





Influence of staple line number and configuration on the leakage of small intestinal functional end-to-end stapled anastomosis: An ex vivo study

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Abstract

Objective: To determine the influence of the staple line configuration on the leakage of small intestinal functional end-to-end stapled anastomosis (FEESA).

Study design: Experimental, ex vivo, randomized study.

Sample population: Jejunal segments ($N = 72$) from 10 mature, canine cadavers.

Methods: Jejunal segments (10 cm) were randomly assigned to a control group (8 segments) and 4 FEESA groups (16 segments/group (8 constructs/group)), according to the number of rows of staples used in the vertical (V) and transverse lines (T), respectively: Control, 2-row V/2-row T (2V/2T), 2-row V/3-row T (2V/3T), 3-row V/2-row T (3V/2T), 3-row V/3-row T (3V/3T). Initial leak pressure (ILP), maximum intraluminal pressure (MIP), and initial leakage location (ILL) were compared.

Results: The ILP (mean \pm SD) for control segments, 2V/2T, 2V/3T, 3V/2T and 3V/3T were 321.38 ± 34.59 , 32.88 ± 7.36 , 50.13 ± 10.46 , 34.38 ± 11.78 , 69.88 ± 21.23 mmHg, respectively. All FEESAs initially leaked at lower pressures than intact segments. The only other differences detected between groups consisted of ILPs that were higher when FEESAs were closed with 3V/3T (69.88 ± 21.23 mmHg) than 2V/2T (32.88 ± 7.36 , $P < .001$). Initial leakage occurred predominantly from the transverse staple line rather than the anastomotic crotch ($P < .001$).

Conclusion: Placing 3 rows of staples in the transverse line (with or without a third row in the vertical staple line) improved resistance to leakage of FEESAs in normal cadaveric specimens.

Clinical significance: The addition of a third row of staples in the transverse line (with or without a third row in the vertical staple line) in FEESAs should be further investigated as a strategy to reduce intestinal leakage clinically.

1 | INTRODUCTION

Intestinal resection and anastomosis is commonly performed in small animals affected by devitalized bowels

secondary to foreign material, intussusception, and neoplasia.^{1,2} A hand-sutured (HS) technique for intestinal anastomosis is widely available and recommended for end-to-end small intestinal anastomosis in dogs.¹⁻⁶

Recently, novel materials and devices for intestinal surgery have been reported in the veterinary literature and include barbed knotless suture,^{3–5} disposable skin staples,⁷ bipolar vessel sealing,⁸ and linear stapling devices.^{2–4,9–13} Functional end-to-end stapled anastomosis (FEESA) has been reported as a safe method for small intestinal anastomosis in dogs,^{9,10,14–17} which can be performed by nonexpert surgeons.¹⁰ Although comparable dehiscence rates are described between traditional HS and FEESA techniques,^{14,18,19–20} an HS anastomosis has been considered a risk factor for dehiscence in dogs with preoperative septic peritonitis.¹⁴ Additional advantages of a FEESA compared to HS anastomosis include a substantially shorter time to perform anastomosis, consistency, reduced tissue trauma, increased strength, and ability to resolve extreme luminal disparity, which is common in chronic bowel obstruction.^{2,3,6,9–11,17,18}

A FEESA can be performed using a gastrointestinal anastomosis (GIA) stapler to create a side-to-side anastomosis (vertical staple line) followed by a thoracoabdominal stapler (TA) applied across the remaining stoma (transverse staple line).^{2,9} The GIA stapler deploys 2 rows of staggered staples on either side of a linear cutting blade whereas the TA stapler deploys 2 rows of staggered staples.^{2,9} The GIA stapler produces mucosal inversion, considered ideal for the intestinal healing, whereas the TA stapler results in an everted pattern that has been associated with increased adhesions, greater inflammatory response, and delayed wound healing.^{13,17} The TA transverse staple line has been considered a possible weak point of a FEESA, with increased incidence of leakage at this location.^{6,9,12,13,17} Reinforcing the TA transverse staple line with a suture oversew in a clinical setting has been associated with a decreased incidence of postoperative dehiscence following FEESA in dogs.¹⁵

An alternative method, as described by White,¹¹ utilizes a GIA stapler in both locations of the FEESA which has the advantage of using only 1 stapler type. The *in vitro* bursting strength of a FEESA created using the GIA stapler in both staple lines has recently been reported to be similar to a FEESA created with the GIA and a TA stapler.^{3,11}

A GIA stapler for open surgery that deploys 3 rows of staggered staples on either side of a linear cutting blade has recently been introduced with the aim of reducing complications associated with FEESA.^{21,22} Along with the additional row of staples, in comparison with traditional 2-row GIA staplers, purported advantages related to this novel stapler include the ability to select staple closure height (1.5 mm, 1.8 mm, 2.0 mm) with a single cartridge and a 2-sided firing mechanism that can be easily manipulated by both right- and left-handed surgeons, respectively.²¹

The bursting strength of a FEESA with a 3-row GIA stapler applied in either the vertical or transverse staple lines or both has not previously been studied. As the use of FEESA is gaining popularity in veterinary medicine, it is necessary to investigate alternative devices that may result in greater functional strength. The objective of this study was to compare initial leakage (ILP), maximum intraluminal pressure (MIP), and initial leakage location (ILL) for FEESA constructs created with fresh, cooled canine cadaveric small intestine using combinations of 2- and 3-row staples for use in the creation of transverse and/or vertical staple lines. We hypothesized that FEESA constructed with 3 rows of staples in both the vertical and transverse staple lines will have a greater ILP and MIP than FEESA produced with 2-row staple lines in both locations. Our secondary hypothesis was that the MIP and ILL would vary between FEESA groups of various staple line configurations.

2 | MATERIALS AND METHODS

2.1 | Tissues samples

Cadaveric jejunal tissue was obtained from healthy dogs immediately following euthanasia as part of an unrelated research study not involving the abdominal cavity (Animal Utilization Protocol #4091). Ten mongrel foxhounds between 12 and 22 months of age weighing between 22 and 26 kg were included. Immediately following euthanasia, the entire jejunum was harvested and flushed with sterile saline (0.9% NaCl solution) to remove any intraluminal material. The mesentery was excised from the mesenteric border. The jejunum was isolated and 10 cm segments were created using Metzenbaum scissors. All bowel segments were kept in sterile saline at 4 °C and tested within a maximum period of 5 h following harvesting.^{13,23} The small intestine was not harvested in dogs that had exhibited vomiting or diarrhea prior to euthanasia or if any pathology of the small intestine was grossly evident.

2.2 | Functional end-to-end stapled anastomosis technique

Jejunal segments were randomly allocated to control (8 segments) (group 1) or 1 of 4 treatment groups (with 16 segments or 8 constructs per group) using a randomization program (www.randomizer.org). Four types of FEESA were created according the number of rows used for vertical (V) and transverse line (T), respectively: 2-row V / 2-row T staple lines (2V/2T), 2-row V/3-row T (2V/3T), 3-row V/2-row T (3V/2T), 3-row V/3-row T (3V/3T).

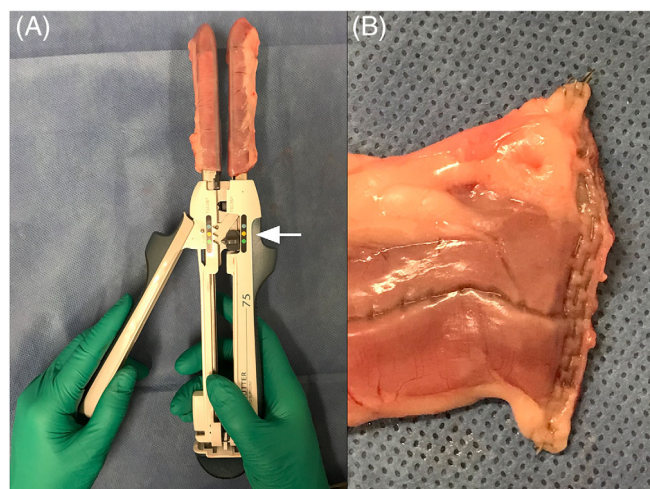


FIGURE 1 Novel linear 3-row stapler. (A) Partial closed novel linear 3-row stapler with cadaveric jejunal segments loaded onto each stapler fork. The arrow indicates the lever setting for the selection of closed staple height (green 1.5 mm, yellow 1.8 mm, blue 2.0 mm). (B) Functional end-to-end stapled anastomosis construct with 3-row linear stapler closure of transverse staple line

Two gastrointestinal anastomosis (GIA) staplers were utilized in this study: (1) 75 mm linear stapler, 2 rows of staples deployed in staggered fashion on either side of a linear cutting blade, with 76 total staples (Proximate TCL75, Ethicon, Cincinnati, Ohio), and (2) 75 mm linear stapler, with 3 rows of staples deployed in a staggered fashion on either side of a linear cutting blade with 118 total staples (NTLC75, Ethicon). (Figure 1).

The FEESA technique was performed as previously described with stapler selection according to group allocation.¹¹ Briefly, an intestinal segment was loaded onto each limb of the selected GIA stapling device, ensuring that the antimesenteric borders were apposed. The stapler limbs were connected, locked, and then deployed creating a side-to-side anastomosis (vertical staple line). The appropriate GIA stapler was loaded and then deployed along the anastomosis stoma (transverse staple line) following staggering of vertical staple lines of the side-to-side anastomosis, 5 mm proximal to the intestinal edge.^{11,13} When the 3-row stapling device was used, the staple closure height of 1.5 mm was selected for each use. A single, simple interrupted suture of 3–0 poliglecaprone suture (Monocryl, Ethicon, Somerville, New Jersey) was placed in the anastomotic crotch formed in the antimesenteric border of the side-to-side anastomosis.¹¹ All constructs were created by a single, small animal surgeon experienced in the use of linear stapling devices (JGQ), under the supervision of a diplomate of the American College of Veterinary Surgeons (AS).

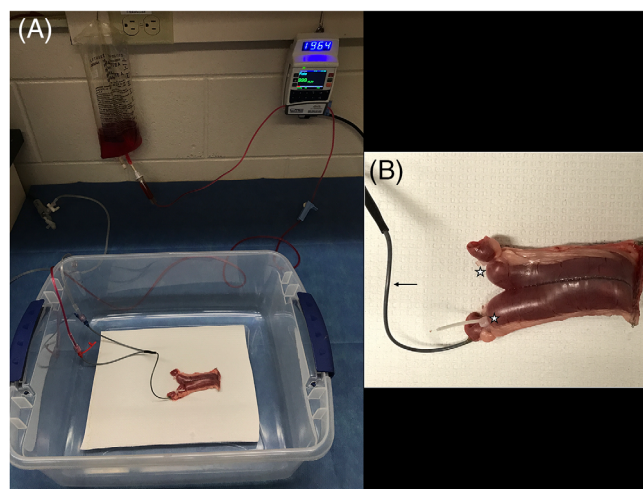


FIGURE 2 Burst pressure testing. (A) Experimental setup for burst pressure testing with an instrumented FEESA construct placed on an absorbent pad. An infusion pump, pressure transducer and red colored dye saline solution are included. (B) Functional end-to-end stapled anastomosis construct instrumented with a double-lumen catheter (black arrow) with cable-ties (white star) sealing both intestinal lumens

2.3 | Pressure testing

Functional end-to-end stapled anastomosis construct testing was performed immediately after creation, which resulted in a total storage time for the intestinal tissue of <5 h. The technique for pressure testing was modified from previous studies.^{5,24} Briefly, a 6 Fr double lumen catheter was placed into 1 intestinal lumen of the FEESA, which was then occluded using a cable tie (Canadian Tire). The remaining lumen was similarly occluded using a cable tie. The catheter was connected to an infusion pump (Vet Pro VIP 2000, Caesarea Medical Electronics, Caesarea, Israel) and a piezoresistive pressure transducer (Gould P2310 transducer, Gould Inc., Rhode Island), coupled to a preamplifier (ADInstruments, Sydney, Australia) that was connected to a computerized data acquisition system (PowerLab from ADInstruments) and analysis software (LabChart from ADInstruments) (Figure 2). The construct was placed into a plastic tray with an absorbent liner (Fisherbrand Absorbent Surface Liners from Thermo Fisher Scientific, Mississauga, Ontario, Canada). Calibration of the system was performed using manufacturer guidelines for each construct. Red food coloring (1 ml/L) (Club House Red Food Color Preparation from McCormick & Company, Mississauga, Ontario, Canada) stained saline solution was infused at a rate of 999 ml/h. The ILP and the MIP were recorded. Initial leak pressure was defined as the pressure at which the red dyed saline solution was first observed

TABLE 1 ILP, MIP, and ILL determined during pressure testing of control segments and 4 FEESA treatment groups

Group	ILP	MIP	ILL		
			TL	Crotch	VL
Control	—	321.38 ± 34.59	—	—	—
2V/2T	32.88 ± 7.36	108.00 ± 14.97	7	1	0
2V/3T	50.13 ± 10.46	101.13 ± 9.85	5	3	0
3V/2T	34.38 ± 11.78	116.63 ± 27.80	8	0	0
3V/3T	69.88 ± 21.23	143.38 ± 21.79	8	0	0

Note: ILP and MIP values are means ± standard deviations, in mmHg. The ILL values represents the number of FEESA constructs distributed according the leakage location.

Abbreviations: —, not applicable; 2V/2T, FEESA 2-row vertical/2-row transverse staple lines; 2V/3T, FEESA 2-row vertical/3-row transverse staple lines; 3V/2T, FEESA 3-row vertical/2-row transverse staple lines; 3V/3T, FEESA 3-row vertical/3-row transverse staple lines; ILL, initial leakage location; ILP, initial leak pressure; MIP, maximum intraluminal pressure; TL, transverse line; VL, vertical line.

extraluminally by a single investigator (JGQ). Once the ILP was recorded, testing was continued until the intraluminal pressure reached a plateau for 5 s or until it acutely dropped. This 5 s pressure plateau or pressure prior to acute drop (if it occurred) was defined as the MIP.²⁴ The leakage location was recorded and defined as being along the vertical or transverse staple line or at the anastomotic crotch. The MIP and leakage location were observed and recorded by a single, nonblinded investigator (JGQ).

2.4 | Statistical methods

An a priori sample size analysis for changes in ILP between FEESA treatment groups was performed using an open source statistical software (G*Power - <https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html>). A minimum sample size of 3–5 FEESA constructs per group was calculated to be able to detect a difference in ILP and MIP, with an alpha of 0.05 and a power of 80%. The study was performed using 8 constructs per group to further increase statistical power.

The differences in ILP and MIP between groups were investigated using a 1-way ANOVA. Residuals were evaluated for normality, homoscedasticity, and the absence of outliers using quantile plots of the residuals. Post hoc tests were performed with a Tukey adjustment. Differences between groups for ILL were investigated with a Fisher exact test. Values of $P < .05$ were considered significant. R (R foundation for statistical consulting, Vienna, Austria) was used for statistical analysis.

3 | RESULTS

Seventy-two segments of jejunum were used over the course of 3 experimental sessions. All jejunal samples

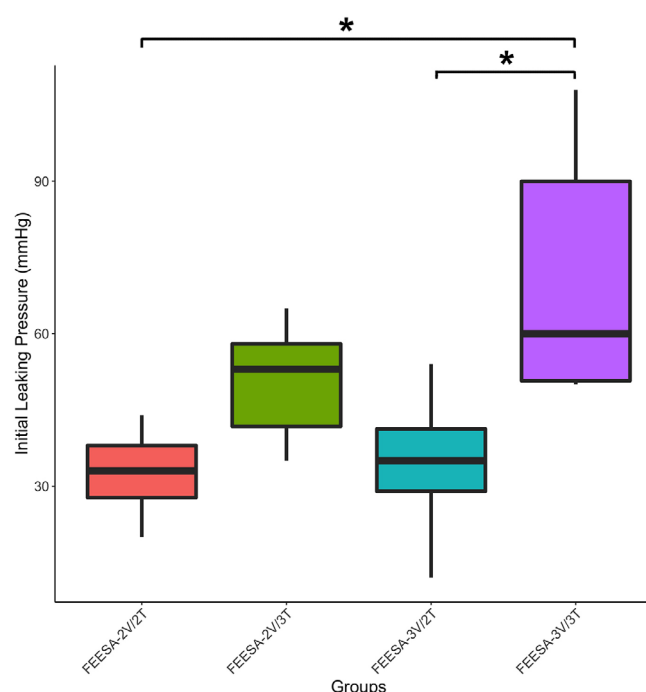


FIGURE 3 Box plot depicting ILP (mmHg) for the FEESA constructs evaluated in this study. FEESA-2V/2T, FEESA 2-row vertical/2-row transverse staple lines; FEESA-2V/3T, FEESA 2-row vertical/3-row transverse staple lines; FEESA-3V/2T, FEESA 3-row vertical/2-row transverse staple lines; FEESA-3V/3T, FEESA 3-row vertical/3-row transverse staple lines. * Indicates a statistically significant difference between groups

harvested were used for FEESA creation and experimental testing. All FEESAs were created successfully and pressure testing was performed without technical challenges.

The mean ± standard deviation ILP, MIP, and the ILL for each FEESA group are depicted in Table 1. All FEESAs initially leaked at lower pressures than intact segments ($P < .001$) (Table 1). The only other differences

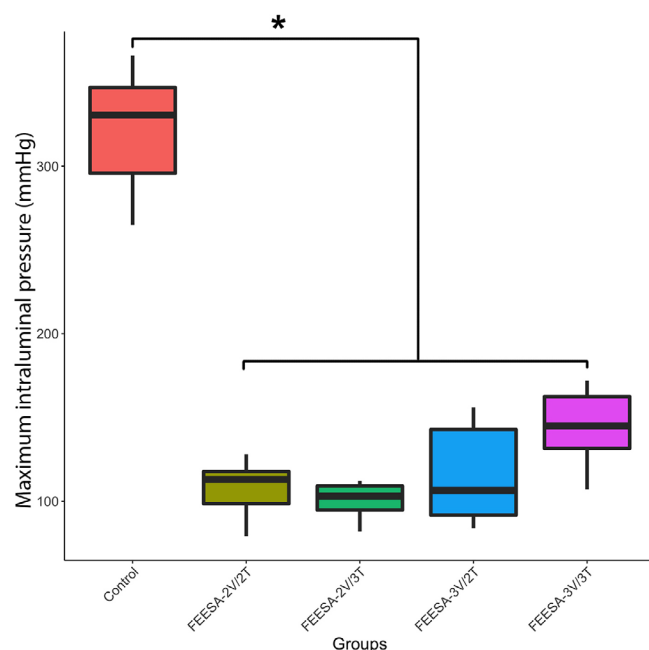


FIGURE 4 Box plot depicting MIP (mmHg) for the control jejunal segments and FEESA constructs evaluated in this study. FEESA-2V/2T, FEESA 2-row vertical/2-row transverse staple lines; FEESA-2V/3T, FEESA 2-row vertical/3-row transverse staple lines; FEESA-3V/2T, FEESA, 3-row vertical/2-row transverse staple lines; FEESA-3V/3T, FEESA, 3-row vertical/3-row transverse staple lines. * Indicates a statistically significant difference between groups

detected between groups consisted of ILPs, which were higher when FEESAs were closed with 3V/3T (69.88 ± 21.23 mmHg) than 2V/2T (32.88 ± 7.36 , $P < .001$) and 3V/2T (34.38 ± 11.78 , $P < .001$) (Figure 3). The intact segments had a higher MIP compared with FEESAs ($P < .001$) (Figure 4).

In all FEESAs, when ILL was detected, it was noted from the transverse staple line or from the anastomotic crotch. No leakage was detected from the vertical staple line. There was no difference in leakage location between FEESAs. However, ILL was more frequent at the transverse staple line in comparison with the anastomotic crotch ($P < .001$).

4 | DISCUSSION

Fresh, cooled, canine cadaveric jejunum was used to test the influence of FEESA staple line configuration on ILP, MIP and ILL. The primary hypothesis was accepted as FEESA constructs with 3 rows of staples in the transverse staple line alone or transverse and vertical staple lines were found to have higher ILP compared to FEESA constructs with 2 rows of staples in the transverse and/or vertical staple line(s). The secondary hypothesis was

rejected as there was no difference in MIP or leakage location between FEESA groups, however, ILL was more frequent at the transverse staple line.

All FEESA constructs tested in the present study had mean ILP and MIP greater than the reported range of intraluminal jejunal pressure in nonanesthetized, healthy dogs of 15–25 mm Hg.²⁵ Based on these data, any of the FEESA constructs evaluated in the present study would be able to withstand peak small intestinal intraluminal pressures in the healthy, live dog. The addition of a third row of staples to the transverse staple line alone or transverse and vertical staple lines of a FEESA increased ILP in comparison with a FEESA constructed with 2 rows of staples. Interestingly, an increase in ILP did not occur when a third row of staples was placed in only the vertical staple line of a FEESA ($P < .0010$). Several recent studies have experimentally and clinically evaluated the utility of suture oversew of the transverse staple line in FEESA constructs.^{3,12,15} In an ex vivo study, the ILP was raised 1.8× in 2V/2T FEESA constructs with a suture oversew of the transverse line compared to a construct without the suture oversew.¹² A possible explanation for the increased ILP in the present study in FEESA constructs with a third row of staples in the transverse line could be that the additional row of staples provides additional support. Further in vivo study comparing the outcomes and complication rates in 2-versus 3-row FEESA with and without suture oversew is required.

The MIP in the intact (control group) segments of this study was consistent with previously reported values in the veterinary literature where similar methodology was used.^{3,4,13} The intact (control) segments had a higher MLP compared with the FEESA groups ($P < .001$). There was no difference in MIP between treatment groups.^{3,8,12}

The most commonly reported technique for FEESA creation is with a GIA stapler for the vertical staple line (side-to-side anastomosis) and a TA stapler for the transverse staple line.^{2,9,17} Using this technique in both in vivo (clinical)^{10,11,15,16,19} and ex vivo studies,^{3,4,8,12,13} the transverse staple line has been reported as the most common site of leakage. It has been hypothesized that possible causes for increased leakage at the transverse staple line (created with a TA stapler) may include its everted orientation resulting in a prolonged inflammatory phase, delayed mucosal re-epithelialization, and/or the potential for staple malformation at the intersection of the GIA/TA staple lines.^{13,17,26} While clinical evaluation has not been performed in a large number of FEESA created with a GIA stapler for both vertical and transverse staple lines, the results of this ex vivo study indicate that the GIA stapler created transverse staple line is also the most common initial site of leakage.

Oversewing the FEESA transverse staple line created with a TA stapler has been shown to increase¹² or have no effect³ on bursting strength in ex vivo studies and has reduced incidence of FEESA dehiscence clinically.^{15,19} Some possible disadvantages associated with oversewing the transverse staple line include prolongation of surgical time and compromise of the lumen diameter of the FEESA construct.³ Clinical and ex vivo comparison, including time to construct completion, of a 3V/3T or 2V/3T FEESA compared with a 2V/2T FEESA, with and without transverse staple line oversew is required before considering 3V/3T as an in vivo alternative to transverse staple line oversew.

The novel stapler used in the present study has the ability to select a closed staple height of 1.5 mm, 1.8 mm and 2.0 mm while using a single cartridge. In the present study, 1.5 mm closed staple height was selected in all FEESA constructs regardless of whether 2 or 3 staple rows were deployed. This closed staple height is consistent with previously published reports.^{2-4,12,13} Further ex vivo and clinical study with this novel stapling device should consider the use of a FEESA-3V/3T or 2V/3T constructs with 1.8 and 2.0 closed staple heights in dogs between 15 and 30 kg.³

Caution must be exercised when extrapolating these data for clinical decision making. Primary outcome measures in this ex vivo study were related to ILP and MIP and ILL. However, bursting strength of an anastomosis is likely only one of several factors involved in small intestinal anastomosis healing and is not an accurate predictor of dehiscence. Limitations of the present study include the use of cadaveric tissues. Although small intestinal tissues were harvested immediately after euthanasia and tested within 5 h of harvesting, these tissues likely have varying biomechanical properties compared with live dog small intestine tissues with and without disease. Additional limitations to this study include the lack of selection of closed staple height on tissue thickness, lack of histopathological evaluation of intestinal tissues, lack of additional procedures to evaluation staple line integrity (eg probing)²⁷, and use of a single, simple interrupted crotch suture to augment the FEESA.²⁸

In summary, a FEESA constructed with a 3-row linear stapler for the vertical and transverse staple line or transverse staple line alone, using a 1.5 mm closed staple height, increased ILP compared to construct creation using 2-rows. There was no difference in MIP or leakage location between FEESA groups. Initial leakage location was more frequent at the transverse staple line compared to the anastomotic crotch. Additional ex vivo and clinical studies are required to further evaluate the 3-row linear stapler at various closed staple heights, with and without suture oversew in canine small intestine.

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CONFLICT OF INTEREST

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

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