

The comparison of the suture materials on intestinal anastomotic healing: an experimental study

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ABSTRACT

BACKGROUND: This experimental comparative study was to evaluate the local effects of three different suture materials on intestinal anastomosis healing.

METHODS: Ethical approval was obtained from the University of Ethical Committee (E-60758568-020-176720). A prospective, experimental comparative analysis was conducted on 24 rats. They were divided into three equal groups; Group 1 underwent colonic anastomosis with Vicryl suture material, Group 2 underwent colonic anastomosis with polypropylene suture; and Group 3 underwent colonic anastomosis with polydioxanone (PDS) suture. The second operation underwent the 7th post-operative day. Adhesion score, anastomotic leakage, anastomotic bursting pressure, hydroxyproline levels, and histopathologic examination were evaluated.

RESULTS: All animals survived, and no leakage, intestinal obstruction, or wound infection was observed during the experiment. The adhesion score was evaluated according to the Diamond classification and same in all groups. Median anastomotic bursting pressure was 125.75 mmHg (10–241) in the Vicryl group, 159.25 mmHg (113–190) in the polypropylene group, and 154.50 mmHg (20–212) in the PDS group. Hydroxyproline tissue concentrations were in the Vicryl group 1699.92 ± 220.8 ng/mg (range: 1509.81–2186.47), in the polypropylene group 1126.24 ± 607.12 ng/mg (range: 53.22–1815.63), and 1547.86 ± 335.2 ng/mg (range: 973.66–1973.2) in PDS group. There was no difference among groups regarding the inflammatory response evaluated by histopathology. There was no statistical significance in all variables evaluated.

CONCLUSION: This experimental study demonstrates that suture materials did not worsen tissue healing during intestinal anastomosis. Absorbable, slowly-absorbable, and non-absorbable suture materials could be used safely in every situation.

Keywords: Anastomotic healing; anastomotic leak; animal experiments; surgical anastomosis; suture techniques.

INTRODUCTION

The importance of intestinal anastomosis continues in current surgical techniques. The methods used in intestinal anastomosis have improved significantly over the years. Despite the widespread use of automatic surgical techniques; hand-sewn intestinal anastomoses continue to be crucial in today's clinical

settings, especially in pediatric and neonatal surgery whom the intestinal lumen diameter is small or when the automatic stapler is not an option.^[1] Although there are many technical and practical considerations that might be used in intestinal anastomoses, research is still yielding promising outcomes.^[2] Leakage, stenosis, and dysfunction, being the most frequently reported problems and causes of morbidity and mortality af-

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ter intestinal anastomosis, have been investigated in the literature.

The strength of intestinal anastomosis is influenced by a variety of regional and systemic factors. The most common local considerations include surgical suturing technique, the characteristics of the suture material used, the tension of the anastomosis, the presence of peritonitis, microcirculation, and more.^[3,4] The healing of the anastomosis and the recovery from surgical damage are both impacted by the suture materials' structure. Both absorbable and non-absorbable suture materials have certain benefits and drawbacks that are well-known.^[5] While non-absorbable suture materials are infrequently favored, absorbable and slowly-absorbable suture materials are regularly preferred for intestinal anastomoses. The outcomes of using polydioxanone (PDS) and polypropylene suture materials vary in the literature while no recent study that compares the suture materials could be found in the literature. Hence, the aim of this study was to compare the effects of three different suture materials on anastomosis durability and healing.

MATERIALS AND METHODS

Study Design

All experiments have complied with the National Institutes of Health Guide for the care and use of laboratory animals. After obtaining IACUC protocol E-60758568-020-176720 from the Pamukkale University Institutional Animal Care and Use Committee, age and weight-matched Wistar Albino rats were used. There were only female rats as participants in each

group. The rats were maintained in standard cages, at a temperature of $21 \pm 1^\circ\text{C}$ and in accordance with the 12-h night-day cycle. The nutrition and water need of the rats were arranged as ad libitum. Natural diets continued and they were not left hungry preoperatively. It was observed that all subjects were healthy after the first operation. After the first operation, the maintenance of all subjects was carried out in separate cages.

Groups

The subjects were examined in three groups according to the suture materials used. In the first group, the suture material was polyglactin (910-Vicryl® group), while, in the second group, it was (PDS group), and it was polypropylene (Prolene® group) in the third group. Each group consisted of eight samples.

Surgical Procedure

The rats have administrated 50 mg/kg ketamine hydrochloride (Flaxes, Pfizer pharma, GmbH Germany) and 10 mg/kg xylazine hydrochloride (Rompun 2% Flacon; Bayer, Istanbul) through the intraperitoneal injection. The abdominal wall hair was cleaned, and the surgical field was cleaned with an antiseptic solution. A 3 cm median incision was performed to reach out to the intra-abdominal cavity. The ascending colon was found and cut through scissors, after which an end-to-end anastomosis was performed. The anastomosis was completed with a total of 10–12 separated and full-thickness 6/0 sutures where all knots stayed outside the lumen under 3.5× magnification optical glasses (Fig. 1). After intestinal anasto-

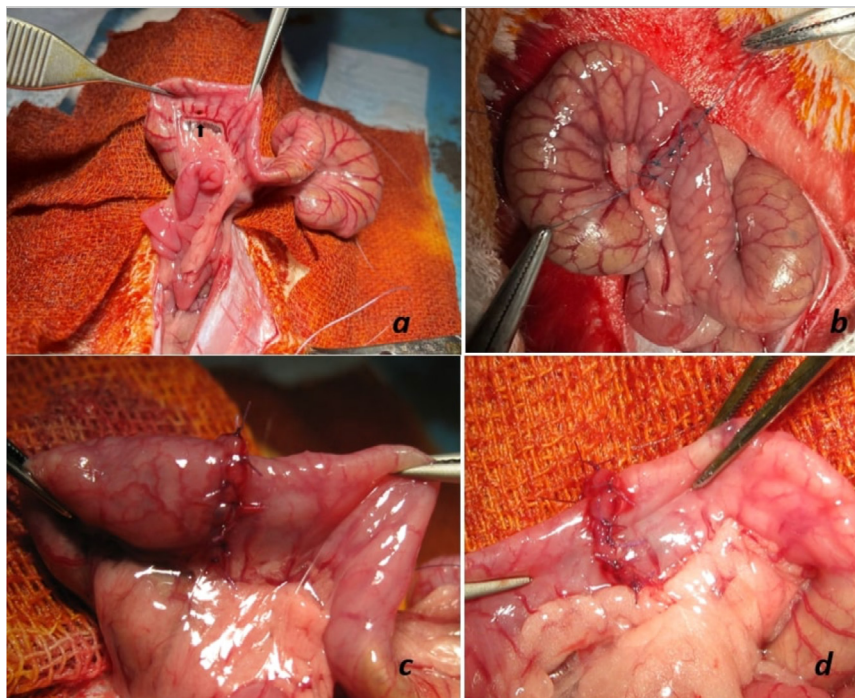


Figure 1. The colonic transection performed without damage of microvascular perfusion (arrow) (a). The anastomosis was completed with 10–12 separated and full-thickness 6/0 sutures with three different suture materials (b) prolene suture, (c) PDS suture, (d) vicryl suture.

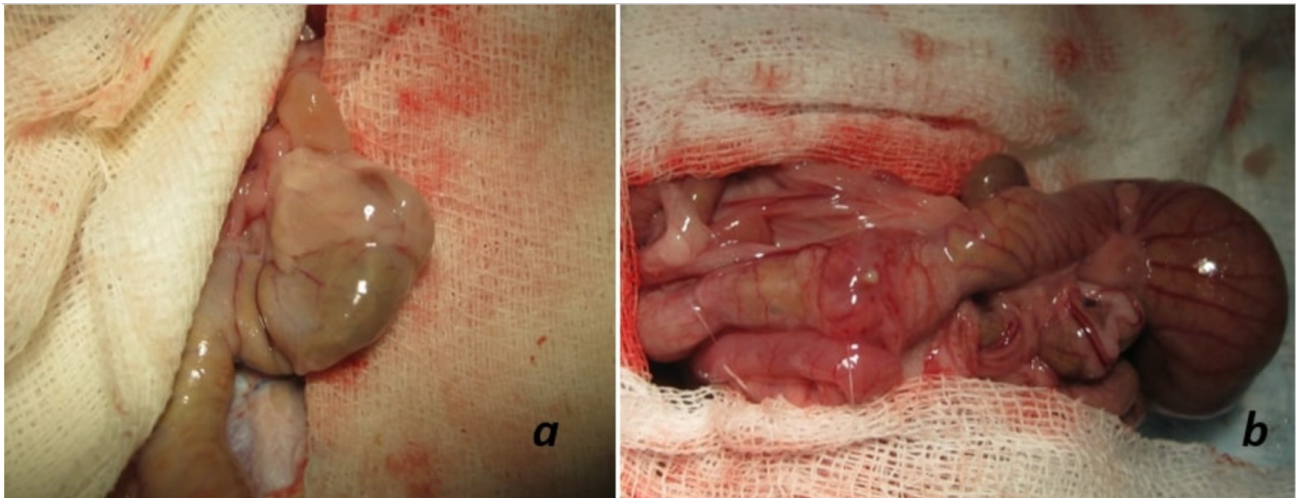


Figure 2. Diamond adhesion classification score 1 (a) and score 2 (b).

mosis was completed, 10 mL of physiological saline was administered into the intra-abdominal cavity. The abdominal wall was closed in accordance with its anatomy. On the 7th post-operative day, re-laparotomy was performed and anastomosis and any intestinal adhesions were examined macroscopically (Fig. 2). The evaluation of the intestinal adhesions was done per the classification by Diamond et al.^[6] Ten centimeters of the intestinal segment including the anastomotic segment were excised for bursting pressure measurement and histological analysis. The euthanization of the animals was done by an intra-cardiac puncture.

Anastomotic Bursting Pressure Measurement

The lack of leakage from the anastomosis line is an accurate demonstration of the durability of the anastomosis. In humans, the evaluation is with the demonstration of the normal passage through the intestinal system while, in animal studies,

it is analyzed by in vitro techniques such as anastomotic bursting pressure measurement which was preferred in this study. The lumen of the excised intestinal segment was cleaned from fecal content with saline. One side of the excised intestinal segment was put and firmly fixated into methylene blue dye while the other end was connected to the invasive manometer (Fig. 3). The methylene blue dye was infused into the lumen at a 1 mL/min infusion rate. The pressure at which there is a leakage from the anastomotic line or intestinal wall was measured as anastomotic bursting pressure.

Histopathologic Examination

After the bursting pressure measurement, 1 cm³ of the intestinal segment containing the anastomosis line was reserved for histopathological examination. Resected specimens were fixated with buffered formaldehyde solution. Five-micron thick sections were taken from paraffin-embedded tissue



Figure 3. Anastomotic bursting pressure measurement with invasive monitoring technique (a, b).

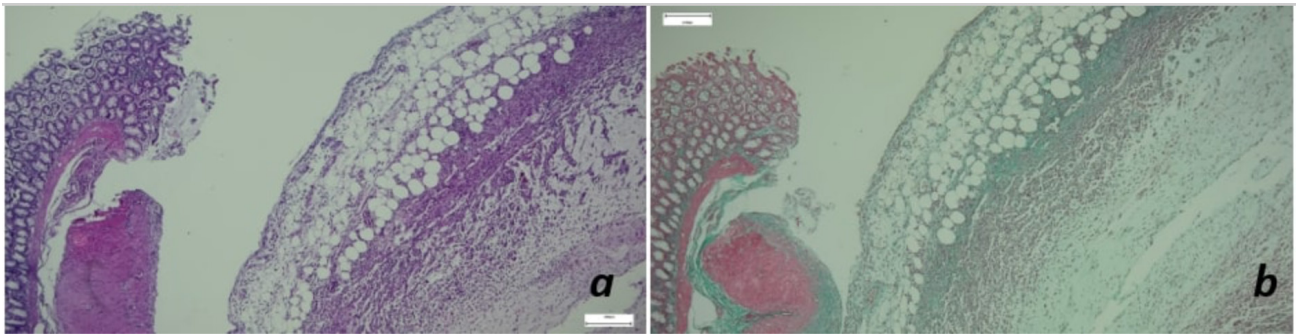


Figure 4. Immature granulation tissue is largely composed of edema and inflammatory cells, score 2 (H&E, $\times 100$) (a), and there is no collagen deposition (Masson Trichrome, $\times 100$) (b).

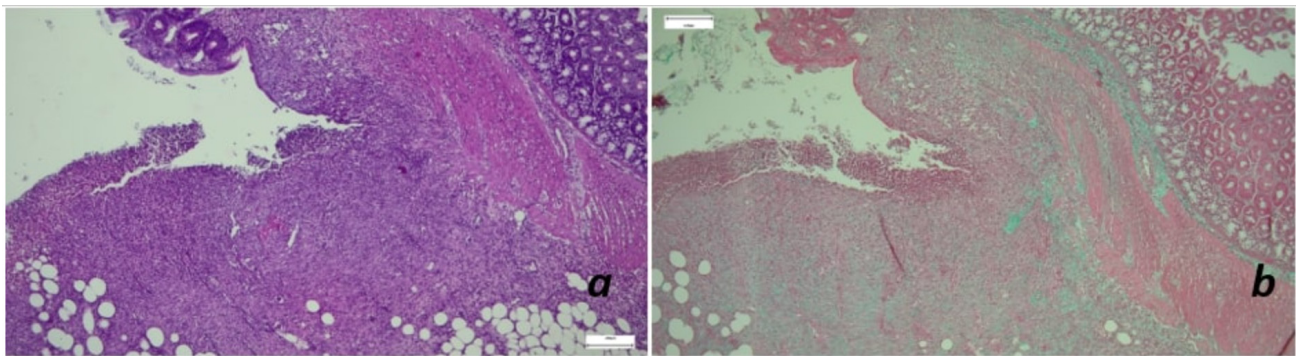


Figure 5. Significant neovascularization and mild re-epithelialization, score 3 (H&E, $\times 100$) (a), and there is moderate collagen deposition (Masson Trichrome, $\times 100$) (b).

samples and were stained with hematoxylin and eosin and Mason Trichrome stains. Light microscopic examination was performed by a single-blinded pathologist. Tissue healing and fibrosis were scored using the Greenhalgh method.^[7] Accordingly, “1” was evaluated as no granulation tissue or epithelial cell migration; “2” immature granulation tissue rich in inflammatory cells; “3” moderately thick granulation tissue, mild re-epithelialization, marked neovascularization, moderate fibroblast, and collagen synthesis; and “4” thick granulation tissue, marked collagen synthesis, and partial/complete re-epithelialization (Figures 4 and 5).

Biochemical Analysis

Intestinal tissue was frozen in liquid nitrogen and stored at -80°C . On the day of analysis, tissues were thawed and homogenized. Homogenization was done in 10 mL of cold 20 mM HEPES buffer and centrifuged at 1500 g for 5 min at 4°C . Hydroxyproline quantity was determined from the supernatant using the enzyme immunoassay method through a commercial ELISA kit (USCN Life Science Inc., Wuhan, China).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 22.0 (IBM Corp, Armonk, NY). Statistics are presented as weighted mean \pm standard deviation. The Kolmogorov–Smirnov test was performed to assess normal distribution. Parametric variables were analyzed by independent t-test and non-parametric variables by Mann–Whitney U-test. One-way ANOVA was used for the homo-

geneity of the variables. If P-value was < 0.05 , relationships between variables were regarded as statistically significant.

RESULTS

There were 24 rats that were included in and survived throughout the study. None of the rats needed to be terminated throughout the study and none had any feeding problems or was observed any body weight loss. The surgery time was similar in all groups. All the rats had an adhesion in the abdominal cavity. The Vicryl group was found to have lower adhesion scores when compared to the rest; however, no statistical significance could be shown ($P=0.161$) (Table 1). Pre-anastomotic dilated bowel was not observed in the second operation and none of the rats had anastomotic leakage, surgical site infection, intra-abdominal infection, sepsis, or intestinal obstruction at the second laparotomy (Table 1).

Anastomotic bursting pressures were 125.75 ± 97.3 mmHg, 154.5 ± 63 mmHg, and 159.25 ± 29.1 mmHg in Vicryl, PDS, and polypropylene groups, respectively, which however, did not reach statistical significance. The histological analysis demonstrated that all groups’ scores were either poor or moderate which was also statistically insignificant (Table 1) ($P=0.491$). The tissue reaction and healing were similar between the groups.

Tissue hydroxyproline levels were 1699.92 ± 220.8 ng/mg in the Vicryl group, 1547.86 ± 335.2 ng/mg in the PDS group, and 1126.24 ± 607.1 ng/mg in the polypropylene group. While the

Table 1. Comparison of results between three different suture material

	Vicryl group (n: 8)	PDS group (n: 8)	Polypropylene group (n: 8)	P-value
Anastomotic bursting pressure (mmHg)	125.75±97.3	154.5±63	159.25±29.1	0.803
Adhesion score (Diamond classification)				
1. Easily lysed Filmy, no vessels (transparent)	0 (0%)	1 (12.5%)	3 (37.5%)	0.082
2. Lysed with traction Opaque, no vessels (translucent)	5 (62.5%)	2 (25%)	1 (12.5%)	0.085
3. Required sharp dissection, Opaque small vessels present grossly	2 (25%)	2 (25%)	1 (12.5%)	0.763
4. Opaque, larger vessels present grossly	1 (12.5%)	3 (37.5%)	3 (37.5%)	0.411
Tissue hydroxyproline levels (ng/mg)	1699.92±220.8	1547.86±335.2	1126.24±607.1	0.188
Survival	8 (100%)	8 (100%)	8 (100%)	
Intestinal obstruction	0	0	0	--
Tissue healing score				
Score 1–3 (absent)	0	0	0	
Score 4–6 (poor)	4 (50%)	4 (50%)	2 (25%)	0.491
Score 7–9 (moderate)	4 (50%)	4 (50%)	6 (75%)	0.491
Score 10–12 (good)	0	0	0	

polypropylene group had the lowest value, it did not reach statistical significance ($P=0.188$), (Table 1).

DISCUSSION

The most common preferred approach in the management of intestinal anastomosis is with absorbable suture material.^[8] Preventing the leakage from the anastomosis is the most important cause of this approach.^[9] Being one of the most common and major complications, leakage and stricture are the common problem that impresses the researchers that work in the field.^[10] The incidence of leakage highly varies between studies in the literature between 6% and 22%.^[3,11] That's the main reason why the experimental studies in the literature mostly seek prevention measures from leakage and improve the healing process.^[2]

The tissue damage that happens during the suturing of the anastomosis affects the healing process especially in premature infants.^[12] There are various local factors that affect the healing process one of which is the different types of suture material.^[13-17] Some of the research in the literature is designed as ischemia-reperfusion studies.^[18-20] This is the first arousal of the presented study since nothing could be found about the correlation between tissue healing and the use of different suture materials. To evaluate alternative suture options, the structural and absorbable properties of each suture material were questioned on the physical, biochemical, and histological consequences on intestinal anastomosis healing.

One of the local factors affecting anastomosis leakage is the quality of the suture material used. Suture materials have been reported to cause tissue reactions, microabscess, circulatory disorders on the intestine wall, and anastomosis leakage.^[21-22]

Intestinal anastomoses made by applying different suture materials or suture techniques used in experimental studies have been reported to have different rates of intestinal anastomotic leakage such as 0–20%.^[4-5,23-26] In the presented study, none of the samples had anastomotic leakage or stricture. The most probable reason for this success is the use of microsurgery with the prevention of damage in the microvascular perfusion.

The anastomotic bursting pressure technique determines the durability and healing of the anastomosis.^[25] This method is an indirect sign of intestinal anastomotic healing. It helps researchers to get crucial findings about the strength of anastomosis both in experimental and human studies.^[4,27] There are many local and systemic factors that maintain the strength of the intestinal anastomosis line and the results have been reported in experimental studies.^[23-24,28-29] In this study, the effects of intestinal anastomosis on the healing process were evaluated as a result of local tissue trauma of a single factor. Therefore, it can conclude that different suture materials can be used safely due to the lack of significant difference in anastomotic bursting pressure values where only suture materials are examined without changing local and systemic factors of experimental animals ($P=0.803$).

Intestinal adhesions are one of the early and late complications of intra-abdominal surgery. The presence of intra-abdominal infection and local-systemic factors are predisposing factors for intestinal adhesion.^[23,26,30] The spread of the fecal content into the abdomen when performing intestinal anastomosis is one of the causes of the source of local infection. Moreover, foreign body reactions, local abscesses, and adhesions could be encountered due to the type of suture material.^[21,23] There are controversies about the type of suture materials to be used and their effects on tissue healing in

experimental studies.^[21,31,32] While the Vicryl group had the best scores, there was not any statistical difference in the adhesion scores between groups ($P=0.161$). Although the polypropylene and PDS group had higher adhesion scores, given that there are no symptoms and signs of intestinal adhesions speculates the idea that healing of the anastomosis will be affected by the type of suture material which is unmeaningful.

Hydroxyproline plays an important role in the synthesis and stability of collagen, the main component of major structural proteins.^[33] Collagen degranulation occurs in the early phase of the healing period and suture materials, like many factors, also affect this phase.^[10] Therefore, the measurement of hydroxyproline concentration in tissue is an objective indicator of tissue healing and anastomotic strength. In previous studies examining suture materials in terms of anastomosis safety, the level of hydroxyproline could not be evaluated; Arikanoğlu et al. applied double-layer anastomosis in their experimental study examining only suture materials.^[23] They found that the hydroxyproline level was significantly higher in the Vicryl-plus group. Another study done by García-Osogobio et al. on dogs also found that hydroxyproline levels were similar.^[34] The single-layer anastomosis was performed in the current study with both absorbable and non-absorbable suture materials. Given the fact that hydroxyproline levels and tissue healing were similar between groups, one can easily state that the adverse effects of the suture material in the prognosis of the anastomosis are very limited if there is any.

Histopathological evaluation of cellular and structural changes gives important information about the negative effects of anastomosis and the negative effects of foreign body reactions on tissue healing. It has been observed that tissue healing is better in suture materials covered with antibiotics or protective film.^[23,35] In this study, in which only the effects of the suture material were observed; the histopathological results of tissue reactions formed by absorbable, non-absorbable or monofilament, multifilament suture materials in the anastomosis line were similar, showing that wound healing due to suture materials was not adversely affected.

There were certain limitations in the study. In real life, the anastomosis is not always performed on the otherwise healthy intestine; the effects of local and systemic factors could not be measured. Another drawback is the short post-operative following time which is specifically important for anastomotic dysfunction and stricture formation.

CONCLUSION

This study led to a finding that sutures of all types – absorbable, non-absorbable, monofilament, and multifilament – could be used in intestinal anastomosis without hesitation.

Ethics Committee Approval: This study was approved by the University of Health Sciences Pamukkale University, School of Medicine Ethics Committee (Date: 07.03.2022, Decision No: E-60758568-020-176720).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: O.U.; Design: O.U.; Supervision: O.U., E.A.; Resource: O.U.; Materials: O.U.; Data collection and/or processing: O.U., E.Ç., A.A.; Analysis and/or interpretation: O.U., H.Ş.; Literature search: O.U.; Writing: O.U., E.A.; Critical review: O.U., E.A.

Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - ÖZ

Farklı dikiş materyallerinin bağırsak anastomoz iyileşmesi üzerine etkileri: Deneysel bir çalışma

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AMAÇ: Deneysel ve karşılaştırmalı çalışmada, üç farklı dikiş materyalinin intestinal anastomoz iyileşmesi üzerindeki lokal etkilerinin değerlendirilmesi amaçlanmıştır.

GEREÇ VE YÖNTEM: Üniversite Etik Kurulu'nun (E-60758568-020-176720) etik onayı alındıktan sonra çalışmaya başlandı. 24 sıçan üzerinde prospektif, deneysel bir karşılaştırmalı analiz yapıldı. Denekler üç eşit gruba ayrıldılar; Grup 1'e vikril dikiş materyali ile kolon anastomozu yapıldı, Grup 2; polipropilen dikişle kolonik anastomoz yapıldı ve Grup 3'e; polidoksanon (PDS) dikişle kolonik anastomoz uygulandı. İkinci operasyon postoperatif 7. günde yapıldı. Adezyon skoru, anastomoz kaçağı, anastomoz patlama basıncı, hidroksiprolin düzeyleri ve histopatolojik incelemeler değerlendirildi.

BULGULAR: Tüm hayvanlar hayatta kaldı ve deney sırasında anastomoz kaçağı, bağırsak tıkanıklığı veya yara enfeksiyonu gözlenmedi. Adezyon skoru Diamond sınıflamasına göre değerlendirildi ve tüm gruplarda aynıydı. Medyan anastomoz patlama basıncı vikril grubunda 125.75 mmHg (10-241), polipropilen grubunda 159.25 mmHg (113-190), PDS grubunda 154.50 mmHg (20-212) bulundu. Hidroksiprolin doku konsantrasyonları; vikril grubunda 1699.92±220.8 ng/mg (1509.81-2186.47), polipropilen grubunda 1126.24±607.12 ng/mg (53.22-1815.63) ve PDS grubunda 1547.86±335.2 ng/mg (973.66-1973.2) bulundu. Histopatoloji ile değerlendirilen enflamatuvar yanıt açısından gruplar arasında fark yoktu. Değerlendirilen tüm değişkenlerde istatistiksel anlamlılık görülmedi.

SONUÇ: Bu deneysel çalışma, intestinal anastomoz ve doku iyileşmesinin dikiş materyallerine bağlı farklı sonuçları olmadığını göstermektedir. Emilebilen, yavaş emilebilen ve emilemeyen dikiş materyalleri her durumda ve güvenle kullanılabilir.

Anahtar sözcükler: Cerrahi anastomoz; hayvan deneyleri; anastomoz iyileşmesi; dikiş teknikleri; anastomoz kaçağı.

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