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# The comparison of fibroblast count and collagen density score in ileal anastomosis with continuous and interrupted suture technique on New Zealand Rabbit



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## ABSTRACT

**Introduction:** Intestinal anastomosis is carried out with the continuous and simple interrupted sutures technique. Simple interrupted suture is the standard technique in intestinal anastomosis nowadays<sup>1,2</sup>. Continuous suture has better serosal apposition, shorter processing time and triggers the formation of hypoxia-inducible factor (HIF) which can stimulate the production of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) that stimulate migration and proliferation of mesenchymal cells, especially fibroblast cells which support the synthesis and increasing collagen density in anastomosis tissue. The aim of current study is to compare the anastomosis technique with continuous and simple interrupted sutures reviewed from fibroblast count and collagen density score in rabbit intestines.

**Method:** This study is an experimental study. The sample of this study consisted of 36 rabbits divided into two groups continuous and simple interrupted sutures. Randomization is done by permuted randomization block. The inclusion criteria in this study were rabbit strain *Oryctolagus cuniculus* aged 8-9 months, weighted 2000-2500 grams, healthy and active. The independent variable in this

study is the ileal anastomosis technique and the dependent variable is the fibroblasts count and the collagen density score formed on the anastomosis tissue assessed histopathologically.

**Results:** This study found the average age of the rabbit was 8 months, with an average weight of 2400 grams, the number of fibroblasts in the group of rabbits which received continuous suture was  $47 \pm 12.679$  fibroblasts per visual field with collagen score was  $1.88 \pm 0.781$ , whereas in rabbits with interrupted sutures, the fibroblasts count was  $42.76 \pm 12.47$  fibroblasts per visual field with collagen score was  $1.82 \pm 0.728$ . However, from the analytic testing showed that the rabbits treated with continuous suture were not significantly different in fibroblast count and collagen density score compared to simple interrupted group ( $p > 0.05$ ), but in continuous rabbit group have higher collagen density scores and fibroblasts count than rabbits who get simple interrupted suture

**Conclusion:** This study proves that continuous suture results in higher fibroblast count and collagen density score than interrupted suture yet statistically insignificant.

**Keywords:** Intestinal anastomosis, continuous suture, interrupted suture, fibroblast, collagen

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## INTRODUCTION

Intestinal anastomosis is the procedure of connecting the intestine that is separated to divert the continuity of the intestinal tract. Manual bowel anastomosis performed using the technique of continuous suture and interrupted sutures. Interrupted suture is now the standard in intestinal anastomosis.<sup>1,2</sup>

The continuous suture has the advantage of a better serosal apposition and shorter processing time. Furthermore, it has the advantage of water-tight anastomosis, but a higher risk of tissue hypoxia due to higher tension in the entire tissue. Hypoxia in the tissue seems to increase leakage, yet hypoxia can induce HIF, a protein transcription factor that can increase the production of VEGF and PDGF.<sup>3</sup>

VEGF will increase vascularization and angiogenesis, which ultimately increases fibroblast

proliferation and helps collagen synthesis in anastomosis tissue. PDGF activities stimulate migration and proliferation of mesenchymal cells, mainly fibroblast cells.<sup>4</sup> HIF also plays a role in modulating the immune system primarily the intestinal mucosa, and helps the formation of the extracellular matrix that supports the synthesis of collagen furthermore increasing collagen density in anastomosis tissue.<sup>3</sup>

Continuous sutures have the advantage of can be applied on tissues with minimal tension, a lower risk of mucosal prolapse, faster and fewer knot. But it has weaknesses, the risk of stenosis is greater, and dehiscence in the knot will result in overall dehiscence.<sup>5</sup> The Memon study in 174 patients in Pakistan showed anastomotic leak rates in continuous sutures (8.4%) is higher than interrupted sutures (6.9%).<sup>6</sup> Another study by Irwin in

466 patients showed a bowel anastomosis leak rate of 1.2% using an interrupted anastomosis technique, while Sarin's study reported a leakage rate of 4.5% in continuous anastomosis technique.<sup>5,7</sup>

Previous research carried out on rabbits by Iwan et al. showed no differences in rates of anastomotic leakage, tensile strength, degree of tissue reaction, and degree of intraperitoneal adhesion between continuous and simple interrupted polyglactin multifilament.<sup>8</sup> The advantages of simple interrupted suture include a more even distribution of tensile strength, lower tissue ischemia, lower risk of leakage and if one of the knot loose, can still be maintained with another knot. However, simple interrupted suture has the disadvantage of longer processing time.<sup>2</sup> Greater risk of tissue hypoxia in continuous suture seems to increase leakage rate. However hypoxia can induce the formation of HIF which acts to stimulate VEGF formation thereby increasing neovascularization in the anastomotic tissue. Vascularity is important in collagen formation in wounds.<sup>3,4</sup>

This difference of opinion underlies our research on rabbits by dividing two groups with interrupted suture and continuous suture. On day 7 the rabbit will be sacrificed and the edge of the anastomosis tissue is cut to assess wound healing histopathologically by evaluating fibroblast count and collagen density score.

## METHOD

This study is an experimental study on rabbits. Performed on New Zealand rabbits when compared to humans, this rabbit has a similar anatomical structure of the abdomen, liver, and intestine. Anatomically, physiologically, and biochemically in the digestive system have similarities with humans.

Rabbits will be divided into two groups; groups of interrupted knot suture (I) and groups of continuous suture with absorbable threads (II), with each of the groups has same number of samples. Each rabbit is numbered on the ear. Randomization is done by permuted randomization block. The registrar will first create the randomization block. Then

randomly selected randomization blocks will be applied for the first group (four samples). This will continue until the entire sample has occupied the two groups so that a total of 36 rabbits are recorded (a total of nine randomization block groups) which are divided into two groups randomly and can be equal in number. When taking action on an experimental animal, the operator is only given instructions by the registrar to do it without knowing the purpose of the study (blinding). Documentation is done by the registrar without the operator's knowledge. On the seventh day, the operator also did not know the previous intervention on the rabbit — only the registrar know and record. During the examination process at the Anatomy Pathology Laboratory, the examiner was only told the sample number but did not know whether the specimen included a group of continuous or interrupted suture. The results will be obtained and recorded by registrar. From the results of recording, data analysis is carried out.

## RESULTS

There were 36 *Oryctolagus cuniculus* rabbits aged 8-9 months, with a bodyweight between 2000-2500 grams. Then divided into two groups as many, both groups performed resection on 20 cm proximal from ICJ and performed anastomosis with different techniques, Group 1 with simple interrupted suture and Group 2 with a continuous suture. The characteristics of the study sample that met the inclusion criteria and did not drop out showed that there were no differences in characteristics between age, weight, hemoglobin level and albumin levels. Likewise with the length of operation in the two groups of samples there was also no difference. Rabbits used in both groups and duration of operation were homogeneous ( $p > 0.05$ ), which meant there were no statistically significant differences in the samples obtained from the Faculty of Veterinary Medicine, Airlangga University. The basic data of the study sample is illustrated in [Table 1](#).

Rabbits were sacrificed on the seventh day by decapitation. Then the fibroblasts count was

**Table 1** Subject Characteristic

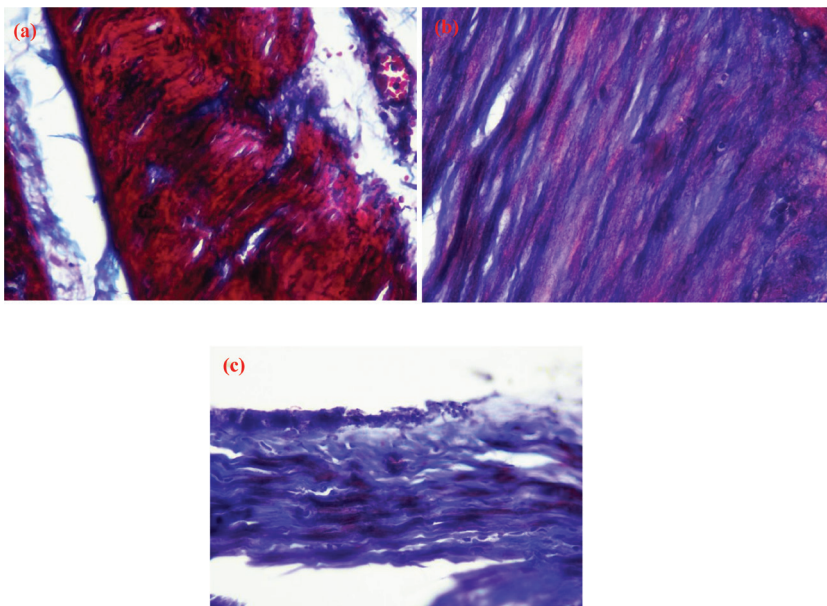
| Characteristics          | Anastomosis Technique |                  | p-value |
|--------------------------|-----------------------|------------------|---------|
|                          | Continuous            | Interrupted      |         |
| Age (month)              | 8.53 ± 0.45           | 8.56 ± 0.46      | 0.838   |
| Weight (grams)           | 2306.47 ± 116.56      | 2282.24 ± 128.15 | 0.692   |
| Albumin (g/dL)           | 3.84 ± 0.38           | 3.85 ± 0.40      | 0.916   |
| Operating Time (minutes) | 22.18 ± 1.62          | 26.58 ± 1.75     | 0.069   |
| Haemoglobin (g/dL)       | 10.46 ± 0.30          | 10.54 ± 0.29     | 0.434   |

**Table 2** The difference of the number of fibroblasts between the continuous anastomosis group and the simple interrupted anastomosis group

|         | Anastomosis Technique |               | p-value |
|---------|-----------------------|---------------|---------|
|         | Continuous            | Interrupted   |         |
| Lowest  | 31                    | 22            | 0.334   |
| Highest | 68                    | 61            |         |
| Mean    | 47 ± 12.679           | 42.76 ± 12.47 |         |

**Table 3** The difference in collagen density score between the continuous anastomosis group and the simple interrupted anastomosis group

|         | Anastomosis Technique |                 | p-value |
|---------|-----------------------|-----------------|---------|
|         | Continuous (n)        | Interrupted (n) |         |
| Score 1 | 6 (35.3%)             | 6 (35.3%)       | 0.838   |
| Score 2 | 7 (41.2%)             | 8 (47.1%)       |         |
| Score 3 | 4 (23.5%)             | 3 (17.6%)       |         |
| Mean    | 1.88 ± 0.781          | 1.82 ± 0.728    |         |

**Figure 1** (a). Score 1; (b) Score 2; (c) Score 3

evaluated per field of view in the continuous anastomosis technique and the simple interrupted anastomosis group.

The normality of the data evaluated using the Shapiro-Wilk test with results  $p=0.388$  ( $p>0.05$ ) which shows that of the number of fibroblasts classified as normally distributed.

The results of the independent T-test showed no statistically significant difference in the number of intestinal fibroblasts carried out by anastomosis using continuous technique with simple interrupted technique  $p=0.334$  ( $p>0.05$ ). But in the continuous group, the average number of fibroblasts was

higher, which was  $47 \pm 12.679$  compared to the interrupted anastomosis group with an average of  $42.76 \pm 12.47$ . (Table 2).

Collagen was obtained by biopsy on the edge of the anastomosis tissue on the seventh day. The difference test results showed that there was no statistically significant difference in intestinal collagen density scores performed by anastomosis using continuous technique with simple interrupted technique  $p=0.838$  ( $p>0.05$ ). But in the continuous group the average collagen density score was higher, which was  $1.88 \pm 0.781$  compared to the interrupted anastomosis group with an average of  $1.82 \pm 0.728$ . (Table 3). Histopathological morphology of score 1, 2, and 3 can be seen in figure 1a, 1b, and 1c.

## DISCUSSION

In this research it is essential to avoid bias because the characteristics of the sample will significantly influence the research, it is necessary to make the samples as homogeneous as possible. Experimental animals were obtained and selected from the same place (research animal unit Faculty of Veterinary Medicine, Airlangga University) and were given the same food. In this study 36 New Zealand rabbits were selected, 18 rabbits performed continuous anastomosis (treatment group) compared to interrupted anastomosis (control group). During the research, one rabbit was found in each group that died during quarantine. One rabbit in the interrupted anastomosis group died on third day and one rabbit in the continuous anastomosis group died on fourth day.

It was found that the fibroblasts count increased in the continuous group compared to the interrupted group but it was not statistically significant ( $p>0.05$ ). This increase in the number of fibroblasts can be caused by the increase in HIF in the continuous anastomosis group. The higher number of fibroblasts in the continuous group caused the continuous group to synthesize more collagen than the interrupted group so that collagen in the continuous group was denser compared with the interrupted group, but it was not statistically significant ( $p>0.05$ ). A study by Jiborn et al. conducted a study comparing the post-op levels of hydroxyproline at days 14 and 28, in interrupted anastomosis and continuous showed that there was a statistically significant difference in the levels of hydroxyproline where the continuous suture had higher levels than the interrupted suture. This study was conducted on experimental animals at Sprague Dawley using ticon suture material (non-absorbable braided polyester). Our study gives different results, this can be caused by the period of rabbits being sacrificed (days 7 vs days 14 and 28) were in our study

the increased fibroblasts and collagen were not too high due to the lysis process was higher than the synthesis process, and the parameters examined were different.

Based on the results of this study it was concluded that there were no significant differences in the fibroblasts count and collagen density between the treatment groups and the control group. Clinically the two groups have same wound-healing effect.

The results of this study are in accordance with a study from Iwan in 2001 which showed no significant differences in anatomic leakage rates and tensile strength in the comparison of intestinal anastomosis techniques in a continuous and simple interrupted.<sup>8</sup>

## CONCLUSION

This study proved that intestinal anastomosis in rabbits with the continuous suture technique produced a higher fibroblast count and collagen density scores compared to interrupted techniques but there was no statistically significant difference.

## SUGGESTION

Further research is needed by evaluating the fibroblasts count and collagen density at longer times as on the 7<sup>th</sup> and 14<sup>th</sup> days. And further research needs to be done to reduce bias and more objective measurements.

## ETHICAL CLEARANCE

This study was approved by the Ethics Committee before this research was conducted. And all experimental animals are treated according to the rules of the Animal Care and Use Committee of Airlangga University.

## STATEMENT OF CONFLICT OF INTEREST

The author states that there is no conflict of interest in this study.

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## CONTRIBUTION OF WRITERS

All authors have contributed to all the processes in this research, preparation, preparation, review and approval of this text.

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