Research Article

Protective Effect of An Amnion Patch Against Postoperative Pancreatic Fistula (Popf) Following Pancreatic Anastomosis in Rats: An in Vivo Experimental Study

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ABSTRACT

Postoperative pancreatic fistula (POPF) remains a major complication following pancreatic anastomosis and is associated with substantial postoperative morbidity. The amniotic membrane possesses both barrier properties and biologically active components that may promote tissue healing and preserve anastomotic integrity. This study aimed to evaluate the protective effect of an amnion patch against POPF following pancreatic anastomosis in rats.

This in vivo experimental study used a posttest-only control-group design involving 24 male Sprague–Dawley rats randomized into a control group (K-1; n=12) and an amnion-patch group (K-2; n=12). All animals underwent pancreaticojejunostomy, with additional amnion-patch application in K-2. Outcomes included POPF occurrence, macroscopic anastomotic integrity, intra-abdominal adhesion score, and peritoneal amylase and lipase levels.

Four of 12 animals in K-1 and 11 of 12 in K-2 reached the final evaluation. POPF occurred in 4/4 (100%) K-1 animals and 2/11 (18.2%) K-2 animals (p=0.011). The amnion-patch group demonstrated better macroscopic anastomotic integrity and significantly lower adhesion scores [median 0 (IQR 0–1) vs 1.5 (IQR 1–2); p=0.006]. Peritoneal amylase and lipase levels were also significantly lower in K-2 (p=0.013 and p=0.006, respectively).

Among animals reaching the final evaluation, amnion-patch application was associated with a lower incidence of POPF, reduced intra-abdominal adhesion severity, lower peritoneal pancreatic enzyme levels, and improved macroscopic anastomotic integrity.

Keywords: amnion patch, pancreaticojejunostomy, pancreatic anastomosis, postoperative pancreatic fistula, POPF.

Introduction

Postoperative pancreatic fistula (POPF) remains one of the most consequential complications following pancreatic surgery despite continuing advances in pancreatic reconstruction. Leakage of pancreatic secretions from the anastomotic site may result in enzymatic injury to the surrounding tissues and trigger local inflammation, intra-abdominal fluid collections, abscess formation, hemorrhage, sepsis, and, in severe cases, organ failure. Consequently, POPF contributes substantially to postoperative morbidity, prolonged hospitalization, and the need for additional therapeutic interventions (Rennie et al., 2024; Nebbia et al., 2024).

Various strategies have been developed to preserve pancreatic anastomotic integrity and reduce the risk of POPF, including modifications of pancreaticojejunostomy techniques, pancreatic duct stenting, and the application of anastomotic reinforcement materials. Nevertheless, POPF remains a persistent clinical challenge, highlighting the need for adjunctive approaches that not only provide mechanical protection but may also promote local tissue healing.

The amniotic membrane is a biologically active biomaterial with properties that may be advantageous for anastomotic reinforcement. Its extracellular matrix contains collagen, laminin, fibronectin, hyaluronic acid, growth factors, and other bioactive mediators. In addition to providing a structural scaffold for cellular adhesion, migration, and proliferation, the amniotic membrane has been reported to exert anti-inflammatory, immunomodulatory, and antifibrotic effects that may facilitate tissue repair and regeneration (Wassmer et al., 2020; Torres et al., 2023; Rouzaire et al., 2024). Experimental studies of gastrointestinal anastomoses have further demonstrated the potential of amniotic membrane application to enhance anastomotic healing and integrity (Uludag et al., 2010). However, evidence regarding its use as a biological patch for pancreatic anastomoses remains limited.

Given its potential dual role as a physical barrier and a biologically active scaffold, an amnion patch may provide additional protection to the pancreaticojejunostomy site during the early phase of anastomotic healing. Therefore, this in vivo experimental study aimed to evaluate the effects of amnion patch application on POPF occurrence, macroscopic anastomotic integrity, intra-abdominal adhesion formation, and peritoneal amylase and lipase levels in a rat model of pancreaticojejunostomy.

Material and Methods

Sample

Male Sprague–Dawley rats aged 10 weeks and weighing 300–350 g were used in this study. The animals were acclimatized for at least 7 days before the experimental procedures and maintained under controlled environmental conditions with a 12-h light–dark cycle, an ambient temperature of 21–25°C, and relative humidity of 37–50%. Standard laboratory chow and water were provided ad libitum. Only animals in good general clinical condition were included in the study. Eligible animals were active, exhibited normal feeding behavior, and showed no evidence of active infection, systemic illness, or anatomical abnormalities of the abdomen or pancreas that could potentially interfere with the surgical procedure or outcome assessment.

Materials

The human amniotic membrane was obtained from a donor undergoing elective cesarean delivery under aseptic conditions. The membrane was thoroughly washed with saline solution containing streptomycin (50 µg/mL) and penicillin (50 µg/mL), followed by blunt separation of the amnion from the chorion. The isolated amniotic membrane was subsequently immersed in basal epithelial medium (BEM), stored at 4°C, and processed within 24 h of collection. Additional materials used in the experimental procedures included isoflurane and pentobarbital for anesthesia, meloxicam for perioperative analgesia, and Polyglactin 910, silk, and nylon sutures for the surgical procedures.

Study design

This in vivo experimental study employed a posttest-only control-group design. Animals were randomly allocated using a computer-independent random-number table prepared before surgery. Group allocation was disclosed to the surgeon only after induction of anesthesia to minimize selection bias. Animal cages and specimen tubes were coded with unique identifiers, and outcome assessments were performed blinded to group allocation. The primary outcome was the occurrence of pancreatic leakage or POPF. Secondary outcomes included macroscopic anastomotic integrity, the presence of perianastomotic fluid collections and abscesses, the severity of intra-abdominal adhesions, and peritoneal fluid amylase and lipase levels.

Pancreaticojejunostomy and amnion patch application

Animals were fasted for 12 h before surgery. Anesthesia was induced with 1–5% isoflurane delivered in oxygen at a flow rate of 0.8–1.0 L/min and subsequently maintained with intraperitoneal pentobarbital. Meloxicam (1 mg/kg) was administered subcutaneously 1 h before surgery for perioperative analgesia. Following laparotomy, the pancreas was carefully mobilized at the junction between the proximal portion and the gastrosplenic segment while preserving the surrounding vascular structures and biliopancreatic duct. The pancreas was sharply transected, and the caudal segment was preserved as the pancreatic remnant for reconstruction. Pancreatic reconstruction was performed using a modified invagination (dunking) pancreaticojejunostomy adapted to rat anatomy. The pancreatic remnant was mobilized approximately 1–1.5 cm, and two stay sutures were placed at its superior and inferior margins. A 3–4-mm jejunostomy was created, and the pancreatic remnant was gently invaginated into the jejunal lumen. The anastomosis was secured using a 6-0 Polyglactin 910 purse-string suture supplemented by interrupted sutures between the jejunal wall and pancreatic capsule to achieve stable pancreaticojejunostomy. In the amnion patch group (K-2), a 2 × 5 cm human amniotic membrane patch was applied circumferentially around the completed pancreaticojejunostomy and secured with interrupted 6-0 Polyglactin 910 sutures. No amniotic membrane was applied in the control group (K-1). The abdominal cavity was subsequently irrigated with warm saline, followed by layered closure of the abdominal wall.

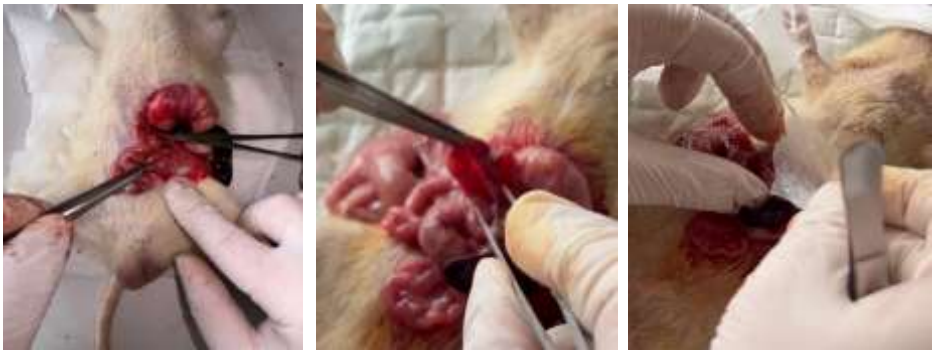

Figure 1. Surgical procedure

Postoperative care and evaluation

Following surgery, the animals received active warming and were closely monitored until spontaneous ambulation was restored. Meloxicam (1 mg/kg) was administered every 24 h for 48 h, while benzylpenicillin sodium (100,000 IU/kg) was administered intramuscularly every 24 h for 48 h. Immediately after surgery, each animal received 2 mL of warmed Ringer's lactate solution and was fasted for 24 h. Oral intake was subsequently reintroduced in a stepwise manner, beginning with a glucose–sodium chloride solution, followed by a soft diet and then standard solid chow. Animals were monitored daily for clinical condition and mortality. For animals that died before the scheduled endpoint, postmortem examination was performed to assess the presence of free intra-abdominal blood, the source of hemorrhage, anastomotic discontinuity or dehiscence, perianastomotic fluid collections, and intra-abdominal adhesions.

Amylase and lipase measurements

Peritoneal fluid was collected from the area surrounding the pancreaticojejunostomy at the time of terminal evaluation. The samples were centrifuged at 3,000 rpm for 30 min and stored at 4°C until biochemical analysis. Amylase and lipase activities were measured and expressed as U/L. The levels of both enzymes were compared between the control (K-1) and amnion patch (K-2) groups as biochemical indicators of pancreatic secretion leakage.

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 26.0. Categorical variables are presented as frequencies and percentages, whereas continuous variables are expressed as mean ± standard deviation (SD) for normally distributed data or as median and interquartile range (IQR) for non-normally distributed data. Data distribution was assessed using the Shapiro–Wilk test. Between-group differences in POPF occurrence were evaluated using Fisher's exact test because of expected cell counts <5. Adhesion scores and peritoneal amylase and lipase levels were compared between groups using the Mann–Whitney U test. All statistical tests were two-sided, and a p value <0.05 was considered statistically significant.

Results

Animal characteristics and allocation

A total of 24 male Sprague–Dawley rats were randomly allocated in a 1:1 ratio to the control group (K-1; n=12) or the amnion patch group (K-2; n=12). Mean baseline body weight was comparable between groups (324.17 ± 14.91 g vs. 325.00 ± 14.64 g, respectively; p=0.956). All animals underwent pancreaticojejunostomy according to their assigned intervention. In K-1, 4/12 animals (33.3%) survived to the 72-h postoperative endpoint, whereas 8/12 (66.7%) died before terminal evaluation. Three deaths occurred on postoperative day (POD) 0 and were associated with intra-abdominal hemorrhage. The remaining five deaths occurred between POD 1 and POD 3 and were accompanied by anastomotic discontinuity/dehiscence and perianastomotic fluid collection. In K-2, 11/12 animals (91.7%) reached the terminal endpoint, while 1/12 (8.3%) died on POD 2 with anastomotic dehiscence and perianastomotic fluid collection. Overall, 15 of 24 animals reached the 72-h terminal evaluation (Figure 2).

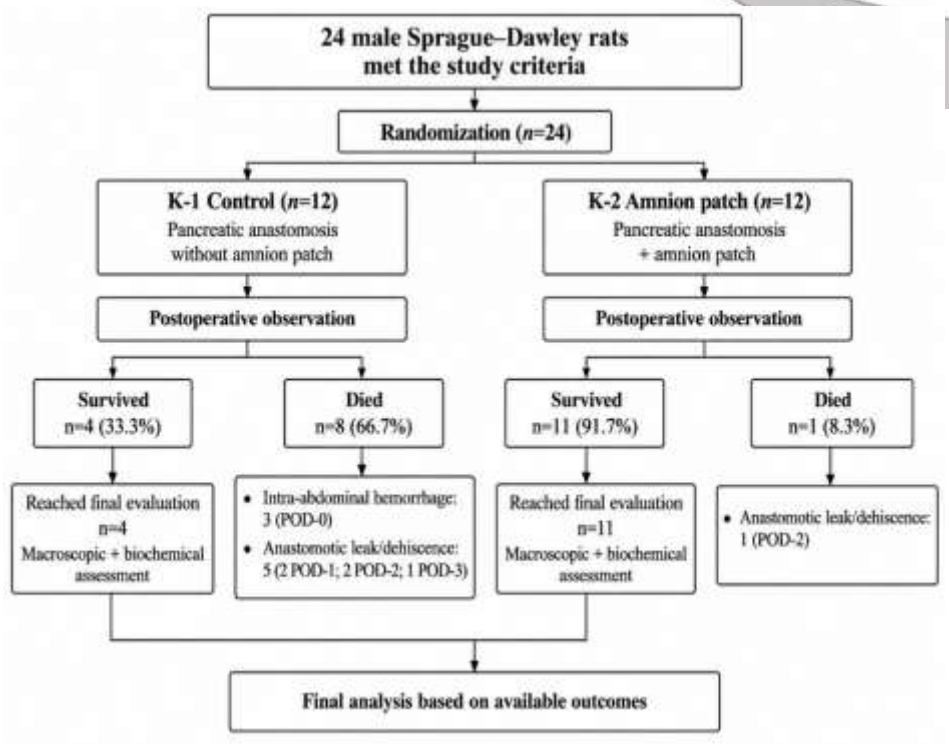

Figure 2. Allocation and flow diagram of the experimental animals

The amnion patch group demonstrated a higher survival rate and a lower mortality rate than the control group.
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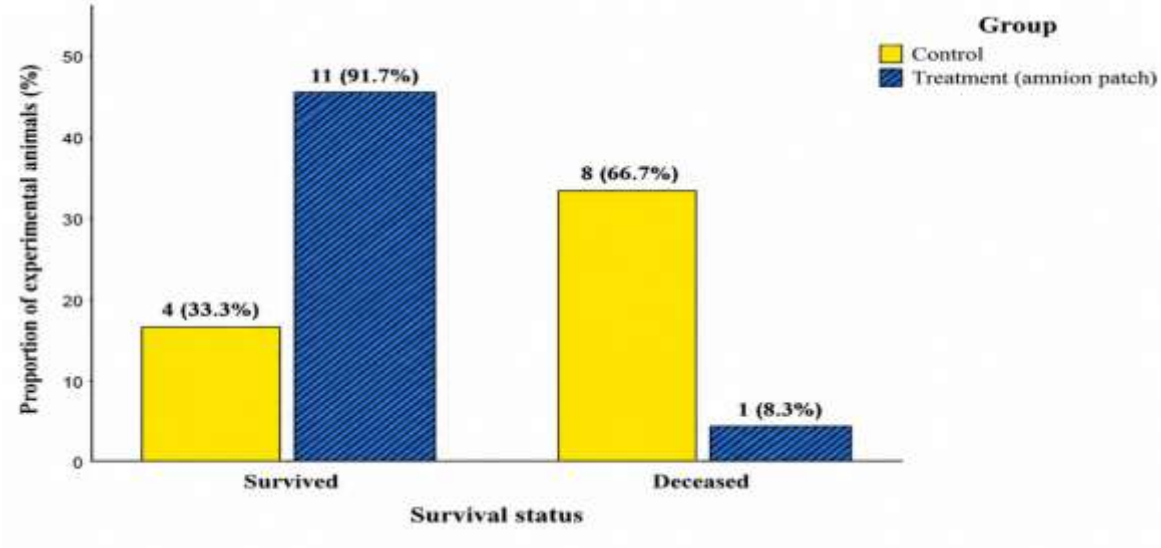

Figure 3. Distribution of survival status among experimental animals

Macroscopic evaluation and intra-abdominal adhesions

Macroscopic evaluation was performed in the 15 animals that reached the 72-h postoperative endpoint (K-1, n=4; K-2, n=11). The control group exhibited anastomotic dehiscence accompanied by intra-abdominal adhesions and perianastomotic abscess formation (Figure 4A). In contrast, most animals in the amnion patch group demonstrated preserved anastomotic integrity without perianastomotic fluid collections or abscesses (Figure 4B). Nevertheless, anastomotic dehiscence with perianastomotic fluid collection was still observed in a subset of animals in the amnion patch group (Figure 4C).

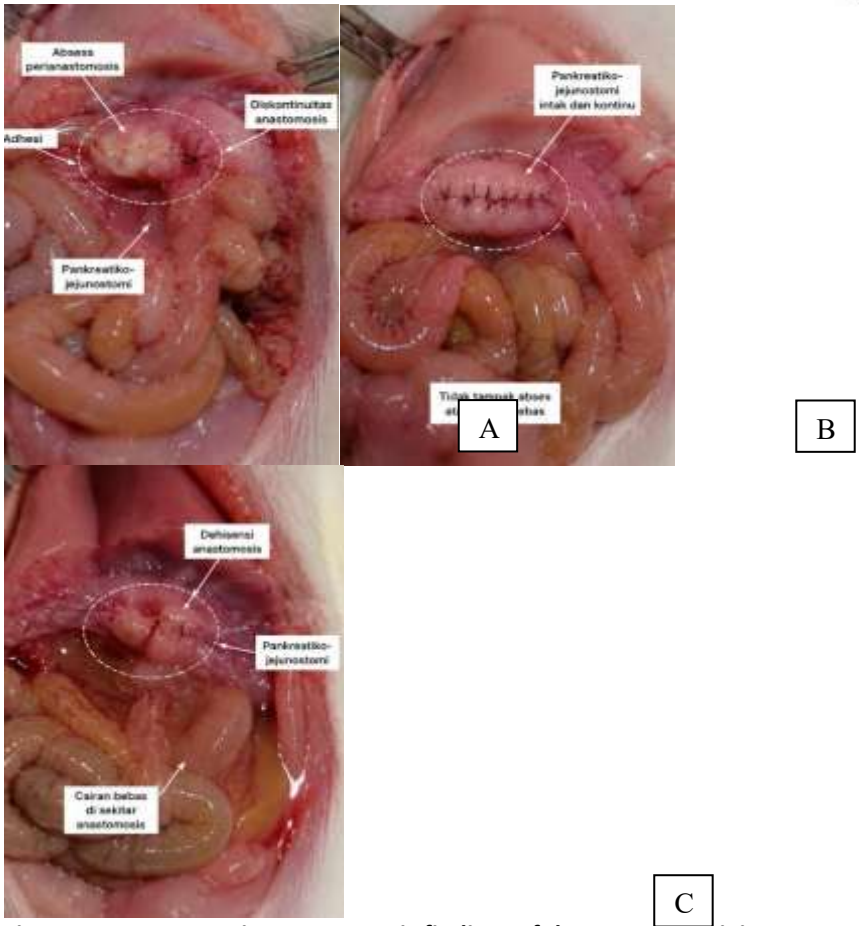

Figure 4. Representative macroscopic findings of the pancreaticojejunostomy site at the final evaluation

All animals in K-1 developed intra-abdominal adhesions (50% with grade 1 and 50% with grade 2), whereas in K-2, 72.7% had no adhesions and 27.3% developed grade 1 adhesions (Figure 5).

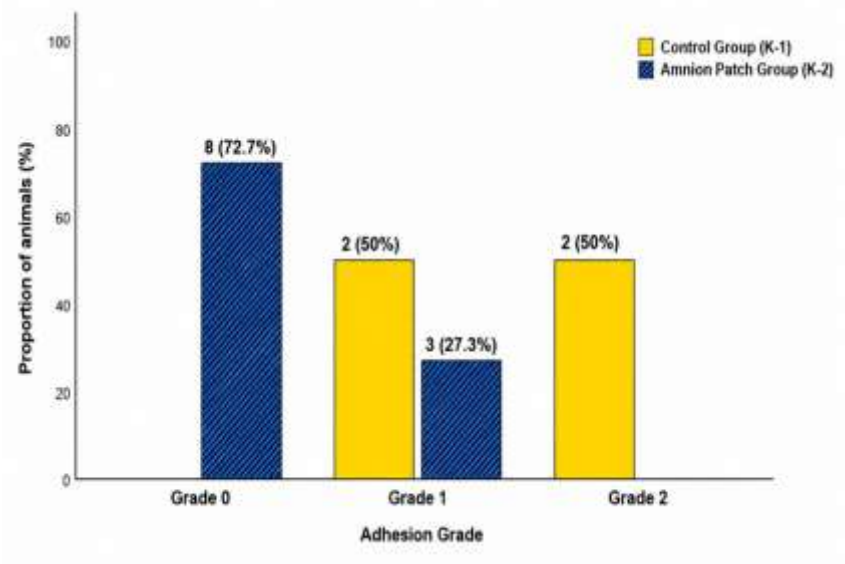

Figure 5. Distribution of intra-abdominal adhesion grades

The median adhesion score was significantly lower in K-2 than in K-1 [0 (IQR, 0–1) vs. 1.5 (IQR, 1–2); p=0.006] (Table 1)

Table 1. Comparison of adhesion scores between the control and amnion patch groups at the final evaluation

Variable	K-1	K-2	p-value
Adhesion score, median (IQR)	1.5 (1-2)	0 (0-1)	0,006

Individual macroscopic findings for each experimental animal that reached the final evaluation are presented in Table 2.

Table 2. Macroscopic and biochemical findings of the pancreatic anastomosis

Rat	Anastomotic discontinuity /dehiscence	Perianastomotic fluid	Perianastomotic abscess	Adhesion	Adhesion score	Amylase/lipase (U/L)	Amylase/lipase elevation
K1-02	+	+	–	+	2	406/390	++++
K1-03	+	+	+	+	2	444/377	++++
K1-08	+	+	–	+	1	421/356	++++
K1-10	+	+	+	+	1	438/368	++++
K2-01	–	–	–	–	0	209/99	++
K2-02	–	–	–	–	0	277/114	++
K2-03	–	–	–	–	0	212/126	++
K2-04	–	+	–	+	1	233/132	++
K2-05	–	–	–	–	0	226/121	++
K2-06	–	–	–	–	0	254/98	++
K2-07	–	–	–	–	0	211/121	++
K2-09	–	+	–	–	1	277/130	++
K2-10	–	–	–	–	1	281/130	++
K2-11	+	+	+	–	0	418/342	++++
K2-12	+	+	+	–	0	432/365	++++

Effect of the Amnion Patch on POPF Occurrence

Among animals that reached the final evaluation, POPF occurred in 4/4 (100%) animals in K-1 and 2/11 (18.2%) animals in K-2, whereas 9/11 (81.8%) animals in K-2 did not develop POPF (Figure 6).

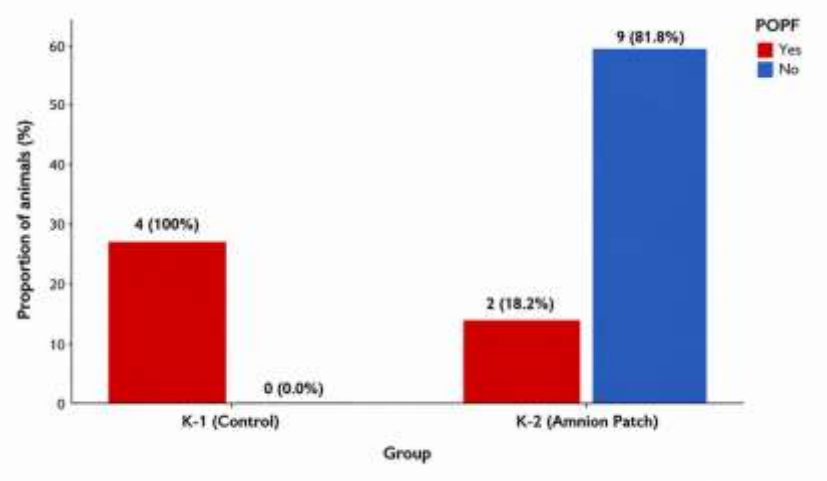

Figure 6. Distribution of POPF occurrence

The amnion patch group exhibited a significantly lower proportion of POPF than the control group. Comparison of POPF occurrence between K-1 and K-2 using Fisher's exact test demonstrated a statistically significant difference (p=0.011) (Figure 7).

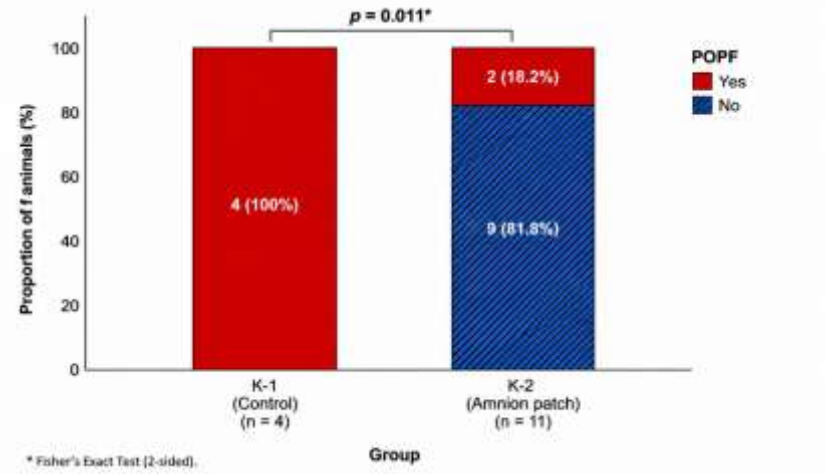

Figure 7. Comparison of POPF occurrence between the control and amnion patch groups

Effect of the amnion patch on peritoneal amylase and lipase levels

Among animals that reached the final evaluation, the amnion patch group exhibited significantly lower peritoneal amylase and lipase levels than the control group (amylase, p=0.013; lipase, p=0.006; Mann–Whitney U test), as shown in Figure 8.

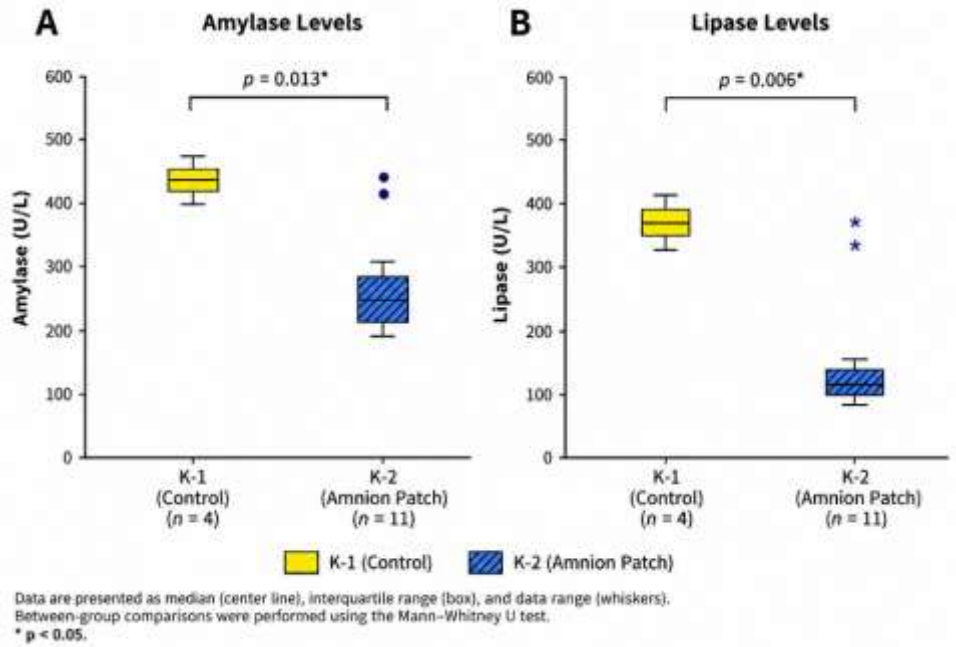

Figure 8. Comparison of peritoneal amylase and lipase levels between the control and amnion patch groups

Discussion

The present study demonstrates that reinforcement of pancreaticojejunostomy with an amnion patch was associated with more favorable early anastomotic outcomes among animals that reached the 72-h postoperative endpoint. Compared with the control group, animals receiving the amnion patch exhibited a substantially lower incidence of postoperative pancreatic fistula (POPF), reduced intra-abdominal adhesion severity, and lower peritoneal amylase and lipase levels. These biochemical findings were accompanied by better preservation of macroscopic anastomotic integrity. Collectively, these observations suggest that the amniotic membrane may provide both mechanical and biological support to the pancreatic anastomosis during the

vulnerable early phase of healing. Nevertheless, these findings warrant cautious interpretation because of the marked differential attrition between groups.

Baseline characteristics were comparable between the two groups. The mean initial body weight was 324.17 ± 14.91 g in the control group and 325.00 ± 14.64 g in the amnion patch group (p=0.956), minimizing the likelihood that differences in postoperative outcomes were attributable to baseline disparities. Ensuring comparable pre-intervention characteristics is particularly important in experimental studies, as it strengthens the attribution of subsequent differences to the experimental conditions rather than pre-existing biological variability (Chu et al., 2023).

The postoperative course differed considerably between groups. Only 4 of 12 control animals reached the 72-h terminal evaluation, whereas eight died during the postoperative period. Three deaths occurred on postoperative day (POD) 0 and were associated with intra-abdominal hemorrhage without evidence of anastomotic dehiscence or perianastomotic fluid accumulation. Conversely, the five deaths occurring between POD 1 and POD 3 were accompanied by anastomotic discontinuity/dehiscence and perianastomotic fluid. In the amnion patch group, 11 of 12 animals reached the terminal endpoint, while one animal died on POD 2 with anastomotic dehiscence and perianastomotic fluid. The very early hemorrhagic deaths in the control group likely reflect perioperative or technical complications rather than pancreatic leakage. Pancreaticojejunostomy in small-animal models is technically demanding, and anastomotic integrity may be influenced by tissue manipulation, pancreatic–jejunal apposition, suture depth and tension, and local perfusion independently of the intervention under investigation (Parmar et al., 2025). Accordingly, although survival was descriptively higher in the amnion patch group, the present findings should not be interpreted as evidence that amnion patch application directly reduces postoperative mortality.

Macroscopic assessment further supported a potential protective effect of amnion reinforcement. Control animals more frequently exhibited anastomotic discontinuity or dehiscence, perianastomotic fluid accumulation, adhesions, and abscess formation, whereas most animals treated with the amnion patch maintained an intact pancreaticojejunostomy without perianastomotic fluid or abscess formation. The biological plausibility of these findings is supported by the structural and bioactive properties of the amniotic membrane. Its extracellular matrix and basement membrane contain collagen, laminin, fibronectin, hyaluronic acid, and other bioactive constituents capable of providing a scaffold for cellular adhesion and migration while potentially modulating inflammatory responses and tissue regeneration (Fenelon et al., 2021; Ingraldi et al., 2023). In a rat colonic anastomosis model, Uludag et al. similarly reported that amniotic membrane reinforcement was associated with increased anastomotic bursting pressure, enhanced neoangiogenesis and fibroblast activity, and greater collagen deposition. Although these mechanisms were not directly assessed in the present study, they provide a biologically plausible framework for the improved macroscopic integrity observed following amnion patch application (Uludag et al., 2010)

A pronounced difference was also observed in intra-abdominal adhesion formation. All control animals that reached terminal evaluation developed adhesions, whereas 72.7% of animals in the amnion patch group had no macroscopic adhesions. Accordingly, the median adhesion score was significantly lower in the amnion patch group [0 (IQR, 0–1) vs. 1.5 (IQR, 1–2); p=0.006]. Postoperative adhesion formation is initiated by peritoneal injury and fibrin deposition; when fibrinolysis is inadequate, the persistent fibrin matrix facilitates fibroblast migration, extracellular matrix deposition, collagen organization, and neovascularization, ultimately resulting in mature fibrous adhesions (Arung et al., 2011). The amniotic membrane may mitigate this process by functioning as a temporary physical barrier between the injured anastomotic surface and adjacent intra-abdominal tissues. In addition, attenuation of pancreatic leakage may reduce enzymatic tissue injury and the associated inflammatory response, thereby further limiting adhesion formation (Hassanabad et al., 2021). This interpretation is consistent with Kaneko et al, who demonstrated less pronounced peritoneal adhesions accompanied by lower ascitic amylase and lipase levels following cell-sheet therapy in an experimental rat model of pancreatic fistula (Kaneko et al., 2017)

The most clinically relevant experimental finding was the marked difference in POPF occurrence. Among animals that reached terminal evaluation, POPF was identified in all control animals (4/4, 100%) but in only 2 of 11 animals (18.2%) receiving the amnion patch, yielding a statistically significant between-group difference (p=0.011). Importantly, POPF in the present study represents an experimentally adapted endpoint rather than a direct equivalent of clinically relevant POPF in humans. The International Study Group of Pancreatic Surgery (ISGPS) definition was developed for patients undergoing pancreatic surgery and incorporates drain-fluid amylase concentrations together with clinical consequences attributable to the fistula (Bassi et al., 2016). In the present animal model, pancreatic enzyme activity was therefore interpreted in conjunction with macroscopic evidence of anastomotic disruption and perianastomotic fluid accumulation. Such a composite anatomical–

biochemical approach is more appropriate for experimental assessment because elevated pancreatic enzyme activity alone does not necessarily establish structural failure of the anastomosis.

The reduction in POPF observed with the amnion patch may reflect complementary mechanical and biological mechanisms. Mechanically, the patch may provide an additional covering around the anastomotic interface and potentially limit the dissemination of pancreatic secretions following minor disruption. Biologically, the extracellular matrix of the amniotic membrane may provide a favorable scaffold during early tissue repair and may modulate the local inflammatory milieu (Fenelon et al., 2021; Ingraldi et al., 2023). However, two animals in the amnion patch group still developed anastomotic dehiscence accompanied by perianastomotic fluid accumulation, underscoring that the intervention does not confer absolute protection. An amnion patch cannot be expected to compensate fully for primary mechanical failure arising from inadequate tissue apposition, excessive suture tension, pancreatic parenchymal injury, or compromised local perfusion. Its potential role should therefore be regarded as adjunctive reinforcement of a technically sound pancreaticojejunostomy rather than a substitute for meticulous anastomotic technique.

The biochemical findings were concordant with the macroscopic observations. Peritoneal amylase and lipase levels were significantly lower in the amnion patch group than in controls (amylase, p=0.013; lipase, p=0.006). Notably, the two animals in the amnion patch group with higher enzyme concentrations also exhibited anastomotic dehiscence and perianastomotic fluid accumulation, whereas most animals with lower enzyme concentrations did not meet the experimental criteria for POPF. This concordance between anatomical disruption, perianastomotic fluid accumulation, increased pancreatic enzyme activity, and POPF strengthens the interpretation that these parameters represent interrelated manifestations along a spectrum of impaired anastomotic integrity.

These findings are broadly consistent with previous experimental studies investigating biologically active reinforcement strategies for pancreatic leakage. Kim et al reported that mesenchymal stem-cell sheet patches in a rat pancreatic model were associated with reduced abdominal fluid accumulation and lower amylase levels (Kim et al., 2018). Similarly, Kaneko et al. (2017) demonstrated lower ascitic amylase and lipase concentrations following application of an adipose-derived stem-cell sheet compared with untreated animals (Kaneko et al., 2017). Li et al. further showed that reduced intra-abdominal exposure to pancreatic secretions was associated with attenuated inflammatory changes and less severe adhesion formation (Li et al., 2021). Taken together, these observations provide a plausible mechanistic link between the lower pancreatic enzyme concentrations, reduced POPF occurrence, and diminished adhesion burden observed in the amnion patch group. The reduction in adhesion formation may therefore reflect not only the direct barrier function of the amniotic membrane but also an indirect consequence of reduced exposure of surrounding tissues to enzyme-rich pancreatic secretions.

The interpretation of pancreatic enzyme concentrations nevertheless requires consideration of the experimental sampling strategy. Amylase and lipase were measured only at the terminal 72-h evaluation, and the total volume of peritoneal fluid was not quantified. Pancreatic leakage is a dynamic process, and previous experimental work has demonstrated temporal changes in both abdominal fluid volume and amylase activity across POD 1, POD 3, and POD 7 (Kim et al., 2018). Consequently, the enzyme concentrations measured in the present study represent the biochemical milieu at the terminal assessment rather than the cumulative magnitude or temporal trajectory of pancreatic leakage throughout the postoperative period.

Limitations

Several limitations should be acknowledged. Most importantly, differential postoperative mortality resulted in a markedly imbalanced terminal sample, with only four control animals compared with 11 animals in the amnion patch group. Several control animals that died before the scheduled endpoint had already developed anastomotic dehiscence and perianastomotic fluid accumulation. Consequently, analyses restricted to animals surviving to 72 h are inherently susceptible to differential attrition and survivorship bias, potentially affecting both the magnitude and precision of the estimated intervention effect. The small number of terminal control animals further increases the sensitivity of proportional outcomes to individual observations.

Second, pancreaticojejunostomy in rats is technically challenging, and variability in tissue manipulation, pancreatic–jejunal apposition, suture placement and tension, parenchymal trauma, and local perfusion may have influenced anastomotic outcomes independently of amnion patch application. Third, anastomotic integrity and adhesion severity were evaluated macroscopically and may therefore retain an element of observer-dependent variability. Fourth, pancreatic enzymes were measured at a single postoperative time point without quantification of total peritoneal fluid volume, precluding assessment of the temporal dynamics and total burden of pancreatic leakage. Finally, neither histopathological evaluation of anastomotic healing nor direct assessment of mechanical strength, such as bursting pressure, was performed. The present study therefore

cannot establish whether the observed benefits of amnion patch reinforcement were mediated predominantly through a physical barrier effect, modulation of inflammation, enhancement of tissue repair, or a combination of these mechanisms.

Overall, these findings provide preliminary experimental evidence supporting the amniotic membrane as a potentially useful biological reinforcement strategy for pancreaticojejunostomy. However, confirmation in larger, methodologically standardized studies incorporating serial biochemical assessment, histopathological evaluation, and direct measurement of anastomotic mechanical strength will be necessary before the magnitude and mechanisms of this effect can be more definitively established.

Conclusions

Amnion patch application to pancreaticojejunostomy was associated with a lower incidence of POPF and intra-abdominal adhesions, better preservation of anastomotic integrity, and lower peritoneal amylase and lipase levels compared with the control group. These findings support the potential role of the amnion patch as a biological reinforcement strategy for pancreatic anastomosis.

Ethics Approval

This study was approved by the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. All animal procedures were conducted in accordance with established ethical guidelines for the care and use of laboratory animals.

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