Use of intraluminal protection in colonic anastomosis in dogs[1]

Uso de protetor intraluminal em anastomose colônica em cães

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ABSTRACT

Purpose: To test the use of intraluminal protection in colonic anastomosis without intestinal cleansing. The intraluminal liner was fashioned from porcine submucosa preserved in glycerin and then fixed 10 cm anteriorly to the anastomotic site. This technique was compared with the one used in termino-terminal colonic anastomosis without intraluminal protection. **Methods**: Twenty-eight dogs were divided into two groups of fourteen animals each. Clinical and histopathological tests were performed on the fourth and twenty-first postoperative days. **Results**: The morbidity and mortality rates were higher in animals that did not receive the intraluminal liner. Histopathological examinations in animals in which the intraluminal liner was used showed better healing, characterized by milder inflammation and increased amount of collagen. **Conclusion**: It can be concluded that the use of intraluminal protection decreases complication rates in colonic anastomosis and promotes better healing.

Key words: Anastomosis, Surgical. Colon. Suture Techniques. Wound Healing. Dogs.

RESUMO

Objetivo: Testar o uso da proteção intraluminal na anastomose colônica sem preparo intestinal. O protetor intraluminal usado foi confeccionado a partir da submucosa de suíno conservada em glicerina, e fixado a 10 cm cranialmente ao sítio anastomótico. Essa técnica foi comparada com a técnica de anastomose colônica término-terminal sem uso do protetor intraluminal. **Métodos**: Foram utilizados 28 cães divididos em dois grupos de 14 animais cada. A avaliação foi através de exames clínicos e histopatológicos. A avaliação anatomo-patológica foi realizada no quarto e vigésimo primeiro dias de pós-operatório. **Resultados**: Um maior número de casos de morbi-mortalidade foi observado nos animais operados sem o protetor intraluminal. O exame histopatológico dos animais nos quais foram usados os protetores intraluminais mostrou melhor cicatrização, caracterizada por processo inflamatório mais discreto e maior quantidade de colágeno. **Conclusão**: O uso do protetor diminui o número de complicações em anastomoses de cólon e melhora a cicatrização.

Descritores: Anastomose Cirúrgica. Colon. Técnicas de Sutura. Cicatrização de Feridas. Cães.

Introduction

Throughout history, surgeons have struggled against the frequent complications that result from intestinal anastomosis, particularly in colon surgery, because of the high rates of morbidity and mortality. Various studies have been conducted with the purpose of finding a method which may prevent such complications[1]. In large bowel surgery (anastomosis in particular), the consequences of anasomotic dehiscence are disastrous. Anastomotic dehiscence is more often observed in the large bowel than in the small one. Studies have shown that collagen is synthesized more rapidly in ileum wounds than in colon wounds. On the first days after colonic anastomosis, collagen degradation exceeds collagen synthesis, which is due to rapid metabolism of insoluble collagen. Resistance to traction is thus lower until the third and fourth days after surgery, and increases around the seventh day, when collagen synthesis exceeds degradation[2]. The direct contact between stool and the suture is also associated with complications that result from colonic anastomosis. Complications arise as a result of bacterial contamination[3] and stress exerted on the suture by luminal contents[4]. It has been suggested that a device for intraluminal protection be used to isolate the suture from stool, which would promote faster healing. It would also provide additional protection against wound dehiscence and consequent extravasation and fistula formation during collagenolysis[5]. It is extremely important that the protecting device be kept at the anastomotic site for at least five days, which is the critical period for cicatrization of colon wounds. Between seven and 11 days after the procedure, resistance of the intestinal anastomosis

is similar to the intestinal resistance of unoperated rats[6]. In this study, we used an intraluminal protection device fashioned from the intestinal submucosa of pigs obtained from an abattoir and preserved in glycerin. We believe that this device might reduce inflammation after colonic anastomosis by preventing contact between stool and the suture, which increases the risk of infection. The present study is relevant because it proposes a new technique in colon surgery – a low-cost, easy alternative to current methods. In addition, the relatively high risk of complications resulting from colonic anastomosis, such as wound dehiscence, abscess, fistula, peritonitis or death, increases the relevance of this study. The purpose of this study was to analyze, both clinically and histopathologically, the results of colonic anastomosis using an intraluminal protection device fashioned from a biological membrane.

Methods

A total of twenty-eight female mongrel dogs (*Canis familiaris*) weighing 5-20 Kg were used. Said dogs were donated by the kennel of the Veterinary Hospital, School of Medical Veterinary, Dom André Arcoverde Foundation, Valença, RJ. The animals were divided into two groups with 14 animals each. The surgical procedures were carried out at the Laboratory of Experimental Surgery, School of Medicine, Federal University of Rio de Janeiro. The animals included in this study had good general health. The dogs were observed for a period of 30 days, when they were given antiparasitic drugs and prophylactic treatment for infectious diseases. During this period, animals adapted well to feeding and management. Laboratory examinations were performed: complete blood count, serum urea and creatinine tests, and stool tests for parasites. All test results were considered normal. Animals were not fed 48 hours prior to the surgical procedure. After the study, the animals were donated. No dog was sacrificed. A device was fashioned from porcine submucosa (intraluminal liner) to protect the anastomosis by avoiding contact between stool and the suture site. The device was placed inside the intestinal lumen, and fixed to the portion anterior to the anastomosis. Porcine submucosa was obtained from an abattoir, Santa Isabel Ltd., which was inspected by the State Inspection Service (SIS, protocol number 428) of Alcântara, city of São Gonçalo, Rio de Janeiro. The animals from which the submucosa was obtained were duly inspected and did not have signs of infectious or neoplastic diseases. After being obtained, the submucosa was immediately put in 98% bidistilled glycerin. The intraluminal liner was kept in glycerin, at room temperature, for at least 30 days prior to its use. Then, it was washed with saline solution for glycerin removal and rehydration. For the surgical procedure, preanesthetic medication was intravenously injected (0.1 mg/Kg of acepromazine). Anesthesia was induced with intravenous sodium thiopental, and semiclosed anesthesia was maintained with halothane. The anterior portion of the abdomen was shaved, and antisepsis was performed using povidone-iodine. A midline incision of approximately 5 cm was made below the umbilicus. After reaching the abdominal cavity, the left colon was exposed. Ligation of the vessels of the area followed, and transverse enterotomy of the colon was performed. Procedures were then carried out differently for both groups.

- Group 1: after exposure, approximately 10 cm of the proximal portion of the colon was everted. The intraluminal liner was cleaned with saline (Figure 1) and placed on a syringe plunger to make its positioning easier (Figure 2). The liner was sutured to the mucosa/submucosa with 3-0 polyglactin 910, with interrupted stitches (Figure 3) to bring the intestinal segments close together for anastomosis.

- Group 2: The intraluminal liner was not used (control group).

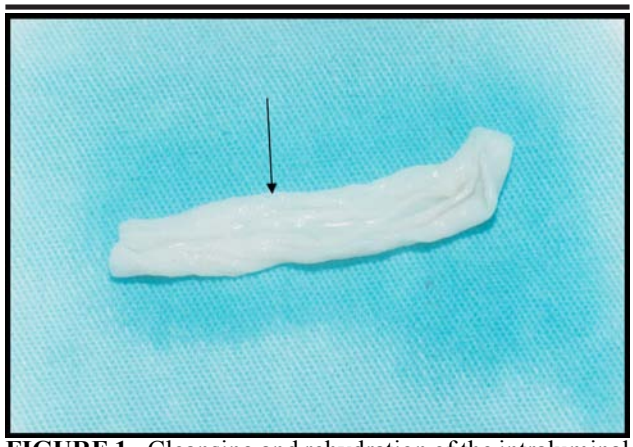

FIGURE 1 - Cleansing and rehydration of the intraluminal liner with saline solution

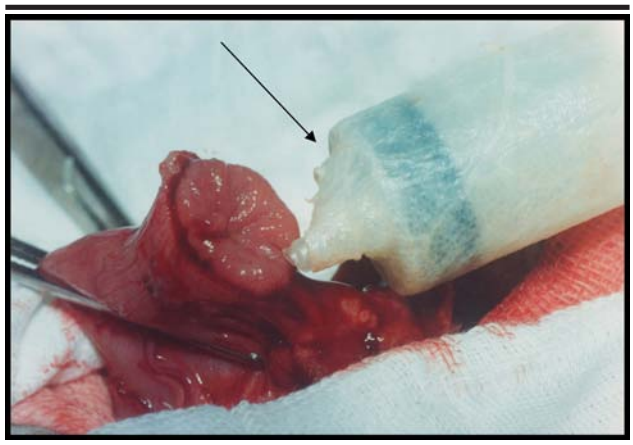

FIGURE 2 - Intraluminal liner being placed on a 10 ml syringe plunger

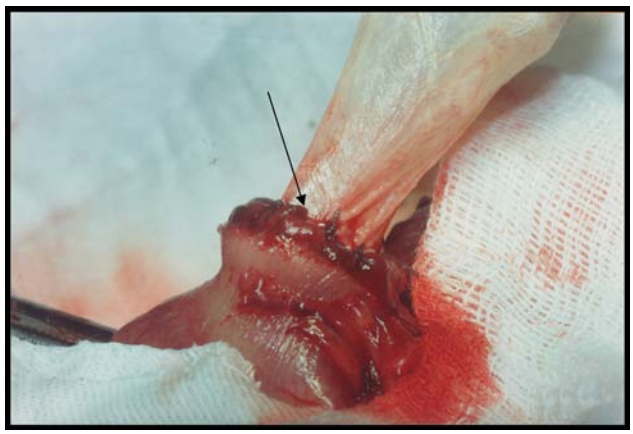

FIGURE 3 - Suturing of the intraluminal liner to the proximal portion of the everted colon

After that, the procedure was the same for both groups. Termino-terminal enteroanastomosis was performed with continuous suture using 3-0 poliglactin 910. Laparorrhaphy was performed with interrupted stitches using 2-0 mononylon suture, followed by similar suturing of the subcutaneous tissue and skin. After surgery, the animals were placed in individual cages. The surgical wound was covered with bandages and surgical tape. In addition, the animals were dressed with cloth shirts to protect the wound. The dogs had no food or water for 12 hours after surgery. After this period of time, dogs had water *ad libitum*. Twenty-four hours after surgery, the animals were fed with liquid food (made with commercial dog food), three times a day. Seven dogs from each group were clinically evaluated every day until the fourth day after surgery. Then, these dogs were reoperated on. The remaining animals were observed for 21 days after surgery and were then reoperated on. Skin stitches were removed seven days after surgery. Clinical parameters to assess surgical complications were observed during the evaluation period: rectal temperature, respiratory rate, heart rate, mucosal color, abdominal pain, surgical wound, vomiting, appetite, and stool examination. Samples from the anastomotic site were collected during reoperations for anatomic pathology analysis on the fourth and 21st days after surgery. These samples were fixed in 10% formalin. The collected material was routinely processed, embedded in paraffin and stained with hematoxylin/eosin. Antibiotic prophylaxis was carried out using penicillin (40,000 U/Kg) and analgesia was maintained with dipyrone (25 mg/kg), for three days. The results were submitted to statistical analysis, considering the mean, mode, variance, standard deviation, and variance correlation of the analyzed data.

Results

The results are presented according to the complications observed: abscess, bleeding, abdominal wound dehiscence, symptoms of intestinal obstruction probably caused by stenosis, leakage and/or fistula at the anastomotic site. All these complications could lead to morbidity and/or mortality.

Clinical evaluation

No significant body temperature changes were observed in group 1 animals. In group 2, increased body temperature was observed in dogs no. 20, 21, 25 and 27. Dog no. 21 died two days after surgery. Autopsy revealed anastomotic dehiscence and peritonitis. Normal heart rate was observed in all animals. Increased respiratory rate, which suggested postoperative pain, was observed in animals no. 1 and 6 from group 1, and animal no. 25 from group 2. Postoperative examination revealed normal mucosal color in all animals. Animals no. 2, 4, 5, 6, 9, 13 and 14 (group 1) had abdominal discomfort or pain, which disappeared over the postoperative period. The same signs were observed in animals no. 16, 18, 20, 21, 24, 25, 26, 27 and 28 (group 2). Only one animal from group 2 (animal 18) vomited on the first postoperative day. All animals had normal appetite during the entire postoperative period. The intraluminal liner (group 1) was found in the feces of three animals five days after surgery and in the feces of four animals eight days after surgery.

Anatomic pathology

Group 1

Macroscopic examination was performed during the reoperation of animals both four and 21 days after the first procedure. In animal no. 14 (4 days), a fistula was observed at the anastomosis. In animals operated on 21 days after the first procedure, adhesion formation between the anastomosis and the greater omentum was observed. In these animals, leakage, fistula, abscess and stenosis were not macroscopically observed (Figure 4).

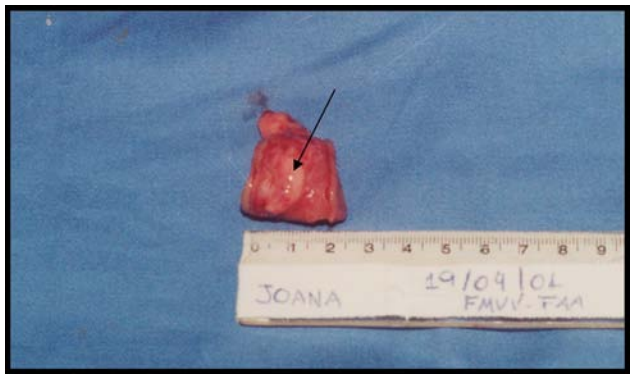

FIGURE 4 - Adhesion to the greater omentum in a dog reoperated on after 21 days (group 1)

Microscopic examination of group 1 revealed formation of scar tissue, mainly on the mucosa and submucosa, with rapid formation of granulation tissue, in animals that were reoperated on after four days. Angiogenesis and fibroblast proliferation were evident in all specimens. Fibroblast activity was intense, with synthesis of extracellular matrix components – mainly collagen (Figure 5). Inflammation was observed, with the presence of mononuclear cells (Figure 6) and few neutrophils. This inflammation, however, was milder than that observed in the control group.

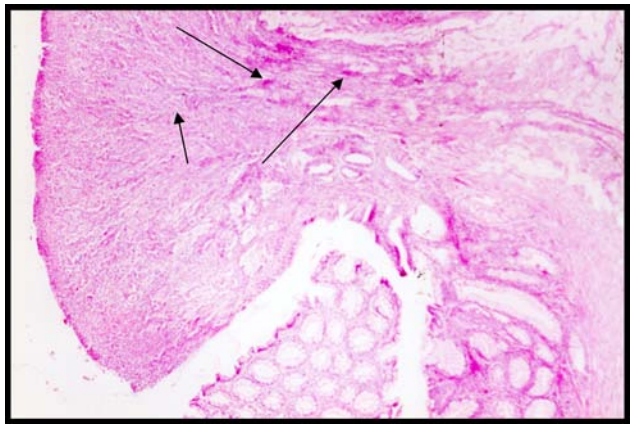

FIGURE 5 - Photomicrograph – Group 1 dog four days after surgery – Colon – Collagen formation (smaller arrow) and angiogenesis, evidence of regeneration (larger arrows). HE 100X

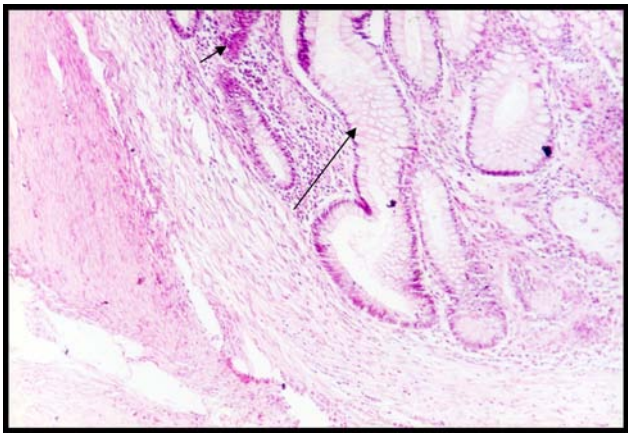

FIGURE 6 - Photomicrograph – Group 1 dog four days after surgery – Colon – Infiltrate of mononuclear inflammatory cells in the mucosa (smaller arrow) and hyperplasia of goblet cells (larger arrow). HE 100X

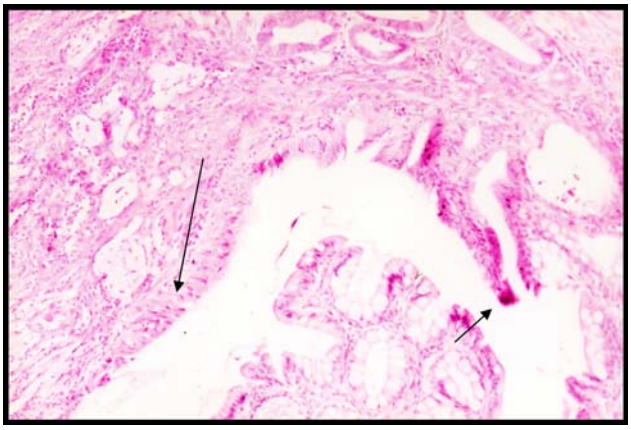

FIGURE 7 - Photomicrograph – Group 2 dog 21 days after surgery – Colonic mucosa with necrotic areas (smaller arrow) and severe inflammation (larger arrow). HE 100X

Connective tissue proliferation and intense fibroblast activity were observed in the animals that were reoperated on after 21 days. Slight inflammation with glandular cystic dilatation, moderate bleeding, and proliferation of connective tissue were observed seven days after surgery, with complete regeneration and no neutrophilic infiltrate. Bleeding, congestion, connective tissue proliferation, and inflammation were observed. Tissue regeneration with preservation of the submucosal glands was also observed.

Group 2

Macroscopic examination was performed during the reoperation of animals both four and 21 days after the first procedure. In animals that were reoperated on after four days, the following were observed: a bruise in animal no. 24; subcutaneous abscess, anastomotic dehiscence and peritonitis in animal no. 25; leakage in animal no. 26; a fistula in animal no. 27; and leakage and peritonitis in animal no. 28. Adhesion formation between the greater omentum and the anastomosis was observed in animals that were reoperated on after 21 days. In these animals, no leakage, fistula, abscess and stenosis were macroscopically observed. Microscopic examination of group 2 samples from animals that were reoperated on after four days revealed cicatrization, with formation of granulation tissue and collagen slower than that observed in group 1. Inflammation was more severe, mainly of the mucosa and submucosa (Figure 7). Numerous giant cells were observed in the muscularis externa and submucosa of animals that were reoperated on after 21 days. These cells were mainly found around the stitches. Breakdown of the tunica muscularis was observed, with the presence of mononuclear infiltrate and fibrous tissue that spread to the submucosa causing glandular dilatation, with cyst formation and secretion retention in some cases (Figure 8). The inflammatory process often affected the serosa. Necrosis of the mucosa and the presence of neutrophilic infiltrate were observed in two cases.

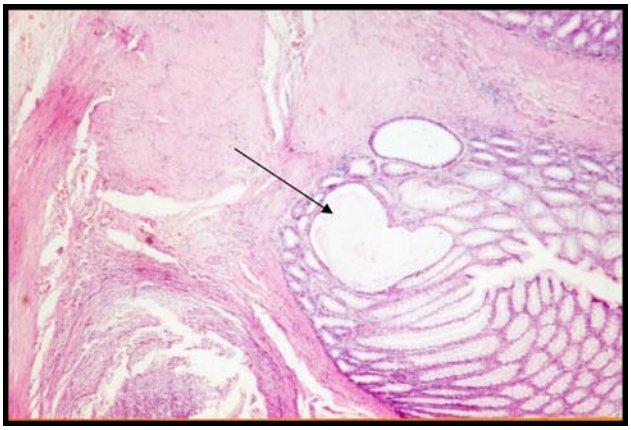

FIGURE 8 - Photomicrograph – Group 2 dog 21 days after surgery – Colon – Cystic formation (arrow) next to fibrosis and granuloma observed in the submucosa. HE 100X

Discussion

Surgery of the large intestine has proved difficult because of the possibility of complications. Among these, the most feared complication is anastomotic dehiscence, with subsequent peritonitis and abdominal sepsis. There is a consensus among surgeons with regard to the need for preparing the colon with preoperative antibiotic therapy, mechanical cleansing and the use of a cathartic, especially in cases of lesion of the left colon. Abdominal trauma is one of the conditions which may hinder preoperative colon preparation. Another difficult condition is intestinal obstruction caused by colon tumor, since the administration of a cathartic is dangerous in this case. Today, there is an alternative technique for preoperative colon preparation. It consists in the continuous irrigation of the large intestine with saline solution using a Foley catheter, which is inserted after appendectomy and drains into a bag. Despite the current techniques, many complications resulting from

colon surgery are still observed, which justifies the present study. Various techniques have been proposed to obtain better results for colonic anastomosis, such as aseptic anastomosis, described by Tassara (1994) and many other authors. Despite the benefits reported, the techniques do not prevent contact between stool and the suture, increasing the risk of contamination and inflammation. The intraluminal liner is used to reduce this risk. In our study, risk reduction was observed in group 1, in which the liner was kept in place for at least 5 days. No deaths were observed among animals from group 1 (intraluminal liner), which contributes to a positive evaluation of the protecting device. After studying 1703 cases, researchers reported[2] that the mortality rate from colonic anastomosis was 33%. This rate may vary according to different factors, but it has always been reported as high. The statistical analysis of our study showed the advantages of using an intraluminal liner to protect the anastomotic site. Complication rates were statistically higher when the device was not used, which is corroborated by the related literature. Infection affects collagen metabolism (both synthesis and degradation), probably reducing intracellular production of collagen[3]. In our study, a significant increase in collagen was observed in group 1 (intraluminal liner), regardless of the presence of infection. This suggests that the presence of bacteria at the suture site somehow reduces collagen synthesis or increases collagen degradation. Since the amount of collagen is reduced when there is infection, some authors[3] have suggested that the preparation of the intestine prior to colon anastomosis is fundamental. According to them, this would increase collagen concentration. In addition, this preparation would reduce the risk of complications resulting from colonic anastomosis[7]. In our study, the liner avoided contact between stool and the anastomosis, reducing the number of complications and increasing collagen concentration, even though intestinal preparation was not carried out. Thus, the use of the device can yield similar results to the preparation procedures recommended by these authors. The presence of granulocytes, macrophages and fibroblasts at the site three to seven days after surgery has been reported in the literature. The continued presence of granulocytes probably suggests collagenolysis[8]. These cells were observed in the present study. In the control group, inflammation was more severe and collagen concentration was reduced. The use of synthetic material for intraluminal protection has been described in the related literature. An experimental study conducted in 2002, however, reported the use of a biological membrane[9]. In our study, the intraluminal liner was fixed to the intestinal wall in a different manner, since the device was positioned 10 cm anteriorly to the anastomosis. Thus, the liner protected the anastomosis for at least five days. This was not observed in other studies, in which the device was prematurely evacuated. In this study, a biological membrane that is easy to obtain was used as an effective, low-cost alternative to the various materials and devices for anastomotic protection[10]. The technique for intraluminal protection with porcine submucosa used in group 1 animals was effective, with no cases of morbidity or mortality. Other protection devices have also been used with good results, e.g. the polyglycolic acid and barium sulfate device[11], the anastomosis ring device composed of plastic and springs[12], the coloshield, proposed by Ravo in 1987, and a condom[13], with a technique similar to the one presented in our study. Elimination of the protection device in group 1 animals occurred between five and eight days; thus, the anastomotic site was protected during the most critical period of colon caicatrization. No cases of morbidity or mortality occurred in group 1, which is in accordance with the results reported in another study[14]. However, the aforementioned study reported that the device was eliminated between 8 and 14 days or 10 and 16 days. It is believed that if the liner remains in place for only three days, its use will not be advantageous, since there will be contact between stool and the anastomosis during a critical caicatrization period. When the liner is fixed next to the anastomotic site, it is evacuated too soon, and the results obtained are similar to those obtained with the traditional procedure[9]. According to the statistical analysis, the correlation between the time the liner was kept in place (in days) and the caicatrization period in group 1 was 1.51. This showed a positive correlation between these two variables: the longer the protector is kept in place, the better the healing. This result was considered high, which might be explained by the fact that seven animals were submitted to a new procedure four days after the first surgery. Standard deviation was low, indicating sample homogeneity. The correlation between the time the liner was kept in place (in days) and the caicatrization period in group 1 was also analyzed disregarding animals reoperated on after four days and including animals that eliminated the liner spontaneously. In this case, the results showed no correlation between the two variables. In addition, no correlation between the two variables was observed when all animals that participated in this study were analyzed. A negative correlation was observed between the rate of complications and when they occurred, i.e., complications occurred more frequently during the four days immediately after surgery. A negative correlation was observed between the rate of complications and the time the liner was kept in place. Therefore, we may state that the longer the device is kept in place, the fewer the complications resulting from colonic anastomosis.

Conclusions

The intraluminal liner placed 10 cm anteriorly to the colonic anastomosis is effective in protecting the site against the adverse effects of fecal contamination. Thus, the rate of complication and inflammation is reduced and mucosal repair at the site is faster when compared with colonic anastomosis without the use of intraluminal protection.

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