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Influence of barbed suture oversew of the transverse staple line during functional end-to-end stapled anastomosis in a canine jejunal enterectomy model

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Abstract

Objective: To evaluate the influence of barbed suture oversew of the transverse staple line during functional end-to-end stapled anastomosis (FEESA) in dogs.

Study design: Randomized, experimental, ex vivo.

Animals or sample population: Grossly normal jejunal segments from 14 adult canine cadavers.

Methods: Ninety-eight jejunal segments (n = 14/FEESA group, n = 14 controls) were harvested and randomly assigned to a control group, FEESA + monofilament suture oversew, FEESA + unidirectional barbed suture oversew or FEESA + bidirectional barbed suture oversew. Oversew techniques were performed using a Cushing suture pattern. Initial (ILP) and maximum leakage pressure (MLP), repair time (s), and location of observed leakage were recorded.

Results: No differences were detected in ILP (p = .439) or MLP (p = .644) respectively between experimental groups. Repairs times using barbed suture were ~ 18% faster (~25 s faster; p < .001) compared to monofilament suture. There was no difference between barbed suture types (p = .697). Mean ILP (p < .001) and MLP (p < .0001) were 6.6x and 5.1x greater respectively in the control group. Leakage location occurred predominately at the crotch of the FEESA in all groups.

Conclusion: FEESAs closed with a transverse staple line oversew using barbed suture, regardless of barb orientation, were completed faster and resulted in similar resistance to anastomotic leakage compared to monofilament suture.

Clinical significance: Oversewing the transverse staple line following FEESA using barbed suture offers similar resistance to anastomotic leakage, and may

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made. © 2022 The Authors. *Veterinary Surgery* published by Wiley Periodicals LLC on behalf of American College of Veterinary Surgeons. be associated with decreased surgical times in dogs compared to monofilament suture. Further studies are necessary to determine the benefits of barbed suture use in both open and laparoscopic gastrointestinal surgical applications following FEESA in dogs.

1 | INTRODUCTION

Enterectomy is a common procedure in dogs to remove areas of diseased bowel following foreign body obstruction, neoplastic disease, intussusception, diverticulum or penetrating trauma.¹ Functional end-to-end stapled anastomosis (FEESA) is routinely performed due to the benefits of decreased surgical time, less necessity for handling of the small bowel, preservation of the enteric blood supply, and reliability and consistency of staple placement compared with the traditional handsewn anastomoses.^{1–5} Although no difference among dehiscence rates has previously been reported between handsewn and stapled techniques^{6,7} a study by DePompeo et al., showed the odds of dehiscence were significantly greater for sutured end-to-end anastomoses compared to FEESA.⁸ Dogs undergoing surgery for previous dehiscence of the small bowel were more likely to experience a subsequent dehiscence using hand sutured techniques.⁸

During FEESA completion, the use of a gastrointestinal anastomosis stapler (GIA) or thoracoabdominal (TA) stapling device is employed to seal the top of the anastomosis. thus creating a transverse staple line.^{2–4} The resultant transverse staple line that is created is an everting closure, causes mucosal exposure and prolongation of the inflammatory response, delayed healing and predisposition to adhesion formation.⁹⁻¹¹ The transverse staple line following FEESA is the most common site of extraluminal leakage, which can predispose to abscess formation and the occurrence of subsequent peritonitis.^{3,9,11} Leakage from the anastomosis is a devastating complication following FEESA, occurring in up to 13% of dogs^{3,9,10,12} with mortality rates up to 85%.^{10,13,14} Leakage from anastomosed bowel segments typically occurs during the lag phase of intestinal healing, caused by a reduction in wound strength compared to immediately postoperatively.¹³ Reinforcement using a sutured oversew of the transverse staple line technique has been shown to be protective against leakage in both ex vivo¹⁵ and in vivo models.¹⁶ A retrospective study by Sumner et al., highlighted the importance of oversewing the transverse staple line following FEESA, with a reduced incidence of postoperative dehiscence seen in the large cohort of dogs where an oversew was performed.¹⁶ In the same study, the frequency of observed peritonitis caused by FEESA leakage fell from $\sim 15\%$ with a mortality rate of 14%

without use of a sutured oversew compared to 0% with no observed leakage in the oversewn group.¹⁶ This highlights the importance of a sutured oversew of the transverse staple line, justifying further evaluation of this technique following FEESA in dogs.

Barbed sutures are created by automated processing that cuts the core suture strand to create protrusions (barbs) on the sutures surface that then interact with, and anchor within apposed tissues.¹⁷ Due to the distribution of tension along the entire length of the suture line. this mitigates the need for initial suture knotting while decreasing the time for pattern completion.^{17,18} Due to these inherent characteristics, barbed suture was initially designed for use in minimally invasive and laparoscopic surgery in both human and veterinary patients.^{17,19,20} There was no difference in mean leakage pressures using knotless barbed suture compared to monofilament suture when applied for laparoscopic gastrointestinal closure in a canine model.²¹ Within the veterinary literature barbed suture has been applied for use in open gastrointestinal, urogenital, and tendon repair.^{18,22-26} Hansen et al., demonstrated that use of barbed glycomer 631 had higher initial leakage pressures (ILP) with no difference in maximum intraluminal pressures compared to monofilament 3-0 and 4-0 USP glycomer 631 for canine small intestinal anastomoses.²⁷ Fealey et al., showed no difference in ILP between 3-0 unidirectional barbed suture and 4-0 monofilament glycomer 631 on ILP in cadaveric canine jejunum.²² To date, use of barbed suture for oversew of the transverse staple line following FEESA has not been evaluated. Barbed suture is an attractive option for gastrointestinal closure due to its ready availability and increased familiarity among small animal surgeons.²⁸ Oversewing of the transverse staple line using barbed suture following FEESA to establish a watertight seal is critical to determine the safety and efficacy of these sutures. This information is important to allow informed suture use for gastrointestinal applications in dogs.

The objective of this study was to evaluate the influence of barbed suture use for oversew of the transverse staple line during FEESA on ILP and maximum leakage pressure (MLP), repair time (S), and location of observed leakage in fresh canine jejunum. Our null hypothesis was there would be no difference in ILP and MLP among experimental groups. Our secondary hypothesis was barbed suture use would be associated with shorter surgical times compared to monofilament suture for pattern completion.

2 | MATERIALS AND METHODS

Due to the secondary use of cadavers unrelated to this study, this research was deemed exempt for requiring IACUC approval by North Carolina State University, College of Veterinary Medicine. A total of 14 adult mixed breed dogs weighing between 17-35 kg were obtained immediately following humane euthanasia. Dogs were obtained from a local small animal shelter whose sex was not recorded. Dogs were included if they had no history or signs of gastrointestinal disease. Dogs were euthanized following IV infusion using 1 ml/5 kg of sodium pentobarbital (Euthasol, Virbac AH, Inc., Fort Worth, Texas). Dogs were excluded if there was a prior history of gastrointestinal disease, dietary indiscretion or if they were being administered any medications within 1 month of collection or if the bowel was grossly abnormal upon visual inspection.

Jejunal segments were harvested and tested within 4 h following euthanasia. The mesentery of the small bowel was sharply transected using straight Metzenbaum scissors, 5 mm from the antimesenteric border of the jejunum. All jejunum were harvested and visually inspected by a single investigator (DJD). Following extirpation, jejunum were milked to clear luminal contents of any ingesta and were flushed with tap water until the water ran clear. Segments were then divided in to 10 cm segments using Metzenbaum scissors, measured using a ruler (Surgical Ruler, Medline, Illinois) and then submerged within room temperature sterile saline (0.9% NaCl) until the time of testing.²⁴

2.1 | Experimental groups

A standard FEESA was created as previously described² using two fresh jejunal segments from each respective cadaver with each dog contributing one FEESA to each experimental group. The FEESA constructs were then randomly assigned to one of three experimental groups (n = 14 FEESA/group) using a random sequence generator (https://www.randomizer.org). Single jejunal segments from each dog were used within the control group (n = 14 segments) and were left intact/ unaltered for validation of intestinal integrity and testing methodology. A 3.8 mm (Blue) staple cartridge (Medtronic, Massachusetts) was loaded on a 60 mm GIA stapling handpiece (DST, Series, Medtronic, Massachusetts). Each limb of the GIA stapler was fully inserted into each respective

jejunal segment, aided by placement and traction on two individual stay sutures using 4-0 USP polydioxanone (PDS, Ethicon, New Jersey). The antimesenteric borders of each jejunal segment were then closely apposed before the handpiece was closed. After verifying correct positioning of the apposed segments, the GIA was locked and engaged by pushing the integrated firing knob to create a side-to-side anastomosis following placement of two rows of DST staples to create a stoma. The transverse staple line was then created using a reusable 60 mm TA staple device (DST TA Series; Medtronic, Massachusetts) loaded with a blue 3.5 mm staple cartridge (Medtronic, Massachusetts), orientated perpendicularly to the GIA staple line, 5 mm away from the edge of the jejunal luminal opening following offset of the longitudinal staple lines.²⁹ Following FEESA completion a single simple-interrupted crotch suture was placed using 4-0 USP glycomer 631 (Biosyn, Medtronic, Massachusetts) equidistant between jejunal limbs to prevent anastomotic separation at this site.

Following FEESA completion, oversewing of the transverse staple line was performed using one of three methods. All groups were repaired using a Cushing suture pattern placed to engage the submucosa with bites placed 2-3 mm apart and 3 mm from the staple line. Repairs were completed using conventional instrumentation in an open setting. The first group were repaired using 3-0 USP glycomer 631 (Biosyn, Medtronic, Massachusetts), using an SH 22 mm ¹/₂ circle taper needle termed FEESA + monofilament suture oversew. During the oversew completion, the transverse staple line was inverted as necessary using the tips of curved mosquito forceps. A square knot followed by three additional throws was used at the beginning and end of the suture line (Figure 1A). The second group was performed using 3-0 USP knotless unidirectional barbed polyglyconate (copolymer of glycolic acid and trimethylene carbonate; V-Loc 90 absorbable wound closure device, Medtronic, Massachusetts) using a swaged V-20 26 mm 1/2 circle taper needle termed FEESA + unidirectional barbed suture. For this group the initial suture bite was taken 5 mm from the staple line with the needle then passed through the preconstructed effector loop and the loop tightened against the serosal surface. At the end of the suture line an additional 3 suture bites were applied past the point of the last staple and the suture cut to 3 mm (Figure 1B).¹⁸ The third group was performed using 3–0 USP knotless bidirectional polydiaxonone barbed suture (PDO; Surgical Specialties Corporation, Pennsylvania) using a 26 mm ¹/₂ circle taper needle termed FEESA + bidirectional barbed suture. For this group an initial suture bite was taken 5 mm from the beginning of the staple line and continued for an additional three bites at the end of the stapled line. Control specimens were tested whole and in their unaltered form. All constructs were



FIGURE 1 (A) Photograph of a completed FEESA using fresh canine jejunum. The FEESA has been augmented using a suture oversew of the transverse staple line in an inverting Cushing pattern using glycomer 631 monofilament suture. Note the partial offset of the longitudinal staple lines. (B) Photograph of a FEESA followed by suture oversew of the transverse staple line using 3–0 USP knotless bidirectional polydioxanone in a Cushing pattern. Two 18-gauge catheters have been inserted into each jejunal limb in to the lumen proximal to the occluding intestinal forceps. The catheter to the top right of the image is connected to a fluid pump containing dyed solution, while the catheter at the bottom right is connected to a pressure transducer. A millimeter ruler (Medline, Illinois) can be seen to the bottom of each image. Abbreviation: FEESA, functional end-to-end stapled anastomosis

performed by a single board-certified surgeon (DJD) using optical magnification (3.5x Loupes, Surgitel, General Scientific Corp, Ann Arbor, Michigan) under surgical lighting.

2.2 | Leakage pressure testing

The technique for leakage pressure evaluation followed those described in detail by other investigators.^{7,15} For all experimental groups, two straight Rochester-Carmalt forceps were used to occlude the open limb of each jejunal segment. Two newly opened 18-gauge, 1.2 inch IV catheters (Insyte, Franklin Lakes, New Jersey) were then inserted into each respective jejunal lumen at a slight angle immediately proximal to each forcep. For control segments, two straight Rochester-Carmalt forceps were placed on each respective end of the jejunal lumen and two IV catheters inserted into the jejunal lumen from each antimesenteric border. In both experimental groups and for control specimens, one catheter was connected to a 5 L bag of Hartmann's solution (Vetivex, Dechra Veterinary Products, Overland Park, Kansas) which was thoroughly mixed with 8 ml of methylene blue (Kordon, Hayward, California) administered using a fluid line (Lifeshield Plumset, Hospira, Lake Forest, Illinois). The fluid rate was controlled using a fluid pump (Plumb A+, Hospira). The other

respective catheter was connected to a fluid line (Lifeshield Plumset, Illinois) primed with sterile saline and connected to the pressure transducer (Logical, Smith Medical, Dublin, Ohio) and a multiparameter pressure monitor (Passport 2, Mindray, Mahwah, New Jersey). Dyed fluid was then infused at a constant rate of 999 ml/h as previously described.²⁷ During testing constructs were suspended and monitored from the sides and above for extraluminal leakage of dyed solution by a single study investigator (Y-JC). The ILP was defined as the intraluminal pressure at which dyed solution was first observed to leak extraluminally. The MLP was defined as the maximum pressure reading during testing or when intraluminal pressures plateaued for at least 6 s or there was complete failure of the repair. Leakage location was observed to occur at the crotch, longitudinal or transverse staple line, or due to serosal tearing of the intestinal segment. In all groups repair time in seconds (s) was evaluated from the time of the initial suture bite to the time of oversew completion when the suture was cut, which was measured using a digital stopwatch (Iphone XR, Apple, California).

2.3 | Statistical analysis

A pilot study determined the method of barbed suture placement, pressure monitoring, and definition of

TABLE 1 Mean ± SD (mmHg) ILP, MLP and repair time (s) for oversew of the transverse staple line following FEESA in dogs

Experimental group	ILP (mmHg)	MLP (mmHg)	Repair time (s)
FEESA + monofilament suture oversew	62.64 ± 9.41^{a}	96.93 ± 9.21^{a}	$159.14 \pm 10.56^{\mathrm{a}}$
FEESA + unidirectional barbed suture oversew	65.43 ± 7.07^{a}	99.00 ± 4.07^{a}	133.71 ± 4.56^{b}
FEESA + bidirectional barbed suture oversew	64.21 ± 7.96^{a}	96.71 ± 9.52^{a}	135.00 ± 7.90^{b}
Control (intact) jejunal segments	428.17 ± 62.61^{b}	495.33 ± 11.43^{b}	N/A

Notes: Superscript letters denote significant differences between groups ($p \le .05$).

Abbreviations: FEESA, functional end-to-end stapled anastomosis; ILP, initial leakage pressure; MLP, maximal leakage pressure; s, seconds.

observed leakage locations. No specimens were rejected at the time of testing with all samples included in the final statistical model. An a priori power analysis concluded that ≥ 12 FEESA/group would provide an 80% power to detect a difference of 22 ± 4 mm Hg at a 5% alpha error rate. A total of 14 FEESA per group were tested to ensure statistical power. Data was evaluated for a parametric distribution using a Shapiro Wilk test for normality. Continuous variables were normally distributed and ILP (mmHg) and MLP (mmHg) were reported as mean \pm SD. Differences in group means between oversew patterns were assessed using a mixed linear model controlling for cadaveric contribution (dog) to each group with experimental group as fixed effects and cadaver as a random effect. Pairwise comparisons of least square means were conducted with Bonferroni adjustment for multiple comparisons. Leakage location was assessed using a Fisher's exact test. Analyses were performed using statistical software (Stata, v.15.0, Stata Corp, College Station, Texas) with a *p*-value of \leq .05 considered statistically significant.

3 | RESULTS

3.1 | Leakage pressure evaluation

No differences were detected in ILP (p = .439) or MLP (p = .644), respectively, between the experimental groups. (Table 1). Mean ILP (p < .001) and MLP (p < .0001) were 6.6x and 5.1x greater, respectively for specimens in the control group compared to other experimental groups.

3.2 | Repair time

Repair times differed among groups (p < .001) (Table 1). Repair times for FEESA + unidirectional barbed suture oversew were 25 s faster (19% faster; p < .001) and FEESA + bidirectional barbed suture oversew were 24 s faster (18% faster; p < .001) compared to FEESA + monofilament suture oversew respectively. There was no difference between barbed suture types (p = .697).

3.3 | Leakage location

Leakage from the anastomotic crotch occurred in 11/14 (78.57%) FEESA + monofilament suture oversew, 12/14 (85.71%) FEESA + unidirectional barbed suture oversew and 12/14 (85.71%) FEESA + bidirectional barbed suture oversew. One construct (1/14; 7.14%) leaked from the transverse staple line in both FEESA + monofilament and bidirectional barbed suture oversew groups. All remaining constructs leaked at the longitudinal staple line. In the majority of control specimens, no leakage occurred during testing (12/14, 85.7%) with only one jejunal segment leaking by serosal tearing (2/14, 14.3%).

4 | DISCUSSION

In this study, we evaluated the influence of barbed suture and its effect on leakage pressures following oversew of the transverse staple line following FEESA. In support of our hypotheses, there was no difference in ILP and MIP among experimental groups regardless of suture type. Repair times to complete the oversew were decreased when barbed suture was used. FEESA oversew performed using barbed suture had similar ILP and MLP that is comparable with use of monofilament suture while requiring less time for oversew completion. Oversewing the transverse staple line following FEESA using barbed suture may offer a viable alternative to conventional monofilament suture for use in dogs.

The physiological intraluminal pressure previously documented in the normal nonanesthetized dogs has been reported to range between 15–25 mm Hg.³⁰ Therefore to be effective against resistance of the FEESA to leakage, the intraluminal pressure of any intestinal anastomotic technique must be equal to, or greater than, intraluminal pressures encountered in vivo to decrease the risk of dehiscence postoperatively.^{2,9,10} In our study,

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all oversew techniques leaked at supraphysiological pressures and may represent an effective option for oversew of the transverse staple line. Duffy et al., evaluated oversew augmentation of the transverse staple line using 3-0 USP monofilament polydioxanone in a Cushing pattern. They showed that oversewing the transverse staple line increased ILP by 1.8 times compared with nonoversewn FEESA.¹⁵ In the present study, there was no difference in ILP between 3-0 glycomer 631 monofilament suture and two commercially available barbed suture materials when applied in a Cushing pattern. Use of barbed suture for oversew of the transverse staple line conferred equal resistance to leakage from the FEESA. Oversew of the transverse staple line during FEESA has been shown to reduce the occurrence of postoperative dehiscence.¹⁶ It should be noted, however, that a wide variety of suture patterns including simple continuous, Cushing, simple interrupted, cruciate, interrupted horizontal mattress, and Lembert patterns using a variety of suture materials to oversew the transverse staple line were used.¹⁶ In that aforementioned report, one patient had two jejunal FEESA; one anastomosis where the transverse staple line was oversewn while the other was not. Nonoversewn FEESA leaked and required revision surgery while all oversewn FEESA remained intact.¹⁶ Based on the results of our study, use of barbed suture may represent a viable alternative while providing similar resistance to leakage to monofilament suture for suture oversew of the transverse staple line. The influence of barbed suture oversew on rates of dehiscence and postoperative leakage are currently unknown and represent an area for future exploration.

Barbed sutures are manufactured by machined processing and cutting into the core suture shaft at a geometric angle to create barbs with either a unidirectional (polyglyconate) or bidirectional (polydioxanone) design and orientation.¹⁷ An important drawback encountered during the manufacture of barbed suture is a reduction in the functional diameter of the suture shaft, which may inadvertently weaken the suture itself.¹⁷ The tensile strength of barbed suture is equivalent to the same size or 1 USP size smaller when using the same monofilament suture material.^{31,32}In the present study, there was no difference in ILP and MLP when using barbed suture material used regardless of barb orientation for completion of the oversew. Giusto et al., showed no difference in the mean bursting pressures when using barbed polyglyconate (150 \pm 16 mm Hg) or barbed polydioxanone (145 \pm 22 mm Hg) for use in hand-sewn end-to-end jejuno-jejunal anastomosis in a porcine model.³³ The results of this aforementioned study agree with our study findings demonstrating equivalence among these barbed sutures regarding their ability to confer similar resistance to leakage.33

Due to their knotless design, barbed sutures can facilitate decreased repair times compared with the use of traditional monofilament sutures.^{17,31,33} Barbs are easily pulled through anastomosed tissues in a single direction only, that then self-engage and interact with the submucosa when tension is applied to the suture line. This effect is unique to barbed sutures and removes the need for continual tension to be placed on the suture strand during suturing.³⁴ Our results are in agreement with those of prior investigators, with use of barbed sutures resulting in decreased repair times.^{17,33,34} In our study, repair times on average were $\sim 18\%$ shorter when compared to time for suture oversew using monofilament suture. Although subjectively assessed, we found both barbed sutures types easy to handle, with minimal tissue drag when taking each consecutive suture bite through apposed jejunal tissues. Use of bidirectional barbed suture has a decreased number of barbs per unit length and greater spacing between adjacent barbs compared with unidirectional barbed suture.³³ This may aid in less tissue resistance that may be appreciable when longer lines of suture material or tissue approximation is required. It should be noted that verification of suture and needle placement during oversew of the transverse staple line is required, as backward retraction of the suture is not easily accomplished after an initial tissue bite is taken.

When the FEESA is not oversewn, leakage is predominantly seen at the transverse staple line in both ex vivo¹⁵ and in vivo studies in dogs.^{5,10,16} Reasons for leakage in this area include failure of normal staple deployment and closure or incorrect tissue engagement at the intersection of GIA and TA staple lines, increased gastrointesthickness or staple conflict/overlap. Sutured tinal oversew of the transverse staple line during FEESA may obviate the occurrence of leakage from this location.^{7,15} In an ex vivo study by Duffy et al., leakage occurred predominantly from the crotch of the anastomosis between jejunal limbs following use of a suture oversew of the transverse staple line. Therefore the anastomotic crotch may represent a possible weak point in the oversewn FEESA construct.¹⁵ To reinforce and decrease the occurrence of leakage from this location, the addition of a crotch suture has been recommended to reduce tension between each apposed jejunal limbs and prevent separation in this area.^{4,29} In our study, over 80% of leakage, regardless of suture material used for oversew of the transverse staple line, occurred at the crotch of the anastomosis which is in agreement with the results of previous investigators.^{4,15,29} When using barbed suture material the suture hole and track through tissues may be larger in size compared to use of an equivocally sized monofilament suture. Protruding barbs on the sutures

surface were wider than the core suture shaft potentially resulting in a greater degree of iatrogenic tissue trauma.³² In our study, no leakage occurred at the level of the suture hole when using barbed suture regardless of group. Different locations of leakage may be seen if a different suture or pattern was used for oversew completion.

Limitations of this study include its ex vivo design which prevents assessment of the normal inflammatory response encountered during wound healing, revascularization, edema or adhesion formation, tissue viability and tearing which may contribute to construct failure. In cases of neoplastic, infiltrative or inflammatory disease the jejunal thickness may differ which may affect the integrity of apposed tissues. In dogs affected by gastrointestinal pathology, multiple factors may predispose to dehiscence postoperatively such as intestinal foreign body obstruction or serum albumin concentrations <2.5 g/dL.¹⁴ We used a Cushing suture pattern; however, different results may be seen when other patterns are used for oversew. Engagement of the submucosa was only subjectively assessed and based on surgical experience. In our study, we used healthy adult dogs devoid of visual gastrointestinal pathology but results may differ in toy or giant breed dogs which may require use of a different stapling device. We used sutures from different manufacturers composed of different materials. This was, however, purposeful to represent what is clinically available for use in our tertiary referral hospital. Our study did not evaluate additional techniques to reinforce the anastomosis such as omental wrapping, serosal patch placement or different methods of anastomotic crotch augmentation which may be used intraoperatively to augment the FEESA.^{35,36} Lastly, we did not leak test the anastomosis using an infusion of saline. This may have led to additional sutures being placed which may indirectly have led to changes in ILP, MLP or repair times.

In conclusion, oversewing the transverse staple line following FEESA using barbed suture, regardless of barb orientation had similar ILP and MLP compared to use of monofilament suture but was associated with decreased repair times. Oversewing the transverse staple line following FEESA using barbed suture may offer a viable alternative to use of conventional monofilament suture in dogs. Further studies are necessary to determine the benefits of barbed suture use for both open and laparoscopic suture applications following FEESA in dogs.

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Yi-Jen Chang: Contributions included acquisition of data, interpretation of the data, writing and revising the manuscript, and approval of the final version to be published.

George E. Moore: Contributions includes statistical analysis, interpretation of data, review of the manuscript and approval of the final version to be published.

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CONFLICT OF INTEREST

The authors have no disclosures or conflicts of interest to report.

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