more substantial stenosis. As we have demonstrated, a better approach may be a vertical incision extended down across tracheal rings and the removal of a 2 × 1 cm portion of multiple rings. This approach minimizes pressure-strain to the tracheal wall while ensuring a sufficiently large stoma for intubation.

Airway Maintenance

During surgery, the dermis was secured to the tracheal mucosa in such a manner that skin covered the sternohyoid muscles and grew in flush with the tracheal mucosa at the stomal opening. Barking, coughing, and use of the tongue for thermoregulation were not affected. Contrary to previous reports [18,19], animals did not require humidification of the vivarium ambient air. To intubate the animal, no local anesthetic was required—only a lubricant. Animals displayed little reaction to intubation and in most cases resting measurements could be taken within 5 minutes. After training, dogs would lay unrestrained in a cubicle for repeated 5 or 6 hour respiratory measurement during aerosol administration. Results of respiratory and systemic effects of air pollution (which include 4 of the dogs reported in this paper) have been published [27-29].

Viscous, mucoid tracheo-bronchial secretions were noted lining the outside of the stoma, especially after prolonged intubation. These secretions did not cause complications such as pulmonary obstruction. On occasion, wood shaving bedding became stuck to the tracheobronchial secretions surrounding the stomas and posed risk of inhalation and pulmonary obstruction. These observations necessitate daily cleaning of the stomas to prevent complications.

In groups of dogs from previous studies from our laboratory [28,29], rarely, a few dogs, when excited, would occlude the stomal opening with the posterior membranous portion of the trachea causing apoplexy which immediately relieved the obstruction.

Respiratory Measurement

In the past, respiratory measurement in the conscious dog has been made with a face mask, head tent, or tracheostomy intubation [1]. The former two have been sources of dead space and gas leakage. Additionally, they tended to excite the animals and cause hyperventilation. Permanent tracheostomy cannulation was introduced as an alternative, however, this high-maintenance technique became associated with pressure necrosis, tracheal stenosis, and a high mortality rate [13,14]. As we have shown, cannula-free tracheostomy in conjunction with intermittent cuffed endotracheal tube intubation during long-term experimentation is a useful alternative which prevents major complications associated with tracheostomy intubation.

The presence of a tracheostomy has been shown to have no effect on airway mechanics [25]. Our finding of lack of pathological changes in the trachea and only minimal chronic inflammation at the stomal site is further evidence that tracheostomy as used in our studies is unlikely to alter lung physiology.

Conclusions

During long-term respiratory studies, this practical and dependable tracheal stoma provides a means for examining acute and long-term effects of environmental and pathophysiological influences on the respiratory systems of conscious dogs. With minimal post-operative and maintenance-related complications, individual subjects could be used for chronic experimentation for an ongoing period of 10 years.

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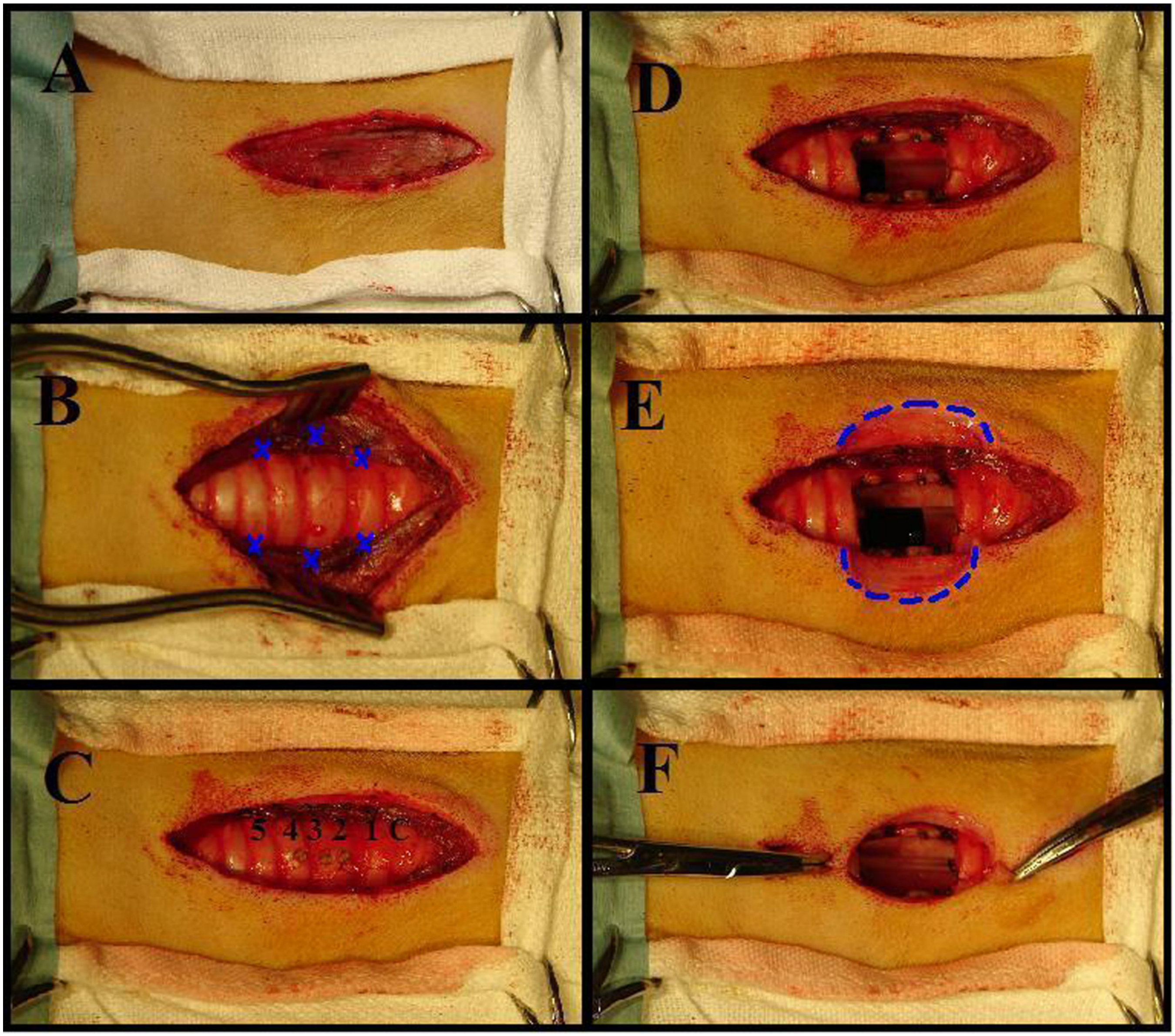

Figure 1.

A: A 6 cm incision is made parallel to the trachea on the anterior neck. **B:** The trachea is exposed and the sternohyoid muscles are sutured to the lateral tracheal wall. Blue X's mark the location of sutures. **C:** Tracheal rings 2,3,4 are identified and marked with electro-cautery. **D:** Rings 2,3,4 are removed creating a rectangular stoma, 2 × 1 cm in the anterior wall of the trachea. **E:** A four-leafed clover shaped incision was formed by removing a 2 cm × 1 cm semicircle of skin from either side of the tracheal opening (Dotted Blue Line). **F:** The edges of the four-leafed clover shaped incision approximate to the edges of the stomal opening.

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Figure 2.
Final surgical result. Fourteen 3-0 Prolene stitches were used to suture the anterior neck skin (four-leafed clover incision) to the edges of exposed tracheal cartilage.

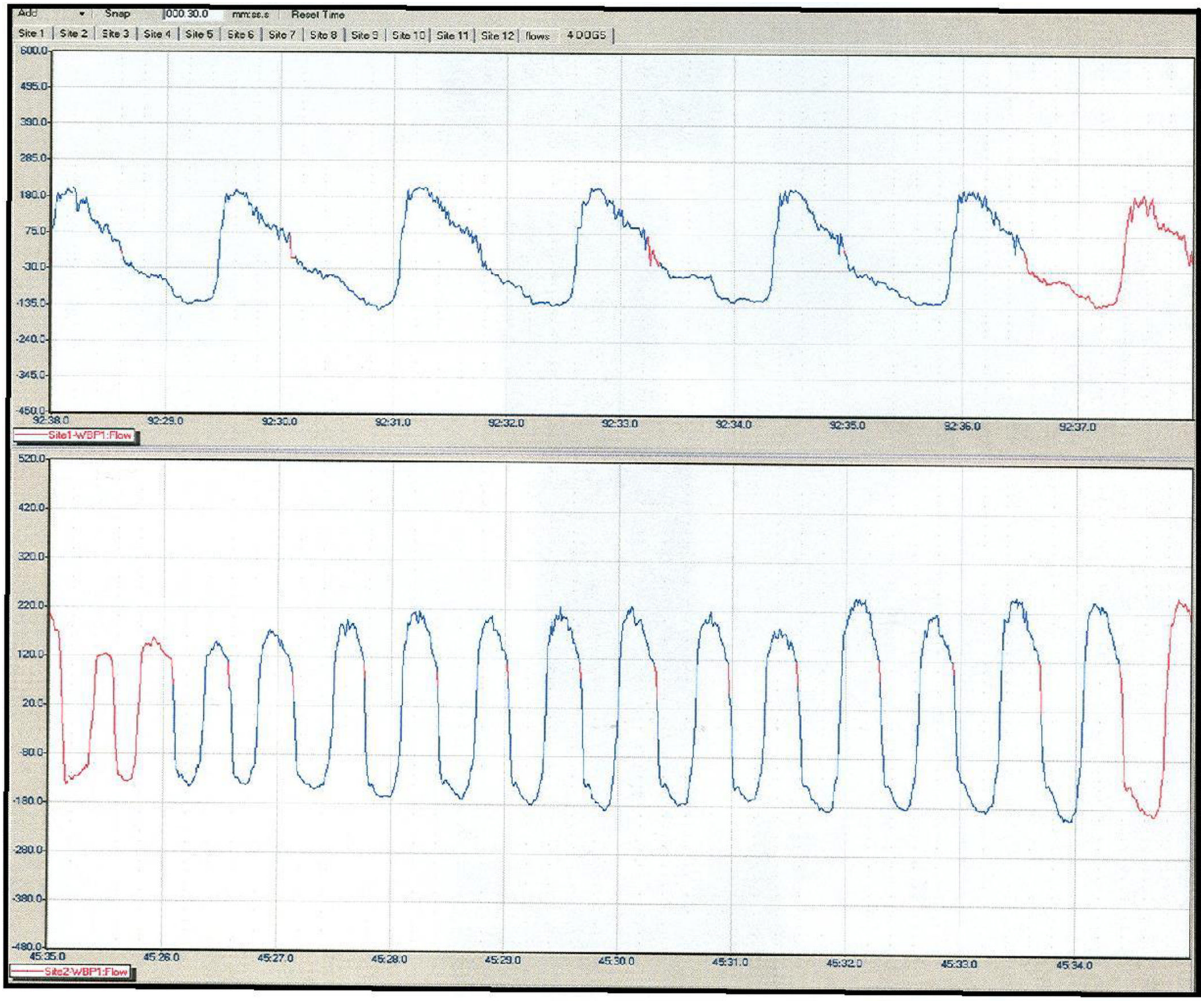

Figure 3.
Example of respiratory data collected from intubated tracheal stoma.

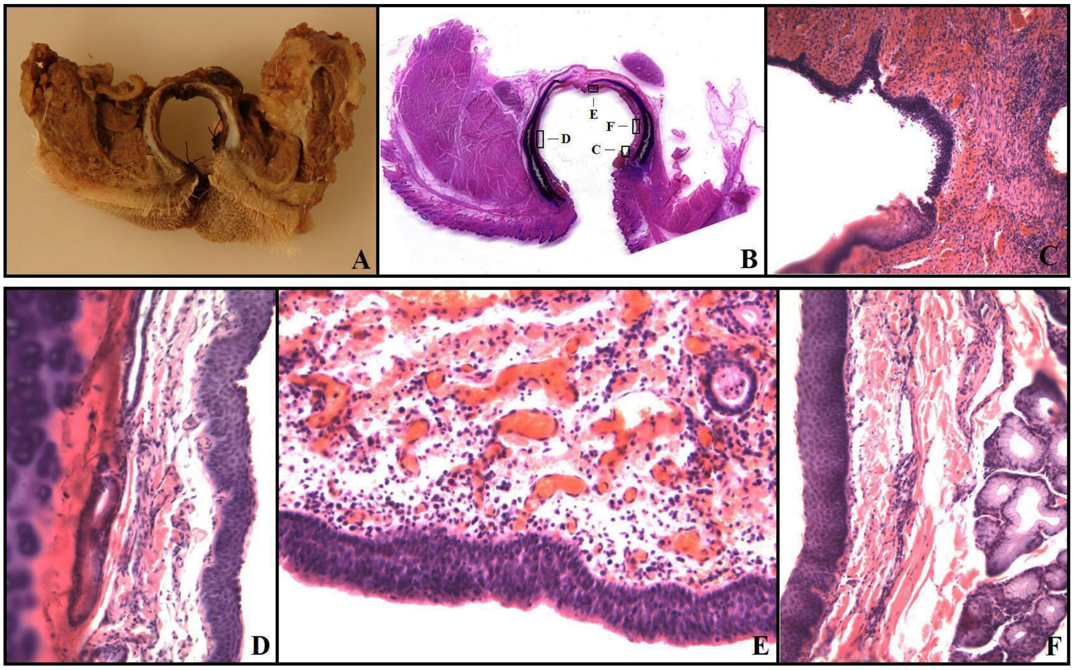

Figure 4.

Histopathology. **A,B:** The typical gross appearance of a cross-section at the stoma. **A:** The cross-section with fixation. **B:** A whole-mount large histological section showing the trachea, stoma, cutaneous mucosal junction, and the lateral muscle-trachea approximation. The boxes highlight areas illustrated at higher magnifications in the photomicrographs shown in C-F. **C:** The cutaneous-mucosal junction showing the transition from stratified squamous epithelium to respiratory mucosa. There is minimal chronic inflammation in both the subcutaneous tissue and the lamina propria of the trachea. Magnification 40X. **D:** The later tracheal wall. The respiratory mucosa has slight squamous metaplasia, but the lamina propria between the mucosa and cartilage has no pathological changes. Magnification 200X. **E:** The posterior tracheal wall opposite the stoma. Slight chronic inflammation is present in the lamina propria. This was the most severe chronic inflammation seen in any section of the trachea. The respiratory mucosa has no pathological changes. Magnification 200X. **F:** Lateral tracheal wall with squamous metaplasia, no chronic inflammation, and normal tracheal mucosal glands. There was neither an increase in tracheal mucosal gland thickness in the lamina propria, nor a pathological shift in the normal serous/mucous gland proportions. Magnification 200X.