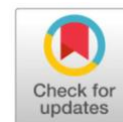




Original Research



Analysis of continuous and figure of eight suture techniques on the healing of duodenal perforation



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Abstract: Duodenal perforation is a rare but potentially life-threatening gastrointestinal emergency. This study aimed to evaluate the healing of duodenal perforations both macroscopically and microscopically in rats sutured with figure-of-eight and continuous techniques compared with interrupted sutures. A total of 24 rats underwent a 10-mm longitudinal incision in the small intestine and were divided into four groups: sham, control with interrupted sutures, treatment group 1 (continuous technique), and treatment group 2 (figure-of-eight technique). Healing was assessed macroscopically by measuring bursting pressure and microscopically through fibroblast count and collagen density on histopathological examination at day 7. The figure-of-eight group demonstrated a significantly higher bursting pressure (177.5 ± 86.7 mmHg) compared with the control group (85.3 ± 55.7 mmHg, $p < 0.05$). Both the continuous and figure-of-eight groups showed significantly higher fibroblast counts and collagen density compared with the control group ($p < 0.05$). However, no significant difference in bursting pressure was observed between the continuous and control groups ($p > 0.05$). These findings indicate that both the figure-of-eight and continuous suturing techniques enhance microscopic healing parameters of duodenal perforation compared with the interrupted technique, while at the macroscopic level only the figure-of-eight technique provides significantly greater wound healing strength.

Keywords: Continuous suture; Figure-of-eight suture; Interrupted suture; Bursting pressure; Fibroblast; Collagen.

INTRODUCTION

Duodenal perforation refers to a full-thickness defect in the wall of the duodenum, which can result in leakage of intestinal contents into the abdominal cavity¹. Duodenal perforation is recognized as a rare yet potentially life-threatening gastrointestinal emergency, with a reported mortality rate between 4% and 30%². The management of this condition poses significant challenges due to the elevated rates of reperforation and complications³.

In the surgical management of duodenal perforations, two commonly utilized techniques are the continuous suturing technique and the figure-of-eight suturing technique. The choice of either method can have a substantial impact on healing and patient outcomes, including the potential to reduce complications such as anastomotic leaks^{4,5}. This study aims to evaluate the healing of duodenal perforations sutured using figure-of-eight and continuous suture techniques in comparison to interrupted sutures. Interrupted suture was chosen as control,

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because it is a common and conventional technique suture for gastrointestinal perforation repair.

In assessing the effectiveness of our suturing techniques, measuring bursting pressure has become an integral aspect. Bursting pressure refers to the intraluminal pressure at which a surgical anastomosis or suture line fails. This metric is crucial, as it reflects the ability of the sutured site to withstand physiological pressures. Higher bursting pressures are generally associated with stronger suturing techniques and a reduced risk of postoperative complications⁶.

At the microscopic level, the significance of fibroblasts and collagen in the wound healing process is paramount. Fibroblasts play a crucial role in collagen synthesis, which is essential for the formation of new tissue and the repair of the damaged duodenum. Assessing fibroblast counts and collagen density is important for evaluating the healing progress of duodenal perforations following surgical intervention⁷.

The assessment will be carried out macroscopically by measuring bursting pressure and microscopically through fibroblast count and collagen density analysis. Given the ongoing debate over the optimal suturing technique for duodenal perforations, it is important to analyse and compare both techniques' healing progress. Based on available literatures, no prior animal study has compared these techniques against conventional interrupted suture while evaluated both mechanical strength and histological healing. This comparison is essential for determining the most effective suture technique and reduce complications.

MATERIAL AND METHOD

Animal Model

The Ethics Committee granted approval for all animal experimental protocols. A total of twenty-four male Wistar rats, each weighing between 250 and 300 grams, were acclimated in polypropylene cages within a standard room maintained at temperatures ranging from 23 to 35°C. The environment was kept on a 12-hour light-dark cycle with humidity levels between 40 and 70% for a duration of seven days. The rats were provided with unrestricted access to AIN 76A standard food and water. Following a 12-hour fasting period, the rats were anesthetized through intraperitoneal injection of 80 mg/kg of ketamine. A midline abdominal incision was made to expose the duodenum, followed by a 10-mm longitudinal incision at the first part of the duodenum. The duodenal perforation was then disinfected and closed using a 6/0 polypropylene (Ethicon) non-absorbable suture, applied at 2-mm intervals. As explain in flowchart in Figure 1.

Research Design

The research employed a posttest-only control group design to assess the treatment's impact on the experimental group in comparison to the control group, ensuring equivalent pre-treatment conditions between the groups. While the study groups were balanced at baseline, random allocation was not performed. Instead, non-probability sampling methods, specifically consecutive sampling, were utilized in the sampling process, and a comparison control was established. Despite ensured equal group sizes, it may not as applicable as randomized allocation. The two pathologists conducting the histological assessment in this study were blinded to ensure objectivity. The research has been reported in compliance with the ARRIVE guidelines⁸.

Animal termination

The rats were assigned to four experimental groups, each receiving different suturing techniques to close the duodenal perforation by solo operator. The first treatment group (T1) underwent continuous suture technique. Second treatment group (T2) received a figure-of-eight suture. The control group was treated with an interrupted technique. The fourth group served as a sham control, which did not undergo laparotomy or suturing. A total of 24 rats, 6 from each group

(Sham, Control, T1, T2) were terminated on day 7 using a cocktail (Ketamine 50 mg/kg, Xylazine 10 mg/kg, and Acepromazine 2 mg/kg). The duodenum was harvested for measurement of pneumatic bursting pressure and histological assessment.

Measurement of Pneumatic Bursting Pressure

The duodenum was collected for the assessment of pneumatic bursting pressure, serving as a macroscopic indicator of wound healing. Both the distal and proximal sections of the duodenum were secured using 3.0 silk threads, with the proximal end connected to a catheter for the introduction of air pressure. A separation of 4 cm was maintained between the two securing points. The duodenum was submerged in a sterile saline solution, and air pressure was gradually introduced using a syringe. The bursting pressure was recorded with a manometer until evident signs of air leakage appeared. The presence of the exhausted air bubble indicates the healing strength of duodenal perforation. Pressure measurements were documented in millimeters of mercury (mmHg).

Histological assessment

Paraffin-embedded samples were cut into 4- μ m sections and stained with hematoxylin and eosin according to established protocols as follows:

- a. The specimen was placed in a glass container and immersed in xylol I solution for 2 minutes, followed by xylol II for an additional 2 minutes.
- b. It was then submerged in absolute alcohol I for 2 minutes, followed by absolute alcohol II for another 2 minutes.
- c. The specimen underwent a 2-minute immersion in 95% alcohol, followed by 80% alcohol, and was then rinsed with running water.
- d. After staining with hematoxylin for 10 minutes and eosin for 2-3 minutes, the specimen was dried.
- e. The specimen was sequentially dipped in 95% alcohol for 10 times, followed by absolute alcohol for 10 dips, and finally soaked in absolute alcohol II for 2 minutes.
- f. The specimen underwent a 1-minute soak in xylol I, followed by a 2-minute soak in xylol II.
- g. Permount adhesive was applied to the specimen and covered with a cover slip.

The painted preparations were then recorded using an Optilab Advance Plus camera with Optilabviewer 4.0 software connected to an Olympus CX22 microscope for each preparation. Histological assessment was conducted by two independent blinded pathologists. The quantification of fibroblast count and collagen density was conducted under a light microscope with 400x magnification.

Statistical Analysis

Statistical analyses were conducted to determine the mean, mode, and median of the data. The normality of the data was assessed using the Shapiro-Wilk test due to the sample size being less than 50, while the homogeneity was tested using the Levene Statistics test. If the data was found to be normally distributed and homogeneous, the independent t-test test was utilized to assess differences among treatment groups. In cases where the data was not normally distributed, transformations were applied for confirmation, with the Mann Whitney test being used if normality could not be achieved. A p-value of <0.05 was considered significant. Data processing and analysis were performed using SPSS 25.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Macroscopic measurement

Bursting pressure measurements were conducted for all groups (Sham, Control, T1, T2) following the termination on the seventh day. The highest bursting pressure results were observed in the Sham group, with a pressure of 300 ± 35.6 mmHg (95% CI: 262.6-337.4). Subsequently, the figure-of-eight suture group was recorded a

pressure of 177.5 ± 86.7 mmHg (95% CI: 86.5-268.5). Afterward, the continuous suture group was recorded a pressure of 141.3 ± 96.9 mmHg (95% CI: 39.6-243.1). Finally, the control group (interrupted suture) demonstrated the lowest bursting pressure of 85.3 ± 55.7 mmHg (95% CI: 26.8-143.8) (Figure 2).

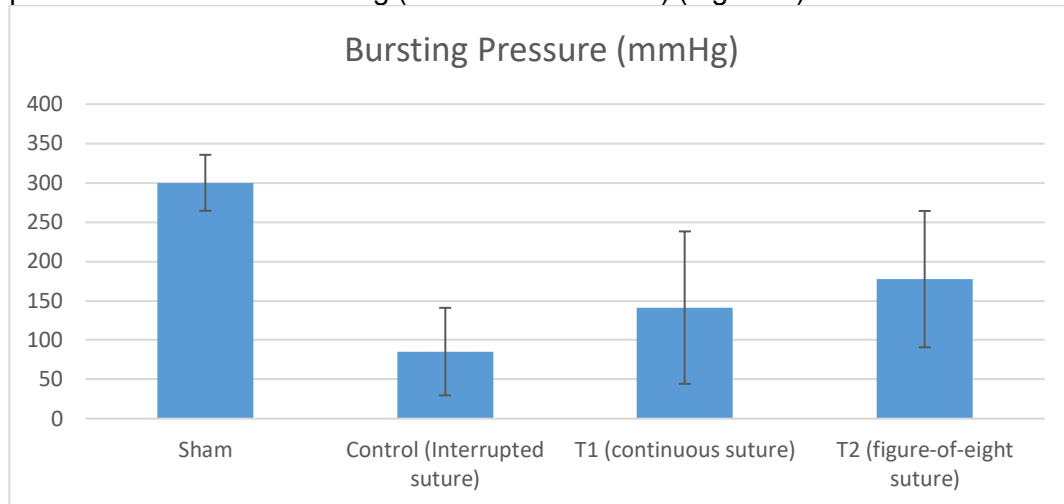


Figure 2. Average bursting pressure measurement for all groups.

The Shapiro Wilk and Levene tests showed normal and homogeneous data ($p > 0,05$), so it could be continued with parametric tests (Table 1-2). The result of the t-test showed an insignificant difference between the continuous suture technique bursting pressure and the interrupted suture techniques bursting pressure measurement ($p=0,248$), while there is a significant difference of bursting pressure between the figure-of-eight suture technique and the interrupted suture technique ($p= 0,042$)(Table 1-2).

Table 1. Bursting pressure on first treatment group (T1)

	Sham	Control (Interrupted suture)	T1 (continuous suture)	P value
Mean \pm SD	300 \pm 35.6	85.3 \pm 55.7	141.3 \pm 96.9	
Shapiro-Wilk		0.709*	0.302*	
Lavene Test				0.082**
Independent T-test				0.248***

Note: *Normality $p > 0.05$ **Homogenous $p > 0.05$ ***Insignificantly different $p > 0.05$

Table 2. Bursting pressure on second treatment group (T2)

	Sham	Control (Interrupted suture)	T2 (figure-of-eight suture)	P value
Mean \pm SD	300 \pm 35.6	85.3 \pm 55.7	177.5 \pm 86.7	
Shapiro-Wilk		0.709*	0.200*	
Lavene Test				0.088**
Independent T-test				0.042***

Note: *Normality $p > 0.05$ **Homogenous $p > 0.05$ ***Significantly different $p < 0.05$

Microscopic measurement

Histopathological examination of the duodenal wound site was conducted using a microscope with a magnification of 400x on all groups (Sham, Control, T1, T2). Fibroblast counts were recorded across five fields of view, and the results

were averaged for each preparation. Additionally, the density of collagen fibers was evaluated as a percentage ranging from 0 to 100%, also observed at the wound site using the same magnification. Each preparation was analysed in five fields of view, with the results were subsequently averaged. The findings regarding fibroblast and collagen observations were presented in figure 3.

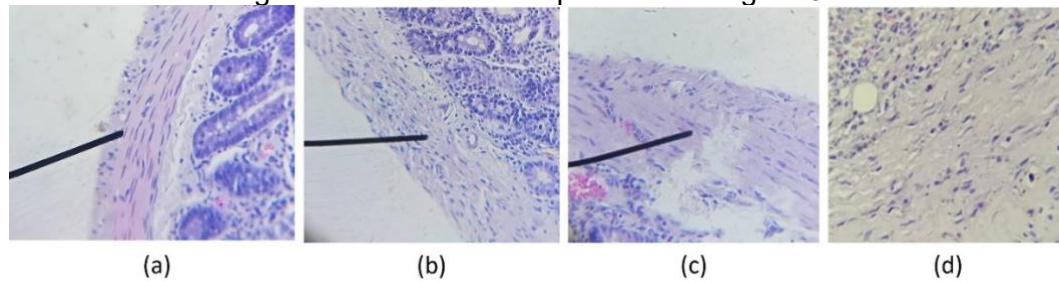


Figure 3. Histopathology of duodenal tissue of various study groups using hematoxylin and eosin staining showing fibroblast count and collagen density. *a: Normal duodenal tissue; b: Duodenal tissue with continuous suturing technique; c: Duodenal tissue with interrupted suturing technique; d: Duodenal tissue with figure-of-eight suturing technique. White bar: 10 μ m. (x400 magnification).*

The highest fibroblast count results were observed in the Sham group at 23,43 \pm 0,26 (95% CI: 23.1-23.7). Subsequently, the continuous suture group was second highest fibroblast count which was recorded at 22,47 \pm 1,09 (95% CI: 21.3-232.6). Afterward, the figure-of-eight suture group was showed fibroblast count at 21,70 \pm 0,62 (95% CI: 21.0-22.3). Finally, the control group (interrupted suture) demonstrated the lowest fibroblast count of 18,67 \pm 0,84 (95% CI: 17.8-19.5) (Figure 4).

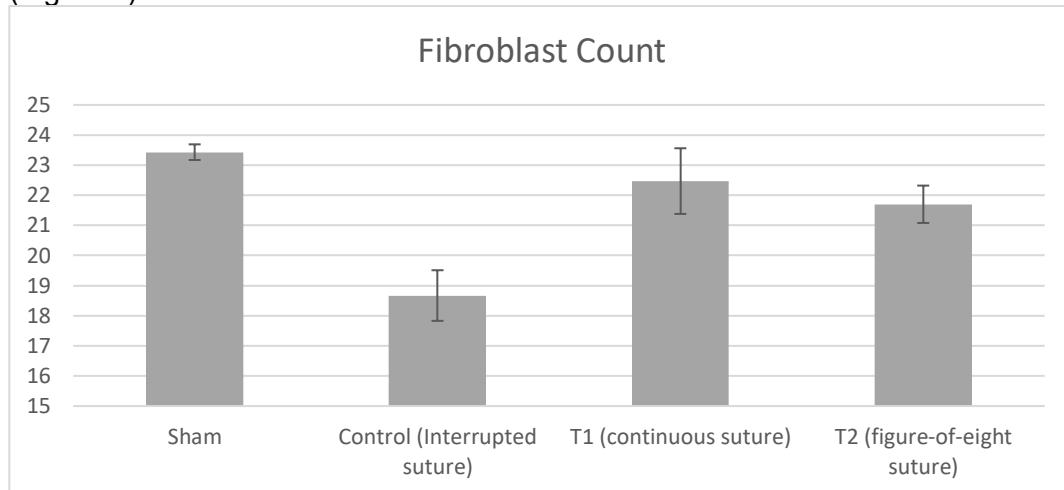


Figure 4. Average fibroblast count for all groups

The Shapiro-Wilk and Levene tests indicated that the data met the assumptions of normality and homogeneity ($p > 0,05$), allowing for the continuation with parametric tests (Table 3-4). The results of the t-test revealed a significant difference in fibroblast count between the continuous suture technique and the interrupted suture technique ($p = 0,00$)(Table 3). Additionally, a significant difference in fibroblast count was observed between the figure-eight suture technique and the interrupted suture technique ($p = 0,00$)(Table 4).

Table 3. Fibroblast count on first treatment group (T1)

	Sham	Control (Interrupted suture)	T1 (continuous suture)	P value
Mean ± SD	23.43±0.26	18.67±0.84	22.47±1.09	
Shapiro-Wilk		0.092*	0.080*	
Lavene Test				1.000**
Independent T- test				0.000***

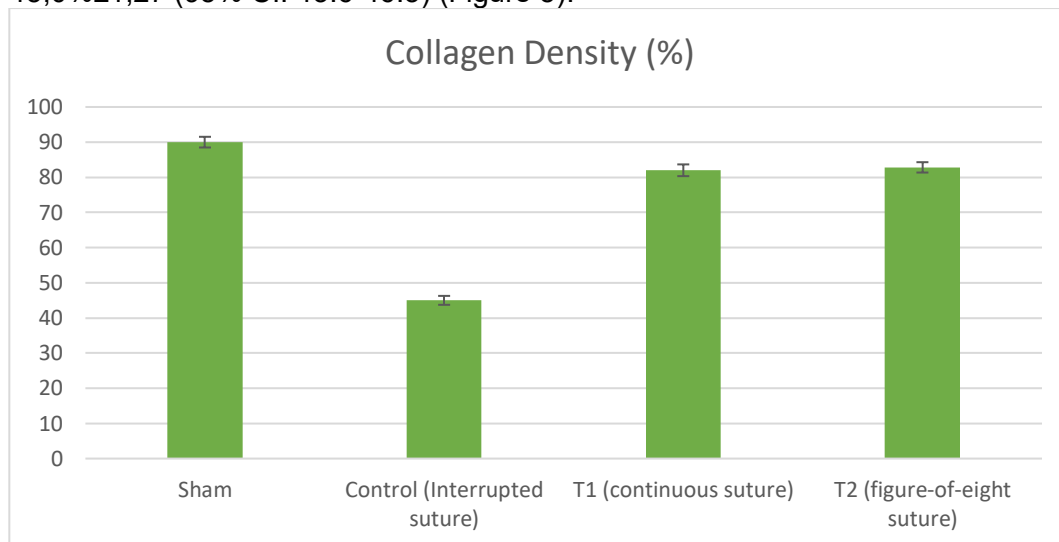
Note: *Normality $p > 0.05$ **Homogenous $p > 0.05$ ***Significantly different $p < 0.05$

Table 4. Fibroblast count on second treatment group (T2)

	Sham	Control (Interrupted suture)	T2 (figure-of- eight suture)	P value
Mean ± SD	23.43±0.26	18.67±0.84	21.70±0.62	
Shapiro-Wilk		0.092*	0.854*	
Lavene Test				0.163**
Independent T- test				0.000***

Note: *Normality $p > 0.05$ **Homogenous $p > 0.05$ ***Significantly different $p < 0.05$

The highest collagen density results were observed in the Sham group at $90\% \pm 1,52$ (95% CI: 89.7-90.3). Subsequently, the second highest collagen density group was the figure-of-eight suture group which was observed at $82,83\% \pm 1,47$ (95% CI: 81.3-84.4). Afterward, the continuous suture group which collagen density was recorded at $82,00\% \pm 1,67$ (95% CI: 80.2-83.7). Finally, the lowest collagen density demonstrated in the control group (interrupted suture) at $45,0\% \pm 1,27$ (95% CI: 43.6-46.3) (Figure 5).

**Figure 5.** Average collagen density results for all groups

The Shapiro-Wilk and Levene tests indicated that the data met the assumptions of normality and homogeneity ($p > 0,05$), allowing for the continuation with parametric tests (Table 5-6). The results of the t-test revealed a significant difference in collagen density between the continuous suture technique and the interrupted suture technique ($p = 0,00$) (Table 5). Additionally, a significant difference in collagen density was observed between the figure-eight suture technique and the interrupted suture technique ($p = 0,00$) (Table 6).

Table 5. Collagen density assessment on first treatment group (T1)

	Sham	Control (Interrupted suture)	T1 (continuous suture)	P value
Mean ± SD	90±1.52	45.00±1.27	82.00±1.67	
Shapiro-Wilk		0.101*	0.252*	
Lavene Test				0.243**
Independent T-test				0.000***

Note: *Normality $p > 0.05$ **Homogenous $p > 0.05$ ***Significantly different $p < 0.05$

Table 6. Collagen density assessment on second treatment group (T2)

	Sham	Control (Interrupted suture)	T2 (figure-of- eight suture)	P value
Mean ± SD	90±1.52	45.00±1.27	82.83±1.47	
Shapiro-Wilk		0.101*	0.804*	
Lavene Test				0.356**
Independent T-test				0.000***

Note: *Normality $p > 0.05$ **Homogenous $p > 0.05$ ***Significantly different $p < 0.05$

Perforation is a medical condition characterized by damage to the gastrointestinal wall, leading to the leakage of intraluminal contents into the peritoneal cavity. This can result from a variety of underlying causes⁹. Duodenal perforation is associated with significant morbidity and mortality, making timely and appropriate treatment essential. Management of this condition can be performed using various techniques, but it typically involves the closure and suturing of the wound employing continuous, figure-of-eight, or interrupted suture methods¹⁰⁻¹².

The initial approach to managing gastrointestinal perforation involves surgical intervention or mechanical reapposition. Once the wound has been closed, the healing process commences. Typically, wound healing consists of overlapping phases at the cellular level, which include haemostasis and inflammation, proliferation, and maturation and remodelling. These mechanisms are interrelated and persist from the onset of the injury until complete healing occurs. All wounds progress through these phases to restore tissue integrity¹¹⁻¹³.

The healing process of duodenal perforation wounds can be evaluated through bursting pressure examinations, which measure the strength of sutures in relation to potential leakage in colonic repair performed in experimental rats. This measurement provides insights into the healing of duodenal perforations by utilizing air pressure to assess the strength and integrity of the wound healing process facilitated by sutures¹⁴. The integrity of the duodenal lumen is evaluated by applying pressure, as this measurement is a comparison of force to the measured area. The pressure recorded serves as an indicator of the duodenal lumen's integrity during the healing process, expressed in mmHg units on a manometer^{6,15,16}. The initial integrity of the repair relies on the formation of fibroblasts and collagen, which are essential for ensuring a watertight seal and providing adequate suture retention in the intestinal wall, particularly within the submucosal layer⁷. Consequently, the density of fibroblasts and collagen will also be a focus in the assessment of wound healing.

The group that received figure-of-eight sutures demonstrated the highest bursting pressure in this study, followed by the continuous suture group and the interrupted suture group. The continuous suture group also exhibited the highest fibroblast count, followed in order by the figure-of-eight suture group and the

interrupted suture group. Additionally, the collagen density measurements indicated that the figure-of-eight suture group achieved the highest density results, followed by the continuous suture group and the interrupted suture group.

The figure-of-eight suture provides a robust method for securing tissue layers, allowing for less tension on the edges of the repair. This technique is characterized by crossing the suture over itself in a figure-of-eight pattern. Measurements of bursting pressure, fibroblast count, and collagen density in the figure-of-eight suture group revealed significant differences compared to the interrupted suture group. This finding supports the hypothesis that figure-of-eight sutures serve two crucial functions in enhancing wound healing. Firstly, they act as haemostatic sutures, preventing bleeding at the anastomosis line. Secondly, they effectively align and connect both sides of the mucosa, which is essential for maintaining the structural integrity and minimizing leakage at the anastomosis site. There is a widespread agreement on the criteria for an optimal anastomosis, which should ideally be well-vascularized, impermeable to fluid, straightforward to execute, resilient under pressure, and free from leakage. In gastrointestinal tract anastomoses, suturing is typically performed on the seromuscular layer in an inverted fashion, ensuring a transmural stitch from the exterior to the interior of the tissue. This technique has become the standard in most surgical practices and was utilized by the surgical operators involved in this study. The figure-of-eight suture technique is associated with a reduced risk of bleeding at the anastomosis line due to its hemostatic characteristics, which facilitate enhanced vascularization of the surrounding tissue and contribute to collagen synthesis from fibroblasts. Additionally, this technique effectively aligns both sides of the mucosa, thereby ensuring the strength and integrity of the duodenal wall layer^{4-7,17-19}.

A comparison between continuous sutures and interrupted sutures revealed significant differences in the fibroblast count and collagen density; however, no notable differences were identified in bursting pressure examination. The integrity of duodenal which reflected to bursting pressure was dependent on the activity of fibroblast and collagen, as well as the thickness of the tissue, accuracy of stitches placed, and tension distribution along the suture line. The small sample size and biological variations may have limited the ability to identify a significant difference in bursting pressure. Notably, while not statistically significant, the continuous suture group exhibited an average bursting pressure of 141.333 mmHg, compared to 85.33 mmHg in the interrupted suture group. This trend suggests that, on average, continuous sutures may offer superior tissue healing strength relative to interrupted sutures^{20,21}.

Previous research supports this notion, indicating that continuous sutures present a lower risk of suture leakage, making them a preferred choice among surgeons for intestinal anastomosis procedures^{12, 22, 23}. Continuous suture techniques are associated with a reduced risk of ischemia, as they require fewer knots, only at the beginning and end of the suture line⁴. As Eickhoff et al study previously conducted, they found continuous sutures significantly reduce anastomotic leak rate than interrupted suture in colonic anastomosis^{13, 24}. The enhanced blood flow facilitated by continuous sutures allows macrophages, which originate from circulating monocytes, to effectively reach the wound area through blood vessels, thereby supporting the healing process, particularly in terms of fibroblast formation, collagen synthesis, and angiogenesis^{7,11}.

In contrast, interrupted sutures involve cutting the suture thread at each knot, resulting in a greater number of knots and increased tension, which heightens the risk of tissue ischemia. Moreover, continuous sutures present a lower risk of lumen shrinkage^{15, 25}. The suture thread placement in continuous sutures ensures a more uniform distribution of pressure across the anastomosis or wound site, contributing to a reduced rate of suture leakage compared to interrupted sutures. The consistent pressure distribution provided by continuous sutures helps maintain the integrity of the anastomosis or wound closure, facilitating improved tissue

healing outcome^{4,13, 26-28}. These results offer potential future research combination of suturing technique with minimally invasive surgery, since one of the aims of minimally invasive surgery is to reduce post operative complication. It is worth exploring that optimal suturing method with minimally invasive surgery could enhance post operation result and decrease morbidity rates in long term theoretical implications^{28, 29}.

The limitation of this study is that it was conducted at one time, preventing the observation of potential complications, such as strictures. Additionally, the sample size was relatively small, indicating a need for further research with a larger sample population. Moreover, this experiment has not rule out the possibility of operator variability in suturing methods and duodenal sample consistencies. Finally, this study focused on comparisons with interrupted suture group as control, comparison between all three suture techniques will emphasize cleared result. Future studies should include more detailed biomolecular assessments, such as evaluations of TGF- β , TNF- α 3, VEGF, EGF, and lactate, which influence the development of fibroblasts and collagen. Additionally, while bursting pressure remains a widely accepted and practical indicator of anastomotic integrity, it is limited as a sole mechanical marker. It does not assess other biomechanical properties such as elasticity, tensile strength, or long-term durability, nor does it directly evaluate tissue remodelling or vascularization, which are critical for sustained healing^{29,30}.

CONCLUSION

The figure-of-eight suture technique demonstrated superior average integrity in the healing strength of duodenal perforation wounds, both macroscopically and microscopically, when compared to the interrupted suture technique. Additionally, the continuous suture technique showed enhanced average integrity in microscopic assessments of duodenal perforation wound healing strength relative to the interrupted suture, although macroscopic evaluations did not reveal a significant difference. Additional studies with bigger sample sizes and more thorough examination are advised for future research.

AUTHORS' CONTRIBUTIONS

Eko Setiawan: Supervision, Validation, Conceptualization, Methodology, Software, Writing- Original draft preparation, Visualization. **Dimas Nabih:** Conceptualization, Methodology, draft preparation, Visualization, Reviewing and Editing. **Akmal Prahendra:** Conceptualization, Methodology, Writing- Original draft preparation, Data curation and Visualization **Kiki Wijaya:** Conceptualization, Methodology, Writing- Original draft preparation, Data curation and Visualization, **Asa Ahmad:** Conceptualization, Methodology, Writing- Original draft preparation, Data curation and Visualization, **Affiq Salsabil:** Conceptualization, Methodology, Writing- Original draft preparation, Data curation and Visualization, **Wildan Abdillah:** Conceptualization, Methodology, Writing- Original draft preparation, Data curation and Visualization.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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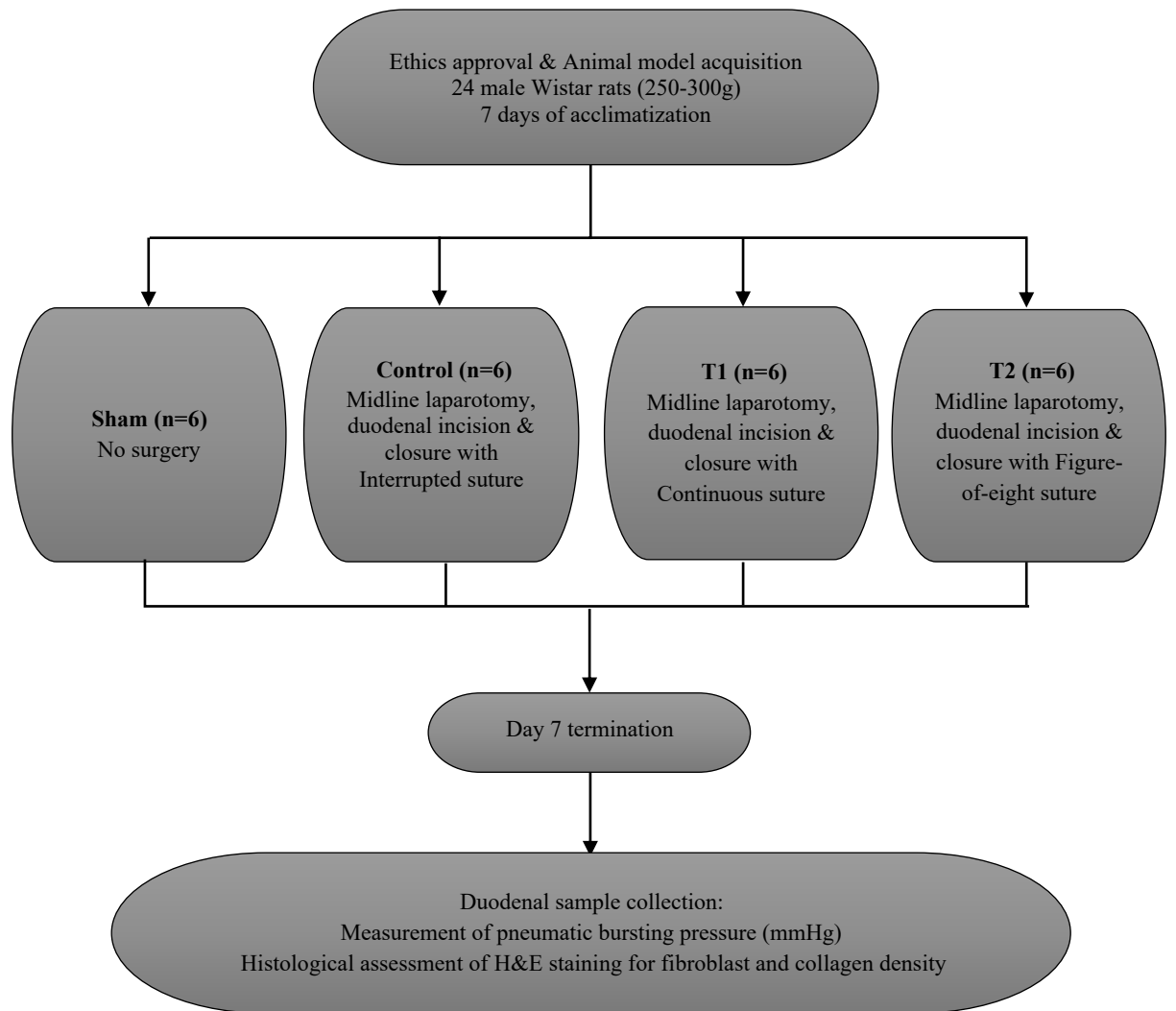


Figure 1. Flowchart of study design