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RESEARCH ARTICLE

Laparoscopic Versus Conventional Surgery for Colotomy Closure; Molecular Evaluation

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Abstract

The goal of this study was to compare between laparoscopic and conventional surgery on the healing of colotomy closure by using cruciate mattress suture pattern. Twelve adult local breed bucks (male goat), were used, it was divided into two equal groups. In group A (Conventional group), the colotomy incision was created and closed by cruciate mattress suture pattern, while in group B (Laparoscopic group), the colotomy closure by same pattern intracorporeally using 3-0 Polygalactin-910. Molecular assessment at zero time, one and three weeks post operation by vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) gene expression to evaluate the colotomy healing process. Molecular evaluations indicate that the highest value of vascular endothelial growth factor was noted in group A whereas the lower value in group B at one-week post operation. The highest value of fibroblast growth factor-2 was noted in group B while the lower value in group A at one-week post operation. In conclusion the study clearly demonstrated that the healing process in cruciate suture laparoscopically superior to that occur in conventional technique.

Keywords: *Enterotomy, Colotomy, Laparoscopy, Cruciate Mattress Suture Pattern, Molecular, VEGF, FGF-2, Quantitative PCR, Colon, Bucks. Goat.*

Introduction

Enterotomy is an incision into the intestine is most often indicated for removal of foreign bodies and diagnostic or supportive procedures such as biopsy, culture, cytology and feeding tubes. Laparoscopic surgery is also called minimally invasive surgery, keyhole surgery or patient friendly technique. Laparoscopic surgery is commonly used in the diagnosis and managed of a variety of colorectal and gastric surgeries, gastrointestinal pathologies, pelvic conditions and all kinds of hernias.

It is also widely used to carry out surgical procedures as well as removal of diseased or damaged tissue and biopsies [1]. Laparoscopic surgery has spread worldwide these days and many digestive procedures have become the standard practice. Numerous reports favoring this approach have been published [2]. Laparoscopy in the veterinary medicine take place a different purpose such as, experimental purposes like intestinal anastomoses in dog [3], Cholecystectomy and liver biopsy in dog [4], Cholecystectomy in goats [5], abomasopexy in bovine [6] colopexy in dog [7], overiohysterectomy in goat [8]. There was a sparse research about laparoscopic intestinal surgery in small ruminant thus the goal of this study was to made compassion between the laparoscopic and conventional colotomy suture using cruciate mattress suture pattern in relation to post-operative healing of colonic wound closure based on the measurement of the levels of FGF-2 and VEGF at zero, one and three-weeks post operation.

Materials and Methods

Experimental Animals Design

In current study, 12 adult healthy bucks (2-3) years and weighing (23 ± 2.0) kg were used. Two weeks prior to experiment, all animals were housed in pens of farm animals at the

College of Veterinary Medicine/ University of Baghdad, they were dewormed against parasite using ALbendazole (10 mg/kg) and Ivermectin (Kepro) at a dose of 0.2mg/Kg., B.W., subcutaneously injected and vaccinated with Co-Baghdad vaccine (Alkendy/Iraq) administered subcutaneously with a dose of 5 ml to evade the enterotoxaemia.

Laparoscopic Equipment

The basic equipment for minimal invasive technique was used in this study manufactured by Karl Storz (Germany).

Surgical Technique

The experimental animals were divided randomly into two equal groups (6 bucks/ group), The bucks in all groups were fasted for feed and water for 24 and 12 hours respectively. The right flank region of group (A) was subjected to aseptic prepared, while the wide ventral abdominal area from xiphoid cartilage into the pre-pubic area and laterally into the right and left flanks in all the bucks in group (B) were prepared aseptically.

Buck was pre-medicated with an intramuscular injection of xylazine 2% 0.05 mg/kg B.W, IM and then anesthetized using an intravenous injection of thiopental sodium at a dose of 15 mg/Kg. B.W. for induction. The trachea was intubated and anesthesia was maintaining with a mixture of halothane (1.5- 2.5 %) and oxygen (2-3 %) with closed circular anesthetic system. In group (A), the anesthetized buck was laid on left lateral recumbence and the right flank region of each buck was properly draped, while in the group (B) buck was placed in dorsal recumbence with all the legs were tied to the surgical table apart, the surgical area was properly draping.

Surgical Procedure in Group (A)

A vertical skin incision was made in the middle of the para lumbar fossa extending from 3 to 5 cm ventral to the transverse process of the lumbar vertebrae for a distance of 8 - 10 cm was extended distally (Figure1.A), then the incision was made through external, internal and transverse abdominus muscles for entrance into the peritoneal cavity, following this the colotomy was performed. In order to optimize exposure of the colon, it is helpful to displaced the small bowel from the abdomen to the right of the abdominal incision then the exteriorize of colon (Figure1.B).

Digesta of extra abdominal part of the colon are gently milked into the intra-abdominal part of the colon, 2.5 - 3 cm enterotomy incision was made at the anti-mesenteric surface, About of 0.5 cm length and width were obtained from the colon incision site which mixed with TRIzol® reagent for gene expression ,then the incision was closed by cruciate suture (Figure1.C) by using 3-0 Polygalactin-910 (Vicryl; Ethicon, UK), the exteriorized colon was continuous wet with pre-warmed 0.9% saline solution and rinsed with copious amounts of this solution prior inserted into abdominal cavity.

After that the bowel was replaced into normal abdominal position. The peritoneum and transverse abdominal muscle were closed with simple continuous sutures pattern using same suture materials mentioned above. The internal and external abdominal oblique muscles were closed at the same manner. Care was taken to ensure firm apposition of the fascia in the external abdominal oblique muscle (Figure1.D). The skin was closed with non-absorbable sutures silk (No.1) in a horizontal mattress suture pattern (Figure1.E).

Surgical Procedure in Group (B)

Animal was placed in dorsal recumbence with the fore and hind limbs were tied to the surgical table, three ports were used, first ports for the laparoscope (telescope) while the second and third ports for laparoscopic instruments. First port was induced in the midline area between umbilicus and xiphoid cartilage, 12 cm from umbilicus and 4 cm caudal to the xiphoid cartilage.

Second port in the left para median area was about 12 cm caudal to the last rib and 7 cm dorsal to umbilicus and about 13 cm lateral to the first port, while the third port was induced in the left para median area was 11 cm caudal to the last rib and 3-4 cm dorsal to umbilicus and about 7 cm lateral to the first port. Approximately 1 cm of mini laparotomy incision was performed to allow the passage of the first laparoscopic port and with a screwing motion, an (10-mm) trocar-cannula unit was passed through the abdominal wall and directed caudally with a 30º angle inclination. Then the insufflators tube (CO_2) was connected to the laparoscopic portal

cannula for establishing and maintaining the pneumoperitoneum (8-12 mmHg) at a rate of (3.5 L /min.), after that the trocar was removed and the telescope (10 mm, 30° and 45 cm long) with camera attached was introduced through the rubber seal on the cannula. The peritoneal cavity immediately explored by the telescope to ensure the insertion of the other port under vision and don't caused any trauma or bleeding to the abdominal viscera and to note the presence of any abnormalities.

The grasping forceps was introduced into the abdominal cavity through the second and third cannula under vision, which used for manipulate the abdominal organs, lifting the greater omentum and to identify the colon. After identification of colon, grasping the operated area and the stay suture was placed on the colon in order to suspend it into the ventral abdominal wall caudal to the umbilical area in view of the laparoscopic telescope. Silk suture (No. 0) was passed through the abdominal wall into the peritoneal cavity about 10 cm superior to the colotomy site.

The surgical needle was passed into the right quadrant by the assistant and pierced into the peritoneal cavity intra corporeally, the needle was passed through colon that was grasped by the forceps and returned back out of the abdomen at the point of insertion and the silk was clamped extra corporeally on the outer surface of the abdomen wall (Figure 2A). Approximately 2.5 - 3 cm incision was performed by the use of laparoscopic scalpel blade No.10 in the exposed colon wall; the incision line was orientated perpendicularly to the long axis of colon (Figure 2B). About of 0.5 cm length and width were obtained from the colon incision site which mixed with TRIzol® reagent for gene expression (Figure 2C). The surgical site was examined for hemorrhage and leakage. Then the probe of sucker was used to wash the surgical area. The colotomy incision was closed by using cruciate suture (Figure 2D) using vicryl (No. 3-0), then the stay suture was removed extra corporeally in order to allowing the suspend part of the colon to return to the position.

After completion of the procedure, the laparoscopic port instrument and the laparoscope were retracted, pnemoperitoneum (CO_2) was evacuated by opening the cannulas, then removed of all ports. The laparoscopic portal sites were closed through suturing of the muscles and peritoneum with vicryl (No. 0) in a simple continuous suture pattern, and the skin was closed with silk (No. 1) in simple interrupted suture.

Postoperative Care

Systemic antibiotic (penicillin-streptomycin) was administered at a dose rate of (10.000 I. U/Kg B.W. and 20mg/kg B.W.) respectively for 5 days postoperatively. During the two-day post operation, the pre-warmed fluid therapy (Sodium chloride 0.9 % and glucose anhydrase 5 %) (ADWIC. Egypt) was administered at a dose of (10-20 ml / Kg B.W.), then soft food was offered at the third day postoperatively and return to normal food at the 7 days postoperatively. The skin sutures were removed on day 12th post operation.

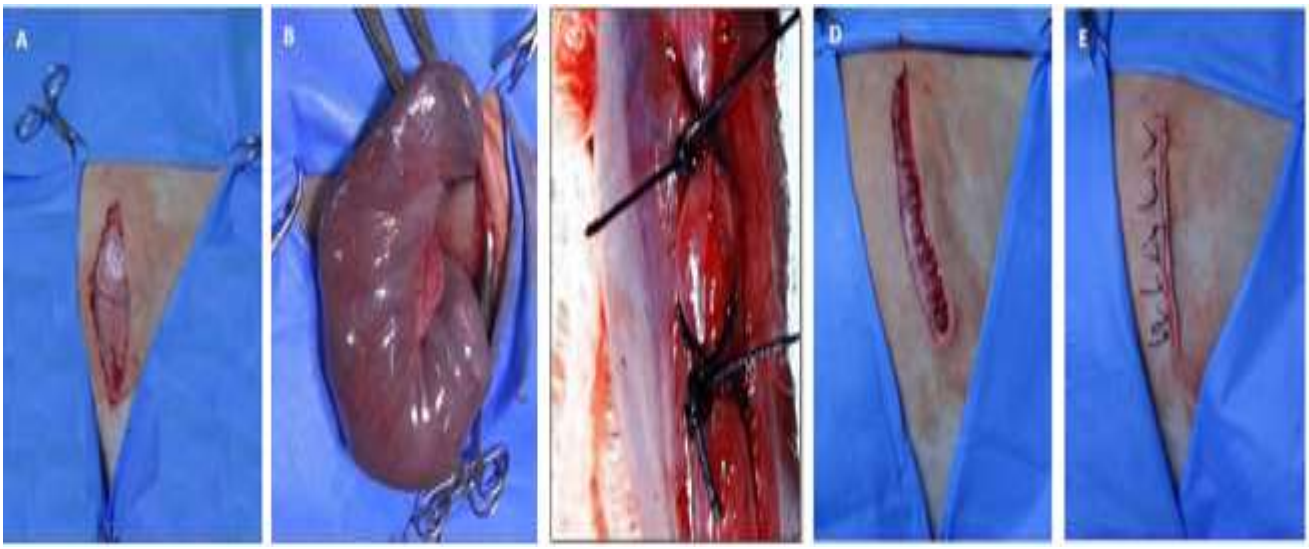

Figure 1: Shows vertical skin incision in right flank region (A), exteriorize of part of colon outside the abdominal wall (B), colotomy closure by cruciate mattress suture pattern (C), muscles closure by simple continuous suture pattern (D), skin closure by horizontal mattress suture pattern (E)

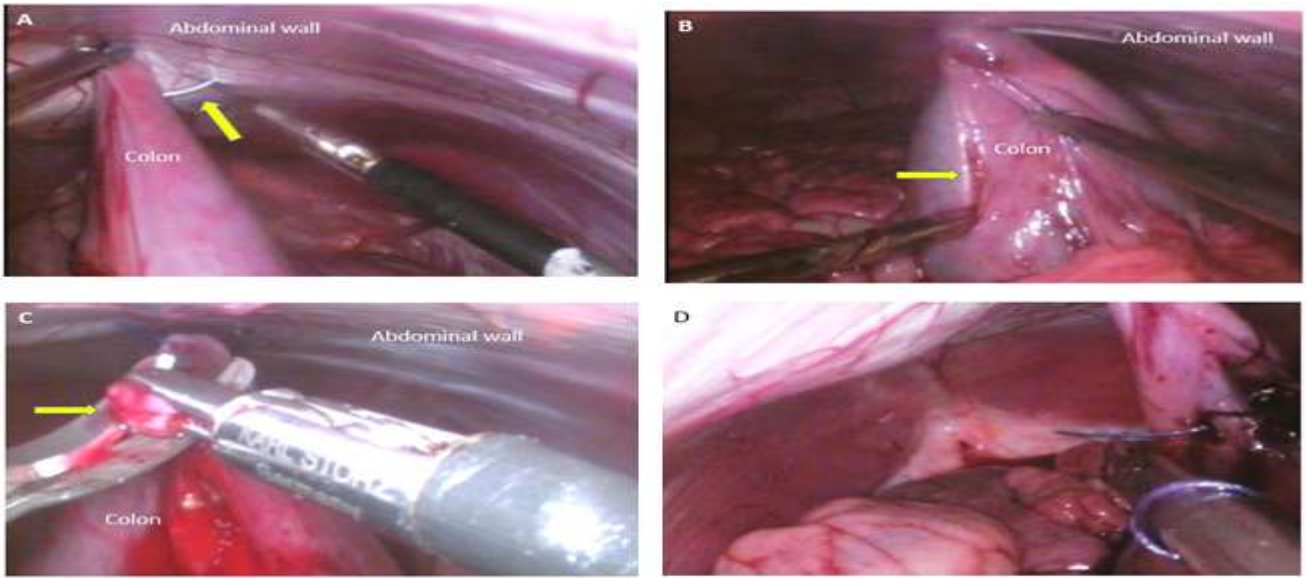

Figure 2: Show the first step of laparoscopic colotomy procedure include suspended of colon and stay suture (arrow) was placed to fixation of colon (A), the incision of colon by using of laparoscopic scalpel blade (arrow) (B), the sample obtain from the colon for gene expression (arrow) (C), the colotomy suture by intracorporial cruciate mattress suture pattern (D)

Molecular Evaluation

The biopsies from colon operative site were collected in zero day which recorded as a control, then at 1 and 3 weeks post-operative (3 animals for each period) and these biopsies were immerged in Trizol Regent (Thermo Scientific, U.S.A.), and kept in freezing, then evaluated using Real Time Polymerase chain reaction (R.T.P.C.R) technique for VEGF and FGF-2.

Materials: Kits, Instruments and Primers

The components of the kits were mentioned in (Table, 3).

Table 3: Details of kits components used in this study

Kits	Company/ Origin
TRIzol Reagent	Thermo Scientific, USA
GoTag qPCR Master Mix, GoTaq® 1-Step RT-qPCR System, MgCL2, Nuclease Free Water, Quantiflor RNA System.	Promega, USA
Isopropanol,70% Ethanol	ROMIL pure chemistry,UK
Chloroform	LiChrosolv, Germany

Primers

The primer used in this study including the specific gene of goats which including Anterior Reverse Fibroblast Growth Factor2 (AR-FGF-2), Anterior and Reverse Vascular Endothelial Growth Factor (AR-VEGF), genes primers that were used as target genes (Table, 5). The primers were used in quantification of gene expression by using Real-Time quantification Polymerase Chain Reaction (RTqPCR) techniques based BRYT Green DNA binding dye (Promega-USA).

Table 5: The details of primers used in the study

Primer Name	Seq.	Product Size	Annealing Tem.
FGF2-F	5`-AGTGTGTGCAAACCGTTACCTTGC-3`	107	60
FGF2-R	5`-ATACTGCCCAGTTCGTTTCAGTGC-3`		
VEGF-F	5`-GTGCGGGGGCTGCTGTAATGA-3`	100	
VEGF-R	5`TCACCAGGAAAGACTGACACA-3`		

Methods and Workflow

RNA Purification

RNA was isolated from sample according to the protocol of TRIzol™ Reagent and processed according to the steps mention in kit leaflet.

Quantitation of RNA

Quantus Florometer was used to detect the concentration of extracted RNA or cDNA in order to detect the goodness of samples for downstream applications. For 1 µl of RNA, 199 µl of diluted Quanty Flour Dye was mixed.

After 5 min. incubation at room temperature in dark place, RNA concentration values were detected.

Absolute Quantification by the Standard Curve

The standard curve method employs a dilution series of known template copy number in the qPCR assay. Linear regression of log concentration (copy µl-1) versus C_T gives the standard curve, and this is then used to calculate template concentration (copy µl-1) of the sample.

Reaction Setup and Thermal Cycling Protocol

Quantitative PCR (qPCR) master mix was ready with one step RT-qPCR kit that dependent BRYT Green dye discovering of gene amplification in Real-Time PCR system and its contents were listed in (Table, 6). After that, the smart PCR tubes were loaded in the smart cycler instrument using the reaction protocol mentioned in (Table, 7).

Table 6: The quantitative Real-Time PCR (qPCR) master mix Kit

Master mix components	Con.(stock)	Con.(final)	Volume
qPCR Master Mix	2 X	1 X	5
RT mix	50 x	1 x	0.25
MgCl2			0.25
Forward primer	10 µM	1 µM	0.5
Reverse primer	10 µM	1 µM	0.5
Nuclease Free Water			2.5
RNA	10 ng/µl	10 ng/µl	1
Total volume			10
Aliquot per single rxn	9 µl of Master mix per tube and add 1 µl of Template		

Table 7: The protocol in Real-Time PCR system

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:20	40
Annealing		00:20	
Extension	72	00:20	

Statistical Analysis

The results were expressed as Means ± S.E. Parametric data were analyzed by one ways analysis of variance (ANOVA) continued with Least Significant Difference (L.S.D.), and P< 0.01 was considered to be significant. Statistical Package for Social Sciences (SPSS) was used SAS [9].

Results

VEGF

Table 8: Effect of group and weeks in mean of VEGF concentration

Group	Mean ± SE of VEGF conc.		LSD value
	One weeks	Three weeks	
Group A	211.02 ± 8.05	14.47± 1.25	31.45 **
Group B	52.49± 2.47	10.71 ± 0.59	6.75 **
LSD value	30.409 **	4.053 NS	---
** (P<0.01).			

The VEGF in (Table 8) recorded the increase of its gene values at two periods first and third week in group A (211.02 ± 8.05) and (14.47 ± 1.25) respectively, compare with group B values. The significant differences (P<0.01) appeared at 1st week in between both groups, but disappeared at 3rd week.

FGF-2

The FGF-2 in (Table 9) reflected the elevating of its gene values at 1st and 3rd week in group B (281.81±14.62) and (101.07±7.28) respectively, compare with group a values. The significant differences (P<0.01) appeared at 1st week and 3rd week in between both groups.

Table 9: Effect of group and weeks in mean of FGF-2 concentration

Group	Mean ± SE of FGF-2 conc.		LSD value
	One weeks	Three weeks	
Group A	168.82 ±12.54	21.11 ± 1.42	22.85 **
Group B	281.81 ± 14.62	101.07 ± 7.28	19.56 **
LSD value	41.362 **	26.774 **	---
** (P<0.01).			

Discussion

VEGF

The molecular results of the level of VEGF in the current study showed significant difference between both groups along studied period, as well as, the level of VEGF which showed superiority at 1st week and decrease with time at 3th week for both groups. This result may be attributed to the progression of the healing process, in which the VEGF was responsible for angiogenesis to enhance the formation of granulation tissue and remodeling that require less vascularity due to tissue maturity.

Johnson and Wilgus [10] mention the steps of sprouting angiogenesis, new blood vessels are created from established quiescent vessels. Quiescent vessels exhibit a mature phenotype, with phalanx ECs lining the inner surface of the vessel. Quiescent vessels are covered by a basement membrane and are typically surrounded by pericytes. When endothelial cells become activated in response to a proangiogenic stimulus like VEGF, the basement membrane is degraded, pericytes detach, and a specialized endothelial cell called a tip cell is selected.

This cell guides elongation of the new vascular sprout. Stalk cells follow behind the tip cell, migrating and proliferating to lengthen the new vessel. During the resolution phase of angiogenesis, two adjacent new vessels will join one another to establish blood flow and non-perfused vessels will regress. Eventually the basement membrane will be re-created, pericyte coverage will be restored, and the vessels will return to a quiescent state.

In addition to initiate angiogenesis, the role of a high VEGF level in the wound may have another function to temper the local immune response in the injured area, this come in correlate with [11] whom reported that the devitalized tissue and exposed self-antigens in a wound contain an intense immunologic stimulus and the VEGF may act as an immunosuppressive agent that prevents a local autoimmune reaction.

In this study the level of VEGF gene was low in laparoscopic groups when compared to conventional groups at one week, this means the level of VEGF reach the peak at 3-4 days and will progress at one week, this may agree with other stated that, the significantly higher increase of local VEGF during one week in the laparoscopy group *vs.* the laparotomy group was interesting [12].This may be explained by the postoperative effect of local acidosis, from CO2 insufflation on endothelial cells. Furthermore, another study has demonstrated *in vitro* that hypercarbic acidosis induces VEGF expression in bovine aortic endothelial cells [13].

The results showed that the levels of VEGF in the two groups were increased in different degrees at one week after operation, the growth factor levels of the laparoscopic group were significantly lower than the conventional open surgery group, the difference was statistically significant at 1st week, the results of this study are consistent with those reported in previous studies [14], other researchers have confirmed that the laparoscopic surgery was less traumatic and has a relatively mild inflammatory response [15].

The result of molecular evaluation of VEGF gene expression indicate less post-operative pain in laparoscopic group when compared with conventional group, this may be due to small incision and less damage to nerve network and also may be attributed to decrease expression of VEGF level in this group. This corresponded with [16] whom stated that the vascular invasion into the tissue is associated with increased expression of nerve growth factor (NGF) within the vascular channels; the NGF promotes sensory neuron survival, axonal outgrowth and, importantly, is associated with pain sensitization.

FGF-2

The elevated level of FGF-2 in group B reflects more fibroblast proliferation and collagen deposition than in group A at one week and third weeks post operation in the current study.

These results are correlate with previous studies [17] in rat whom suggested that FGF-2 serves as a key factor to accelerate wound healing by promoting fibroblast proliferation and neovascularization and agree with [18] in dog, whom stated that the local application of FGF2-GH induced enormous proliferation of fibroblasts and dense collagen at the pancreaticojejunal anastomosis site after one-week post operation. And also the results of our study are in accordance with previous work by [19] whom stated that the significant expression of FGF-2 in wound tissue begins approximately 8 days after full thickness wounding, and peaks at 12-14 days.

Riswana and Don [20] reviewed that, some FGFs have been detected at the wound site, demonstrating that the endogenous proteins are also regulators of wound healing. To provide functional evidence for the role of FGF-2 in wound repair. Broadley *et al* [21] used a neutralizing polyclonal antibody that was raised against FGF-2. The continuous release of the antibody caused a striking reduction in cellularity and vascularization compared with the granulation tissue formed in the control. In addition, DNA, protein, and collagen levels in the anti-FGF-2 group were reduced by 25–35% relative to control at day 7 after implantation. This study strongly suggested an important role of endogenous FGF-2 in wound repair.

Conclusion

The study clearly demonstrated the benefit of laparoscopic surgery and it has also been proven to be exploration of abdominal cavity. Molecular evaluation shows better healing in laparoscopic groups when comparison with conventional groups.

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