INTRODUCTION

Gastric perforation in neonates is an emergency in neonatology, with a mortality rate of up to 75%. The mechanisms that cause gastric perforation in neonates are spontaneous perforation, ischemia, and trauma. Treatment of gastric perforation in neonates is debridement and primary gastric repair. If the necrosis is extensive enough, repair of gastric perforation can be done using the Graham patch method, by repairing the defect in the gaster using the omentum, which has a good vascular pedicle or by using the modified Graham patch method, by improving primarily first and then applying a flap using the omentum on top of the repair.

Many studies have been carried out to improve tissue healing, including using various surgical techniques and materials, one of which is using human-dried amniotic membrane technology. Therefore, based on those mentioned above, this study aims to evaluate the differences in the number of fibroblasts and collagen density in gastric perforation repair with H-DAM as a biomaterial patch compared to using an omental patch in New Zealand white rabbits.

METHODS

This study was a true experiment with 30 New Zealand rabbits (median 1.7 kg) with a randomized control trial study design. The inclusion criteria for this study are New Zealand White Rabbit male sex, age 6-9 months with a weight of 2-3 kg, healthy and active. The Exclusion criteria for this study are rabbits not fasted for 12 hours. During the 12-hour fasting period, it behaved aggressively and attacked other rabbits, and found surgical site infection. Data were analyzed using SPSS version 25.0 for Windows.

CONCLUSION: There is an increase in the number of fibroblasts and collagen density in gastric perforation repair with H-DAM as a biological dressing compared to primary repair of gastric perforation using an omental patch in rabbit models so that H-DAM can be the repair technique of choice in gastric perforation in the rabbit.

Keywords: Perforation, Gastric, Dried Amniotic Membrane.
On the 7th postoperative day, the rabbits were terminated and then the gastric tissue that had been repaired was taken to examine further the density of collagen and the number of fibroblasts. Data were recorded, statistically analyzed and compared between the control and treatment group with One-Way ANOVA Test and statistically processed using SPSS version 25.0 for the Windows program.

Evaluation of collagen density using a score like in Figure 2. In previous research, it was found that the average score for good collagen density and not causing leakage was 3. The average score was evaluated as follows: 1) Score 0: Density of collagen fibers in the wound area is very low; 2) Score 1: Density of collagen fibers in the wound area is low, 10-25%; 3) Score 2: Density of collagen fibers in the wound area is moderate, 25-50%; 4) Score 3: Density of collagen fibers in the wound area is tight, 50-75%; 5) Score 4: The density of collagen fibers in the wound area is very tight, 75-100%.

RESULTS

A total of 30 samples of rabbits were homogeneous (Table 1) and none of them dropped out. The results showed a significant difference in gastric healing in examining the number of fibroblasts with the Kruskal-Wallis test (p=0.000). As for the collagen density factor, from the One-way Anova analysis, it was found that the three data groups did not have a significant difference with the result $\alpha = 0.234$ p < 0.05.

From the 30 samples, collagen density was examined and scored 0 to 4, with a score of 0 indicating low collagen density to 4 with very dense collagen density. It is known that there is a dominance of the distribution of score 3 in the Control group (2.56±1.07), as much as 40% (4 rabbits), whereas, in the Omental Patch group (2.08±0.97), it is dominated by a score of 2, as much as 70% (7 rabbits) and in the H-DAM group (2.84±0.88) it is dominated by a score of 3 as much as 60% (6 rabbits) but not statistically significant (p=0.234) (Table 2). However, there was a statistically significant difference of fibroblast count among Control (60.80 (58.40-152.20)), Omental Patch (34.00 (23.00-67.20)), and H-DAM (146.00 (38.00-198.00)) groups.

![Figure 1](image1.png)

(A) Rupture in the gastric corpus was carried out by primary suturing; (B) H-DAM measuring 10 cm x 10 cm; (C) Treatment group by placed H-DAM over primary sutures; (D) Treatment group by patching omentum over primary sutures.

![Figure 2](image2.png)

(a) Score 0; (b) Score 1; (c) Score 2; (d) Score 3; and (e) Score 4.
Table 1. Demographic Data of Research Subjects

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Control (n=10)</th>
<th>H-DAM (n=10)</th>
<th>Omental Patch (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Month)</td>
<td>6-9</td>
<td>6-9</td>
<td>6-9</td>
<td>0.934*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>7.13±1.05</td>
<td>6.03±1.02</td>
<td>6.53±1.08</td>
<td></td>
</tr>
<tr>
<td>Weight (gram)</td>
<td>1,200-2,200</td>
<td>1,200-2,200</td>
<td>1,200-2,200</td>
<td>0.820*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1,690.00±258.05</td>
<td>1,720.00±308.02</td>
<td>1,710.00±238.01</td>
<td></td>
</tr>
</tbody>
</table>

*p > 0.05 means the data was statistically homogenous using the Levene test.

Table 2. One Way ANOVA based on Collagen Density

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=10)</th>
<th>Omental Patch (n=10)</th>
<th>H-DAM (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Density</td>
<td>2.56±1.07</td>
<td>2.08±0.97</td>
<td>2.84±0.88</td>
<td>0.234*</td>
</tr>
<tr>
<td>Fibroblast Count</td>
<td>60.80 (58.40-152.20)</td>
<td>34.00 (23.00-67.20)</td>
<td>146.00 (38.00-198.00)</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*aOne-Way ANOVA; bKruskal-Wallis; *Statistically significant if p-value less than 0.05

Figure 3. Macroscopic observations postoperative day 7 after gastric repair with H-DAM. H-DAM remains (black arrows), with no tissue adhesions, pus, or leakage.

Based on the results of the study, when macroscopic observations were made when the surgical wound was reopened, it was found in the group with H-DAM shown in Figure 3, the dried human amniotic membrane used for repair had fused with the gastric tissue, no adhesion was found with the surrounding tissue, the omentum remained intact, and no leakage was found (Figure 3-5).

The difficulty conducting research with experimental rabbits was during postoperative care because many factors affect the healing process. Some rabbits experienced a decrease in appetite after surgery, thus affecting the systemic healing process so that proper and adequate quarantine is needed before and after surgery so that optimal results are expected.

DISCUSSION

In this study, observations were made on the 7th day after the gastric repair procedure, hoping to obtain a proliferative phase by comparing the number of fibroblasts and collagen density in the three groups. There appears to be a significant difference in the mean number of fibroblasts between the control group, the H-DAM group and the omental patch group. The highest average number was found to be dominated by the treatment group with H-DAM, where the amniotic membrane can stimulate migration from the ECM and increase the proliferative phase by increasing the...
IL-β and produce MMP inhibitors which accelerate the healing process.\textsuperscript{47-44} It can be seen that in the HE staining preparation (see Figure 5B), in the amnion group, there were no inflammatory cells. In contrast, in the Omental patch group, there were still inflammatory cells between the tissues and fibroblast cells, as shown in Figure 5C.

This makes H-DAM superior to the Omental patch group because it has anti-inflammatory abilities and stimulates faster fibroblast formation so that the collagen produced is also denser and causes wound healing to occur faster than the other groups.

The difficulty conducting research with experimental rabbits was during postoperative care because many factors affect the healing process. Some rabbits experienced a decrease in appetite after surgery, thus affecting the systemic healing process so that proper and adequate quarantine is needed before and after surgery so that optimal results are expected.

There needs to be further observation on different days to see the peak collagen density, collagen maturation, type of collagen and number of fibroblasts as well as other factors that affect the healing process, such as neovascularization, angiogenesis, and inflammatory mediators in gastric perforation repair with H-DAM as biologic dressing compared with primary repair of gastric perforation using an omental patch in a rabbit model.

**CONCLUSION**

There is an increase in the number of fibroblasts and collagen density in gastric perforation repair with H-DAM as a biological dressing compared to primary repair of gastric perforation using an omental patch in rabbit models so that H-DAM can be the repair technique of choice in gastric perforation in neonates.

**CONFLICT OF INTEREST**

There is no competing interest regarding the manuscript.

**ETHICAL CLEARANCE**

Ethics approval was obtained from the Animal Care and Use Committee.
(ACUC), Universitas Airlangga, Surabaya, Indonesia, with number 2.KEH.140.10.2022 before the study was conducted.

**FUNDING**
None.

**AUTHOR CONTRIBUTIONS**
All authors equally contribute to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

**REFERENCES**


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