An assessment of clinical, biometric, cosmetic and microscopic outcomes of four suture techniques for cutaneous closure of laparotomy wounds: an experimental study in rabbits

Abstract

The purpose of this study is to identify a suture technique that provides superior cosmetic outcomes and proper wound healing for skin closure after midline laparotomy of small animals in a rabbit model. This study also attempts to clarify the role of selected four suture patterns in wound healing, wound complications, cosmetic impacts and histopathology on the laparotomy skin wound. Twenty four female rabbits of white New Zealand and California breeds were used for this experimental study. Four suture patterns were compared for cutaneous closure of a 7-cm midline laparotomy wound, classified as: buried continuous subcuticular–intradermal (BCSID), interrupted cruciate mattress (ICM), running horizontal mattress (RHM), and far–near–near–far (FNNF). The different groups were studied in terms of clinical findings, wound and suture biometrics, cosmetic assessment, microscopic examination, and statistical analysis. RHM presented a ‘very good’ cosmetic grade on a 6-element scale, clinically associated with rapid successful wound healing, and no complications. BCSID was superior in cosmetic terms among the tested groups where it showed an aesthetically ‘excellent’ score. However, the technique was not efficient enough to prevent wound dehiscence in some cases. ICM demonstrated a ‘very good’ cosmetic degree but was not functional enough to prevent invasive contamination or infection in certain instances. FNNF was the inferior among all groups in regards to the cosmetic outcomes but was characterized by successful, slowly-progressive healing. Suture-to-wound length (SL: WL) ratio of all groups seemed to have a correlation with the rate of wound dehiscence as wound dehiscence is unlikely to occur if a SL: WL ratio is more than 4:1. The microscopic results proved that the RHM suture pattern was the favourable technique. RHM can be concluded as the suture technique of choice for cutaneous closure of laparotomy wounds in small animals like rabbits. It serves as a compromise of high-quality cosmesis and optimal wound healing. The assessed suture techniques can be graded in a descending order from the superior to the inferior cosmetically as BCSID>RHM>ICM>FNNF, and histopathologically as RHM>FNNF>BCSID>ICM. Clinical trials are needed to be performed to validate and reproduce the outcomes of this study on canine and feline patients.

Keywords: cosmetic surgery, suture technique, wound healing, laparotomy, histopathology

Introduction

Laparotomy is a commonly performed surgical procedure in small animal practices and referral hospitals. Technical considerations when performing the operation include an appropriate patient preparation, surgical approach and wound closure. It is important to know whether the wound closure by continuous or interrupted suture techniques provide proper wound healing and lower complication rates, so as to allow veterinary surgeons to make an informed decision when choosing a pattern for an abdominal closure. Several suture techniques are routinely used for cutaneous closure of surgical wounds, specifically those implemented in laparotomy operations of small animals as dogs and cats. Surgeons and pet owners have various controversial attitudes on the subject of cosmetic surgery. However, the owners and breeders take special pride in their pet’s appearance for dog/cat show competitions, and are deeply shocked by the poor cosmetic outcome of a given surgery, even if patient function and comfort have been restored or maintained. An ideal suture technique should provide accurate, quick and secure skin edge apposition for a sufficient length of time until complete healing occurs. Major wound complications include wound dehiscence, haemorrhage, infection, evisceration or herniation that may develop to worsen the final results. In non-complicated cutaneous wounds, an in-depth look shows that fibrotic scar deposited during skin wound healing can cause disfiguration and loss of dermal function. The need for rapid, aesthetic, and effective closure of the cutaneous wound has increased over recent years. Useful suture techniques improving cosmetic results include deep dermal sutures with buried knots, simple interrupted sutures, vertical mattress, horizontal mattress, subcuticular sutures, continuous over-and-over sutures and far–near–near–far pulley sutures. A variety of suture materials are also available to select appropriately for each wound. In the past, a general veterinary practice would have stocked only silk, catgut, nylon and perhaps stainless steel, but we now use a range of synthetic absorbable and non-absorbable sutures. The role of sutures in wound repair process is dedicated to provide haemostasis and adequate support for healing tissue. Wound healing is the reestablishment of tissue continuity that occurs through a complex process with well-orchestrated cellular and
biochemical events activated at the time of injury. The process is conceptually divided into three continuous and overlapping phases: inflammation, proliferation, and maturation. There has been an assumption that wound healing is a relatively homogeneous process across species. We need to look into the presentation of wound healing in various veterinary and human surgical studies, and to the fact that almost all knowledge of cutaneous wound healing has been derived from studies on relatively few species as rodent, pig, dog, horse, and human. As the skin of rabbits resembles in a great extent to that of cats, an experimental study in rabbits gives pivotal results that can be more representative and mimic to those of cats. Many researchers in veterinary and human practice have investigated the healing of laparotomy skin wounds with regards to aesthetic impact. Previous studies on dogs, cats, rabbits, and rats have shown that the approach and design of suture technique exerts a clinical, biomechanical, physical, and histological influence on the quality of wound healing. This study aimed to compare between four suture techniques for skin closure of midline laparotomy incisions in order to achieve superior cosmetic results, effective wound closure, lowest postoperative complications, and optimal wound healing. The four suture techniques involved buried continuous subcuticular–intradermal suture pattern (BCSID), interrupted cruciate mattress suture pattern (ICM), running horizontal mattress suture pattern (RHM), and far–near–near–far suture pattern (FNNF). The different suture patterns were assessed in terms of clinical, biometric, cosmetic and microscopic outcomes of the cutaneous wound healing in a rabbit model.

Methods

Animals

Twenty four (24) clinically healthy female rabbits of white New Zealand and California breeds (8 NZ and 16 CA) whose age ranged between 63–132 days (Mean±SEM; 111±5.5days) with a body weight of 1055–2910gm (1965±97.4gm) were used for the study. Recommendations of the Guide to the Care and Use of Experimental Animals were followed to come up with the concepts of animal welfare and proper management. The rabbits were obtained from Poultry Farm of the Faculty of Agriculture, Assiut University (Assiut, Egypt), and clinically examined to ensure good health status and absence of any dermato logical disorders.

Experimental design

The study design was approved by the Board of Veterinary Surgery Department Council and Faculty of Veterinary Medicine Council with a sample size of 20–30 animals considering animal reduction principles. The animal sample size was determined in the current study by a power calculation from a previous study on cats that has used 24 animals. Randomization was assigned by two participants who were blinded to the study design. The animals were chosen randomly for ear identification using a permanent marker. Randomization was carried out via a paper lottery. The animals were divided into four groups, each with two subgroups. Each group involved six rabbits; and each subgroup included three rabbits. The four groups were classified according to the skin suturing pattern. The corresponding subgroups were subdivided based on the time sequence of histopathological samples’ collection on the 10th and 17th postoperative day (POD).

Surgical operations

The surgical operations were performed by the same surgeon at the Small Animal Operation Unit, Department of Veterinary Surgery, Anaesthesiology and Radiology, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. Surgeries were performed under general anesthesia by IM injection of ketamine hydrochloride (Ketamine®, 50mg/ml, Sigma-Tec, Egypt) and xylazine hydrochloride (Xyla Ject®, 20 mg/ml, Adwia Co., Egypt) at the dose rate of 50mg/kg for ketamine, and 10mg/kg for xylazine. The animals were secured in a dorsal recumbency. The operation field was aseptically prepared in a routine manner at the ventral abdominal wall. A 7–cm longitudinal midline incision in the skin, subcutaneous tissue, linea alba and peritoneum was performed. In all groups, the subcutaneous tissue, abdominal muscles and peritoneum were closed in a mass closure fashion with 4 interrupted cruciate mattress sutures using absorbable monofilament 3–0 polydioxanone (PDX) (Unicryl M®, UniMed, KSA) as in Figure 1. The skin incision was sutured in the different groups by four different suture techniques. The four suture patterns involved:

i. Buried continuous subcuticular–intradermal suture pattern,
ii. Interrupted cruciate mattress suture pattern,
iii. Running horizontal mattress suture pattern, and

Figure 1 Plate showing the operative technique of laparotomy in the rabbit. (A) Placement of towels and clamps. (B) Incision of the skin, with dissected subcutaneous tissue. (C) Incision of the linea alba before peritoneal penetration. (D) Exploration of abdominal viscera after peritoneal penetration. (E) Suturing of the subcutaneous tissue. (F) Suturing of the abdominal muscles with four cruciate mattress sutures. (G) Insertion of the needle in the dermis for placement of the initial buried suture in BCSID closure. (H) Burial of the end suture after apposition of the skin edges prior to cutting off the suture thread in BCSID closure.

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Configurations of the suture techniques were illustrated in a schematic diagram (Figure 2). The BCSID suture technique was performed with fully buried knots. The suture materials used for skin closure were:

1. Absorbable monofilament 3–0 PDX (Unicryl M®, UniMed, KSA) in the BCSID group, and
2. Non-absorbable multifilament 3–0 silk sutures (Silk®, Bekon, South Korea) in the ICM, RHM and FNNF groups.

Postoperative care

A long-acting oxytetracycline antibiotic (Alamycin LA®, 200 mg/ml, Norbrook, UK) was IM administered as a single dose following the surgery at the dose rate of 20mg/kg. A protective non-compressive abdominal bandage was applied for 6 days using sterile gauze and replaced after every wound examination post surgery. The rabbits were housed during the entire study period under the same conditions at the Experimental Animal House, Faculty of Human Medicine, Assiut University, Egypt. The animals were allowed free access to food and water ad libitum. A maintenance diet of both commercial feed pellets (Vinos®, Vinos Feed Service Co., Egypt) and fresh vegetables (carrot and parsley) were given in a natural light/dark cycle, with an ambient/warm room temperature.

Clinical assessment

The skin wounds were followed up on postoperative days (PODs) 0, 2, 4, 6, 8, 10, 12, 14, 16 and 17 for clinical findings including; scab (inflammatory exudate), haemorrhage, hyperaemia, purulent exudate, dehiscence, and swelling. Presence of sinus, fistula or incisional hernia was also inspected. Parameters for clinical assessment of wound healing were summarized in Table 1 which constitutes a modified method after Pedrajas.26 The scab color was assessed through a colorimetric index of wound healing continuum.27 Wound dehiscence percentage was calculated by means of dividing the length of dehiscence on the entire wound length per cent.28 The surgery duration of skin closure was also recorded.

Table 1 Parameters for clinical assessment of wound healing: a modified method after Pedrajas

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scab (inflammatory exudate)</td>
<td>Type: serous/sero-sanguineous/sanguineous</td>
</tr>
<tr>
<td></td>
<td>Amount: none/scant/moderate/copious</td>
</tr>
<tr>
<td></td>
<td>Colour: Colorimetric index of wound healing continuum</td>
</tr>
<tr>
<td>Haemorrhage</td>
<td>Amount: none/mild/moderate/severe</td>
</tr>
<tr>
<td>Purulent exudate</td>
<td>Amount: none/scant/moderate/copious</td>
</tr>
<tr>
<td>Swelling (elevation)</td>
<td>Degree: none/mild/moderate/severe</td>
</tr>
<tr>
<td>Dehiscence</td>
<td>Percentage:WD %=(L×100)/E</td>
</tr>
<tr>
<td>Sinus/Fistula/Hernia</td>
<td>Existence: absent/present</td>
</tr>
</tbody>
</table>

WD %, wound dehiscence percentage; L, length of dehiscence; E, entire wound length;
26 Gray et al. 2010
27 Hohenleutner et al. 2000

Wound and suture biometrics

Measurements of the wound length were taken on PODs 0, 5, 7, 10 and 17 for evaluation of wound contraction. The length of consumed suture in surgery was measured for calculation of the suture-to-wound length ratio (SL: WL) as described by Varshney et al.29 This calculation was carried out via a mathematical formula; consumed suture length (cSL)=preoperative suture length (pSL)−remained suture length (rSL). Then, the consumed suture length (cSL) was divided on the preoperative wound length (pWL).

Cosmetic assessment

The scoring system used for cosmetic assessment of the healing wounds was the Stony Brook Scar Evaluation Scale (SBSES) with a slight modification after Rao et al.30 Two assessors performed the
cosmetic assessment; of which one assessor was aware of the different groups (subjective assessment), and one assessor was blinded to the different groups (objective assessment). The parameters of the original SBSES included width, height and colour of the scar, presence of suture/hatch marks, and overall appearance on PODs 10 and 17. Hair development in the cutaneous wounds was evaluated as a new parameter to the modified scale (mSBSES) with 0 for absence and 1 for the presence of any hair on the incision line (Table 2).

**Microscopic assessment**

The histopathological specimens were taken on the 10th and 17th POD from every rabbit in each group. Fresh tissue specimens of rectangular area were collected including the operative line. They were excised carefully to avoid traumatization. The specimens were fixed in 10% neutral buffered formalin. They were dehydrated in a graded alcohol series, cleared with methyl benzoate, embedded in paraffin wax, sectioned at 4-μm thickness, and stained with hematoxylin and eosin for histopathological examination by light microscopy. Histopathological characterization of wound healing in the different groups was blindly assessed by the same pathologist, as described by Kiani et al.†

**Table 2: Cosmetic scoring system of wound scars: Modified Stony Brook Scar Evaluation Scale (mSBSES)**

<table>
<thead>
<tr>
<th>Scar category</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Width</strong></td>
<td></td>
</tr>
<tr>
<td>&gt;2</td>
<td>0</td>
</tr>
<tr>
<td>$\leq$2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td></td>
</tr>
<tr>
<td>Elevated / depressed</td>
<td>0</td>
</tr>
<tr>
<td>Flat</td>
<td>1</td>
</tr>
<tr>
<td><strong>Colour</strong></td>
<td></td>
</tr>
<tr>
<td>Darker than surrounding skin</td>
<td>0</td>
</tr>
<tr>
<td>Same colour or lighter colour</td>
<td>1</td>
</tr>
<tr>
<td><strong>Hatch/suture marks</strong></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>Absent</td>
<td>1</td>
</tr>
<tr>
<td><strong>Hair development</strong></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td><strong>Overall appearance</strong></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
</tr>
<tr>
<td>Good</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6</td>
</tr>
</tbody>
</table>

*SBSES—Standard scale parameters (Singer et al. 2007, Rao et al. 2015)

†mSBSES—A novel parameter added by the present authors to this modified scale.

§Grading system of total mSBSES mean percentage, Excellent: 80–100%; very good, 70–79%; good, 60–69%; satisfactory, 50–59%; poor, 30–49%; very poor, 0–29%.

**Statistical analysis**

Repeated measures two-way analysis of variance (ANOVA) was performed to detect any overall differences between the suture techniques in terms of wound length (WL) that was measured over different time points. In this analysis, the suture technique and the time of wound healing (T) were set as fixed effects, whereas the subject (rabbits) was considered random effects. The one-way ANOVA test was used to find whether the suture techniques are statistically different in terms of suture lengths (SL) and suture-to-wound length (SL:WL) ratio. A follow up analysis was performed using Tuckey’s test for multiple comparison to find which pair of techniques are different using the 95% confidence intervals. To compare the suture techniques in cosmetic scores of mSBSES, Welch’s test was used instead of one way ANOVA. This test assumes unequal variances, which is practically better than assuming equal variances in normal ANOVA and it works well when the response variable takes a few numbers of numeric values. Differences between techniques were considered statistically significant when P<0.05. The data analyses were conducted using Minitab Statistical Package (Version 18.1, Minitab Inc., developed at Pennsylvania State University, USA).

**Results**

**Clinical assessment**

All wounds of BCSID group showed marked hyperaemia of the skin around the incision on POD 0. The terminal ends of all wounds were slightly elevated over the initial and terminal knots. In 1 out of 6 incisions, the wound margins at the entire length were slightly elevated over the skin surface on POD 2. Sanguineous exudate was seen predominantly from POD 2 to 4. The scab sloughed off by POD 4. Two cases out of 6 (33.5%) showed partial dehiscence (WD=33.5%) at the caudal third of incisions, where these wounds eventually healed by second intention. Hair started to grow from POD 10 until it masked the whole incision line at POD 17 (Figure 3). In ICM group, the wound margins were slightly elevated over the skin surface in all animals (Figure 3). Serosanguineous exudate was found in medium quantities confined to the wound bed and partially soiled the protective dressing on POD 2, and the subsequent scab developed from PODs 2 to 10. In 3 cases (50%) on POD 6, there was an increase in the amount of exudates that could stretch the wound margins a bit to render a small filled gap between a couple of sutures. Of such cases, one revealed scant purulent exudate. From POD 12 to 17, the other wounds were seen with variable degrees of hair growth. All wounds of RHM group were slightly elevated on POD 0 (Figure 3). They gradually relieved and eventually flattened with the surrounding intact skin by POD 12. Every single wound did not show any haemorrhage or purulent discharge. Pink wound beds as well as serous scab were characteristic findings in this group from POD 2. Scab started to slough off from POD 6, and completely fell off by POD 10. Hair growth was not noticeable in 2 out of 3 cases (66.7%). Regarding hyperaemia, the skin colour in all wounds was normal except one which was mildly hyperaemic on POD 2 and returned normal on subsequent days. The skin edges in FNNF group were slightly inverted on POD 0 (Figure 3). Minimal dehiscence (WD=10%) occurred at the middle portion of incision in one case (16.7%) on POD 2 due to animal self-mutilation. No haemorrhage was observed in any day. Three cases (50%) were hyperaemic and showed marked allergic signs on POD 4 presented with rashes, denuded or erosive scaly skin in the surrounding area which recovered and appeared normal by POD 8. The suture marks of all wounds of the group left a characteristic railway-like appearance. No sinus, fistula nor incisional hernia developed in any of the four groups. The surgery duration of skin closure lasted for 44-56 (Mean±SEM; 49.5±2.0) minutes in BCSID group, 15–18 (17.2±0.5) minutes in ICM group, 16-19 (17.5±0.6) minutes in RHM group and 15–21 (18.2±0.9) minutes in FNNF group.
Wound and suture biometrics

The wound lengths (WL) of BCSID group decreased from POD 0 to POD 5 then slightly increased on POD 7 and re-decreased successively from POD 10 to POD 17 (Table 3 & Figure 4). The WLs of ICM group decreased from POD 0 to POD 10. Afterwards, the lengths returned to be around the initial WL on POD 17. Measurements of the WLs for the RHM group decreased continuously from POD 0 to POD 10 and then increased again on POD 17. All WLs of FNNF group decreased gradually from POD 0 to POD 7, slightly increased on POD 10 and declined afterwards on POD 17. The measurements for preoperative suture lengths (pSL), remained suture lengths (rSL), consumed suture lengths (cSL), preoperative wound lengths (pWL) and suture-to-wound length ratios (SL:WL) for each group were recorded in Table 4.

Cosmetic assessment

The wound scars of BCSID group on POD 10 had an aesthetic total score 4.5±0.7 out of 6 (where the number 6 is the optimum score of modified Stony Brook Scar Evaluation Scale). Such score of the BCSID group was graded as ‘very good’ as 75%. Later on POD 17, the score increased to 5.0±1.0 out of 6 (excellent, 83.3%) (Table 5 & Figure 5). The cosmetic outcome of the ICM group was initially ‘poor’ (mSBSES=2.8±0.9, 47.2%) on POD 10. Over time, it improved to became ‘very good’ (mSBSES=4.7±0.7, 77.8%) on POD 17. In RHM group, the resulted scars were ‘satisfactory’ with mSBSES score (3.2±0.5, 52.8%) on POD 10. Increasingly, the score had the same outcome of ICM (4.7±0.7, 77.8%) on POD 17. The FNNF group had a ‘very poor’ cosmetic outcome at the first time of evaluation (POD 10; mSBSES=0.5±0.3, 8.3%). On the 17th postoperative day, the aesthetic features of its scars improved but remained ‘poor’ looking and they did not reach a satisfactory level of cosmesis (mSBSES=2.7±0.9, 44.4%).
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Figure 5 Chart illustrating comparative results for the cosmetic outcomes of wound scars expressed by the mean scores of mSBSES for each suture pattern on PODs 10 and 17.

Table 5 Cosmetic scores of each suture pattern represented by mSBSES total points on PODs 10 & 17

<table>
<thead>
<tr>
<th>Group</th>
<th>POD 10 (points)</th>
<th>%</th>
<th>POD 17 (points)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCSID</td>
<td>4.5±0.7</td>
<td>75</td>
<td>5.0±1.0</td>
<td>83.3</td>
</tr>
<tr>
<td>ICM</td>
<td>2.8±0.9</td>
<td>47.2</td>
<td>4.7±0.7</td>
<td>77.8</td>
</tr>
<tr>
<td>RHM</td>
<td>3.2±0.5</td>
<td>52.8</td>
<td>4.7±0.7</td>
<td>77.8</td>
</tr>
<tr>
<td>FNNF</td>
<td>0.5±0.3</td>
<td>8.3</td>
<td>2.7±0.9</td>
<td>44.4</td>
</tr>
</tbody>
</table>

Mean ± SEM; mSBSES, Modified Stony Brook Scar Evaluation Scale; % Percentage of mSBSES total score in relation to the maximum score of the scale (6)

Microscopic assessment

Histopathological characteristics of the skin tissue closed by the BCSID suture pattern varied in both phases of wound healing on the 10th and 17th postoperative day. On POD 10, a detectable amount of immature connective tissue was observed at the deep layers of dermis along the wound incision; afterwards it developed on POD 17 as abundant mature collagen bundles infiltrated with chronic inflammatory cells. Pseudo-healing was observed in one incision and a moderate wound gap was shown between the wound margins with keratinization. Vascular changes expressed by varying degrees of neovascularization were seen in the dermis in one case on POD 10 with haemorrhage (Figure 6A). This reaction was replaced by connective tissue infiltration of the dermis and subcutaneous tissue with a moderate to high number of monocytes, lymphocytes and plasma cells and foreign body giant cells aggregated around the suture (Figure 6B). The wounds closed by the ICM suture pattern demonstrated two cases with inverted wounds. Suggestive inflammation occurred in one of the two inverted wounds. The number of acute inflammatory cells increased in such case on POD 10 leading to pus formation at the epidermal surface and was associated with mild haemorrhage within the dermis (Figure 6C). A mild degree of chronic inflammatory cellular reaction was also observed in all wounds on POD 10 which disappeared later on POD 17. Formation of granulation tissue began to develop at the base of the wounds but the amount was scant and remained constant in both phases of the histopathological changes at the 10th and 17th day post wounding (Figure 6D). On day 10 post-surgery with RHM suture pattern, the epidermal cells began to cross over the injury and underwent partial reepithelialization. The dermis was composed primarily of unorganized dense connective tissue with thick irregular collagenous fibers with infiltration of acute inflammatory cells around the wound edges where scab was also developed on the surface to effectively seal the wound and further collapse (Figure 6E). By day 17, the wounds distinctly demonstrated complete healing as the granulation tissue fully filled the wound gap, and this was accompanied by complete reepithelialization with hyperkeratosis (Figure 6F). The wound gap on POD 10 with FNNF suture pattern showed partial healing. The granulation tissue formation was adequate to fill the entire wound space while the reepithelialization was absent (Figure 6G). On day 17, no change was detected in the amount of granulation tissue but reepithelialization was completely developed (Figure 6H). The comprehensive histopathological scores among all groups at both stages of assessment on PODs 10 and 17 were tabulated and shown in a comparative chart (Table 6).

An assessment of clinical, biometric, cosmetic and microscopic outcomes of four suture techniques for cutaneous closure of laparotomy wounds: an experimental study in rabbits

POD 17
++

POD 10
-

42
+++

39
++

POD 17
+

POD 10
-

RHM
-

B

+++

+-

++++

++

+++

++

++

++++

+-

++++

+++

++++

+-

POD 17
37
+++

11,33,34
++

-

-

Citation:

Table 6: Histopathological results showing the mean scores of wound healing assessed via Histological Scoring System (after Kiani et al) on PODs 10 and 17

<table>
<thead>
<tr>
<th>Variable</th>
<th>BCSID</th>
<th>ICM</th>
<th>RHM</th>
<th>FNNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute inflammation a</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Chronic inflammation b</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Granulation tissue amount c</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Fibroblast maturation</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Collagen deposition</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Re-epithelization d</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a– none, +scarct, ++moderate, +++abundant
b– immature, +mild maturation, ++moderate maturation, +++fully matured
c– none, +partial, ++complete but immature or thin, +++complete and mature
d– none, +up to 5 vessels per high power field (HPF), ++6 to 10 vessels per HPF, +++more than 10 vessels per HPF

Statistical analysis

One-way ANOVA test showed that the four suture techniques are not the same in suturing time (surgery duration), where P<0.05. From Tuckey’s test, it is found that the first technique (BCSID) is different from the other three techniques, FNNF, ICM, RHM, at 5% significance level. BCSID has the highest average time among the four techniques.

The suture patterns were selected based on their prospective cosmetic significance. Clinical findings of this study proved that the favourable technique among tested groups for cutaneous closure of laparotomy wounds was RHM suture pattern as it revealed a compromise of the most desired clinical, cosmetic and histopathological results. Although BCSID provided superior cosmetic outcomes on both PODs 10 and 17, the technique failed to counteract the postoperative factors which led to dehiscence in 33.3% of cases. Even though, the dehisced wounds eventually healed within 17 days postoperatively by mixed intention healing. These findings are in accordance with those of Ghas et al., where the highest number of wound complications occurred in the subcuticular group (46.7% of cases). It had also grade-III infection as a sequel of partial dehiscence. However, Gurusamy et al., reported that superficial wound dehiscence may be reduced by using continuous subcuticular sutures. This difference might depend on whether the sutures were absorbable or non-absorbable, because most of these wound dehiscences were reported in two recent trials in which the continuous skin suture was by absorbable subcuticular sutures. Subcuticular and intradermal suture techniques are commonly used in human plastic surgery.

In veterinary research, subcuticular patterns were also cosmetically favourable in canine, feline, and caprine skin closure. In one study on bovine skin, cruciate mattress suture pattern yielded good cosmetic appearance with fewer suture marks. In a range of studies on human facial skin, RHM suture pattern was concluded as a favourable alternative to traditional simple continuous suture pattern, with smoother and flatter scar than the conventional method. FNNF pattern is considered as a combination of tension and appositional suture pattern. The far component takes up the tension while the near component holds skin margins in apposition. The suture can be applied loosely without tightening to avoid inversion at the incision line. It is known that the more apposition is executed on a wound closure, the more cosmetic results will be achieved.

Discussion

A number of studies have been conducted to assess the effects of different suture techniques and materials on the performance of skin wound healing in laparotomy incisions. Demands for an aesthetic laparotomy closure, within elective or emergency procedures, are increasing in small animal practices. Scarce studies are available in the veterinary practice about the cosmetic impact of wound healing potential and the response to those approaches by which different closure methods and materials would be reliable and practical. In midline laparotomy incisions, there is a close connection between the suture technique and the wound healing process. The closure technique plays a fundamental role in this process and primarily affects both early and late wound complications. Potential complications encountered in cutaneous laparotomy closure were reported in previous studies. Examples of these problems included wound dehiscence, haemorrhage, swelling, inflammation, infection, sinus formation or incisional herniation. The current study attempted to investigate the challenges occurring in laparotomy skin wound healing with a special focus on the aesthetic and clinical outcomes. Four suture techniques were compared after reviewing available literatures and recommendations of each author’s preferred method. The suture patterns were selected based on their prospective cosmetic significance. Clinical findings of this study proved that the favourable technique among tested groups for cutaneous closure of laparotomy wounds was RHM suture pattern as it revealed a compromise of the most desired clinical, cosmetic and histopathological results.

The suture patterns were selected based on their prospective cosmetic significance. Clinical findings of this study proved that the favourable technique among tested groups for cutaneous closure of laparotomy wounds was RHM suture pattern as it revealed a compromise of the most desired clinical, cosmetic and histopathological results. Although BCSID provided superior cosmetic outcomes on both PODs 10 and 17, the technique failed to counteract the postoperative factors which led to dehiscence in 33.3% of cases. Even though, the dehisced wounds eventually healed within 17 days postoperatively by mixed intention healing. These findings are in accordance with those of Ghas et al., where the highest number of wound complications occurred in the subcuticular group (46.7% of cases). It had also grade-III infection as a sequel of partial dehiscence. However, Gurusamy et al., reported that superficial wound dehiscence may be reduced by using continuous subcuticular sutures. This difference might depend on whether the sutures were absorbable or non-absorbable, because most of these wound dehiscences were reported in two recent trials in which the continuous skin suture was by absorbable subcuticular sutures. Subcuticular and intradermal suture techniques are commonly used in human plastic surgery.

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48 The author also prefers continuous pattern in most cases with the understanding of the rational mechanics involved in suturing. Many experienced surgeons, even those who have performed surgeries for many years, have developed poor surgical technique. Suture coding plays an important role in the infrastructure for surgical sutures as every suture pattern has its own code that helps in the structure and method of application. Common complications that occur after abdominal wound closure include, but not restricted to, wound dehiscence, infection, seroma, haemorrhage, peritonitis, or adhesion.

49 One of the reasons that may be countable for wound dehiscence in BCSD group is the abdominal pressure. The load exerted by the viscera on the skin wound may impair the wound healing. The dehisced portions were mostly situated at the caudal half of the incision line. This may be due to settlement of the large intestine in such region. The load exerted by the caudally-located intestine was thought to be greater than the cranially-situated stomach. The healing process was still in primitive stages on the first few days so the injured tissue could not bear the abdominal pressure. Therefore, the BCSD was weak enough to cause dehiscence; this was supported by Adams et al. and Harari.

50 In the present study, cutaneous wound dehiscence of some cases (33.3%) in BCSD group might be attributed to other factors such as the insufficient tissue of that thin skin which was held by the subcuticular sutures, as well as the postoperative traumatization that might occur to the sutured skin. The SC tissue was engaged in BCSD through alternative tissue bites in the beginning, middle and end of the incision. Potential influence of inadequate SC sutures in the middle of the incision (where the tension will be the most) might have a reflection on the BCSD findings of the current study due to the difference in the amount of tissue incorporated. In small young rabbits, the SC tissue is highly fragile in nature and can be easily torn; this may also have a possible influence on wound dehiscence.

51 For the aforementioned reasons, PDX and silk sutures were used in the present study. Silk sutures may not be used for skin closure in small animal practices in the USA. Where cosmetic results are important, closure and prolonged apposition of wounds and avoidance of irritants will produce the favourable result. Therefore, it was advisable to use the smallest inert monofilament suture materials such as nylon or polypropylene; to avoid skin sutures; to close subcuticularly whenever possible; and to secure close apposition of skin edges using a topical skin adhesive. In the UK, a survey was conducted on current practices and influences on the choice of suture material, pattern and size used in commonly performed procedures in small animal practices. For skin closure, the most common suture material used by UK veterinarians was non-absorbable multifilament. Short-acting monofilament was most commonly chosen for subcutaneous closure while long-acting monofilament was most commonly chosen for linea alba. A simple continuous pattern was the most commonly used for subcutaneous and linea alba closure overall. The cost-effective considerations had also an influence on the selection of the suture pattern, material and size. In Australia, the twisted coated products were much more popular among skin sutures than nylon in packets or cassettes. In the opinion of the reporters, this reflects habitual usage rather than superior properties. Simple interrupted was the most popular skin suture pattern, followed closely by horizontal mattress. Few Australian veterinarians used other suture patterns. In Egypt, simple interrupted suture is the most fundamental technique of wound closure used in cutaneous surgery. It possesses greater tensile strength, and has less potential to cause wound oedema and impaired circulation.

52 Simple continuous is used in tissues that are elastic and are not subject to a lot of tension. The horizontal mattress helps in minimizing the wound tension, closing the dead space, and facilitating wound edge eversion. It is both an interrupted and a continuous pattern. Despite the sophistication of current suture materials and techniques, closing a wound still involves the same basic procedure based on Halsted’s principles. Development of good suture technique requires a deep understanding of the rational mechanics involved in suturing. Many experienced surgeons, even those who have performed surgeries for many years, have developed poor surgical technique. Suture coding plays an important role in the infrastructure for surgical sutures as every suture pattern has its own code that helps in the structure and method of application. Common complications that occur after abdominal wound closure include, but not restricted to, wound dehiscence, infection, seroma, haemorrhage, peritonitis, or adhesion.

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In the present study, wound infection was a postoperative complication when ICM was used. The infection was detected in 33.3% of cases. The tension force of the suture threads in this technique was in an oblique direction to the incision line. The effect of tension was possibly lower than the other techniques in which the tension was perpendicular to the incision line. After the knots were tied, each mattress loop was crossed and partially overlapped; leading to tissue misconfiguration. Another reason explains why the ICM wounds were susceptible to infection is the dimensions of the suture pattern. The sutured tissues were held together only at the suture points. In the spaces between suture points, the immediate tensile strength might be effectively zero. The distance between two successive stitches at the intersection point of x-strands was 2cm. If compared to an interrupted horizontal mattress suture, such distance was two-fold greater. This created a passage between the wound margins that paved the way for wound contamination and invasion of microorganisms, as well as excessive fluid leakage which could stretch the wound margins. This interpretation was in accordance with Fried and Walsh. Silk sutures may be questionable for aseptic closure in situations where it represents a predisposing factor for surgical site infection since silk is composed of natural proteins with a braided structure. However, a combination with antibacterials can prevent the invasion of bacteria and reduce the probability of infection. In the present study, the antibacterial activity was induced and sustained by the use of a systemic long-acting broad-spectrum antibiotic (Oxytetracycline LA). The blood levels of the antibiotic persisted for at least five days as prescribed by the manufacturer. To our knowledge, we believe that silk is a smooth suture material acceptable for the delicate lapine skin. Silk is affordable, easily manipulated, and will not interfere with wound healing if handled carefully with a combined antibacterial agent. In the intracutaneous groups (ICM, RHM, FNNF), silk sutures resulted in an overall 83.3% normal closure rate in the rabbit’s skin. With exception for BCSID, the present study revealed no significant difference between RHM, ICM and FNNF groups in terms of the duration of skin suturing. The time lasted ~18 minutes (average) in RHM, ICM and FNNF groups due to ease handling of these techniques where closure of wounds was percutaneous passing through all layers of the skin. In contrast, Homvise found some problems with RHM; of which, it was time-consuming that required ~15 minutes extra time. This result was attributed to the length of sternal wound in such study. BCSID suture pattern in the current study required longer time (~50 minutes) for skin closure as high accuracy was needed to pass the suture thread through the subcutis and the dermis of the rabbit’s delicate skin. In small animals, most previous studies were concerned with wound biometrics in case of second intention healing. The current study compared changes in first intention healing. The wound length plot showed a descending slope into shortest lengths in BCSID and FNNF groups on POD 5. This gradual shortening of WL may return to wound contraction as described by Fossum & Williams who reported that the ‘pull theory’ is the mechanism where fibroblasts and myofibroblasts give motive power for contraction and hence a shortage in WL. Contraction proceeds at a rate of ~0.6 to 0.8mm/day and it stops when the wound edges meet each other, or when tension from the skin around the wound is equal to or greater than the contractile forces created by myofibroblasts, or when there is insufficiency of these cells. In ICM and RHM groups, the WL plot was terminated with an ascending slope toward the preoperative WL. This may be due to synthesis of collagen in the skin with an aim to regain standard tensile strength of the newly formed tissue. In the present study, the suture-to-wound length ratio in BCSID, ICM, RHM and FNNF groups was 2.1:1, 3.6:1, 2.4:1 and 5:5:1 respectively. These results appear to correlate with the incidence of wound dehiscence. It was proven in previous studies that a WL ratio more than 4:1 for laparotomy closure could reduce WD. However, dehiscence did not occur with RHM group of which the ratio was 2.4:1. This may be attributed to the suture material implanted in the full thickness of the skin with an equal tension applied on the wound edges that led to abundance in the remained suture and shortage in the consumed suture. Cosmetic assessments in the current study revealed that the BCSID suture pattern was the superior technique among all groups as it provided the highly cosmetic appearance of wounds on both PODs 10 and 17. Nevertheless, they showed more aesthetic outcomes on the 17th day with the highest mSBSES score. This was consistent with Sylvestre et al. who concluded that the buried continuous subcuticular suture pattern may influence a better cosmetic appearance on a cutaneous closure of canine ovariolhysterectomy at the time of recheck visit. If in cosmetic order, the second cosmetic rank was for RHM, and the third was for ICM, while FNNF technique came at the last as it gave worst cosmetic results. In plastic surgery, the Patient and Observer Scar Assessment Scale (POSAS), and Vancouver Scar Scale are widely used for scar assessment in human patients, but it is difficult to decide which scale can be used typically for each scar condition and treatment method. The assessment of newly developed surgical scars was most commonly performed by the POSAS. Since the patient is involved in the POSAS assessment; this scale cannot be applied to those speechless creatures, the rabbits, but perhaps via the sponsorship of the animal owner. Clinical scar assessment lacks a standardized methodology and a systematic approach. Therefore, studies continue to lack consensus regarding the most appropriate and applicable evaluation instrument. Fearmonti et al. discussed the quantitative and qualitative measurement modalities that are needed to evaluate and monitor scars. They provided a review on available clinical tools and current assessment scales used to subjectively and objectively characterize scars. The authors emphasized that an optimal, universal scar scoring system is necessary to be constructed in order to better characterize, understand and treat pathologic scarring. The Stony Brook Scar Evaluation Scale (SBSES) was proposed by Singer et al. and its reliability was acceptable. It is a 5-element ordinal wound evaluation scale developed to measure short-term cosmetic outcome of wounds in 5 to 10 days after injury up to the time of suture removal. This scale incorporates assessments of individual attributes with a binary response (1 or 0) for each, as well as overall appearance, to yield a score ranging from 0 (worst) to 5 (best). The SBSES has only recently been suggested for use in research, as it was designed to measure short-term rather than long-term wound outcomes. In the current study, we innovated a new parameter added to our modified 6-element SBSES with 0 for absence and 1 for presence of any hair development on the incision line. The novel parameter acted as an indicator for the body’s response in removing the devitalized fatty tissue during the debridement phase. Bohling et al. clarified that extensive debridement of SC tissue may delay wound healing particularly in felines. A higher risk for wound infection may also accompany extensive removal of SC tissues in canines. In conclusion, wound infection and dehiscence were the most important complications that were detected in the present study. The cosmetic assessment was the least affected parameter among the four techniques utilized in the present study. An experimental study in rabbits. J Dairy Vet Anim Res. 2019;8(1):42–53. DOI: 10.15406/jdvar.2019.08.00241

physiological functions with developing hair follicles and its adnexa. The mSBSES cosmetic scores in the current study were consistent with the clinical and microscopic outcomes. The degree of cosmesis in case of RHM group was low and undesirable on POD 10 due to the presence of suture marks at that time. By day 17, the technique gave an enhanced outcome of cosmesis as these marks faded away over time. These results were in accordance to Moody et al. who stated that RHM provides good wound eversion, reduce wound tension, and promote haemostasis. It also has a less constrictive effect because the wound edge pressure is applied only unilaterally which is evenly distributed across the entire wound line. Moreover, RHM is a continuous pattern, thus limiting the stress on the wound edges. A punctate scar (i.e. suture holes) was seen in FNNF group. Dunn explained that this occurs when the skin sutures are not placed with excessive tension and are removed at an early time. The forces that create either crosshatching or suture marks will remain after suture removal. Histopathological findings of the present study were consistent with such explanations where the FNNF group showed notable great amounts of mature collagen fibers. This result was in agreement with Yang et al. who stated that the main histological components of the skin scar are thick epidermis and dermis with tough collagenous fibers. Microscopic examination of BCSISD group revealed formation of an epidermal bridge (stratum corneum) over the incision while the dermis did not develop and presented with a moderate gap. However, the wound appeared normally healed by the naked eye at the same time. Unexpected adverse results can occasionally occur in cats. Of these, Bohling reported such condition and called the phenomenon ‘pseudo-healing’. The sutured skin incision might seem like normally healed at the time of suture removal but after that time, when the animal exerts a normal physiological stress on the closure, such as a cat jumps up from a chair, complete dehiscence occurs. In contrast, integral skin closure was evident in the RHM group on day 10, with partial reepithelialization and scab formation. By day 17, the wounds notably showed complete healing as the granulation tissue entirely filled the wound gap with complete reepithelialization and hyperkeratosis. Cotran et al. highlighted that the quantitative increase in collagen ends in 10 days and the wound is moderately healed at this time. By 14 to 16days, normal wound healing in the rabbit is complete; this was harmonious with Gourley and Gregory. Likewise, such changes occurred in FNNF group, but reepithelialization was absent on day 10. However, the wounds afterwards underwent complete reepithelialization by day 17. In our study, the overall histopathological findings confirmed that the RHM suture technique was superior to other groups in terms of the wound healing scoring system of Kiani et al. The RHM was associated with successful wound healing on both phases of evaluation on day 10 and 17. The dermal tissues showed acute followed by chronic inflammation, complete reepithelialization, full maturation of fibroblasts, and abundant formation of collagen bundles at the end of the study on the 17th postoperative day. Potential limitation of the present study might lay in the differences in the type of suture material used in BCSISD group (PDX) and the other three groups (Silk). It was presumed that BCSISD could provide favourable outcomes in all terms of the assessment on the account of other tested techniques. However, the overall study outcomes showed unexpected results supporting for RHM. Short-term wound monitoring may impose a restriction for expecting later cosmetic impacts, as a longer time would be required for wound examination so as to ascertain no further downsides might develop. The lack of standardized modalities of scar assessment scales, and the subjective/objective nature of these scales might have a different response to the parameters upon clinical trials. Reliability of cosmetic scores is context and content specific, and further testing required to translate them in a heterogeneous setting (different models, raters, and techniques). Future studies also need to be conducted to implement an equal approach for the aesthetic suture patterns using a uniform material and a standardized scale, hence to validate the appropriate use of these suture techniques in clinical practice.

Conclusion

The running horizontal mattress (RHM) suture technique may be considered the favourable suture pattern of choice for closure of the laparotomy skin wound in small animals such as rabbits. The technique was associated with an upper-second grade of ‘very good’ cosmetic outcomes, and rapid successful wound healing without any complication. This suture pattern serves as a compromise of high-quality cosmesis and optimal wound healing. The assessed suture techniques can be cosmetically graded in a descending order from the superior to inferior as:

i. Buried continuous subcuticular-intradermal suture technique,
ii. Running horizontal mattress suture technique,
iii. Interrupted cruciate mattress suture technique, and

In terms of microscopic assessments, they can be histologically graded as:

i. Running horizontal mattress suture technique,
ii. Far–near–near–far suture technique,
iii. Buried continuous subcuticular-intradermal suture technique, and lastly
iv. Interrupted cruciate mattress suture technique.

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Conflicts of interest

Authors declare there are no conflicts of interest.

References

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