

## Comparative study of different techniques of intestinal anastomosis covered with omentum in dogs

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### Abstract

This study was conducted to compare three techniques of intestinal anastomosis in dogs. Twenty four healthy adult dogs were used. Animals were divided into three equal groups. First group (control group) in which single-layer by cross mattress interrupted suture technique was used to approximate all layers of intestine. While in second and third groups, similar fashion as in first group, but the site of anastomosis was covered by free omentum and pedicle omentum respectively. The radiological and histopathological examinations at the periods of 15 and 30 days postoperation revealed that the third group showed lowest mean of degree of anastomotic stenosis (28.5%), while, the highest mean of degree of stenosis was found in the second group (49.6%). The biopsy examination revealed that simple adhesions in the first and second group, while no adhesions were observed in third group. The histopathological examination showed that the healing in the third group occurred by the first intention healing, while in other groups associated with fibrous tissue formation. Ultimately there were no significant differences of bursting pressure in between three groups at (15) and (30) days postoperation.

### دراسة مقارنة لتقنيات مختلفة من تقمم الأمعاء المغطات بالثرب في الكلاب

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### الخلاصة

صممت هذه الدراسة لغرض المقارنة بين ثلاث تقنيات من تقمم الأمعاء في الكلاب حيث استخدم (24) حيوان من الكلاب البالغة السليمة والتي قسمت إلى ثلاث مجاميع متساوية. استخدمت تقنية خياطة المنجد المتصالب المنقطعة لتقريب جميع طبقات الأمعاء باستخدام صف واحد من هذه الخياطة في المجموعة الأولى (مجموعة السيطرة)، تم إجراء نفس هذا النمط من الخياطة في المجموعة الثانية والثالثة ولكن مكان التقمم غطي بالثرب الحر وسويقة الثرب بالتعاقب.

أجريت الفحوصات الشعاعية والنسجية المرضية بعد (15) و(30) يوم من العملية الجراحية والتي أظهرت أقل معدل في درجة التضيق لمنطقة التقمم في المجموعة الثالثة (28.5%) ، بينما المجموعة الثانية فقد أظهرت أعلى معدل في درجة التضيق (49.6%).

أظهر فحص الخزعة التصاقات بسيطة في المجموعة الأولى والثانية، بينما لم تلاحظ التصاقات في المجموعة الثالثة. بين الفحص المرضي النسجي ان الالتئام في المجموعة الثالثة قد حصل بالالتئام القصد الأول، بينما تم تكوين نسيج ليفي في المجاميع الأخرى.

لم توجد فروقات مهمة حصائياً في الضغط الانفجاري بين المجاميع الثلاثة للفترات (15) و(30) يوم بعد العملية.

## **Introduction**

Different techniques were used to intestinal anastomosis, which include inversion, eversion, or opposition, and most of these techniques associated with different complications such as leakage, adhesion, stenosis, and peritonitis, which depending on suture materials, technique, patient's condition and skill of surgeon(1). The intestinal adhesions lead to secondary intestinal obstruction, constriction, incarceration, or it may be hamper the normal passage of food by the stenosis of lumine diameter and on the other hand the serosal or omental adhesions for the intestine may form apoint for intususption or volvulus (2 and 3), intestinal obstruction may be lethal and need urgent surgery, and the anastomotic leakage cause peritonitis and secondary complications like abscess, or fistula (4,5 and 6). The omentum is that it plays a central role in peritoneal defence by adhering to sites of inflammation, absorbing bacteria and other contaminants and providing leukocytes for a local immune response (7). In particular its rich blood supply that supports a high absorptive capacity its pronounced angiogenic activity which may support local tissues and ischemic tissues, its innate immune function, and finally its high concentration of tissue factors which promotes haemostasis(8), so that surgeons have long exploited the unique structure and function of the omentum(9). There are several reports of the use of an omental flap to reconstruct the mediastinum in patients with mediastinitis secondary to open heart surgery(10), and also the omentum was used as free transfer graft for the treatment of chronic ulcers, progressive hemifacial atrophies and contused wounds. The transferred omentum appears to maintain its volume and nature under normal circumstances(11). Because of the unique structure and functions of omentum as mentioned above and the successful of the authors to make intestinal anastomosis by one raw of sutures(12 and 13), so that, it was evaluated the efficiency of intestinal anastomosis by using cross mattress interrupted as one raw and compare it with the same suture technique but it is coated by omentum as free or pedicle graft.

## **Materials and Methods**

Twenty four healthy local breed male dogs, weight (13 – 19 kg) and age from (2 -6 years) were used. The animals were treated for external and internal parasites by using antihelminths (Niclosamide, 300 mg/kg B.W orally and ivermectin 200 ug /kg BW subcutaneously). The animals were housed under similar conditions and feeding. The animals were divided randomly into three equal groups:-

First group (control group):- in which cross mattress interrupted suture pattern was used. Second group:- In which intestinal anastomosis was achieved by single cross mattress interrupted suture technique and the anastomotic site was covered by free omentum. Third group:- In which intestinal anastomosis was achieved by single cross mattress interrupted suture technique and the anastomotic site was covered by pedicle omentum. The animals were fastened from food for about 24hr. and water 12 hours before operation. The animals anesthetized by atropine sulphate at a dose of 0.04 mg/kg BW intramuscularly as premedication fifteen minutes Later, general anesthesia induced by administration of mixture of xylazine HCL 2% and ketamine HCL 5% at a dose of 5mg , 15 mg/kg B.W intramuscular respectively (14). Pencillin – streptomycin at a dose of 10.000.I.u., 20mg intramuscular respectively at half hour before operation as prophylaxis medication. Ventral midline lapratomy were performed in all animals under aseptic routine technique then resection of part on intestine was performed by the following technique:- A loope of jejunum was exteriorized and prepared for resection by application double ligature of blood vessele which supply the resected area. Two doyens intestinal forceps were applied from each ends of the resected part (Fig.1),

scissor was used to cutting between the forceps begin at one side, then complete resection to the other end. In first group:- The intestinal ends were anastomosed by cross mattress interrupted suture pattern with polygalactin 910 size (2/0) (Fig .2) and (diagram1). In second group:- Similar manner as in group one,except that the anastomotic site was covered by free omentum and fixed around the site of anastomosis by simple interrupted with wall of intestine (Fig.3).In third group:- Similar manner as in group one,except that the anastomotic site was covered by pedicle omentum and fixed around the site of anastomosis by simple interrupted with wall of intestine(Fig.4). The abdominal wall and skin closed by a routine manner. Pencillin – streptomycin was given at a dose mentioned above intramuscular for 5 days post- operation. Fluid therapy, dextrose 5%(20 ml/kg BW) was used intravenously daily for about 2 days after operation,then gives soft food for about two days and the days later left free for food and water. Clinical observations were recorded for all experimental animals such as, oedema at the site of operation, activity, defecation and urination. Under general anesthesia and aseptic technique , the anastomosis part of intestine was observed after laparotomy to register any abnormalities at the site of anastomosis such as adhesion between site of anastomosis with mesentery, omentum or with the other loops of intestine or with abdominal wall. Ten to fifteen centimeter segment of intestine were resected which including the anastomotic site to achieve the radiological study, the segment of intestine rinsed with the water to remove all ingesta and the lumen filling with the freshly suspension of barium sulphate (25%). X- rays were taken(kv=45,mAs=3), in order to study the degree of stenosis at 15 and 30 days postoperation in all groups as the following formula:-

$$\text{Degree of stenosis} = 100 (1 - 2A / B + C)$$

A= diameter at site of anastomosis

B and C = diameter 2 cm far from the site of anastomosis proximally and distally (15).

The statistical analysis of the mean degree of anastomosis stenosis was performed by using analysis of a variance (ANOVA).

After taking the radiological image for specimen, the Barium sulphate solution was evacuated and then the one end of the specimen was connected with barometer and the other end was connected with air pump. The intestinal specimen was immersed in water to observe the air bubbles during the bursting of specimen either in the anastomosis or adjacent areas.

In all groups, biopsy were taken from anastomotic site at a period of 15 and 30 days post-operation for histopathological examination. Slides were prepared routinely and stained by haematoxyline and eosin stain (16).

## Results

The clinical examination showed oedema, simple redness of the operation area with light elevation in the temperature of operation area. These signs began to subside gradually during the 3-4 days after operation with improvement of the health state of the animals. The defecation and urination were normal. The segments were taken after 15 and 30 days of the operation revealed that in first group showed adhesion between the site of anastomosis and omentum, mesentery, loops of intestine(Fig .5). In second group showed adhesions similar to those observed in the first group (Fig .6). While in third group showed no adhesions except the attachment between the site of anastomosis and pedicle omentum (Fig .7) . The radiological examinations showed the higher mean for stenosis degree in the second group ( $52.3 \pm 7.46$ ) at 15 day (Fig.8), while less mean of stenosis degree in third group ( $28.5 \pm 1.9$ ) at 30 days (Fig.9)(Table.1).

**Table (1) Show the mean degree of stenosis of intestinal anastomosis and the stander error (S.E.) of three groups at 15 and 30 days**

Group	No. of animals	Stenosis degree at 15 days	Mean of stenosis degree at 15 days	No. of animals	Stenosis degree at 30 days	Mean of stenosis degree at 30 days
First	1	47.6	41.7 A ± 2.09	5	55	40.3 B ± 5.12
	2	38		6	31.4	
	3	41.8		7	39	
	4	39.7		8	36	
Second	1	42.1	52.3 C ± 7.46	5	65.8	49.6 D ± 5.56
	2	74.5		6	40.5	
	3	47.3		7	47	
	4	45.4		8	45.1	
Third	1	51	34.6 E ± 5.81	5	30	28.5 F ± 1.9
	2	25.7		6	28	
	3	35		7	33.3	
	4	27		8	26	

\* LSD = 7.1

\* There is a significant difference between group two(C,D) and group one(A,B)and three(E,F) at the level of  $P \leq 0.05$ . at 15 and 30 days .

\*There is a significant difference between group three(E,F) and group one (A,B)and two(C,D) at the level of  $P \leq 0.05$ , at 15 and 30 days .

\* There is no a significant differences between the periods of 15(A,C,E) and 30 days(B,D,F) in all groups at the level of  $P \leq 0.05$

The results of bursting pressure examination were summarized in(table.2)as the following:

**Table (2) Show the means of bursting pressure ± stander errors for the three groups after 15 and 30 days**

Group	No. of animals	Bursting pressure 15 day	Mean (mHg)	No. of animals	Bursting pressure 30 day	Mean (mHg)
First	1	350	491.25 A ± 61.85	5	530	606.25 B ± 35.78
	2	650		6	700	
	3	500		7	615	
	4	465		8	580	
Second	1	390	582.5 C ± 84.39	5	600	647.5 D ± 73.64
	2	800		6	850	
	3	550		7	500	
	4	590		8	640	
Third	1	750	545 E ± 69.4	5	500	610 F ± 65.9
	2	450		6	800	
	3	470		7	550	
	4	510		8	590	

\* L.S.D = 173.3

\* There is no a significant differences between the groups at 15(A,C,E) and 30 days(B,D,F) at the level of  $P \leq 0.05$

\*These results submitted to statistical analysis by using ( ANOVA variance analysis ) and ( L. S.D ) ( less significant difference) for differentiating between means by ( SPSS)(17).

The results of histopathological findings in First group at 15 days postoperation exhibeted that inflammatory cell infiltration mainly lymphocytes,macrophages and nutrophils in serosa of intestine as well as odema and congested blood vessels (Fig.10-A), also there is inflammatory cell infiltration mainly nutrophils in the subepithelial layer (Fig.10-B) and there is proliferation of fibroblasts which replaced the fibrin network in incision line which covered by vaculated epithelial cells (Fig.10-C). At 30 days postoperation, revealed loose distribution of fibrous connective tissue with

inflammatory cells were seen in incision line which covered by thin layer of epithelial cell (Fig.11-A). While in other section small villi covered by single layer of round epithelial cell covering dilated lamina propria which contained few lymphocytes and macrophages (Fig.11-B). Second group, at 15 days postoperation there is sever inflammatory cells infiltration mainly neutrophils, macrophages, and lymphocytes in muscular layer and submucosal layer (Fig.12-A) also there is sever fibrous connective tissue proliferation around the suture material and in subepithelial layer with inflammatory cells infiltration in addition to that thin layer of epithelial cells covered the incision line (Fig.12-B). At 30 days postoperation showed sever inflammatory cell infiltration mainly macrophages, plasma cell, and lymphocytes in subepithelial layer (Fig.13-A). and moderate thickness of epithelial layer covered the incision. In other section the material suture surrounded by proliferation of connective tissue with inflammatory cells are seen. The epithelial layer cover the incision consist from round villi which arise immature epithelial cell (Fig.13-B). In other section the treated material surrounded by sever inflammatory cell mainly neutrophils with fibrous connective tissue proliferation also were seen (Fig.13-C). Third group:-At 15 day postoperation there is mild fibrous connective tissue proliferation with inflammatory cells infiltration in incision section which is covered by a thin layer of epithelial cells (Fig.14-A), in other section there is proliferation of blood vessels (Fig.14-B). At 30 day postoperation revealed that suture material surrounded by fibrous connective tissue with few inflammatory cells (Fig.15-A). In other section, the plasma cells, macrophages are infiltrated in the subepithelial layer with regenerated complete columnar epithelial layer (Fig.15-B), also a few inflammatory cells were seen between mucosal glands (Fig.15-C), and mature villi lined by many layers of cuboidal cells, supported by heavy matrix (Fig. 15-D).

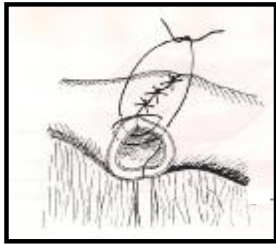


Diagram (1): Show the steps of cross mattress interrupted suture.

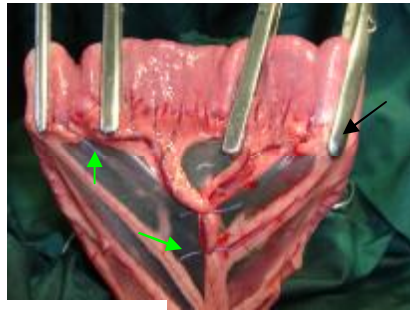


Fig. (1)



Fig. (2)

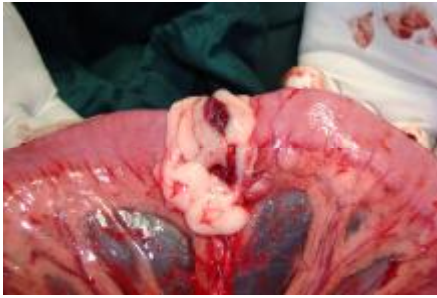


Fig. (3)



Fig. (4)

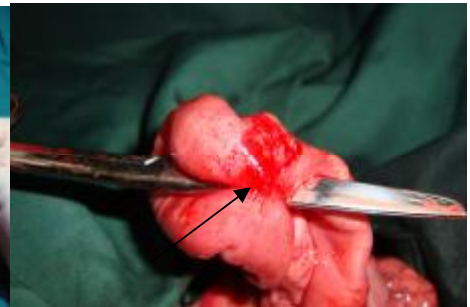


Fig. (5)



Fig. (6)

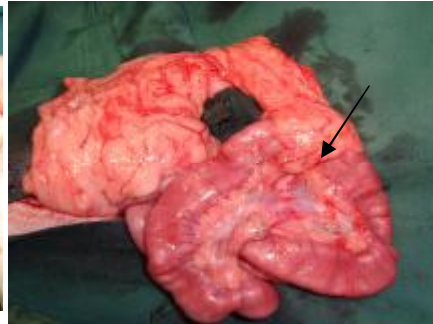


Fig. (7)



Fig. (8)



Fig. (9)

Fig.(1) Application of ligature of mesenteric blood vessels ( ) and four dyone intestinal forceps ( ). Fig.2 Closure of two ends of intestine by cross mattress interrupted suture Technique .

Fig.(3) The anastomosis area of intestine was wrapped by free omentum .



Fig.(4) the anastomosis area of intestine was wrapped by pedicle omentum . Fig.(5) show adhesion in between loop of intestine in one animal of first group at 30 days post operation. Fig.(6) shows adhesion in between omentum and anastomotic site in one animal of second group at 30 days post operation. Fig. (7) shows no adhesion of anastomotic site in one animal of third group at 30 days post operation. Fig. (8) shows stenosis site of intestinal anastomosis in one animal of second group at 15 days post operation. Fig. (9) one animal of third group showed the site of anastomosis at 15 days post operation.

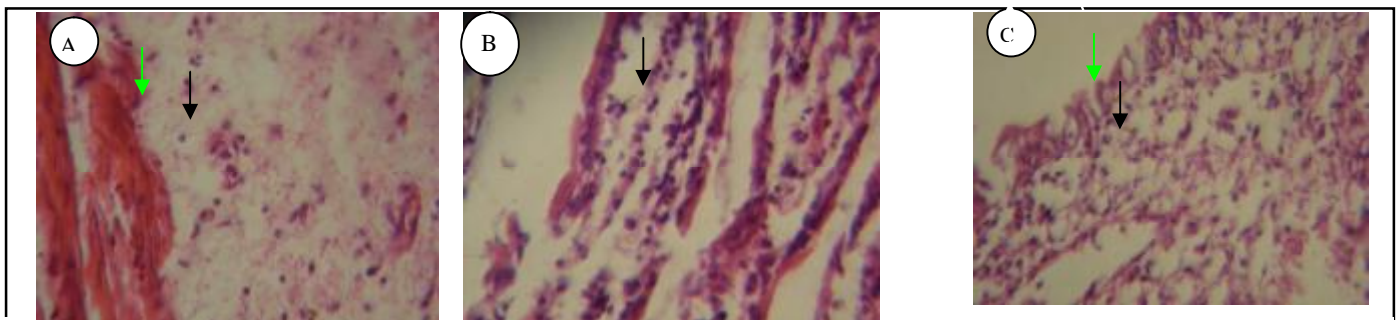


Fig. (10) Histological section in the intestine of one animal in first group at 15 day postoperative showed: A- inflammatory cell infiltration in the serosa with odema B - inflammatory cells mainly neutrophils infiltration in subepithelial layer C- proliferation of fibroblast with inflammatory cells in incision line covered by vacuolated epithelial layer (H and E 40 X).

Fig. ( 11) Histological section in the intestine of one animal in first group at 30 day postoperative revealed:A - loose distribution of fibrous connective tissue → with macrophage infiltration → in the incision line covered by thin layer of epithelial cells . B- small round villi covered by single layer of epithelial cells → (H and E 40X).

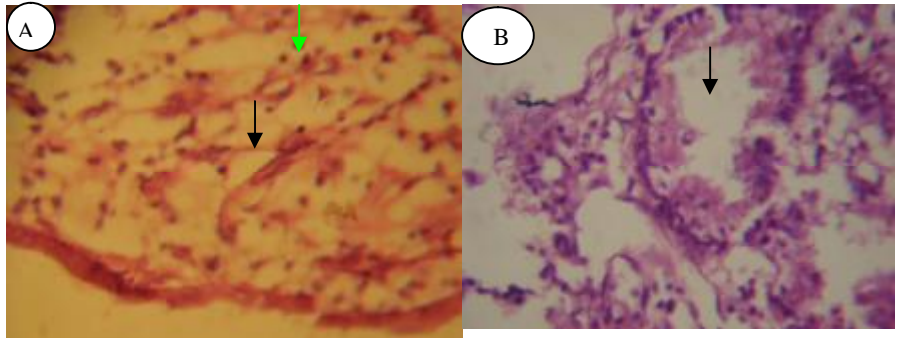


Fig.12 Histological section in intestine of one animal at 15 day treated with free omentum revealed: A- sever inflammatory cell infiltration in muscular layer → and in subepithelial layer → B- sever fibro connective tissue proliferation around the suture material → with thin layer of epithelial lining cells → as well as inflammatory cells in subepithelial layer → (H and E 40X) .

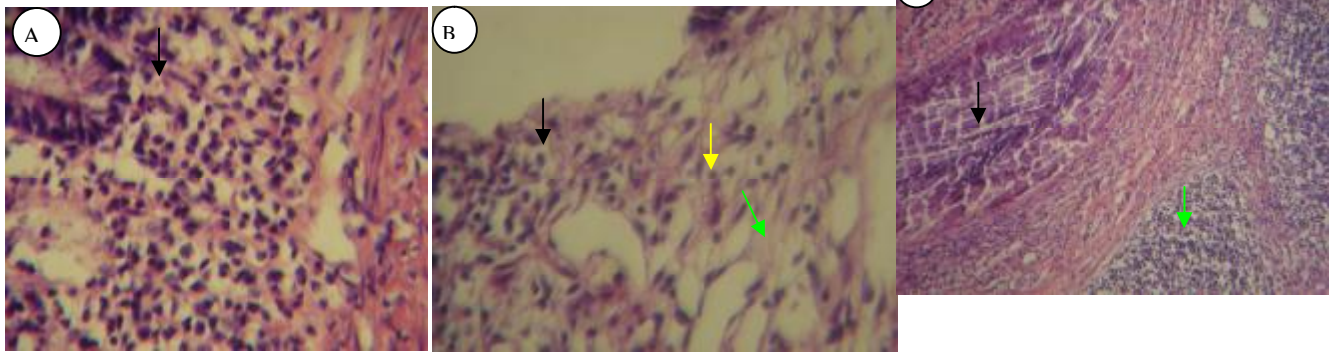
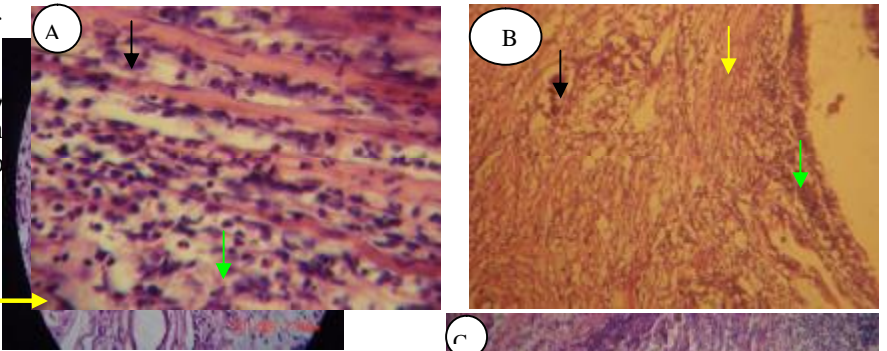
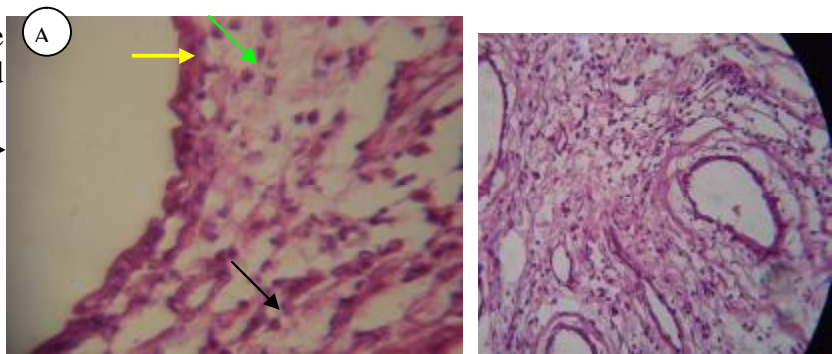


Fig.(13) Histological section in intestine of one animal at 30 day treated with free omentum revealed: A- large amount of mononuclear cells infiltration in subepithelial layer → . B- rounded villi lined by cuboidal epithelial cells covered the incision line → which filled with mature fibrous connective tissue →.infiltrated with mononuclear cells → C- treated material → surrounded by fibrous connective tissue severly infiltrated with neutrophils → (H and E 40X)

Fig.(14)Histological section of intestine of one animal at 15 day posttreated with pedicle omentum revealed:- A- mature fibrous connective tissue → with macrophage infiltration → in the incition line covered by moderate thickness of epithelial cell layer → B- high density of blood vessels → ( H and E 40X)



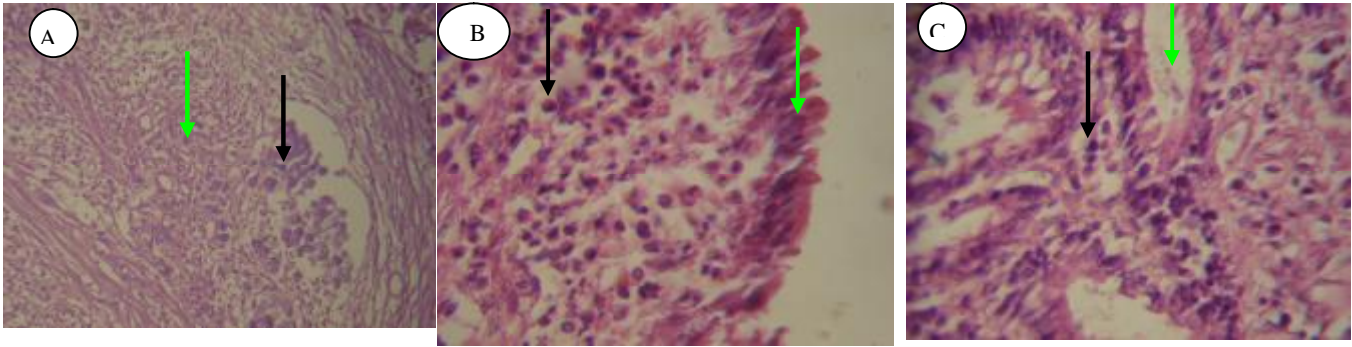
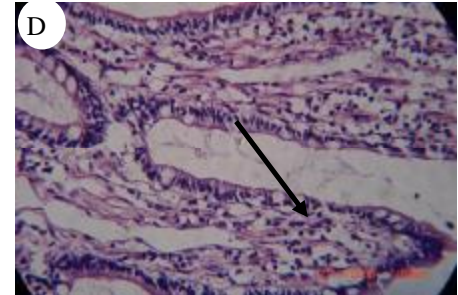


Fig.15 Histological section of intestine of one animal at 30 day posttreated with pedicle omentum revealed:- A-suture material → surrounded by fibrous connective tissue infiltrated with inflammatory cells → B- mononuclear cell infiltration in subepithelial Layer → with complete thickness of columnar epithelial layer covered the incision line → . C- few mononuclear cell infiltration → between mucosal glands → . D- mature villi → ( H and E 40X).



## Discussion

The results of clinical observations were appeared that, slight inflammation on the site of operation, which exhibited that the area around the incision became redness and oedema this agreed with other authors (18), (19). The animals revealed normal defecation after the operation and this coincide with other workers (20), (21), (22). Who proved the successful of perfect intestinal layers apposition with one layer suture pattern. First group showed adhesions more than third group may be due to the anastomotic site covered by pedicle omentum this minimized the adhesion(23).

In second group also showed adhesions more than the third group because the free omentum when it was resected behaved like any tissue subjected to injury by secreting the prostaglandines and also the free omentum produce large amount of macrophage leading to increase the intensity and period of inflammation by producing additional amount of prostaglandines causing the dilatation of blood vessels and increase it is permeability leading to increase the amount of exudate in the area, this phenomena was coincide with other workers(24), or it may be due to the omentum itself represent asource for fibroblasts and formation of fibrosis. While in third group appeared less adhesions because the pedicle omentum reduced the inflammation due to it is ability to absorb the odema, forgin bodies (surgical sutures and bacteria), inflammatory exudates and it is drainage leading to decrease the fibrosis and adhesions, this agree with the author (25). but disagree with (26) who found that use the free or pedicle omentum in intestinal anastomosis in rabbit increase the mortality and this may occurred due to small size of intestine and abdominal cavity in comparison with dogs. Third group showed best result in reduce stenosis degree because the presence of pedicle omentum reduced the odema as well as supply of the anastomosis area with new blood vessels (27) leading to increase of oxygen in the area and reduce the inflammatory reactions and adhesions. This technique of cross mattress suture may reduce the stenosis degree, this agree with (28). The stenosis degree in second group more than in two other groups may be due to the free omentum increase the adhesions around the anastomosis site and determined the dilatation of anastomotic line.

There is no significant differences of bursting pressure between the three groups but the second group showed more than the other two groups because increase in fibrous tissue and collagen fiber amount in anastomosis area and neighboring tissues , this agree with (29). While the strength of bursting pressure in first group lower than

that in other two groups may be due to the use of omentum in other two groups help to formation of fibrous connective tissue, this agree with (23).

The histopathological findings revealed that neutrophils were very few in the third group with the short inflammatory phase leading to presence of sufficient oxygen amount which help the conversion of proline to hydroxyproline and help on the formation of good type of collagen which indicator for healing process degree, this agree with the authors (30) who showed that the apposition sutures enhance the healing. While the neutrophils were found in large amount in first and second groups referring to long inflammatory phase and delay the healing. Moderate thickness of epithelial layer cells in third group at 15 days after operation and complete thickness of columnar epithelial layer covered the incision line after 30 days of operation and presence of regular mature fibrous connective tissue with few amounts of fibroblasts, blood vessels and large amounts of lymphocytes which stimulate and activate the phagocytes to produce excess amounts of growth factors may be lead to increase the healing process. There is Hyperplasia of intestinal glands with formation of many rounded villi lined by many columnar epithelial cells and this agree with the authors (31) who found that the healing of intestinal anastomosis by apposition layers technique occur at least degree of inflammation and fibrosis. So that the healing in this group was occurred by first intention which characterized by few fibrous tissue with formation of new blood vessels (Angiogenesis), this agree with the authors (30). Who found that the apposition layers techniques in intestinal anastomosis produce first intention healing. The follow up of histopathological results of group three revealed that this group achieved the main aims of intestinal anastomosis technique which were referred to them by the authors (30), (31). The above mentioned results refer to that the pedicle omentum contain substances interfere with immunal and inflammatory processes and stem cells have the ability to recognize into different cell types (32) and enhance the healing process by early angiogenesis in the anastomosis area with its ability to absorb the edema, foreign bodies, inflammatory exudates and its drainage, and increase the proliferation of fibroblasts, immunocytes and granulation tissue which make healing in anastomosis area (25), and also the pedicle omentum provide a biologically viable plug to prevent early leakage from anastomosis area (33). While in the first group at 15 days showed irregular and immature loose distribution of fibrous connective tissue covered by thin and vacuolated epithelial layer and there is proliferation of inflammatory cells and fibroblasts and at 30 days showed small rounded villi lined by one cuboidal cell supported by few matrix. In second group at 15 days showed severe inflammatory cell infiltration (neutrophils and monocytes) severe irregular fibrous connective tissue proliferation this occur may be due to that the omentum contain large amount of endothelial and mesenchymal cells which have the ability to convert into fibroblasts (34) and also the omentum contain B cell and macrophages with thin layer of epithelial lining cell. And at 30 days showed small rounded villi lined by one layer of cuboidal epithelial cells. These results in both first and second group refer to that the healing process was lower than that of third group.

The conclusion with this study revealed that the healing of third group better than other groups.

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