



Effect of Peritoneum Graft on Intestinal Anastomosis Healing After Deserosalization in Dogs

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THE trauma and damage of the intestinal serosa layer resulted in serious complications. To solve these problems, many substances were used to cover up the affected serosa. This study demonstrated the possibility of using an autogenous peritoneal layer as an implant to overcome the complications of serosal layer damage at the anastomotic area. The study was conducted on twenty-four healthy adult local breed dogs. The serosal layer of the jejunum was removed and then a complete circumferential cut was made in the intestinal wall. The dogs were randomly allocated into two equal groups (n=12 each). In the control group, end-to-end jejunum anastomosis was done by a simple interrupted suturing technique. In the peritoneal group, the area of the anastomosis was sutured as the first group and wrapped by the peritoneum. After 15 days of surgery, both gross and histopathological investigations were studied. Correspondingly, radiographical investigation and bursting pressure test were performed on the 7th and 15th post-surgery. By comparison with the control group, the application of autologous peritoneal graft revealed an efficacious and beneficial effect on the healing process of the intestinal anastomotic site. The healing process in the peritoneal graft group was characterized by rapid mucosal re-epithelization, enhanced collagen fiber formation, and organization, no leakage, reduced adhesions and stenosis, and increased bursting pressure.

In conclusion, wrapping of intestinal anastomosis area with an autologous peritoneal layer after serosal damage is a beneficial and effectual technique without severe complications.

Keywords: Intestine healing, Serosa defect, Peritoneum, canine intestinal anastomosis

Introduction

Intestinal anastomosis is the most challenging surgical operation for repairing different bowel pathological disorders. Intestinal anastomosis is usually performed in cases of intestinal inflammation, volvulus, strangulation, and bowel perforation [1, 2]. Several factors influence the healing process of the intestinal anastomosis site, including the kind of surgery technique, nutritional support, reperfusion, personal experience, and vascularity of the intestinal segment [3, 4].

Improper healing of the anastomotic site has been associated with severe complications like

adhesions, shock, ileus, leak, and bowel narrowing [3-6]. Regardless of the advances in knowledge and improvements in management and surgical techniques of bowel anastomosis, adhesions and leak remains the most frequent complications that are often associated with increased risk of morbidity [7-9].

Adhesions are fibrous tissues that represent one of the furthest medicinal challenges in surgery. It is considered a major cause of many post-operative surgical complications such as intestinal stenosis and obstruction, infertility, intra-abdominal abscess formation, chronic

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(Received 20/04/2023, accepted 26/07/2023)

DOI: 10.21608/EJVS.2023.206948.1492

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abdominopelvic pain, and ureteral obstruction. Moreover, is often accompanied by extended periods of recovery and increased costs [10,11]. The serosa that covers most of the gastrointestinal tract is the main key to adhesion formation. The main conspicuous function of the mesothelium is the production of a lubricant surface that facilitates relatively free movement of abdominal viscera within the abdominal cavity. Additionally, it plays essential roles in the transport of fluid, resolution of inflammation, tissue healing, fibrin deposits lysis, protection against infecting microorganisms, and prevents adhesion formation [12-15]. Intestinal serosal damage occurs after intra-abdominal trauma and separation of the adhered or infiltrating cancer for the wall of the intestine [14, 16]. If such lesions are unnoticed, the healing processes of the gastrointestinal tract are influenced leading to fibrous attachment formation between visceral organs and tissue or the abdominal wall, or it may be accompanied by tissue necrosis and stenosis [16]. Therefore, recent experiments have been focused on discovering an ideal strategy to diminish or prevent these problems. These experiments included the use of drugs and biological agents that provide a physical barrier to prevent adhesion formation and can accelerate the wound healing process; one of them is peritoneum graft [17, 18].

Peritoneum is a semipermeable membrane consisting of a mesothelium cells layer, basement membrane connective tissue containing elastic and collagen fibers, and numerous cell-like adipose cells, macrophages, and lymphocytes [19]. The peritoneum cells secrete several types of growth factors including fibroblast, transforming, and epidermal growth factors that are enhancing the healing process [19,20]. Therefore, the peritoneum has been used as a protective tissue to limit the escape of content from many internal organs at the perforation site such as the intestine and urinary bladder [21,22]. Yin, 2005 used a peritoneal graft for wrapping the harsh defects in the large or small intestine in 30 patients and it was concluded that such grafting provided a simple, easy, cheap, safe, feasible, and effective substitute for the treatment of serious serosal membrane defects [23]. Castillo *et al.* 2019 demonstrated that using auto-peritoneum to treat duodenal injuries in rabbits was not associated with a formation of adhesion, leak or intestinal perforation [21]. Although the use of peritoneal grafts from different source have been

studied in many studies, there is no available document on the influence of peritoneal graft, either experimentally or clinically, on intestinal anastomosis wound healing after removing serosa. Consequently, The aim of this study to demonstrate the beneficial value of using peritoneal graft for improving the healing process of intestinal anastomosis after experimentally induced serosal damage in dogs.

Material and Methods

This study comprised twenty -four adult healthy local breed dogs weighing approximately 20- 24 kg. The animals were housed in cages individually and allowed to adapt for two weeks before the beginning of the experiment. The operative dogs were assigned randomly to two equal groups; the control and the peritoneal graft-treated group (n=12 each). The research was conducted after the approval of the Animal Care and the Ethics Committee of Mosul University, Iraq. (UM. VET. 2022.011).

Surgical procedure: The dogs were anesthetized by intramuscular injection of 10% ketamine hydrochloride mixed with 2% xylazine at doses of 10mg/kg and 3 mg/kg, respectively [24]. Then, an 8-10 cm ventral midline laparotomy was performed under strict perpetration. The jejunum was carefully exteriorized from the abdominal cavity and moistened with a wet sponge. To make serosal damage, the serosa layer of the entire circumference of the jejunum was scraped at a width of 1 cm with a toothbrush (Figure 1). In the middle of the abrasion site, a complete circumferential cut was made. Then, dogs were randomly assigned into two groups (n= 12 each). Six animals for the assessment of bursting pressure (Bp) and another six animals were utilized for radiographic and micropathological examination. Animals in both groups (n=12 each) were involved in macropathological assessment as follows:

Control group, the animals succumbed to the end-to-end anastomosis with one row of simple interrupted suture pattern using 3/0 Polygalactin suture material only (Figure 2). While, in the treatment group; the anastomotic place was stitched like the control group and then the site was covered by a peritoneal graft (about 2x 5 cm in diameter). The peritoneal graft was secured to the intestine by intermittent stitches using polygalactin suture material (Figure 3). Lastly the abdominal wall and skin were closed routinely.

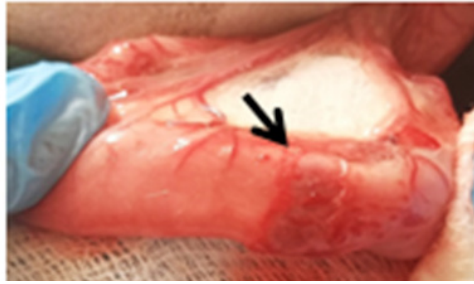


Fig. 1. Showing abrasion of the serosal layer.

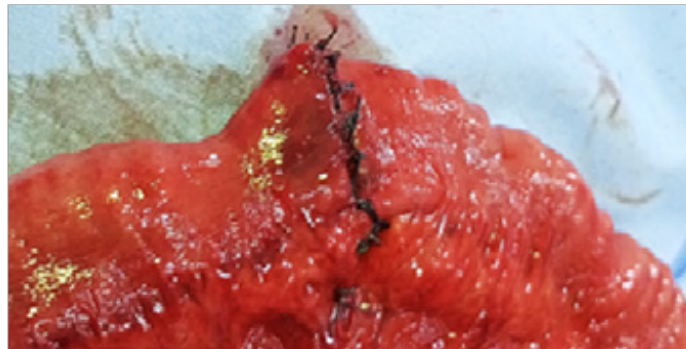


Fig. 2. Showing end-to-end intestinal anastomosis.

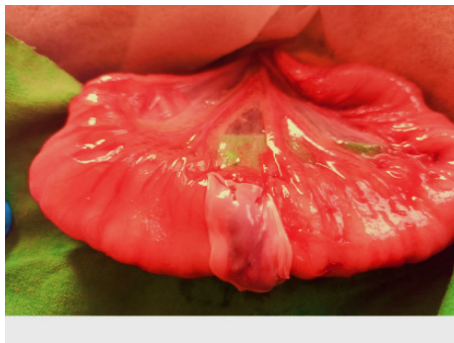


Fig. 3. Showed wrapping of the anastomotic area using a peritoneal graft.

To prevent postoperative dehydration, the animals were given intravenous fluid therapy (dextrose 5%) throughout the operative surgery and for several days after the operation. Then, they were allowed to take soft food. All dogs received penicillin-streptomycin for 5 days postoperatively.

Macro pathological assessment: The animals were observed daily to evaluate any clinical abnormalities. The macropathological assessment of the abdominal wall incision was made, and the abdomen was reopened to inspect and evaluate the healing process at the anastomosis site. The degree of adhesion formation and leakage at 7 and 15 postoperative days for all animals was evaluated. In addition, the adhesion degrees are

scored based on the previously used adhesion score [25], Table 1. The degree of leakage was scored according to the previously reported leakage score [26], Table 2

TABLE 1. Criteria scores employed for evaluating the degree of adhesions formed on the anastomotic area [25].

Score	Standards
0	No adhesion
1	Slight adhesion
2	Mild adhesion
3	Severe adhesion

TABLE 2. Criteria scores employed for evaluating the grade of leakage on the anastomotic area [26] .

Score	Standards
0	No leakage signs on the anastomotic site
1	Anastomotic small abscesses
2	Large abscesses or free pus
3	Fecal peritonitis or visible dehiscence

Radiographical assessment

For the radiographical examination, a piece of intestine (10 cm), including the area of anastomosis, was harvested and washed with water to remove bowel contents. After taking the radiograph; the degree of intestinal stenosis was estimated by using the following formula:

Degree of stenosis = $100 [1 - 2s / (f + t)]$; the diameter of the bowel at the anastomotic place, while f and t; the bowel diameters above and below the anastomotic area at a distance of 2 cm [18].

Histopathological assessment

Following radiological imaging, samples containing the anastomotic site were collected, washed, and cleaned with normal saline; and fixed in 10% formalin for the histological staining using hematoxylin and eosin stain.

Radiographic and histopathological examination of the anastomosis site was performed at 7 and 15 postoperative days for 6 animals from each group (3 animals for each period).

Bursting Pressure assessment

For evaluation, Bursting Pressure to the site of anastomosis at 7 and 15 postoperative days, 3 animals for each period were used. Following anesthetization, re-laparotomy was made through the former incision, and the anastomotic area was identified without any manipulation of adhering tissue. A piece of the intestine (approximately 10 cm) incorporating the area of anastomosis was resected, evacuated from contents, and washed with distilled water. The distal tip of the intestine piece was locked with a clamp; the proximal tip was connected to the air pump of the sphygmomanometer which was firmly secured around the tube with a suture. Then, the intestinal piece was placed in water and gradually was fill up with air. The pressure value necessary to induce leakage or rupture was taken as Bp (mm-Hg) according to Portilla-de et al. [27].

Statistical analysis: Data were expressed as mean (\pm standard error) and subjected to statistical analysis at $P < 0.05$. For adhesion and leakage chi-square was used, while ANOVA and Duncan test were used to evaluate the stenosis and BP estimation. The data were analyzed using the Microsoft Excel application.

Results

Macro pathological assessment

Clinically, through the first day after surgery, all animals showed minor symptoms of discomfort detectible by depression and a decrease in appetite. After that, animals appeared to have normal clinical signs and good appetites without any mortality rate. The abdominal wound healed without any complications. The adhesion score was significantly lowered in the treatment group compared to the control group at $p < 0.05$. Adhesions formation were severely formed in the control group between the sites of anastomosis and surrounding tissue (Grades 2 and 3), while in group two the formation of adhesions was less in severity than in group control (Grades 0,1 and 2), Figures 4 and 5.

Signs of leakage were present in 2 of 12 animals (grade 1) (17 %) in the control group and 0 of 12 dogs (0%) in the treatment group. Generally, leak incidence in the control group was higher than in the peritoneal graft group and the leaked score was insignificant among the two groups (Figure 6).

Radiographical assessment

The radiographic images indicated varying degrees of stenosis in the diameter of the intestinal loop on the anastomotic site. In the control group, there was a significantly higher degree ($P < 0.05$) of stenosis than the peritoneal graft group on the 7th and 15th days post-surgery. Also, in both groups, there was a decrease in the stenosis grade on the 15th day compared with the 7th day, but this difference was insignificant (Figs. 7, 8).

Bursting pressure assessment

Significant variance was not detected in the bursting pressure test between the control group (136 \pm 1.5 mmHg) and peritoneal graft group (142 \pm 0.5 mmHg) on day 7th, while this variation was significant on 15 days, control (145 \pm 2.4 mmHg), peritoneal graft group (156 \pm 1.2 mmHg). The bowel segment treated with a peritoneum sheet demonstrated significantly more resistance pressure than the control group on day 15 post-operation. Both groups expressed higher BP at 15 days than at 7 days and this elevation was significant in the two groups (Figure 9).

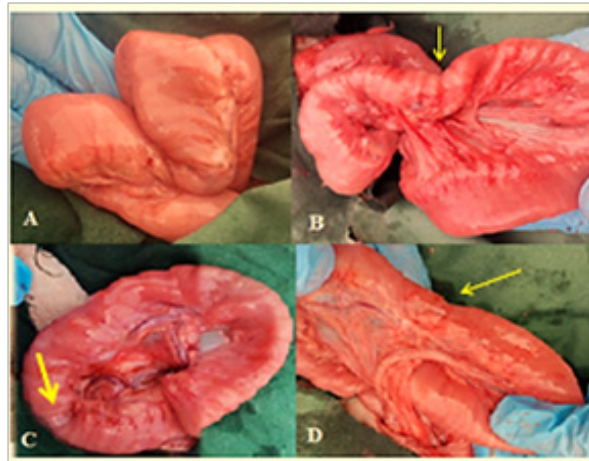


Fig. 4-A, B. Photograph picture showed the anastomotic area adhesion in the control group. A: adhesion between the loops of the intestine and mesentery (grade 3). B: between the intestine and mesentery (grade 2). C, D: showed the anastomotic area adhesion in the peritoneal graft group. (C): there is no adhesion in the anastomotic site (grade 0). D: adhesion between the intestine and omentum (grade 1).

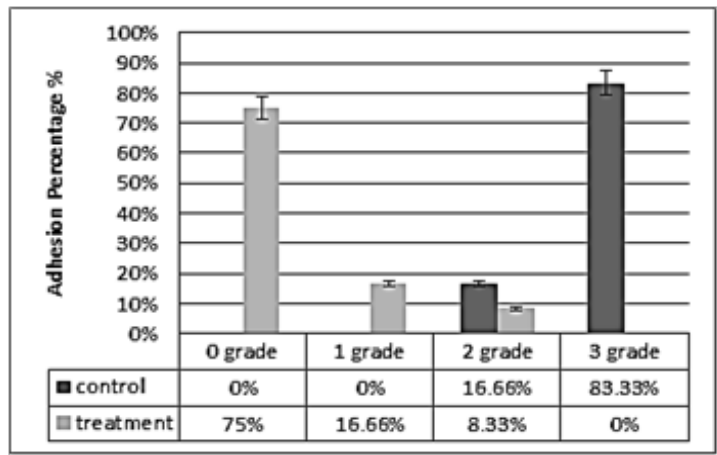


Fig. 5. Graphic diagram showing the adhesion score in groups of study.

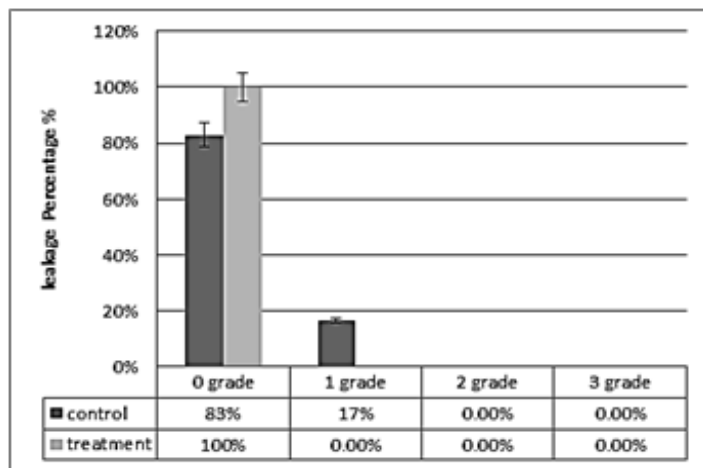


Fig. 6. Graphic diagram showing the leakage score in groups of study.

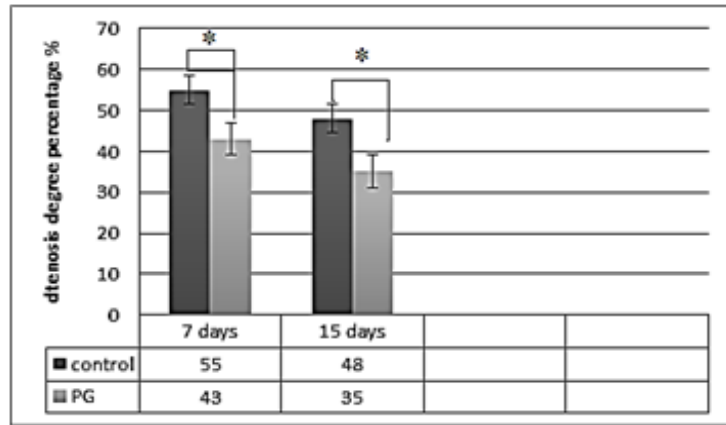


Fig. 7. Graphic diagram showing stenosis in the anastomosis site *: significant at ($P < 0.05$).

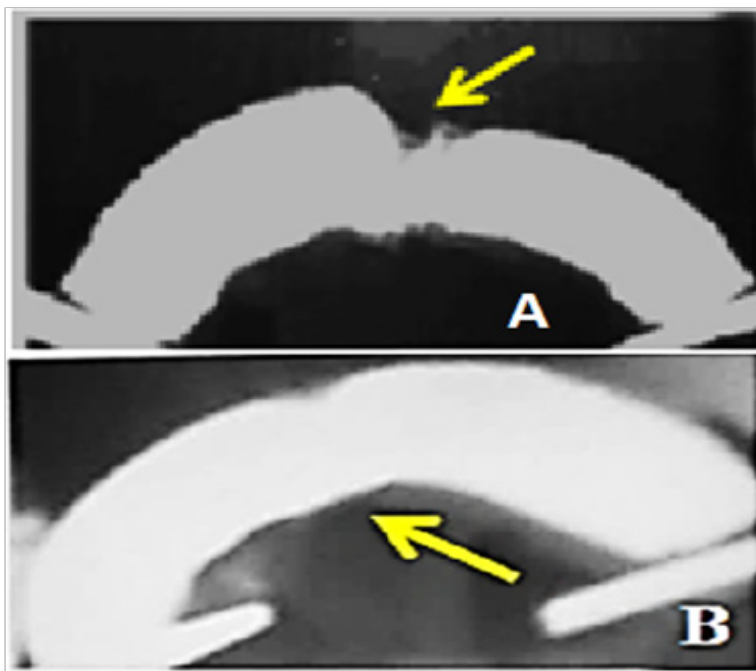


Fig. 8. Radiological picture shows severe anastomosis area stenosis in group one (A) compared to group peritoneal graft (B) at 15th postoperative days.

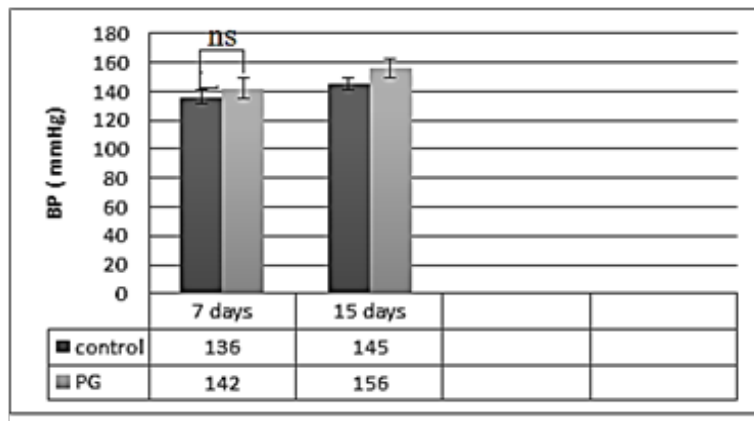


Fig. 9. Graphic diagram showing the BP of the anastomotic site. ns: non-significant at $P < 0.05$.

Histopathological assessment

In group one, the microscopic examination of the anastomotic site indicated severe inflammatory reaction including infiltration of polymorphonuclear and mononuclear cells at 7 days post-surgery, decreasing progressively at day 15 post-surgery. The fibrous connective tissue was evident on the 7th post-operative day, increasing in density on day 15 postoperatively. Also, the formation of granulation tissue and new angiogenesis were obvious on the 7th day postoperative, increasing progressively on day 15 postoperatively. Also, on 7th postoperative day showed severe mesothelial damage. This damage appeared less tensely in all samples on day 15 post-operation with the presence of re-epithelization (Figure 10: A, B, C)

Conversely, in the treatment group, on the 7th postoperative day the histological examination

revealed well-developed granulation tissue manifested by abundant angiogenesis; In addition to the formation of new connective tissue and a medium inflammatory reaction. Additionally, the degree of mesothelium damage was moderate and the starting of mucosal epithelial layer reformation and muscular regeneration was evident. Peritoneal graft destruction and resolution were noted by the clear loss of a border between a newly formed intestinal tissue and the peritoneal graft tissue (Figure 11). While at 15 postoperative days, excellent healing was observed represented by well-developed granulation tissue formation, mild to moderate inflammatory reaction, high angiogenesis, more degree of serosal layer restoration, mucosal re-epithelization and muscular regeneration (Figure 12: A, B, C).

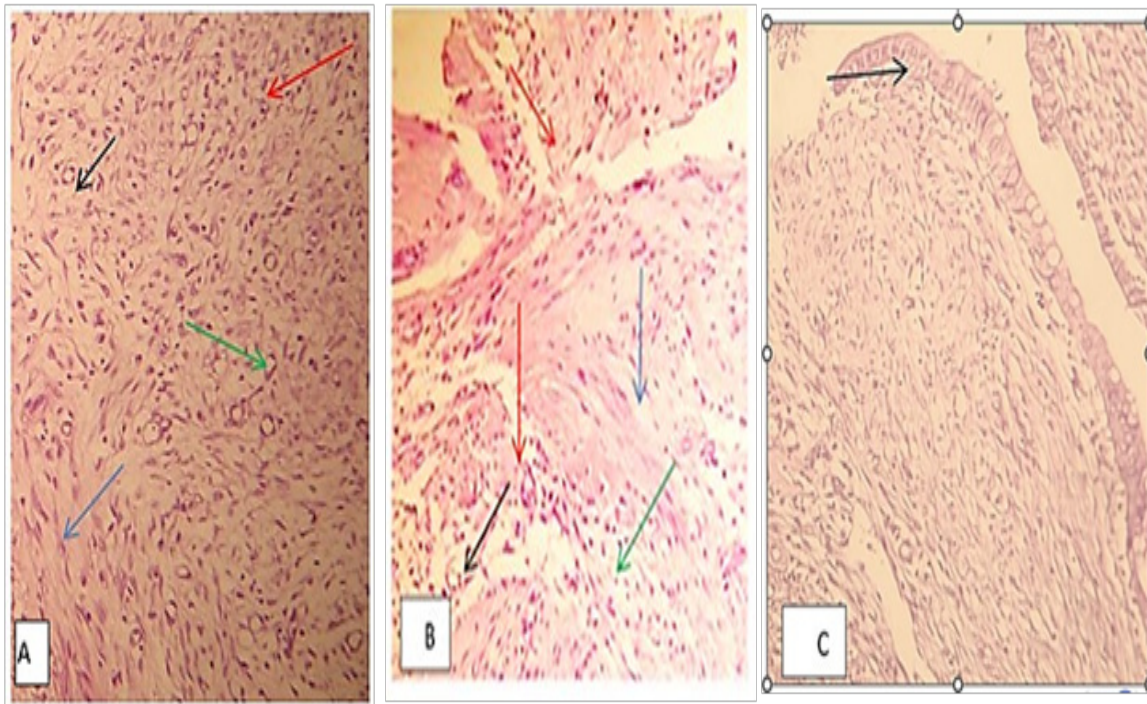


Fig. 10-A. 7day post–operation) A: granulation tissue of the control group shows severe infiltration of inflammatory cells red arrow, the proliferation of fibroblast black row with new blood vessels green arrow, slight collagen deposition blue arrow 10 X5X

Fig. 10-B, C: Microscopic examination of anastomotic area (15 days), A: mature granulation tissue of control group shows medium infiltration of inflammatory cells red arrow deposition collagen fiber blue arrow with new blood vessels black arrow, the slight proliferation of fibroblast green arrow, 10x6.3 X

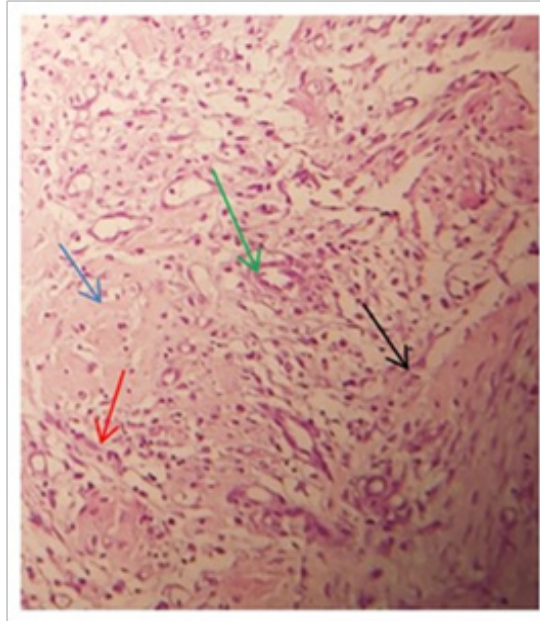


Fig.11. 7 days post–surgery A: granulation tissue of the peritoneal graft group shows medium infiltration of inflammatory cells red arrow, the proliferation of fibroblast black arrow with more new blood vessels green arrow, intensive collagen deposition blue arrow (10 X5.9X).

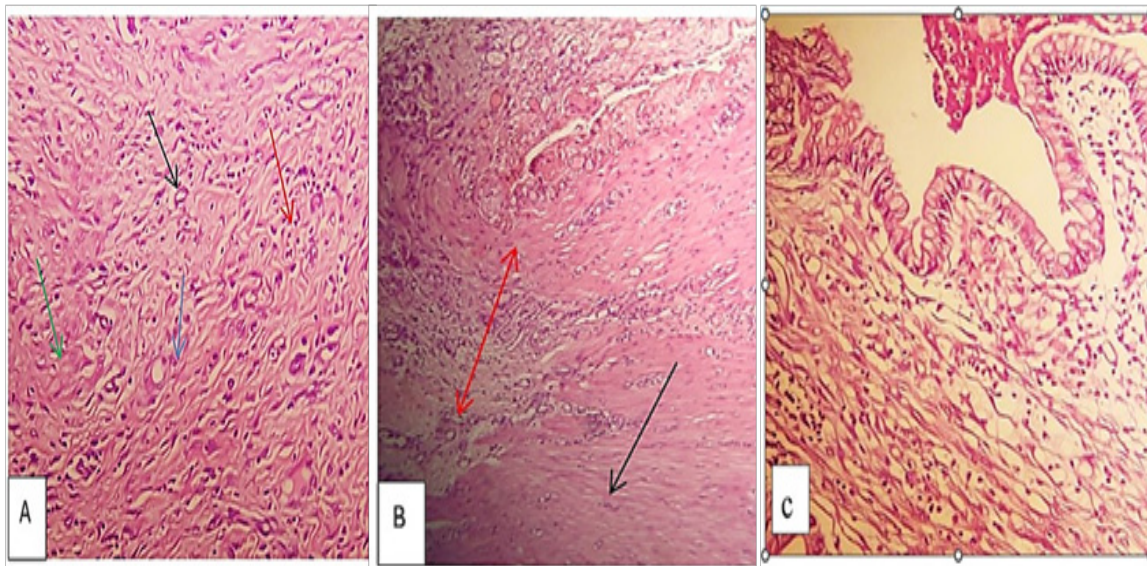


Fig. 12-A. Microscopic examination of anastomotic area (15 days), A: mature granulation tissue of peritoneal graft shows mild infiltration of inflammatory cells red arrow mature bands collagen fibre blue arrow with new blood vessels black arrow, the slight proliferation of fibroblast green arrow, 10x4.5X.

B. Microscopic examination of anastomotic area (15 days), A: mature granulation tissue of peritoneal graft group (red two-head arrow), muscular layer formation (black arrow), 10x3.2X.

C. Microscopic examination of anastomotic area (15 days), A: peritoneal graft group shows epithelial restitution (10x5.2X).

Discussion

Numerous complications may appear due to some mistakes during abdominal surgery, one of which is serosal damage. It has been reported that the traumatic serosal surfaces of abdominal organs resulted in intra-abdominal adhesions. After any injury to the mesothelial layer of the serosa, the wound-healing process starts, during this procedure the fibrin exudes to the outside. This matter if not demolished through a fibrin lysis process, a mass of fibrin is produced, which leads to forming bands of fibrous tissue between the organs in the peritoneal cavity [14, 26]. Serosal layer abrasion of the intestine is the most common result of iatrogenic intra-abdominal trauma. On the other hand, the very widespread style for producing adhesions in experimental research is serosal damage [26]. Toward obtaining damage to the serosal layer numerous ways were used. In this study serosal abrasion has been induced by rubbing the serosa with dental brushes, the serosal abrasion technique used in this study has been approved by other researchers [12, 26].

In a previous experiment, the results indicated that the whole removal of the serosal layer before performing the anastomosis enhances the repairing course, but did not increase the hazard of stenosis built on the hypothesis that inflammatory adherent processes evoked by serosal damage [26]. Also, in another study, the deserosalization in the anastomosis site resulted in improved mechanical strength and collagen deposition [12]. While in rats, it has been suggested that the abrasion of the serosal layer of intestinal edges and anastomosed by inverted technique does not reduce leakage and did not increase the mechanical strength of the anastomotic site [28]. Therefore, we designed this experiment to study the effect of peritoneal graft in decreasing the complication of deserosalization. In the current study, after the removal of the intestinal serosal layer, the anastomotic site was sutured with a simple interrupted apposition technique to evaluate the healing processes of the anastomotic site without and after wrapping it with a peritoneal patch and to assess the role of peritoneum patch on the healing processes, adhesion formation, and leakage prevention. In the present study, the animals in the control group showed severe intra-abdominal adhesions. This result is similar to previous reports, as it indicated that this model of serosal damage reliably leads to the formation of abdominal adhesion [27]. Adhesion is regarded

as one of the largest problems that increase morbidity and death after abdominal surgery [12]. Nevertheless, in this study, the adhesions formed were without undue mortality.

Recently, some studies have been conducted to decrease and prevent adhesion formation following serosal damage. One of these methods is the use of tissue graft [21]. Implants from tissue sources have been utilized commonly to prevent adhesions around several body parts [29]. In our study, using a peritoneal graft reduced adhesion formation at the site of anastomosis, and the same result was recorded by others [30]. Previous studies used peritoneal grafts to repair several tissues such as blood vessels, the urinary bladder, and the intestine. These works found that using of peritoneum fold for repairing led to preventing the development of adhesion and enhanced the healing of tissues. Recently, the peritoneal graft was used efficaciously for the restoration of tissue because it is safe, effective, and rapidly available without causing any defect to other tissues in comparison to other graft, especially in emergency operations. [18,31].

Stenosis formation usually occurs following intestinal resection and anastomosis due to several factors. One of the most common factors is adhesion [18,32]. In this study, the stenosis in group one was higher and this may be owing to the extensive adhesion. The compression generated by adhesion prevents the intestinal lumen from expanding at the site of the suture line and thus its narrowing [18]. Minor stenosis was shown in group two and this may be attributable to the effect of the graft that led to the formation of little adhesions and thus the occurrence of slight stenosis. This result agreed with previous studies [18,33], which indicated that utilizing the biological barrier to cover the anastomotic place reduces adhesions and stenosis. In addition, in my opinion, the degradation and absorption of the graft in the treatment group led to reducing the pressure at the area of anastomosis and thereby reduced the grade of narrowing 15 days after the operation compared with day 7th post-operation. This result agrees with previous work [18] indicating that graft degradation promotes the expression of many inductive proteins that lead to quicker tissue regeneration. Moreover, on day 7 after anastomosis surgery, the mean stenosis of the two groups was lower compared to the 15 days after the operation and this result was similar to that described previously [33].

There have been numerous experimental studies on anastomotic site bursting pressure measurement; Bp is an effective criterion for assessing the anastomotic strength and its healing integrity [33]. The Bp value among the peritoneal graft-coated group and control group at 15th days post-operation was significantly different, which indicates that the coating of the injured site of the anastomosis with peritoneal graft increased the strength of the anastomotic site. These results are in agreement with others who reported a significant increase in strength and Bp of the anastomotic area after reinforcing with tissue graft [28, 34, 35]. However, there were differences in Bp between the 7th and 15th days groups but this difference was not significant. Ditek, et al. have suggested that in the early phase of the healing period, the bursting pressure of the anastomotic area normally decreases because it depends on the inflammation rate and then increases as the strength holding capacity for suture material increases due to increases in the collagen synthesis [35].

By comparison with group one, the second group demonstrated a faster and improved healing process which was characterized by less adhesion formation, less degree of stenosis and leakage, decreased inflammation and increased collagen fiber deposition, enhanced angiogenesis, and the rapid epithelial layer of mucosa with villi formation. This excellent effect may be due to the biological components of the peritoneal graft that has a possibility for tissue regeneration and healing. This effect is agreed with previous works [18, 29, 34] specified that the peritoneal graft has an appositive effect on the healing of the anastomotic site where the graft act as a barrier to prevent adhesion and leakage of content. Additionally, the peritoneum composes of mesothelial cells, which can regulate adhesion molecules such as E-cadherin and vascular cellular adhesion molecule-1. These cellular particles which express via mesothelium cells have two functions in serous layer damage healing. Initially, it facilitates the inflammation cell migration through and along the length of serosal surfaces. Later during the healing of a serosal wound, the adhesion molecules are downregulated, and this regulation is associated with the loss of mesothelium cohesion enabling the mesothelium cells to change toward a further mesenchymal encode. Moreover, mesothelium cells adjust inflammatory reactions through the production and liberating of the hyaluronic compound that plays a critical role in tissue

healing [22]. Additionally, some researchers have noticed the presence of precursor cells identical to stem cells in the peritoneal layer. These progenitor cells have been employed to tissue reconstruction for different body parts [22]. Moreover, the peritoneum contains collagen, growth factors, and other molecules like proteins which play a vital function in the improvement healing process through cell proliferation, stimulation of fibroblast, macrophage recruitment, deposition, and production of collagen, and stimulating angiogenesis [22]. The rapid reepithelization, increased angiogenesis plus tissue maturation in group two are similar to that described previously. This result can be attributed to the biological properties of the peritoneal graft that act like a protecting coat in opposition to adhesions and aids in the curing process of the intestine . [22,18].

Conclusion

Reinforcing intestinal anastomoses after deserosalization with a peritoneum autograft can improve intestinal healing manifested by decreased the stenosis, adhesion, and increased bursting pressure, intestinal re-epithelization, and collagen deposition.

Acknowledgments

I am sincerely thankful and great gratitude to the Veterinary Medicine College, Mosul University, for its support and assistance in carrying out this research. Also, I extend my thanks to my colleague Dr.Osama Azeldeen Abdulla (College of Vet Med, Dep. of Microbiology) for his assistance.

Conflict of interest

There was no conflict of interest

Author's contribution

All authors contributed equally in this work

Funding statement

The current research is financially supported by own authors

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تأثير رقعة البريتون على إلتئام تفاغر الامعاء بعد إزالة المصلية في الكلاب

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تلف الطبقة المصلية للأمعاء ينتج عنه العديد من المضاعفات ولغرض التقليل من هذه المضاعفات، تم استعمال مواد متعددة. ولهذا تهدف هذه الدراسة الى معرفة دور رقعة البريتون الذاتية في تقليل مضاعفات اذى الطبقة المصلية في مكان تفاغر الامعاء. تم استعمال 24 حيوانا من الكلاب البالغة، تم ازالة الطبقة المصلية للصائم وبعدها تم اجراء قطع كامل لمحيط الامعاء. قسمت الكلاب بعدها عشوائيا الى مجموعتين. في مجموعة السيطرة، تم فقط مفاغرة الصائم نهائية ل نهاية بطريقة الخياطة البسيطة المتقطعة. في مجموعة البريتون، خُيط موقع التفاغر بصورة مشابهة لمجموعة السيطرة وبعدها تم لفه برقعة البريتون. تم دراسة عملية الالتئام في المجموعتين عيانيا لمدة 30 يوما نسجيا، شعاعيا اضافة الى اجراء فحص الضغط الانفجاري. للمدد 7 و15 يوما بعد العملية، اشارت النتائج ان استعمال رقعة البريتون له تأثير فعال وذو قيمة على التئام مكان التفاغر في حيوانات المجموعة الثانية مقارنة بالمجموعة الاولى، وتميز هذا الالتئام بسرعة تجدد ظهارة المخاطية، زيادة تكوين الاليف الكولاجينية وتعويضها، قلة الالتصاقات، عدم وجود تسرب وقلة درجة التضيق اضافة الى زيادة في الضغط الانفجاري. نستنتج من هذه الدراسة ان احاطة مكان تفاغر الامعاء برقع البريتون بعد ازالة الطبقة المصلية له تأثير ناجح، آمن وذو كفاءة عالية من دون حدوث مضاعفات. الكلمات المفتاحية: تلف المصلية، البريتون، الرقع، الالتصاق، تفاغر الامعاء.