

# Single-Layer versus Double-Layer Laparoscopic Intracorporeally Sutured Gastrointestinal Anastomoses in the Canine Model

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## ABSTRACT

**Objective:** The objective of this study was to compare the gross and histopathologic changes following 1- versus 2-layer hand-sewn suture techniques in laparoscopic gastrointestinal anastomosis in dogs.

**Methods:** This was an experimental prospective study of 16 healthy mixed breed male and female dogs. Animals were randomly divided into 2 groups. Two-layer side-to-side hand-sewn laparoscopic gastrojejunostomies were performed in group A, so that simple interrupted sutures were placed in the outer layer and simple continuous suture was used in the inner layer. The 1-layer simple continuous anastomosis between the stomach and jejunum was done in group B precisely. Specimen were collected from the sites of anastomosis, and H&E staining was performed for light microscopic studies.

**Results:** All animals survived the surgery. There was no gross inflammation, ischemia, apparent granulation tissue, abscess or fistula formation, leakage or stricture formation, and all sites of anastomosis were patent. Several adhesion formations were found in the abdomen with the higher incidence in the control group. Mean scores of leukocyte infiltration and granulation tissue formation at the sites of anastomosis were statistically insignificant between groups ( $P > 0.05$ ).

**Conclusions:** Gross and histopathologic findings revealed that hand-sewn laparoscopic gastrointestinal anas-

tomosis with the 1-layer suture technique is comparable to the 2-layer suture technique.

**Key Words:** Gastrointestinal anastomosis, Laparoscopy, Histopathology, Canine.

## INTRODUCTION

Gastric out-flow tract disease is an overall term used to describe a variety of diseases that cause reduced or delayed gastric emptying. Definitive treatment is usually surgery, and the purpose of surgical management is to re-establish normal gastric emptying. Numerous surgical procedures have been described to relieve gastric outflow obstruction.<sup>1,2</sup> Gastroduodenostomy (Billroth I) and gastrojejunostomy (Billroth II) are common procedures performed to manage gastric outflow obstruction and are usually performed for resection of gastric adenocarcinoma.

Research indicates that laparoscopic procedures are superior to open surgeries, because they reduce morbidity. One of the advantages of the technique in gastrointestinal surgeries is the reduction in the period of postoperative intestinal paralysis so that gastrointestinal function returns rapidly to its normal status.<sup>3</sup> It also involves a reduced immune response compared with open surgery.<sup>4</sup> This technique not only results in a reduction in tissue desiccation and foreign body contamination but also decreases intraabdominal adhesions. Today, the technique has gained wide clinical acceptance in surgical practice.<sup>5</sup> Some reports on performing laparoscopic gastrointestinal anastomosis have been summarized successively.<sup>6-8</sup> The feasibility and safety of laparoscopic Billroth II gastrectomy in a canine model was reported by Soper et al in 1994.<sup>8</sup> Surgeons agreed that the laparoscopic gastrointestinal anastomosis is superior to the open technique, because of faster recovery, less pain, and better cosmesis.<sup>9</sup>

Multiple studies in the veterinary literature examine the effectiveness of various techniques to create gastrointestinal anastomosis.<sup>10</sup> In addition, gastrojejunostomy has been reported by the use of several suture techniques

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including 2-layer or 1-layer techniques and the use of staplers. Two-layer techniques include simple continuous (both layers), simple interrupted (external layer), simple continuous (inner layer), and an inverting pattern like lembert (external layer), simple continuous (inner layer). One-layer techniques using a full-thickness simple interrupted, continuous inverting, simple continuous, and continuous vertical mattress have also been reported.<sup>11-15</sup> Leakage is an important complication of resection of the intestine that is often followed by serious morbidity or death. If the surgical technique is sound, the single-layer anastomosis will be efficient and safe.<sup>16</sup> In previous studies, we have shown that mean surgical time and mean blood loss are comparable in 1- and 2-layer hand-sewn suture techniques in gastrointestinal anastomosis. Also, contrast radiographs revealed no organ displacement, all anastomosis sites were patent; the 1-layer suture technique is safe and feasible compared with 2-layer anastomosis.

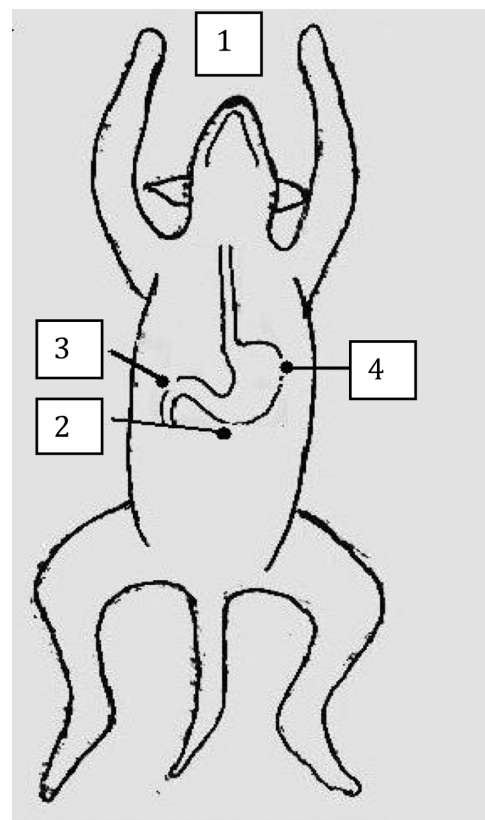
The objective of the present study was to compare the gross and histopathologic changes following 1- versus 2-layer hand-sewn suture techniques in laparoscopic gastrointestinal anastomosis in dogs.

## MATERIALS AND METHODS

### Operative Technique

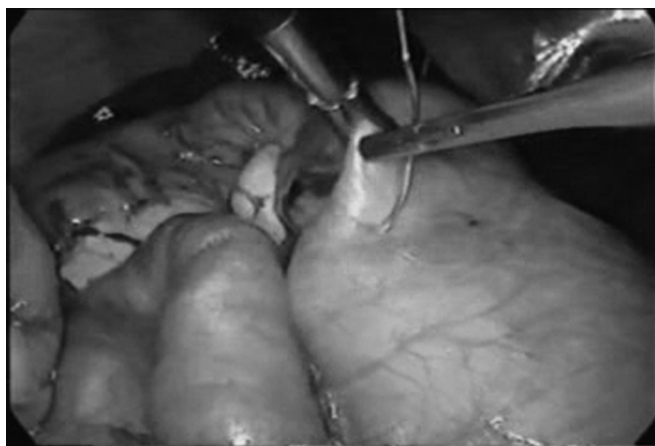
Sixteen healthy adult dogs of both sexes weighing 14kg to 17kg were maintained under similar housing and feeding conditions. Approval was received from the University of Tehran Research Committee according to the rules of ethics issued by the Animal Care Committee Research Council. The dogs were randomly divided into 2 groups: group A (control) and group B (treatment). Cefazolin (20mg/kg, IV) was administered preoperatively, and food was restricted for 12 hours before the surgery. After induction of general anesthesia according to a standard protocol, the abdomen was prepared and draped for aseptic laparoscopic surgery. Dogs were positioned in dorsal recumbency in a 30° Trendelenburg position (head down) for laparoscopic surgery through 3 portals. A variation of the standard closed trocar technique was used for the first trocar, without the use of a Veress needle. The first trocar was placed at the umbilicus after a 10-mm skin incision was made, and the subcutis was prepared until revealing the linea alba. The primary camera trocar was inserted while the ventral abdominal wall was pulled up to avoid trauma to visceral organs then insufflation with

CO<sub>2</sub> was started. An automatic high-flow CO<sub>2</sub> insufflator (Olympus) was connected to the laparoscopic cannula at a pressure of 14mm Hg to 18mm Hg. A 10-mm diameter 0° rigid telescope (29cm length, Wolf, Knittlingen, Germany) connected to a light source and inserted into the peritoneal cavity from the umbilicus, and a 360° scan was performed for evaluation of any abdominal abnormalities. Under direct vision, the second and third trocar 5-mm diameter sheaths, 10-cm in length were inserted through the 5-mm skin incisions. The second trocar was placed in the right lateral subcostal, and the third one was placed in the left lateral subcostal (**Figure 1**). The laparoscopic procedures were recorded on videotape. Surgery started with the surgeon standing between the dog's legs, and the camera operator was on the left side of the dog. At first, the antrum was identified and transversally closed by using a 0 polyglycolic acid (Dexon, Covidien, Dublin, Ireland) suture with a figure 8 technique to occlude the pylorus and outflow tract. Then, the beginning loop of jejunum was identified and brought up to the ventral surface of



**Figure 1.** Port placement for laparoscopic gastrojejunostomy procedure. 1: Monitor, 2: Camera operator, 3 & 4: 5-mm laparoscopic forceps.

the greater curvature of stomach that was decompressed by the esophagogastric tube if needed. A 2-cm side-to-side hand-sewn anastomosis between the greater curvature of the stomach and a proximal loop of jejunum was performed as follows: In group A, seromuscular simple interrupted sutures were placed between the stomach wall and jejunum first (**Figure 2**). Then a monopolar cautery was inserted to the abdomen through the left portal and a 2-cm longitudinal full-thickness gastric incision was made in a hypovascular area of the ventral aspect of the stomach, between the greater and lesser curvatures. A 2-cm longitudinal full-thickness intestinal incision was also made in the antimesenteric border of the selected proximal loop of jejunum. Both incisions were created while the stomach and the intestine were pulled upward by using a 5-mm diameter grasping forceps to prevent abdominal contamination. Then mucosa and submucosa of the stomach were sutured to the intestine by using simple continuous sutures. Another row of simple continuous sutures was placed in the contralateral side of the incision between mucosa and submucosal layers of the stomach and intestine. Finally, the last row of simple interrupted seromuscular sutures was placed to complete the gastrojejunostomy. In group B, after a row of simple continuous sutures was placed between the greater curvature of the stomach and proximal loop of jejunum, both gastric and intestinal incisions were created in the same way using a monopolar cautery. Then a full-thickness stomach wall was sutured to the intestinal wall with a simple continuous suture pattern to perform side-to-side



**Figure 2.** The ski-shaped needle is inserted into the stomach wall (right) while it is grasped upward by the grasping forceps (up). The proximal loop of jejunum (left) is being sutured to the stomach.

gastrojejunal anastomosis. As explained previously in group A, laparoscopic gastrojejunostomy was done by using a 2-layer side-to-side anastomosis technique, and in group B, a 1-layer anastomosis was performed by using the simple continuous pattern including all tissue structures. The suture material was 3-0 polyglycolic acid (Dexon) on a 26-mm taper point needle, formed to ski-needle shape before the procedure. Sutures were placed while the 5-mm needle holder was inserted from the right portal, and the 5-mm endoscopic grasping forceps was inserted from the left portal. All surgeries were performed by the same surgeon. Administration of Oxymorphone (0.1 mg/kg, IV) as analgesic was done, and Cefazolin (20 mg/kg, 3 times daily, IV) was continued 3 days postoperatively. The animals were sacrificed 4 weeks after the operation.

Recorded parameters were as follows:

*Complications* - All complications including intraoperative and postoperative complications were recorded in both groups.

*Macroscopic findings* - including any signs of inflammation, infection, and diameter of the stoma.

*Histologic evaluations* - including scores of infiltration of leukocytes, granulation tissue formation, and formation of a cyst. Scores were interpreted as 0: Negative, 1: Very few, 2: Few, 3: Moderate, 4: Numerous, 5: Very numerous, which is common for nonparametric variables.

## Statistical Analysis

Statistical analysis and comparisons were determined using the Student *t* test and proportions were compared using the Mann Whitney.  $P < 0.05$  were considered statistically significant.

## RESULTS

### Complications

Minor complications were noted in 7 cases for both groups. No major intraoperative complications occurred, except incomplete avulsion of the mesenteric vessels that resulted in bleeding (in 2 patients in group A). Minor injury to the spleen (one in each group) and liver (one in group A) was seen. Also no leakage or stenosis was observed at the site of the anastomoses during the study. There was no need for conversion to open surgery. Skin emphysema was seen in one dog in group A and one dog in group B.

### Macroscopic Findings

There was no gross inflammation, hemorrhage, infection, ischemia, or apparent granulation tissue, abscess, or fistulae formation found on necropsy. There was no evidence of stricture or leakage at the site of the anastomosis and all were patent. The mean diameter of the stoma was ( $17 \pm 2$ mm) in group A, which was less than that in group B ( $18 \pm 3$ mm) ( $P > 0.05$ ). Accumulation of serous, fibrinous, or purulent exudates was not observed. Despite adhesions, there were no reports of organ displacement.

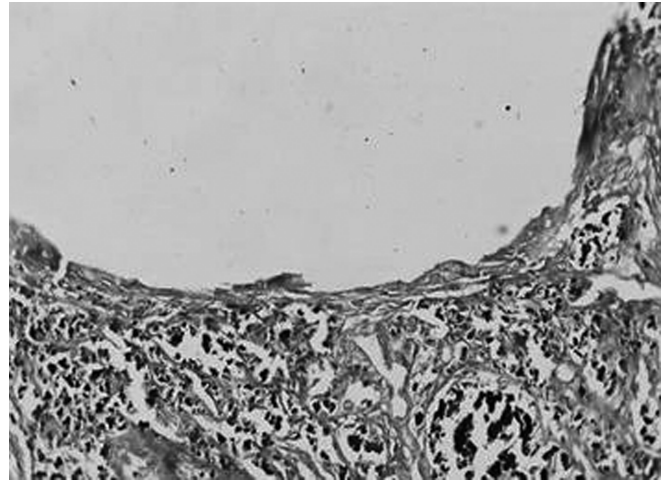
The following adhesions were observed on the macroscopic evaluations:

- between subcutaneous tissue and site of trocar insertion of the camera in one patient in group A,
- between falciform ligament and site of trocar insertion of the camera in 2 patients in group A and 1 patient in group B,
- between omentum and site of trocar insertion of the camera in one patient in group A,
- between omentum and the aponeurosis part of the diaphragm in one patient in group A,
- between omentum and the site of the anastomosis in all patients in both groups,
- between the site of the anastomosis and falciform ligament in one patient in group A and 2 patients in group B,
- between the site of the anastomosis and right medial lobe of the liver in one patient in group B.

Mesenteric lymphadenopathies were found in 2 patients in group A. There was no evidence of gallbladder impaction or stenosis of the common bile duct.

### Histologic Evaluation

Inflammatory reaction ranged from mild to granulomatose around the residues of sutures at the site of the anastomoses (**Figure 3, Table 1**). No evidence was found of edema, hemorrhage, necrosis, or infection in either group, but scars formed in 2 patients in group A. No abscesses or microabscesses were observed. Epithelial migration at the site of the anastomosis was apparent in all cases, and the process of healing was completed in all patients in both groups. Multiple lymph nodules in lamina propria of the sites of the anastomoses were observed only in 2 cases of group A. Scores of infiltration of leukocytes, granulation tissue formation, and formation of a cyst at the sites of the anastomoses were statistically insignificant within and



**Figure 3.** Cross section of suture material residues at the anastomotic site. No inflammatory response is observed in this image (H&E stain; magnification: 400 $\times$ ).

**Table 1.**

Scoring of Parameters in Histological Evaluations of Dogs in 1-layer Suture Technique (Group A) and 2-layer Suture Technique (Group B) in Laparoscopic Gastrointestinal Anastomosis

Parameters	Group A	Group B
Leukocyte infiltration	4	2
Mononuclear cell infiltration	4	2
Granulation tissue formation	5	2
Glandular necrosis	5	3
Scar formation	1	0

\*0=Negative; 1=Very few; 2=Few; 3=Moderate; 4=Numerous; 5=Very numerous.

between groups ( $P > 0.05$ ). Histological evaluation of the repair revealed no significant difference between groups ( $P = 0.156$ ).

### DISCUSSION

Animals with gastric outflow obstruction usually are candidates for re-establishing normal gastric flow from the stomach to the lower parts of the intestine. Very few reports are available in the literature of laparoscopic gastrojejunostomy in animals.<sup>17</sup> Although there are several reports of using different suture pattern techniques in laparoscopic gastrointestinal anastomosis, to the authors' knowledge the safety of the 1-layer suture technique for hand-sewn laparoscopic gastrojejunostomy in dogs and

humans has not been determined. Because laparoscopic-assisted gastrectomy has been increasingly reported as the treatment of choice for early gastric cancer, it was felt that the safety and efficacy of this suturing technique should be demonstrated in an animal model before it is used in human operating rooms.<sup>18</sup>

The purpose of this clinical study was to compare the outcome of the 1-layer suture technique with the 2-layer technique in gastrojejunostomy in dogs. The experimental 1-layer continuous suture technique used for the gastrointestinal anastomosis in this study appears to be safe in dogs, because no patients in group B (treatment group) developed any clinical signs of postoperative leakage or peritonitis. A simple continuous suture pattern was used, because it is faster and easier to apply. Boure et al<sup>19</sup> in 2005 set up the experimental study of usage of laparoscopic 1-layer closure of the bladder in calves. He stated that if the rupture of the bladder occurred it would be at the incision line. During the surgeries, the sutures were placed so that the distance between bites was around 5mm and positioning of the tissue structure was well established. The positive intraabdominal pressure established by pneumoperitoneum prevented abdominal leakage from the intestine or stomach during the procedure. No leakage happened at the sites of anastomosis postoperatively.

The most important complication of anastomosis is leakage, and despite all improvements in noninvasive surgeries, leakage still remains a serious problem with high mortality.<sup>14,17</sup> There are different reports of leakage rates in gastrointestinal anastomosis. Factors such as sufficient blood supply, lack of tension, and well-apposed tissues at the anastomotic site are important issues of the successful anastomosis. Several factors have been mentioned as a predisposing factor for leakage at the gastrointestinal anastomotic site, such as sex (males are more affected than females are), nutritional defects, presence of peritonitis before surgery, trauma, concurrent infections, malignancies, diabetes, corticosteroids, azotemia, hypoproteinemia, and hypoalbuminemia.<sup>17,20,21</sup> Also it has been proved that the most crucial period is 3 to 5 days postoperatively, when the anastomosis site is the loosest due to fibrinolysis and collagen decomposition.<sup>10</sup> In 2003, Ralphs et al<sup>22</sup> reviewed 115 gastrointestinal anastomosis treatments, and he reported 14% anastomotic leakage and that 85% of patients died despite treatment. Soper<sup>8</sup> reported no anastomotic leakage in his experimental laparoscopic gastrojejunostomy. Although training and following principle laparoscopic techniques are important issues in preventing complications like leakage, a study of 200 laparoscopic

gastrojejunostomies in 2006 showed that even highly skilled surgeons may not be an index to eliminate the incidence of leakage.<sup>23,24</sup> In 2005, Fujiwara et al<sup>25</sup> reported a statistically significant lower incidence of gastrointestinal leakage with the laparoscopic compared with the open technique for gastric cancer treatment. We encountered complications during the surgical procedures. Those were all because of injuries by the tips of laparoscopic instruments, which resulted in minor bleeding. These are important events that may happen during placement of laparoscopic portals. There were no complications related to the surgical procedure or general anesthesia. Also in macroscopic evaluations, there were no reports of hernia in portal sites, and all sites healed normally, except for only one adhesion that was seen at the site of trocar insertion at the umbilicus. According to previous studies, the incidence of hernias at the trocar site is around 1% of abdominal laparoscopic procedures, which were due to the use of 10-mm trocars. There were reports of a few treatments that resulted in hernia, hematoma, subcutaneous hemorrhage, or infection at the trocar site.<sup>9-11</sup> Other risk factors related to the incidence of hernia are long operations and insufficient emptying of the pneumoperitoneum.<sup>12</sup> Several adhesions formed in the abdominal cavity that did not lead to organ displacement. Adhesion formation, seen in all patients, occurred mostly between the site of the anastomosis and the omentum. This was on the ventral side of the anastomosis covered by omentum after the operation was completed, lowering the risk of leakage. Many surgeons have recommended this method.<sup>13,14</sup> The incidence of adhesions was higher in group A than in group B, which may be due to the greater manipulation of tissue, trauma, and injuries from the laparoscopic tools during performance of the anastomosis in group A. Soper<sup>8</sup> reported local adhesion formation in laparoscopic gastrojejunostomy in dogs. No adhesions have been reported in laparoscopic gastrojejunostomy in pigs, because of the use of a stapler. Lack of adhesion formation is one of the advantages of using staplers.<sup>15</sup>

The stoma was checked at necropsy. They were all patent and no stricture was observed. This finding is the same as that of Soper<sup>8</sup> in his report on laparoscopic gastrojejunostomy in dogs. But the diameter of the stoma was decreased compared with the diameter created at the time of surgery, because of the contractive phase of healing. There were no significant differences in diameter of the stoma between the 2 groups ( $P>0.05$ ). The ideal diameter of the stoma in gastrojejunostomy has been reported in the veterinary literature to be 20mm in large breed dogs, which is consistent with our findings.<sup>26</sup> The incidence of stricture or stenosis in the lapa-

roscopic technique has been reported to be from 0% to 3%.<sup>9,10,26</sup> Mesenteric lymph node enlargement in 2 patients in group A was probably because of greater manipulation and trauma during the surgery and the presence of more residue from the sutures that worked as a foreign body at the site of anastomoses. A variety of inflammatory reactions were observed around suture material at the anastomotic site. According to histological scoring, there was no significant difference in the healing situation between the 2 groups. Submucosal apposition has been shown to be worse with a 2-layer intestinal closure technique than with 1-layer closure.<sup>19</sup> The proper healing process occurs when the continuity of histological structures of tissues are saved.<sup>27,28</sup>

## CONCLUSION

In the present study, the gross and histopathologic findings revealed well-established healing at the anastomotic sites without any complications, such as stenosis, stricture, gross granulation tissue, infection, or ischemia. Laparoscopic gastrojejunostomy with 1-layer hand-sewn sutures is safe without serious complications and comparable to the 2-layer suture technique. Further work is needed before a 1-layer laparoscopic gastrojejunostomy can be recommended clinically.

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