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Fistula Treatment by Steam Ablation Method in Rats

Ratlarda Buhar Ablasyon Yöntemi ile Fistül Tedavisi

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ABSTRACT

Objectives: Anal fistula is a common condition in surgical practice. Despite its benign course, treatment is difficult, especially in complicated fistulas, and there is no standard treatment method. Anal fistula surgery can cause recurrence or continence disturbances. Ideal treatment should provide low incontinence and recurrence rates. Conventional techniques have a variable success rate; together with the potential risks, the outcome depends on the anatomy of the patient and the fistula and the wishes of the patient. For anal fistulas, various new procedures must be applied in animals to minimize the potential risk to humans. Steam ablation (SA) is an application like laser ablation, which is mainly used in the treatment of varicose veins in the lower extremities in humans. In this study, we aimed to evaluate the efficacy of SA treatment in an experimentally created anal fistula model in rats.

Methods: Two fistula tracts were formed in the anus of 16 Wistar Albino rats. After waiting for 30 days, SA was applied to one of the tracts while the other was left untreated as control. The animals were euthanized 60 days after the start of the experiment and the samples were analyzed by the pathologist by removing the perianal regions. $P<0.05$ value was considered significant in the results.

Results: About 18.8% of the study group completely recovered, 31.3% moderately recovered, 50% did not recover, 0% of the control group completely recovered, 37.5% moderately recovered, and 62.5% did not recover, and there was no statistically significant difference between them ($p>0.05$).

Conclusion: In this study, where we evaluated the effectiveness of SA in the treatment of fistulas created in rats, we compared the treatment group with the control group. Improvement in the SA group was greater than in the control group, but there was no statistically significant difference between the groups.

Keywords: Fistula-in-ano; rats; steam ablation.

ÖZET

Amaç: Anal fistül, cerrahi uygulamada sık görülen bir durumdur. İyi huylu seyrine rağmen özellikle komplike fistüllerde tedavisi zordur. Anal fistül ameliyatı, nüks veya kontinans bozukluklarına neden olabilir. İdeal tedavi, düşük inkontinans ve nüks oranları sağlamalıdır. Geleneksel teknikler değişken bir başarı oranına sahiptir; Olası risklerle birlikte sonuç, hastanın anatomisine, fistüle ve hastanın isteklerine bağlıdır. Anal fistüller için, insanlara yönelik potansiyel riski en aza indirmek için hayvanlarda çeşitli yeni prosedürler uygulanmalıdır. Buhar Ablasyonu (SA), esas olarak insanlarda alt ekstremitelerde varis tedavisinde kullanılan Lazer Ablasyon (LA) gibi bir uygulamadır. Bu çalışmada, sıçanlarda deneysel olarak oluşturulmuş bir anal fistül modelinde SA tedavisinin etkinliğini değerlendirmeyi amaçladık.

Yöntem: 16 adet Wistar Albino sıçanın anüsünde 2 adet fistül traktı oluşturuldu. 30 gün beklendikten sonra traktların birine SA uygulanırken, diğer trakt kontrol olarak tedavisiz bırakıldı. Deneyin başlangıcından 60 gün sonra hayvanlara ötenazi uygulandı ve perianal bölgeleri çıkarılmak suretiyle örnekler patolog tarafından analiz edildi. Sonuçlarda $p<0.05$ değeri anlamlı kabul edildi.

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Bulgular: Çalışma grubunun %18.8'i tam iyileşmiş, %31.3'ü orta düzeyde iyileşmiş, %50'si iyileşememiş ve Kontrol grubunun %0'ı tam iyileşmiş, %37.5'i orta düzeyde iyileşmiş, %62.5'i iyileşememiş olup, aralarında istatistiksel olarak anlamlı bir farklılık bulunmamaktadır (p>0.05).

Sonuç: Sıçanlarda oluşan fistüllerin tedavisinde SA' nın etkinliğini değerlendirdiğimiz bu çalışmada, tedavi grubunu kontrol grubu ile karşılaştırdık. BA grubundaki iyileşme kontrol grubuna göre daha fazlaydı, ancak gruplar arasında istatistiksel olarak anlamlı bir fark yoktu.

Anahtar sözcükler: Anal fistül; buhar ablasyon; fare.

Anal fistula is a disease defined as the abnormal communication of intestinal epithelium (anal canal or rectum) with the skin.[1] Fistula-in-ano has been a disease difficult to treat throughout its surgical history. Disease and surgical instruments used in the treatment of fistulas have historically been well documented. A surgeon's probes for the treatment of this disease were found among the remains of Pompeii, and the difficulty in managing the disease was first recognized by Hippocrates (460 BC).[2] The incidence of non-specific anal fistula is around 8.6–10/100.000 and the male to female ratio is 1.8:1.[3] It usually affects individuals between 30 and 50 years of age.[4] Optimal treatment aims to close the fistula tract, preserve the sphincters, prevent recurrence, and allow the patient to early return to normal life.

Abscesses occur when the drainage of the anal glands[4,5] located in the intersphincteric area is obstructed as a result of fecalitis, inflammation or trauma, and 30–40% of these abscesses can heal with fistula. Other conditions such as inflammatory bowel disease (ulcerative colitis, and Crohn's disease), infections (Tuberculosis, Actinomycosis, AIDS, and Lymphogranuloma venereum), cancer, and surgical trauma make up about 20% of the etiology.[6,7]

Treatment of an anal fistula depends on the location of the fistula and trigger factors. Most fistulas are treated surgically with different procedures depending on external and internal sphincter involvement.[8] If the tract contains more than 30–50% of the external sphincter and the fistula is recurrent or has lateral branches or separate pathways, it can be called complex fistula.[9] Although incontinence rates have been reported following fistula surgery, this rate is highly variable and affected by many factors, incontinence rate after fistulotomy to intersphincteric and distal fistulas appears to be <10%.[10] Complex fistulas have a greater risk. Fistulotomy/fistulectomy, Transanal Advancement Flap Repair (TAFR), seton placement, Ligation of Intersphincteric Fistula Tract (LIFT) procedure, fistula plug and fibrin glue, Video-Assisted Anal Fistula Treatment (VAAFT), Fistula-tract Laser Closure, Adipose derivative Stem Cells (ASC's), and medical therapy (especially in Crohn's disease) are different treatment methods used to date. Recovery rates range from 14% to 81.4%.[11,12]

Despite years of great advances and the emergence of new technologies, there is no consensus on the surgical technique applied and a standard procedure has not developed. Therefore, old techniques are still used in medical practice.[13] There is great interest in new sphincter-sparing treatment methods, especially due to their negative effects on continence. It is thought that the development of experimental anal fistula models for the application of these new treatment protocols will help new studies.[14]

Among the possible advantages of the steam ablation (SA) procedure we used in this planned study, we can count factors such as stable and low temperature application, ease of application of the procedure, high cost effectiveness, low pain scores, and patient satisfaction. Other advantages are the use of natural sterile water[15] and absolute temperature regulation, without the risk of producing harmful exogenous substances (carbonized blood); the steam produced has a constant temperature of 120°C. Since the applied temperature is limited, treatment-related symptoms and complications such as pain may be less.

The aim of this study was to evaluate the application and efficacy of SA on fistulas formed in an experimental rat model by taking laser ablation (LA) as a sample which was firstly used in vascular surgery and then started to be applied in fistula treatment.

Methods

This study was approved by the local ethics committee. Sixteen male adult Wistar albino rats, each weighing approximately 300 g, were used in the study. Male adult Wistar albino rats, each weighing approximately 300 g, were used in the study. All rats were evaluated in supine position. All procedures were performed under antiseptic care and ster-

ile surgical site conditions. The rats were anesthetized with intraperitoneal ketamine and xylazine in the same syringe. The solution consisted of 2 mL of 10% ketamine and 1 mL of 2% xylazine. 0.1 mL of anesthetic solution was infused for each 100 g of body weight. Then, two fistulas were created at 3 and 9 o'clock with a number 1 steel monofilament suture from 1 cm lateral of the anus to the proximal of the pectinate line (Fig. 1). Steel monofilament suture was preferred, as different materials used in previous studies were harmful to rats. The suture was cut, bent, and preserved for 30 days for the maturation of fistula tracts. Analgesia was not required within the first 48 h after the procedure. The animals were daily followed up for signs of extreme distress or severe perianal sepsis. They were kept in individual cages with food and water ad libitum throughout the study. Water and food were changed once a week.

At the end of 30 days, the rats were re-sedated with the anesthetic method mentioned above, and in the examination, it was observed that a clinically visible fistula tract had formed in all of them. SA was applied to one of the two fistulas formed in each rat under sedation while the other was left as control (Fig. 2). After activation of the device, the catheter was advanced through the fistula tract. The catheter emitting steam in small "puffs" was withdrawn step by step. After the catheter was removed from the fistula tract, external compression was applied to obliterate the tract. SA procedure was performed with the VenoSteam system consisting of a steam generator, a handpiece, and a catheter. The system operates by applying thermal energy (steam micro impulses) to the lumen to be treated. The SVS generator receives the special water from the sterile container. Sterile water is then heated and converted into steam micro impulses transferred to the vessel lumen using the cap and catheter. Steam is introduced into the lumen by means of a 60 cm long metal tube with a blunt tip and a special catheter consisting of two lateral openings at the tip. The tube is wrapped in a plastic sheath at 1 cm degrees from the outside. The outer diameter of the catheter is 1.2 mm and the inner diameter (i.e., working channel) is only 100 nm. Compressed steam is the force pushed into this narrow space. After passing through the catheter channel, the steam reaches 120°C, which is the actual temperature of the steam applied to the lumen. A single steam impulse was calculated to release approximately 60 J of energy per 1 cm long vessel segment (Fig. 3). The rats whose procedures were completed were then returned to their cages and waited for another 30 days.

The animals were sacrificed 30 days after SA to histologically examine the fistulas. The drug used for euthanasia was sodium thiopental intracardially administered after sedation with ketamine and xylazine.

After euthanasia, a circumferential incision to include both fistula tracts was made around the anus with Metzenbaum scissors. Dissection was performed by removing the block extending to the rectum.

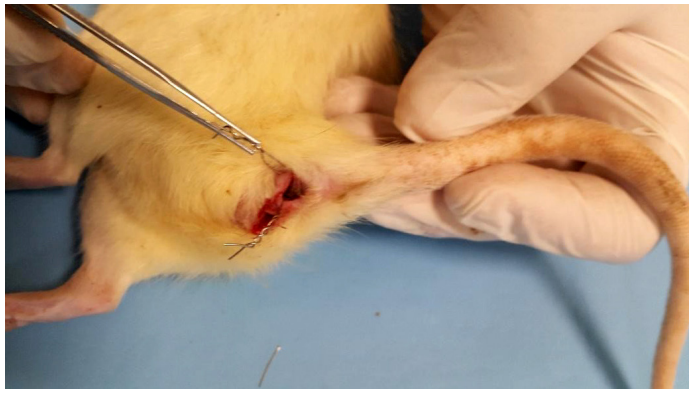

Figure 1. Fistula formation in rats.

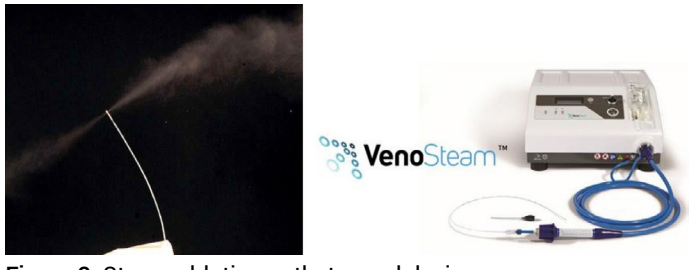

Figure 2. Steam ablation catheter and device.

Figure 3. Application of steam ablation.

Fistula tracts were confirmed histologically in the resected materials. The preparations prepared after staining were analyzed to examine the fistula tract by evaluating both sides of the fistula lumen (inner and outer hole), the presence of lumen, granulation tissue, and epithelization area (Figs. 4-7).

Statistical Analysis

When evaluating the findings obtained in this study, IBM SPSS Statistics 22 (SPSS IBM, Turkey) program was used for statistical analysis. When evaluating the study data, the Fisher-Freeman-Halton test was used to compare qualitative data as well as descriptive statistical methods (frequency). The level of significance was set at $p<0.05$.

Histological Study

Postoperatively, each of the perianal resection materials was encoded and separately taken into containers containing buffered formalin (10% buffered formalin). After resection materials were kept in formalin for 24 h, the marked (studied) and other sides of each specimen were completely sampled by cutting 2–3 mm in thickness, 90° perpendicular to the intestinal tract in a way that one surface is to include skin and the other is to include intestinal wall. The samples were placed on the Tissue Tracker (Leica ASP 300S) as two or three cassettes with encoded sides. After tissue tracking procedure, one slide containing 4-micron sections was prepared with Rotary Microtome

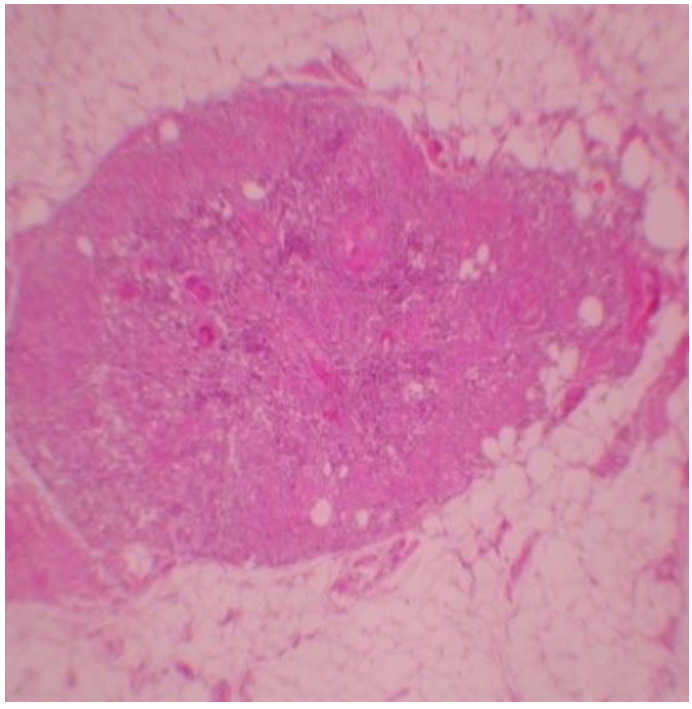

Figure 4. Fistula tract in nodular formation with inflammation and granulomatous reaction in perianal adipose tissue (arrow) H & Ex40.

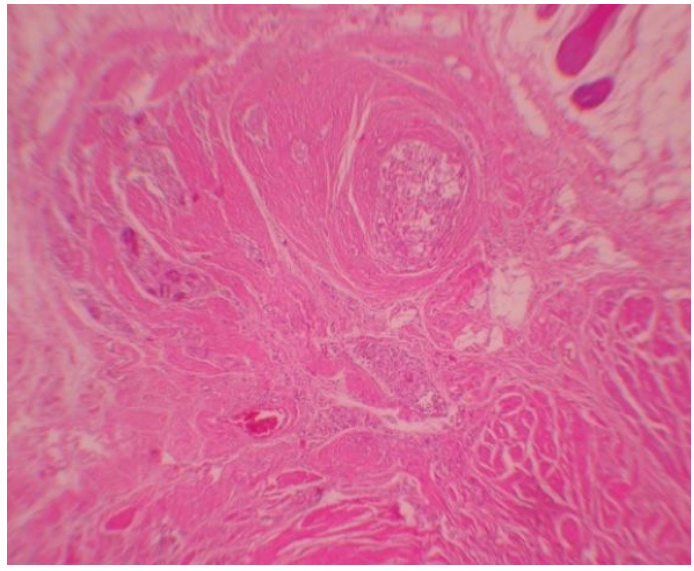

Figure 5. Moderate recovery of fistula tract (Score II); moderate reduction in tract calibration (arrow) and moderate inflammationH & Ex40.

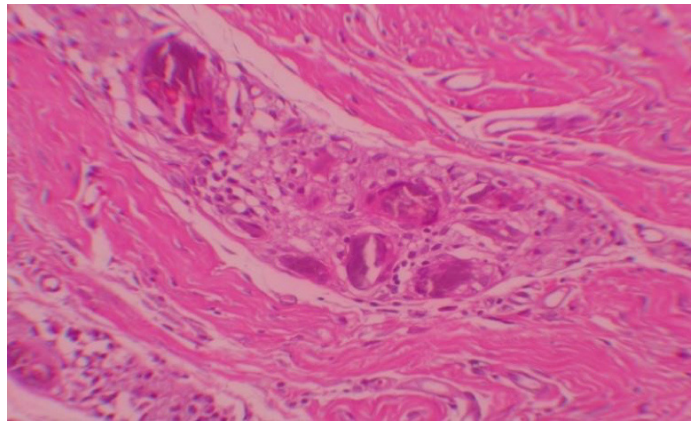

Figure 6. Marked recovery of fistula tract (Score I), significant reduction in tract calibration and foreign body type granulomatous reaction (arrow) H & Ex200.

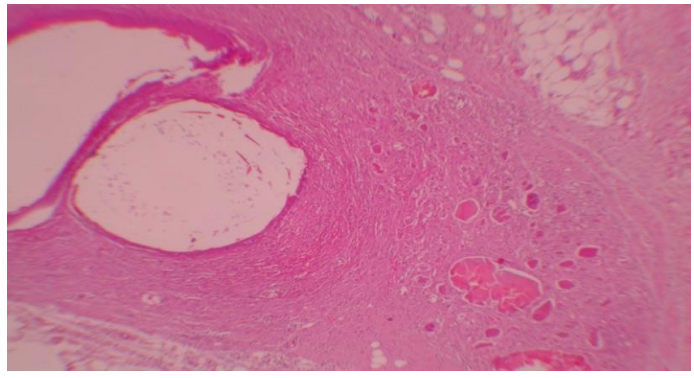

Figure 7. Score III; diffuse inflammation and foreign body reaction around partially re-epithelialized fistula tract (arrow) H & Ex40.

(Thermo) from each of the paraffin-blocked samples using the Tissue Embedding Device (Shandon Histocenter 3). After deparaffinization, the slides were stained with hematoxylin-eosin (Shandon Harris Hematoxylin-Eosin Y -UK) in an automatic staining and coverslipping device (Sakura Tissue Tek Film) in accordance with the protocol. Histopathological examination was performed by a pathologist on a Olympus CX41 RF (Japan) trinocular light microscope and microphotographs were taken with an adapted digital camera.

Histological Evaluation

Recovery of perianal fistula tract was scored in accordance with the following histopathological table:

- Score I: Only vascular proliferation and fibrosis suggestive of disappearance or tract as a result of complete recovery of perianal fistula tract
- Score II: Moderate reduction and moderate inflammation in tract calibration, which is considered to be moderate recovery
- Score III: Lack of reduction in tract calibration or slight reduction and intense inflammation, which is considered to be slight or no recovery.

Results

The study was conducted between January and March 2019 with 16 rats under two groups as the study group of 16 rats (50%) and the control group of 16 rats (50%).

About 18.8% of the study group completely recovered, 31.3% moderately recovered, 50% did not recover, 0% of the control group completely recovered, 37.5% moderately recovered, and 62.5% did not recover, and there was no statistically significant difference between them (p>0.05) (Table 1).

Table 1. Evaluation of scores between the groups

Score	Study group n (%)	Control group n (%)	Total n (%)	p
Complete recovery	3 (18.8)	0 (0)	3 (9.4)	0.325
Moderate recovery	5 (31.3)	6 (37.5)	11 (34.4)	
No recovery	8 (50)	10 (62.5)	18 (56.3)	

Fisher-Freeman-Halton test.

Discussion

In this study, we evaluated the efficacy of SA in the treatment of experimentally formed anal fistulas in rats. When the group treated with SA was compared with the control group, the improvement in the study group was greater, but no statistically significant difference was found.

The aim of anal fistula treatment is to treat perianal sepsis, effective healing of fistula, prevention of recurrence, alleviation of symptoms, preservation of anal sphincter, and rapid patient recovery. For this purpose, different sphincter-preserving surgical techniques have been tried and developed in anal fistula repair. Unfortunately, there are no successful results in the long-term follow-up of many of these new techniques.[16,17] The efficacy of the treatments applied for fistula treatment to date has been limited due to the risk of incontinence. Therefore, minimally invasive procedures continue to be examined in various ways from experimental models to clinical applications.

In the treatment of anal fistula, recovery is achieved in approximately 75–100% of fistulotomy patients; however, there are incontinence rates up to 60% in complicated fistulas.[18,19] Although fistulotomy is a highly effective treatment, it is not a suitable option if it involves a large number of internal and external anal sphincters as in high-located transsphincteric fistulas.[20,21] Fistula Plug is a natural biomaterial obtained from porcine small intestine. Its use is limited to low transsphincteric fistulas due to its difficulty in insertion. In a study conducted at the University of Minnesota,[22] the overall success rate was 31%. Another sphincter-sparing treatment method in complicated anal fistulas is TAFR. In this method, local tissue ischemia or retraction of flaps are the main causes of failure[23] and recurrence rates vary between 7% and 49%.[24-26] Another method is the injection of mesenchymal stem cell suspension (ASC's) extracted from adipose tissue into the fistula tract.[27] Technically, there are difficulties such as the safe sealing of the internal opening and the complete injection of the cell suspension onto the channel. LIFT method is another sphincter preserving technique with a success rate of approximately 81.4%.[28] The difficulty of this procedure is due to the application technique and may impair the vascularity of the anal sphincters and mucosa. In the VAAFT method, the procedure needs to be done gently around the inner mouth of the fistula not to cause an iatrogenic fistula. In addition, irrigation to the fistula tract under pressure may cause the infectious material to spread to normal tissue.[29] As a result, shortening the op-

eration time, lowering irrigation pressure, and preventing fistula formation help to reduce the risk of post-operative perianal sepsis and recurrence. In 2011, Wilhelm described a new sphincter-sparing technique using the radial laser probe, with an average follow-up of 7.4 months and a recovery rate of 81.8%.[30] The disadvantage of this technique was the inability to identify the inner mouth, lateral branches of the fistula, and abscess spaces, and the relative length of the operation time.

For new methods that can be applied in anal fistulas, an experimental model that is equivalent to anal fistulas in humans, cost-effective, and easy to apply is needed. To date, reports only show dogs[31] and pigs as an experimental model in this sense.[32] The reason for using rats as a model is the small size and availability of the animals, the easier the surgical procedure. In addition, rats have the structure of internal and external sphincters similar to humans. Unlike humans, they have simple sebaceous glands that line the anal canal and are located in the submucosal region. Magnetic resonance ımaging findings and histologically proven and therefore the most accepted experimental anal fistula model in the world have been developed in pigs.[32] We believe that although the swine model is useful and successful for studying new techniques, it could be a similar model that could contribute to the development of new techniques for fistula treatment in rats.

Choosing steel wire for fistula development is because it is a material that is difficult to destroy by animals. In our study, the material was well tolerated and the study was terminated at the end of the 13th day without any change in the position or quality of the wire. In this study, lumen and to some extent granulation tissue were observed in all samples, but no epithelization was observed in any of the fistulas. Buchanan et al.[32] did not show epithelization in any of the fistula tracts produced despite 26 days of Seton persistence.

The SA procedure is primarily a method used in endovenous surgery. The procedure is a very similar procedure to LA. Compared to LA, it may be easier to reach side branches that are difficult to access due to the very small and flexible catheter.[33] Temperatures between 600 and 1000°C at the fiber end were determined for LA. This fiber tip has a potential risk of perivenous inflammation and perforation in direct contact with the vessel wall.[15,34,35] The fact that the temperature in SA is much lower than in LA[36] causes less pain and faster recovery. As a result of the rapid recovery, patients return to their daily life earlier. Based on these positive effects in the side effect profile, it is expected that the infiltration depth will be limited and the surgical trauma applied to the anal sphincters will be less with the application of SA with low temperature in fistula treatment. Therefore, it is expected that sphincter damage and pain will be less.

In the fistula model we created, it was aimed to obliterate the fistula tract. In this study, experimentally created fistulas differ in that they are acute fistulas compared to chronic fistulas treated in humans.[11,37] For this reason, perhaps for a realistic comparison, it may be necessary to repeat this study with chronic fistulas. Further evaluation should also be done to determine the optimum SA methodology.

Conclusion

In this study, we evaluated the effectiveness of SA in the treatment of experimentally created anal fistulas in rats. The SA-treated group was compared with the control group, but the improvement in the SA group was greater, with no statistically significant difference between the groups. Further experimental or clinical studies may be useful for the efficacy of SA in anal fistula treatment.

Disclosures

Ethics Committee Approval: T.C. Yeditepe University, Experimental Animals Ethics Committee (7.11.2018-705).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.M.F., A.E.; Design – N.E.B.; Supervision: –A.T. F.; Materials – H.Ç., B.A.; Data collection &/or processing – H.M.A., A.S.; Analysis and/or interpretation – E.S.; Literature search – M.M.F.; Writing – M.M.F., Anıl Ergin; Critical review – B.A.

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