

# *Ex Vivo* Comparison of A UV-Polymerizable Methacrylate Adhesive Versus an Inverting Pattern as the Second Layer of a Two-Layer Hand-Sewn Jejunal Anastomosis in Horses

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## Research Article

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# Abstract

**Background** Jejunal anastomosis in horses with one- or two-layer conventional technique can lead to leaks at the anastomosis site and the possibility of severe septic peritonitis. The objective of this study was to compare the use of a recently designed UV-polymerizable methacrylate adhesive as the second layer of a two-layer anastomosis with a Cushing pattern.

Fifteen fresh harvested jejunum segments were collected from horses euthanized for unrelated reasons and owner consent was obtained for research donation. Each segment was divided in 3 pieces, each assigned to 3 different groups. In 2L-CT group, resection and anastomosis was performed using a double-layer simple continuous/Cushing suture. In 1L-UV-PMA group, resection and anastomosis was performed using a single-layer continuous technique sealed with a UV-polymerizable methacrylate adhesive. Control group was left untouched. Anastomotic construction time of the second layer, Bursting Strength Pressure (BSP), Luminal Diameter Reduction (LDR), and mode of failure were measured and compared between groups.

**Results** The construction time (Mean [95% CI]) was 3.02 min [2.50; 3.55] in 1L-UV-PMA group and 8.09 min [7.59; 8.61] in 2L-CT group. The difference was significant ( $P<0.001$ ).

The BSP (Mean [95% CI]) was 170.47 mmHg [146.29; 194.65] in 1L-UV-PMA group, 175.33 mmHg [156.83; 193.83] in 2L-CT group, and 189.93 mmHg [162.52; 217.34] in the control group. The difference was significant only between the 1L-UV-PMA group and the control group ( $P=0.04$ ).

The LDR (Mean [95% CI]) was 51% [47; 55] in 1L-UV-PMA group and 48% [43; 53] in 2L-CT group. The difference was not significant ( $P=0.26$ ).

Eight segments ruptured on the suture line in the 1L-UV-PMA group and six segments ruptured on the suture line in the 2L-CT group. The difference was not significant ( $P=0.36$ ).

On macroscopic evaluation, the 1L-UV-PMA anastomosis formed a tunnel-like anastomosis. After testing, some of the samples from the 1L-UV-PMA group showed shreds of glue detached from the serosa.

**Conclusions** Complete covering of anastomosis with UV-PMA glue is comparable in terms of leaking pressure and Luminal Diameter Reduction but faster to perform than an inverting suture pattern. Modification of the technique is warranted to decrease tunneling at the anastomosis site.

## Background

Involvement of the small intestine, mainly jejunum, during emergency exploratory laparotomy is reported in about 34% of cases (1). Resection and anastomosis are the method of choice when a segment of devitalized small bowel is found.

Several methods of resection and anastomosis of the jejunum have been described, including hand-sewn techniques (one or two layers using Lembert, Cushing or Gambee patterns, and lately the use of barbed sutures), staples and a biofragmentable anastomosis ring (2–6).

Intestinal suturing involves perforation of the intestinal wall, which can lead to anastomosis leaks and short-term related complications, like peritonitis and death. The survival rates after laparotomy with resection and anastomosis of the small intestine range from 76–88% (7–13). Septic peritonitis has been reported in a minimum of 3% of cases after colic surgery (10). There is no data specifically for anastomosis leaks in horses, but anastomosis leaks are reported in 13–23% of cases of colon anastomoses in human surgery, with subclinical rates reported to be as high as 50% (14).

In human surgery, various methods as staples, patches, or surgical glues have been investigated to prevent anastomosis leakage. Internal surgical glues are used to seal anastomosis to prevent leakages and related complications like bleeding, but more rarely used in veterinary surgery.

Five types of surgical glues are used in human surgery: Fibrins and bovine collagen and thrombine, cyanoacrylates, polyethylene glycol and aldehydes. Fibrins and bovine collagen and thrombine sealants are hemostats (15). They are essentially used in cardiac and vascular surgeries. Their effectiveness and usefulness remain questionable (16). However, they are widely used because they are occasionally the last tool available to treat tricky surgical bleedings (17). Polyethylene glycol and aldehydes are essentially used in aortic suture sealing. Cyanoacrylates are the only surgical glues that can be considered as adhesives. They are only used to suture small skin incisions (< 1 cm) and as bandage over skin essentially because their adhesion is low (18–20). Cyanoacrylate has been described for gastrointestinal anastomoses in rats, pigs and dogs (21–23). Their use in horse abdominal surgery has been limited to inguinal ring closure under laparoscopic guidance (24).

Unfortunately, hemostatic, sealing and adhesive properties of surgical glues are disappointing mainly because of a low adhesion to biological tissues and of a hold over time that is too limited and less than tissues healing time. An efficient surgical adhesive capable to strongly seal and replace traditional suture would be a great tool for surgeons and a major clinical breakthrough because it would simplify surgical procedures, thereby reducing the incidence of numerous surgical complications. Research on surgical adhesives is intense in the development of novel solutions (25–30).

An UV-polymerizable methacrylate adhesive (UV-PMA)<sup>1</sup> is designed to anchor into the biological tissues top surface offering sealant and has adhesive properties up to ten times greater than commercially available soft tissue glues.

The objective of our study was to compare the UV-PMA with a Cushing pattern as the second layer of a two-layer jejunal anastomosis, in terms of feasibility, sealing properties, luminal reduction, and anastomosis time.

We hypothesized that UV-PMA would have the same mechanical properties and be faster to realize than a Cushing pattern.

## Results

Horses (6 males, 9 females) had a median age of 11 years (range, 2–27 years). Breeds were represented as follow: 8 Thoroughbred Cross (Selle français), 3 Thoroughbred, 2 Standardbred, 1 Shetland Pony and 1 Spanish Horse. Because of a technical issue with the ultrasound in the first horse tested, LDR was available only for 14 horses. All data were determined to be normally distributed.

## Construction Time

Mean [95% CI] 1L-UV-PMA construction times (3.02 min [2.50; 3.55]) were significantly lower compared to 2L-CT (8.09 min [7.59; 8.61];  $P < 0.001$ ).

## Bursting Strength Pressure

Both anastomoses groups had a lower BSP compared to the control group (Control: 189.93 mmHg [162.52; 217.34]; 2L-CT: 175.33 mmHg [156.83; 193.83]; 1L-UV-PMA: 170.47 mmHg [146.29; 194.65]), with only the difference between the 1L-UV-PMA group and the control group being statistically significant ( $P = 0.04$ ).

## Luminal Diameter Reduction

No significant difference was found in LDR between suture (48% [43; 53]) and glue groups (51% [47; 55],  $P = 0.26$ , Table 1).

Table 1

Mean values and confidence intervals obtained from the control, 2L-CT, and 1L-UV-PMA groups

	Control [95% CI]	2L-CT [95% CI]	1L-UV-PMA [95% CI]
BSP (mmHg)	189.93 [162.52; 217.34] *	175.33 [156.83; 193.83]	170.47 [146.29; 194.65] *
LDR (%)	N/A	48 [43; 53]	51 [47; 55]
Construction Time (min)	N/A	8.09 [7.59; 8.61] *	3.02 [2.50; 3.55] *
* Significant Difference ( $P < .05$ )			
2L-CT: Double layer anastomosis; 1L-UV-PMA: 1-layer anastomosis and UV-polymerizable methacrylate adhesive application; BSP: Bursting Strength Pressure; LDR: Luminal Diameter Reduction; N/A: Non Applicable.			

## Mode of failure

No significant difference in mode of failure was observed in our study ( $P = 0.36$ ). However, six (Mesenteric Suture:  $n = 3$ ; Non-mesenteric Suture:  $n = 3$ ) and eight (Mesenteric Suture:  $n = 5$ ; Non-mesenteric Suture:  $n = 3$ ) segments in the suture and glue group respectively ruptured on the suture line (Table 2). All anastomoses showed serosal tearing at the point of at least one suture penetration.

Table 2  
Rupture pattern from control, 2L-CT, and 1L-UV-PMA groups

	Control	2L-CT	1L-UV-PMA
Extremities	5	3	2
Mesenteric Mucosa	10	6	5
Mesenteric Suture		3	5
Antimesenteric Suture		3	3
<b>Non-suture site</b>	<b>10</b>	<b>6</b>	<b>5</b>
<b>Suture site</b>		<b>6</b>	<b>8</b>
2L-CT: 2-layers anastomosis; 1L-UV-PMA: 1-layer anastomosis and UV-polymerizable methacrylate adhesive application			

## Macroscopic Evaluation

On macroscopic evaluation, the 1L-UV-PMA anastomosis formed a wider anastomosis site than the 2L-CT anastomosis, that looked like a small tunnel instead of a ring of constriction. After testing, some of the samples from the 1L-UV-PMA group showed shreds of glue detached from the serosa, while all samples from the 2L-CT group showed tearing of the serosa on the second suture line.

## Discussion

This study compared the use of UV-PMA with a Cushing pattern while performing a second layer on jejunal anastomosis. UV-PMA was as effective as ligatures in terms of strength and luminal reduction but quicker to apply.

Instead of over-sewing the first hand-sewn layer we opted for placement of an UV-PMA. Previous reports have shown that a one-layer anastomosis is sufficient in horses to achieve good anastomosis, and some practitioners use this technique (5, 31–35). In our study, a one-layer anastomosis with glue application resulted in a tight-sealed anastomosis that ruptured at equivalent BSP than a two-layer anastomosis. This first layer hand-sewn anastomosis was mandatory to obtain a good apposition between the different segments before glue application.

Values obtained in our study for BSP were close to what has been reported in other studies (3, 5, 31–34). Some of these studies showed a significant difference between anastomosed and healthy intestine (3, 5)

but not all of them had a control group. Although the difference in BSP between the 1L-UV-PMA and the control group was significant, BSP values in our study were far above what has previously been reported to occur clinically in horses (36). Because of its liquid and plastic properties, the UV-PMA glue represents a tighter seal than a Cushing pattern and could therefore limit leakage in the immediate post-operative period.

LDR showed no statistical difference in our study. Values obtained for both anastomosed groups are higher than the maximal 44.6% described in the literature (1). The reason for this high luminal reduction might come from suturing the first layer with a full-thickness continuous pattern instead of a submucosal continuous pattern as is more commonly performed (1).

Almost half of the anastomoses ruptured at the suture line, highlighting the importance of careful surgical technique when performing bowel resection and anastomosis in clinical cases. Nieto et al (31) reported that incorporation of the submucosa in the suture line is the most important step when suturing, and that incomplete incorporation of this layer would cause failure adjacent to the anastomosis. In our study there were no ruptures adjacent to the suture line, but failure at the suture line could be caused by incomplete incorporation of the submucosa as well. The ruptures in our study occurred most of the time at the mesenteric border, as previously reported (5, 33–35). This site of weakness may be induced by improper knot placement or inaccurate seromuscular suture placement.

On a visual assessment, the 1L-UV-PMA anastomosis caused a larger constriction on the anastomosis site and was stiffer regarding distension than the 2L-CT anastomosis. In the immediate post-operative period, a constriction of the anastomosis site may interfere with the passage of ingesta. Intussusception has been shown to appear secondary to inflammation or during the postoperative period in humans and horses (37, 38), and the formation of a constriction ring could create a lead point for an intussusception. Improvement of the technique could be to only use the glue at critical locations, such as the attachment to the mesentery or at the suture knots, to enhance sealing properties and limit contamination, as well as to decrease the risk of a constriction ring.

Intestine anastomoses have been reported to be weakest at 3–7 days after surgery (39, 40). As we opted for an *ex vivo* study, we could only evaluate the strength of the anastomosis at the time of construction. However, evaluation of the long-term healing of the anastomosis site, such as inflammation, or other effect of the glue (e.g. adhesions) within the abdominal cavity could not be obtained.

So far, several changes are necessary on the glue to make it suitable for clinical cases. The first glue component was very fluid and tended to spread all over the bowel and the surgical area. Extra care should be taken not to spill any glue within the abdomen by adding supplementary surgical drapes to isolate the site of anastomosis before application. The second glue component had a more viscous consistency and was easier to apply on the suture line. When submitted to high pressures, the glue kept its initial form and formed a constriction ring, resisting tissue distention at the anastomosis site. Therefore, the tubular shape of the bowel should be considered when polymerizing the glue with ultraviolet light. The UV-PMA is non-resorbable in its current form, and with the formation of a

constriction ring, the use in live animals is therefore limited. A permanent constriction site on a regenerating intestine could lead to permanent stenosis and recurrent colic signs. Being able to manufacture a bioresorbable intestinal glue would be a great improvement, and lead to clinical use in all types of horses, with minimal risk of permanent stenosis after anastomosis. The manufacturer has been notified of these limitations at the end of the study and some details have already been improved.

Although construction time was faster for the 1L-UV-PMA group than for the 2L-CT group, a difference of approximately 5 minutes might not be clinically and economically relevant. However, a liquid sealant for anastomosis used on several key locations such as the mesenteric border or the knots of the suture line could lead to a decrease in post-operative leakage and sepsis from the anastomosis site.

## Conclusion

This study shows that a Cushing pattern or the use of UV-PMA as the second layer of a two-layer jejunal anastomosis have a similar BSP and LDR, and the UV-PMA application is faster to perform than a hand-sewn second layer.

However, the glue layer forms a stiffer and larger anastomosis ring than a hand-sewn anastomosis and has physical properties issues in its current form. Some modifications in the technique of application need evaluation before the possibility of clinical trials.

## Methods

Small intestinal segments were collected from 15 client-owned horses euthanized for reasons unrelated to disease of the gastrointestinal system. Horses were free of any signs of colic within 24 hours before euthanasia and owner consent was obtained for donation for research. Intestinal segments were harvested immediately after euthanasia and anastomosis, bursting pressure test and lumen reduction measurements were all performed within the following 4 hours.

### *Intestinal specimens*

A segment of 1.5 to 2 meters of jejunum was harvested. Three to five centimeters of mesentery was kept on the mesenteric border. The segment was lavaged with tap water to remove ingesta and stored in a saline (0.9% NaCl<sup>2</sup>) solution at room temperature throughout the study except during anastomosis and testing procedures. Three samples of 30 to 40 cm were obtained from each harvested segment to perform a two-layer hand-sewn anastomosis (2L-CT group), a one-layer hand-sewn anastomosis sealed with an UV-PMA layer (1L-UV-PMA group) and a control segment (Control group).

### *Anastomosis techniques*

A two-layer anastomosis and a one-layer anastomosis with application of methacrylate glue were realized on each sampled horse by the same ECVS Resident. All intestinal samples for the suture and

glue groups were transected at mid-distance from each end at about 60° to the mesenteric attachments before performing the anastomosis.

### ***2-layer Anastomosis (2L-CT Group)***

Polyglycolic acid USP 2-0 on a taper cutting needle<sup>3</sup> was used in a hemicircumferential simple continuous full thickness pattern to appose all layers of the intestinal wall, which was interrupted at mesenteric and anti-mesenteric borders to avoid a string-purse effect. Suture bites were taken approximately 5 mm apart and 3 mm from the incised edge. The same suture material was then used in a hemicircumferential Cushing pattern, started at the middle of the first layer sutures (3 and 9 o'clock) in order to avoid knot superposition between the two layers. Bites were placed 3 mm from the first layer and 5 mm apart (Figure 1).

Construction time from the first bite of the second layer to the final knot was recorded.

### ***1-layer Anastomosis and UV-PMA(1L-UV-PMA Group)***

The same technique as in the 2L-CT group was used for the first layer. After this the intestinal sample was dried with a gauze swab, before application of the glue.

The adhesive consists of 2 separate layers and is liquid in its initial form. It solidifies with the aid of UV-light at a wavelength of 395 nm.

The first layer of the surgical glue was applied directly on the suture, on a width of about 5 mm on each side of the suture and was polymerized by the action of the UV LED curing lamp for 30 seconds. Then the second layer was applied over the first one and polymerized by the action of the UV LED curing lamp for 30 seconds. The intestine was rolled between the surgeon's fingers while the UV lamp was applied to polymerize the glue on the entire circumference of the bowel.

Construction time for glue application was recorded from the end of the first suture to completion of the second 30-sec UV-light period.

### ***Evaluation of Luminal Diameter Reduction (LDR)***

Before mechanical testing, each segment was distended to an intraluminal pressure of 20 mmHg, as previously described (5). Ultrasonography was used to determine luminal diameter reduction for 2L-CT and 1L-UV-PMA groups.

Ultrasonography was performed using a 6-15 MHz linear transducer.<sup>4</sup> For each group, longitudinal images on the anastomosis were taken. Care was taken to obtain the largest diameter possible. The samples were held in a linear position by an assistant. Luminal diameter measurements were determined from these images using a linear function of the ultrasound unit, on the anastomosis site (Measurement A) and at 2 cm proximal and distal to the anastomosis (Measurements B and C, Figure 2).

Mean value of measurements B and C served as reference for normal luminal diameter. LDR at the anastomosis site was calculated by dividing the luminal diameter calculated at the anastomosis site (Measurement A) by the mean normal diameter:

$$LDR = \frac{2 \times A}{B + C}$$

### ***Bursting Strength Pressure (BSP) Testing***

After completion of the anastomosis, the intestinal segment was placed in a water tank for bursting strength pressure testing. The same method as previously described was used (3,5,6,31–35). Briefly, each intestinal segment was submerged in 30 L saline solution within a water tank at room temperature. Infusion sets were inserted at each end of the intestine, and a knot was made with a polypropylene string around the intestine to provide a watertight seal between the intestinal wall and the infusion set (Figure 3). One infusion set was connected to a roller pump<sup>5</sup> for fluid delivery. The other infusion set was connected to a T-connector attached to a pressure transducer.<sup>6</sup> The pressure transducer was then connected to a computer to assess real-time pressure within the bowel on a dedicated software.<sup>7</sup> A balanced electrolyte solution (Hartmann's solution) tainted with methylene blue (2mL/L, 0,2%) was infused in the bowel at constant rate (700 mL/min, pump's maximum) until failure occurred. Bursting Strength Pressure (BSP) was determined as the maximal pressure obtained before failure. Failure was first detected as apparition of blue tainted flow inside the saline bath. With time visual rupture of the intestine could be observed. Every trial was video recorded and re-assessed to describe the mode of failure.

Modes of failure were recorded as: "Extremities" when the knots around the infusion sets ruptured before the intestine; "Mesenteric Mucosa" when the mucosa and muscularis ruptured and the intestine ballooned without rupture of the serosa on the mesenteric border of the intestine at a location more than 2 cm away from the suture; "Mesenteric Suture" when the suture line ruptured at the mesenteric border of the intestine, "Non-mesenteric Suture" when the suture ruptured at a location different from the mesenteric border (Figure 4).

The same mechanical testing was performed for the control group.

Macroscopic evaluation of the anastomosed segments was performed before and after testing. The anastomosis before and after BSP testing was observed for abnormalities and shredding of the glue, and the mode of failure was confirmed after BSP test by visual assessment.

### ***Statistical Analysis***

Descriptive statistics were reported as mean [95% CI]. Data was tested for normality with a Shapiro-Wilk test.

A paired *t*-test was realized to evaluate a statistical difference in construction time, BSP and LDR.

Mode of failure was assessed using a chi<sup>2</sup>-test between suture-related failures (Mesenteric and Non-mesenteric Suture types of failure) and non-suture-related failures (Mesenteric Mucosa types of failure).

Statistical analysis was performed using excel software<sup>8</sup> and for all statistical tests a *P*<0.05 was considered significant.

## List Of Abreviations

1L-UV-PMA: One-Layer Ultraviolet Polymerizable Methacrylate

2L-CT: Two-Layer Conventional Technique

BSP: Bursting Strength Pressure

ECVS: European College of Veterinary Surgeons

LDR: Luminal Diameter Reduction

UV-PMA: Ultraviolet Polymerizable Methacrylate Adhesive

## Declarations

### *Ethics Approval and consent to participate*

Not applicable

### *Consent for publication*

Not Applicable

### *Availability of data and materials*

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### *Competing interests*

Perrin B.R.M. is the CEO of Cohesives, the company providing the glue. He had a part in reviewing the report but did not participate in the experimental part and the initial writing.

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Not applicable

### ***Authors' contributions***

AL: Study design, study execution, statistics, writing of the manuscript, final manuscript approval;

BP: Study design, writing of the manuscript, final manuscript approval;

OL: study design, writing of the manuscript, final manuscript approval.

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Not applicable

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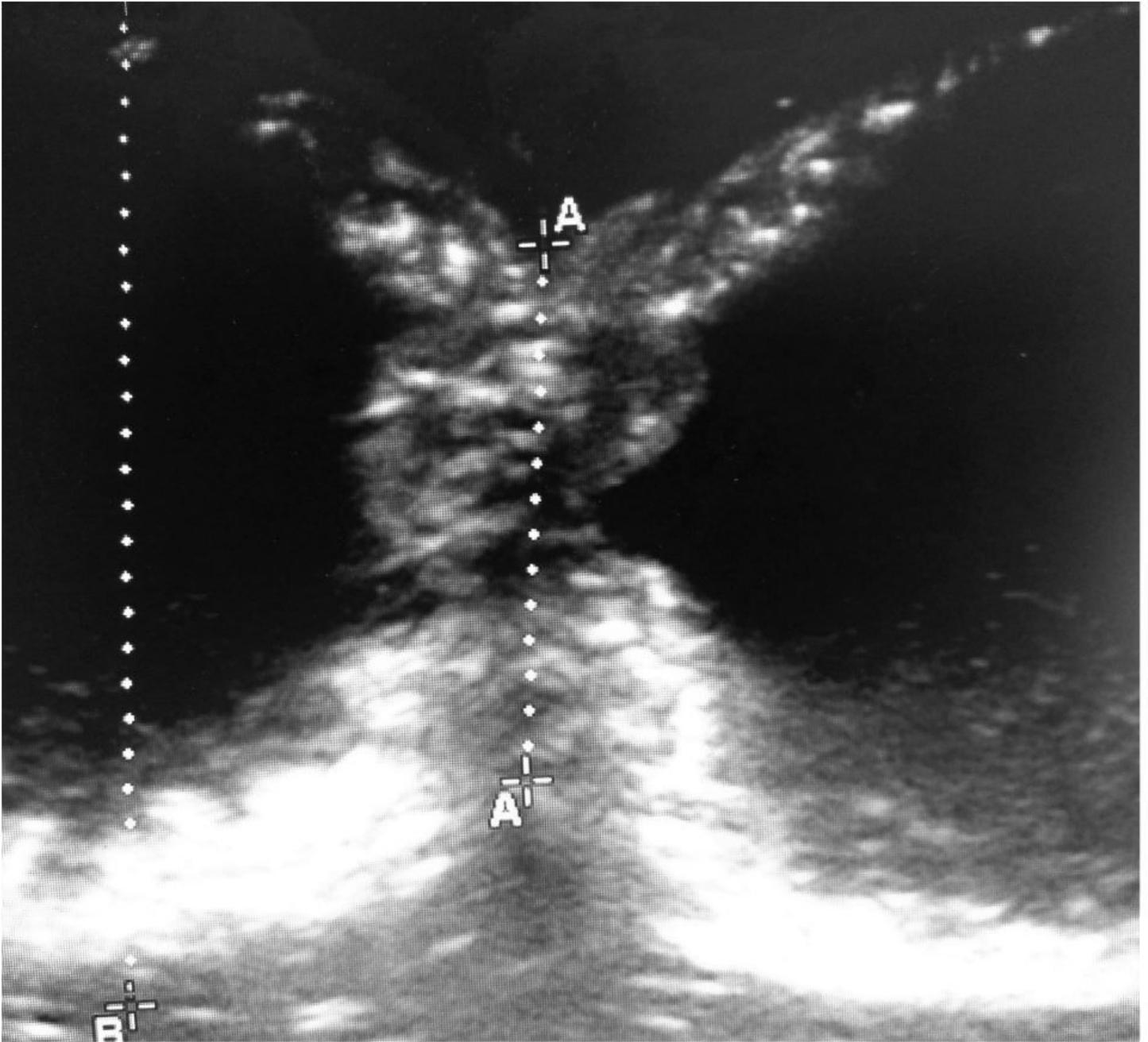
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## Figures



**Figure 1**

Intestinal samples ready for testing. A. Double-layer hand-sewn anastomosis; B. One-layer anastomosis covered by the UV-polymerizable methacrylate adhesive. The star is located at the mesenteric border.



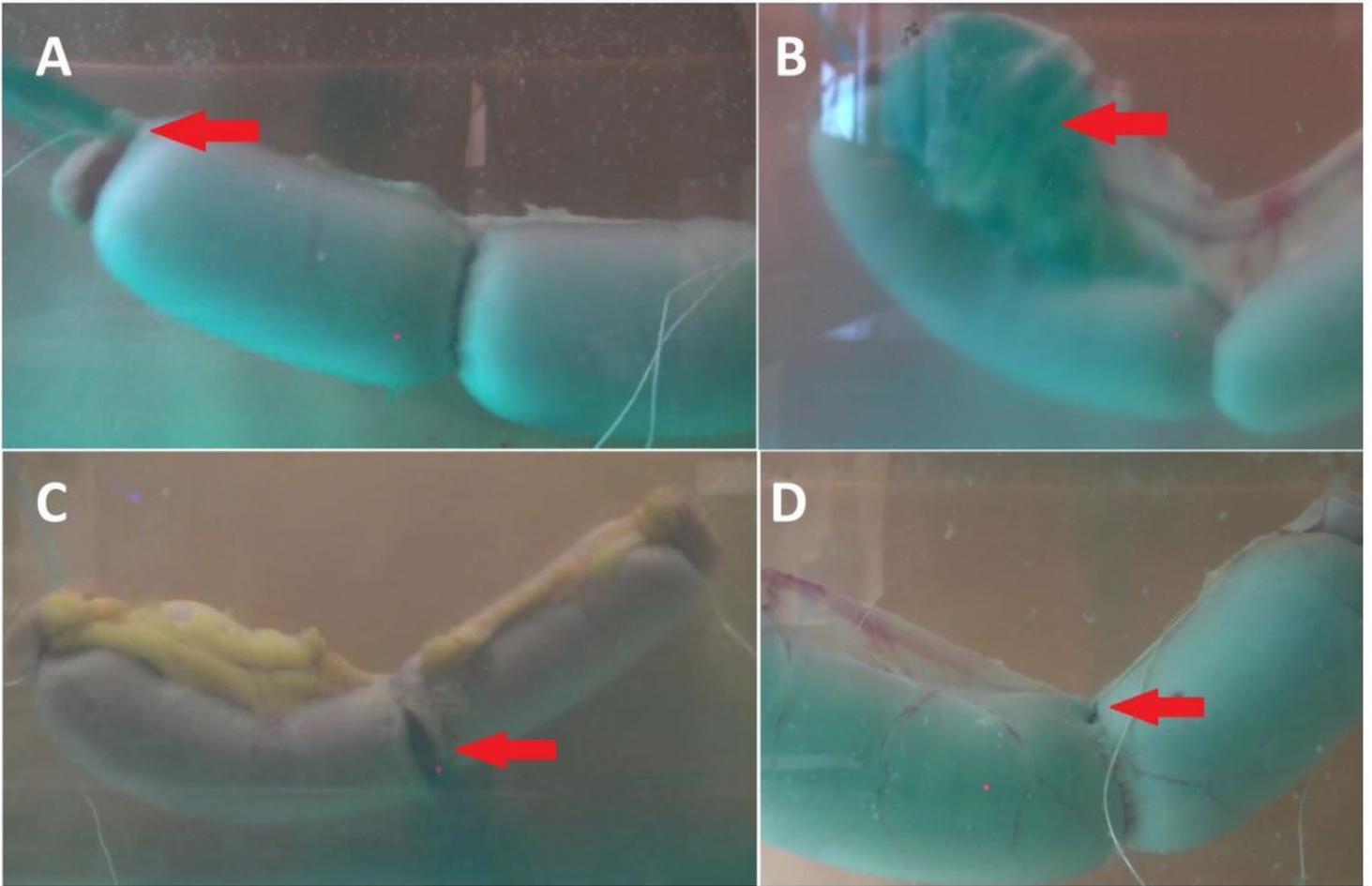
**Figure 2**

Ultrasound image recorded for Luminal Diameter Reduction. The "A" dotted line is at the location of the suture line, the "B" dotted line is positioned 2 cm cranial to the anastomosis. A third measurement is made on the other side of the anastomosis 2 cm caudal to the anastomosis.



**Figure 3**

The intestinal segment (1L-UV-PMA group) is submerged in a water tank, ready for bursting strength pressure testing. The infusion set on the left is connected to the roller pump for fluid infusion, the infusion set on the right is connected to the pressure sensor. Note the tunnel-like anastomosis.



**Figure 4**

Modes of failure after bursting strength pressure testing. The red arrows show the location of the failure. A: "Extremity" mode of failure, 2L-CT group; B: "Mesenteric mucosa" mode of failure, 2L-CT group; C: "Non-mesenteric suture" mode of failure, 1L-UV-PMA group; D: "Mesenteric suture" mode of failure, 1L-UV-PMA group.