J Surg Med. 2021;5(4):371-375.
DOI: 10.28982/josam.901535

Research article

Journal of Surgery and Medicine
e-ISSN=2602-2079

Two natural materials found to reduce adhesion formation in a rat uterine horn model

Erkan Elçi [1], Sena Sayan [2], Gülhan Elçi [3], İbrahim Aras [4], Numan ÇCim [5], Harun Egemen Tolunay [6], Yıldıray Başbuğan [7]

[1] University of Health Sciences, Umraniye Training and Research Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey
[2] Marmara University, Faculty of Medicine, Department of Obstetrics and Gynecology, Istanbul, Turkey
[3] University of Health Sciences, Sancaktepe Training and Research Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey
[4] Yüzüncü Yil University, Faculty of Medicine, Department of Pathology, Van, Turkey
[5] Istanbul Bilim University, School of Medicine, Department of Obstetrics and Gynecology, Istanbul, Turkey
[6] Etlik Zübeyde Hanım Maternity and Women's Health Teaching and Research Hospital, Department of Perinatology, Ankara, Turkey
[7] Yüzüncü Yıl University, Faculty of Veterinary Medicine, Van, Turkey

ORCID ID of the author(s)
EE: 0000-0002-9792-6592
SS: 0000-0001-6758-1127
GE: 0000-0003-2350-2567
İA: 0000-0003-4911-3922
NC: 0000-0003-0983-3204
HET: 0000-0002-8922-4400
YB: 0000-0001-5124-7853

Corresponding Author
Erkan Elci
University of Health Sciences, Umraniye Training and Research Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey
E-mail: dr.erkanelci@gmail.com

Ethics Committee Approval
The study was approved by the local ethics committee of the Yüzüncü Yıl University Faculty of Medicine Department in Van, Turkey for the use of laboratory animals and was performed at the Experimental Surgery Training and Education Center at the same hospital (approval number: 2015/06-12).

Conflict of Interest
No conflict of interest was declared by the authors.

Financial Disclosure
The authors declared that this study has received no financial support.

Published
2021 April 16

Abstract

Background/Aim: Post-operative pelvic adhesions cause various problems in patients, pose surgical difficulties to clinicians, and an increase in health costs. We compared the effectiveness of two natural materials, Trehalose (TRZ), Human Amnion Fluid (HAF), with oxidized regenerated cellulose (ORC) in terms of adhesion prevention after gynecological operations.
Methods: In this controlled experimental study, twenty-four female Wistar Hannover rats were divided into four groups: Control, TRZ, HAF, and ORC. The control group received medication used for the surgical procedure only. 3% TRZ, cell-free HAF, and 1 cm^2 ORC (interceed®) were laid on the tissue on the antimesenteric side of each uterine horn damaged with a 10-Watt bipolar cautery. Adhesions were scored 30 days after the first surgical procedure.
Results: The extent, severity, degree, total adhesion, inflammation, and fibrosis scores of the control group were significantly higher than those of the TRZ and HAF groups (P<0.05 for each). There was no significant difference between the Control and ORC groups in terms of inflammation (P=0.055), but all other parameters were significantly higher in the control group compared to the ORC group (P<0.05). The TRZ group had lower total adhesion scores (P=0.019) and histopathological scores (P=0.015, P=0.001) than the ORC group.
Conclusions: TRZ and HAF may be useful in preventing pelvic adhesions.

Keywords: Adhesions, Human amnion fluid, Interceed, Oxidised regenerated cellulose, Trehalose

How to cite: Elçi E, Sayan S, Elçi G, Aras İ, Çim N, Tolunay HE, Başbuğan Y. Two natural materials found to reduce adhesion formation in a rat uterine horn model. J Surg Med. 2021;5(4):371-375.

Introduction

Postoperative adhesion is a common complication after abdominal surgery with an incidence of 60-90% [1]. It is known that post-operative peritoneal adhesions occur in 90% of patients who undergo gynecological surgery. In gynecology, postoperative intra-abdominal adhesions may cause infertility, organ damage due to adhesions, intestinal obstruction, and chronic pelvic pain in subsequent operations [1, 2]. It is associated with mortality ranging from 3-30% in severe adhesions [3].

The abdominal peritoneal cavity consists of two sections, visceral and parietal, which are covered with a single layer of mesothelial cells [4]. The mesothelium is very fragile and can be easily damaged due to various reasons (cutting, cauterization, ischemia, desiccation, or abrasion) [5, 6]. After surgery or injury, reperitonealization starts within 8 to 24 hours and ends in 7-10 days. Therefore, the optimal agent should be effective for at least 1 week to prevent adhesions [6-8].

Many agents have been tried to reduce adhesions. Steroidal and non-steroidal anti-inflammatory medications are used to reduce inflammation, recombinant tissue plasminogen activator [t-PA], anti-coagulants, commercial or natural barriers (absorbable material/solution/gel, liquid paraffin, human amniotic membrane) and vitamins are used to reduce fibrin formation [5]. However, no method or agent has yet been proven to completely prevent adhesion formation after surgery.

Oxidized regenerated cellulose (ORC) (Interceed) is the first generation mechanical barrier agent used on the damaged visceral peritoneum and was approved by the US Food and Drug Administration (FDA) for the prevention of post-surgical adhesions [5]. Many experimental studies have shown it to have a protective effect against adhesion formation [9], making it a frequently used clinical agent [10]. Its total absorption time from the abdomen is 2 weeks [11-14].

Trehalose (TRZ) is a natural disaccharide consisting of two glucose molecules [11, 12]. It is used for organ preservation during transplantation, treatment of dry eye syndrome [12, 13] and in different sectors such as the food and cosmetics industry [13]. The protection of biological molecules and the cell membrane can be explained by 3 theories: Water replacement, glass transformation, and chemical stability [15]. In experimental studies, it has been said to have a protective effect against adhesion. Absorption time from the abdomen is thought to be 1 week [13, 15].

Human Amniotic Fluid (HAF) contains stem cells, dead tissue cells, hyaluronic acid (HA), growth factors (fibroblast growth factor (FGF), insulin like growth factor I and II (IGF-I and IGF-II), epidermal growth factor (EGF) etc.), fibrinolytic factors (Plasminogen activator inhibitor -1 (PA-1), and tissue plasminogen activator (t-PAI), etc.) [16, 17]. It is said that the densities of these factors, proteins and cells change according to the gestation period [18]. Various experimental studies have been carried out on adhesions and wound healing in tissues such as the nerves, tendons, sclera, and the abdomen [19, 20]. However, no clear information was published about the absorption time of HAF from the abdomen.

In this study, we comparatively investigated the effects of intraperitoneal ORC, TRZ, and HAF use on adhesion prevention after gynecological operations.

Materials and methods

This study was approved by the local ethics committee of the Yüzüncü Yıl University, Faculty of Medicine in Van, Turkey for the use of laboratory animals and performed at the Experimental Surgical Training and Education Center at the same hospital (Approval number: 2015/06-12 and Date: 30.04.2015)

Animal maintenance and treatment

In this study, twenty-four healthy adult (aged 8-10 weeks) female albino rats weighing 190-240 grams were used. Per the institutional review board's guidelines for animal care, all animals were kept at 22-28ºC degrees, with 14-hour light and 10-hour dark cycles. They had access to as much clean water and food as they wanted. Fresh water and standard rodent food pellets were always made available. A power analysis was performed to calculate the minimum sample size required for this study (alpha error = 0.05 and 1-beta =0.8), which revealed that at least 12 uterine horns were required for each study group.

Twenty-four rats were divided into 4 groups using a computer-based system. Animals were anesthetized intramuscularly with a mixture of ketamine hydrochloride (i.m 40 mg/kg 10% Alfamine) and xylazine hydrochloride (i.m 2 mg / kg 2% Alfazin). Before the surgery, the skin of the abdomen was shaved off and cleaned with 10% povidone iodine solution. The abdomen was entered through a 3 cm vertical incision starting from the top of the urethral opening.

As described by Kaya et al. [21] five standard lesions were made on the antimesenteric side of each uterine horn with a 10-Watt bipolar cautery [22, 23]. The same surgical procedure was performed in all rats. In Group 1 (Control), 2 ml of distilled water was poured into the abdomen and waited 1 minute to prevent possible dry air damage. In Group 2 (ORC), 1 cm^2 Oxidized Regenerated Cellulose adhesion barrier (Interceed; Ethicon Inc., Somerville, New Jersey) was placed on the damaged site of the uterus and tuba, then moistened and fixed by adding 1 drop of distilled water. In Group 3 (TRZ), 2 ml of pre-prepared 3% trehalose solution was poured onto the damaged area with a waiting time of 1 minute. In Group 4 (HAF), 2 ml of the prepared human amniotic fluid was poured on the damaged area with a waiting time of 1 minute. The abdominal incision was closed in two layers in all groups. The musculoperitoneum and fascia were closed with simple separated sutures of 4/0 polyglactin (Vicryl; Johnson and Johnson Co, Ethicon Limited, UK). The skin was closed with simple separated sutures of 4/0 polyglactin. After the animals recovered from surgery, they were housed separately under controlled temperatures of 22–28°C with 14-hour light and 10-hour dark cycles with food and water *ad libitum*. The surgery was limited to approximately 10 minutes for each rat to prevent the tissue from drying at room temperature [21, 23]. All surgical procedures were performed by the same surgeon (N.C), who has 10 years of experience in gynecology and obstetrics, and an Obstetrics and Gynecology resident.

Preparation of Oxidized Regenerated Cellulose, Human Amniotic Fluid and Trehalose Solution

Before the surgery, 1 cm^2 layers were prepared using the commercial ORC brand (Interceed; Ethicon Inc., Somerville, New Jersey) for each rat. Murine amniotic fluid is considered to inhibit xenogeneic antigen response. However, we used human amniotic fluid in this study because murine amniotic fluid is difficult to centrifuge and protect from contamination. Human amniotic fluid (HAF) was obtained in a sterile manner from seronegative, voluntary pregnant patients during their elective cesarean delivery in the 3rd trimester after obtaining informed consent in accordance with the principles of the Helsinki Declaration. Amniotic fluid was centrifuged at 3000 rpm for 15 minutes to separate the cell component. The smears were prepared to ensure that the cells were extracted, stained with the standard Giemsa method, and examined under a light microscope. Samples were frozen at -80 ºC and stored [24] with Trehalose dihydrate ($C_{11}H_{22}O_{11}\cdot 2H_2O$, MW: 378.3 g/M), purchased from a biochemical laboratory (Merck Co., Istanbul, Turkey). Trehalose (30 g) was dissolved in 1000 ml distilled water with electrolytes (0.2 g/L of calcium chloride hydrate, 0.3 g/L of potassium chloride, 6.0 g/L of sodium chloride, and 3.1 g/L of sodium lactate) and prepared as stated in the previous study [25]. These solutions were autoclaved and used under sterile conditions. The percentage of trehalose in solution (3%) was selected based on a previous study [25].

Adhesion scoring, tissue sample collection and histopathological analysis

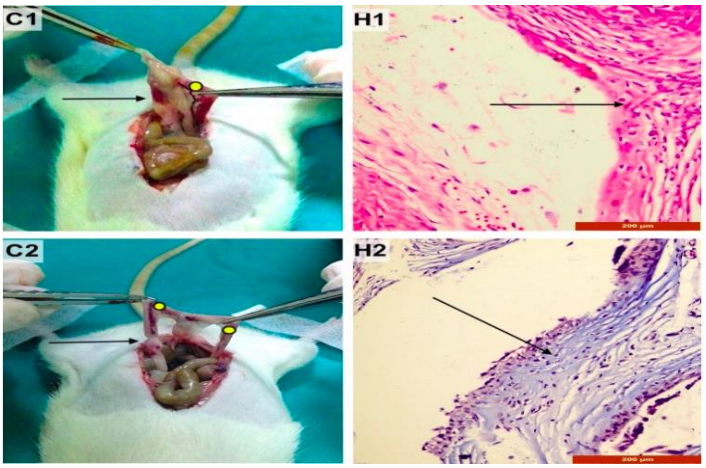
The animals were sacrificed 30 days later by the decapitation method. Leach et al. [26] adhesion scoring system was used for macroscopic (clinical) adhesion scoring (Table 1, Figure 1 (C1, C2). The researcher with an experimental research certificate (an obstetrician with 10 years of experience) who was blinded to the group assignments of the animals evaluated the adhesions according to the spread, severity, and degree of adhesions between the uterine horn and adjacent tissues. Adhesion spread between the uterine horn and adjacent intra-abdominal tissues were scored as follows: 0= no uterine adhesion; 1 = 1%–25% involvement; 2 = 26%–50% involvement; 3 = 51%–75% involvement; and 4 = 76%–100% involvement. Adhesions were further characterized on gross examination for severity as follows: 0 = no adhesions; 1= film-like avascular; 2 = vascular or opaque; and 3 =cohesive attachment of uterine horns to each other or other abdominal structures. The degree of adhesion formation was evaluated with the following adhesion scores: 0 = no adhesions; 1 = adhesion separated from the tissue with gentle traction; 2 = requiring moderate traction; and 3 = requiring sharp dissection.

Adhesions were also examined histopathologically by a pathologist with 10 years of experience and graded for inflammation and fibrosis using previously published grading scales [27] (Table 1, Figure 1) (H1, H2). The edges of adhesion tissue were fixed in 10% formaldehyde for 72 hours, underwent a routine tissue preparation procedure, and were embedded in paraffin. The tissues were examined in 4-micron-thick sections. All sections, after staining with Hematoxylin & Eosin and Masson trichrome, were investigated under a light microscope (Zeiss axioskop 40 Carl Zeiss Göttingen, Germany) and photographed (AxioVision 3,1 Zeiss axioplan 2 imaging Germany, Göttingen). Inflammation was scored as follows: 0 = no inflammation; 1 = presence of giant cells, occasional lymphocytes, and plasma cells, 2 = presence of giant cells, plasma cells, eosinophils, and neutrophils, and 3 = presence of many inflammatory cells and micro-abscesses. The amount of fibrosis was scored as: 0 = no fibrosis; 1 = minimal, loose; 2 = moderate; and 3 = florid dense. The main outcome measures were spread, severity and degree of adhesions, total adhesion scores, number of adhesion-free uterine horns, and histopathological characteristics (inflammation and fibrosis) of adhesions.

Table 1: The extent, severity, degree, inflammation and fibrosis scoring system of the adhesions

	Macroscopic Adhesion Score			Histopathological Score	
Score	Extent	Severity	Degree	Inflammation	Fibrosis
0	No adhesion	No adhesions	No adhesions	No inflammation	No fibrosis
1	1–25%	Filmy avascular	Separated from tissue with gentle traction	Giant cells, occasional lymphocytes and plasma cells	minimal, loose
2	26–50%	Vascular or opaque	Requiring moderate traction	Giant cells, plasma cells, eosinophils and neutrophils;	moderate
3	51–75%	Cohesive attachment	Requiring sharp dissection	many inflammatory cells and microabscesses	florid dense
4	76–100%	-	-	-	-

Figure 1: C1 and C2 show examples of clinic adhesion of uterine horn (arrows) in the control group (yellow dot: uterine horn). H1 shows many inflammatory cells with minimal infiltration at the edge of adhesion (hematoxylin and eosin, Bars = 200 µm). In H2, increase of fibrosis is seen at the edge of adhesion (Masson Trichrome, Bar = 200 µm)



Statistical analysis

Statistical analysis was performed using IBM SPSS (Statistical package for Social Sciences for Windows v22.0 licensed to University of California Davis, USA). Descriptive statistical methods (mean, standard deviation, median, min–max measurements) were used for data analysis. The Kruskal–Wallis test was used to compare differences in scores between the groups, and the Bonferroni corrected Mann–Whitney U test was used to compare differences between the subgroups. A *P*-value of <0.05 was considered statistically significant.

Results

Total macroscopic (clinical) adhesion formation scores were evaluated for all groups. The total clinical adhesion scores were 10.1 (1.72) in the control group, 6.5 (1.64) in the ORC, 3.8 (1.16) in the TRZ, and 4.8 (0.75) in the HAF group. The extent ($P<0.05$), severity ($P<0.05$), degree ($P<0.05$) and total adhesion ($P<0.05$) scores of the control group were significantly higher than those of the ORC, TRZ and HAF groups. Extent ($P=0.007$) and total adhesion ($P=0.019$) scores significantly differed between the ORC and TRZ groups, while severity ($P=0.136$) and degree ($P=0.760$) scores were similar.

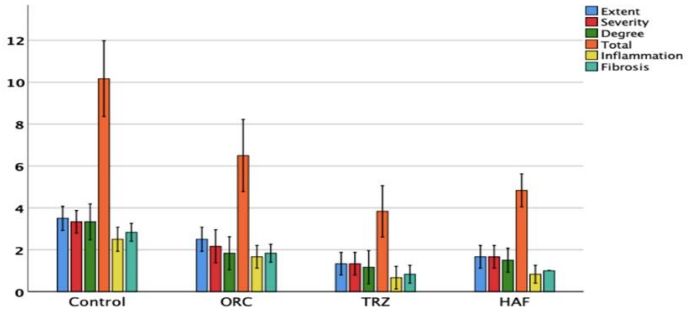
Table 2: The differences of macroscopic (clinic) and histopathological scores between the study groups

	Group 1 (Control)	Group 2 (ORC)	Group 3 (TRZ)	Group 4 (HAF)	*P*-value					
Number of uterin horn	12	12	12	12	I-II	I-III	I-IV	II-III	II-IV	III-IV
Macroscopic Adhesion Score	mean (SD) (range)	mean (SD) (range)	mean (SD) (range)	mean (SD) (range)						
Extent	3,5 (0.54) (3-4)	2.5 (0.54) (2-3)	1.3 (0.51) (1-2)	1.6 (0.51) (1-2)	0.024*	<0.001*	<0.001*	0.007*	0.081	0.99
Severity	3.3 (0.51) (3-4)	2.1 (0.75) (1-3)	1.3 (0.51) (1-2)	1.6 (0.45) (1-2)	0.015*	<0.001*	<0.001*	0.136	0.924	0.99
Degree	3.3 (0.81) (2-4)	1.8 (0.75) (1-3)	1.1 (0.75) (0-2)	1.5 (0.54) (1-2)	0.011*	<0.001*	0.002*	0.760	0.99	0.99
Total	10.1 (1.72) (8-12)	6.5 (1.64) (5-8)	3.8 (1.16) (3-6)	4.8 (0.75) (4-6)	0.001*	<0.001*	<0.001*	0.019*	0.295	0.99
Histopathological Score	mean (SD) (range)	mean (SD) (range)	mean (SD) (range)	mean (SD) (range)						
Inflammation	2.5 (0.54) (2-3)	1.6 (0.51) (1-2)	0.6 (0.51) (0-1)	0.8 (0.4) (0-1)	0.055	<0.001*	<0.001*	0.015*	0.55	0.99
Fibrosis	2.8 (0.40) (2-3)	1.8 (0.4) (1-2)	0.8 (0.40) (0-1)	1 (0) (1)	<0.001*	<0.001*	<0.001*	0.001*	0.003*	0.99

* $P<0.05$ was considered statistically significant. ORC: Oxidized regenerated cellulose, TRZ: Trehalose, HAF: Human Amniotic Fluid

The mean histological inflammation scores in the control, ORC, TRZ and HAF groups were 2.5 (0.54), 1.6 (0.51), 0.6 (0.51) and 0.8 (0.4), respectively. Inflammation was significantly increased in the control group compared to the other groups ($P<0.05$), except the ORC group ($P=0.055$). Fibrosis score was higher in the control group compared to the other groups ($P<0.05$). There were significant differences between the ORC and TRZ groups in terms of inflammation ($P=0.015$) and fibrosis scores ($P=0.001$). Inflammation and fibrosis scores of the TRZ and HAF groups were significantly lower compared with those of the control and ORC groups (Table 2, Figure 2).

Figure 2: Subgroup analysis of the study groups for macroscopic and histopathological scores (Error bars represent 95% confidence intervals)



Discussion

Abdominal adhesions occurring after gynecological operations not only cause morbidity and mortality, but also increase health expenses [3, 28]. Complications caused by pelvic adhesions in the United States are estimated to cost $ 1.3 billion annually [28]. These economic costs and complications have directed surgeons to use more advantageous surgical (laparoscopic, robotic) and medical methods (barrier, steroid, anti-inflammatory agents) to reduce adhesions. The cost of synthetic materials (synthetic barrier, gel) currently used in the clinic to reduce adhesions is remarkably high. For this reason, research for more efficient or similar but cheaper methods must continue.

In the literature, ORC, which we also use in our clinic, is reported to reduce adhesions in intrauterine, uterine horn, intestinal, pericardial, pleural and tendon repair models, although the same results were not obtained for adhesions between the thyroid and the skin [9]. Its adhesion prevention potential has been confirmed by many studies [5, 8-10, 26, 29]. However, in the presence of blood, some disadvantages have been reported, as their anti-adhesion effects are reduced and they can slip from the damaged area [29]. In our study, adhesion was decreased in the ORC group.

The autophagy feature of TRZ reduces myocardial fibrosis and conjunctival fibrosis in myocardial infarction [5, 13], while it has no effect on inflammation [30]. It has been reported that 7% TRZ used in the rabbit hysterectomy model is effective in minor injuries but not in major injuries [14, 31]. After dry air damage in human mesothelial cell culture, 3% TRZ was shown to reduce mesothelial cells and adhesions [25]. We also used 3% TRZ in this study and observed that TRZ reduced adhesions. At the end of 30 days, inflammation and fibrosis were the lowest in the TRZ group compared to other groups. Inflammation is expected to decrease in all groups within 30 days; however, we think that the reduction of fibrin gel, which is permanent after inflammation, and the decrease of fibrosis as a result, are important preventors of adhesion. Furthermore, TRZ's crystal structure may have increased its effectiveness by creating a barrier on the tissue.

Cells, proteins, and small molecule factors are found in HAF. Most of its protein content is made up of hyaluronic acid [17, 32]. HAF has been reported to contain hyaluronic acid stimulating activators (HASA) as well [17, 19] and reduces fibroblast activity and fibrin deposits in serosal surface injuries

[18]. In a rat study, HAF decreased intra-abdominal adhesions [33]. In another study, amniotic fluid spilled during a cesarean section did not have an adhesion preventing effect [34]. It has been reported that adhesions are reduced with rat amniotic fluid after hysterectomies in rats [18]. In the adhesion model made in rats, cattle amnion fluid was used and tried in three ways: Raw form, cell-free form, and cell and protein-free form. After the second surgery, the score was the same as the first surgery in all groups. After the third surgery, adhesions increased in the cell-free amniotic fluid group compared to the second scoring, and the adhesions in the cell and protein-free amniotic fluid group was decreased [24]. Cell-free HAF was used in our study, and we observed that HAF and TRZ decreased the adhesions equally. The adhesion scores of all groups were less than that of the control group, and the total adhesion score of TRT was lower than that of ORC. The TRZ and HAF groups were similar in terms of clinical and histopathological adhesion scores ($P>0.05$). We think that HA in HAF acts as a barrier and provides anti-adhesion with the proteins and factors it contains.

Limitations

In this study, we did not fully describe the most active substance in the HAF used. In addition, we used human amniotic fluid, which is genetically different from that of rats. Also, if ORC slides from the surface it adheres to, its effectiveness decreases because it is in film form. Unlike ORC, TRZ and HAF were liquids. Most of the previous studies took 7 and 14 days. We think that our study lasting 30 days is useful in evaluating late-stage adhesion.

Conclusion

While the 3% TRZ reduces adhesion clinically and histologically with its autophagic feature and the crystal barrier on the tissue, HAF does the same by acting as a barrier due to hyaluronic acid and anticoagulant-anti-inflammatory factor balance. We think that further studies on the combination of TRZ and HAF may show improved adhesion prevention properties.

References

1. Monk BJ, Berman ML, Montz F. Adhesions after extensive gynecologic surgery: clinical significance, etiology, and prevention. Am J Obstet Gynecol. 1994;170:1396-403.
2. Liakakos T, Thomakos N, Fine PM, Dervenis C, Young RL. Peritoneal adhesions: etiology, pathophysiology, and clinical significance. Dig Surg. 2001;18:260-73.
3. Ellis H. The clinical significance of adhesions: focus on intestinal obstruction. The European journal of surgery Supplement:= Acta chirurgica Supplement. 1997:5.
4. Mutsaers SE, Wilkosz S. Structure and function of mesothelial cells. Peritoneal Carcinomatosis: Springer; 2007. p. 1-19.
5. Schnüriger B, Barmparas G, Branco BC, Lustenberger T, Inaba K, Demetriades D. Prevention of postoperative peritoneal adhesions: a review of the literature. The American Journal of Surgery. 2011;201:111-21.
6. Çim N, Elçi E, Elçi GG, Almalı N, Yıldızhan R. Are the skin scar characteristics and closure of the parietal peritoneum associated with pelvic adhesions? Turkish journal of obstetrics and gynecology. 2018;15:28.
7. Holmdahl L, Eriksson E, Eriksson BI, Risberg B. Depression of peritoneal fibrinolysis during operation is a local response to trauma. Surgery. 1998;123:539-44.
8. Medicine PCotASfR. Control and prevention of peritoneal adhesions in gynecologic surgery. Fertil Steril. 2006;86:S1-S5.
9. Li J, Feng X, Liu B, Yu Y, Sun L, Liu T, et al. Polymer materials for prevention of postoperative adhesion. Acta Biomater. 2017; 61:21-40.
10. Zhang Y, Liu Q, Yang N, Zhang X. Hyaluronic acid and oxidized regenerated cellulose prevent adhesion reformation after adhesiolysis in rat models. Drug Des Devel Ther. 2016; 10:3501.
11. Collins J, Robinson C, Danhof H, Knetsch C, Van Leeuwen H, Lawley T, et al. Dietary trehalose enhances virulence of epidemic Clostridium difficile. Nature. 2018; 553:291-4.
12. Chou L-F, Cheng Y-L, Hsieh C-Y, Lin C-Y, Yang H-Y, Chen Y-C, et al. Effect of trehalose supplementation on autophagy and cystogenesis in a mouse model of polycystic kidney disease. Nutrients. 2019; 11:42.
13. Wu N, Chen L, Yan D, Zhou M, Shao C, Lu Y, et al. Trehalose attenuates TGF-β1-induced fibrosis of hSCFs by activating autophagy. Mol Cell Biochem. 2020.
14. Fujino H, Lee S, Suzuki S, Chung U-i, Mochizuki M, Nishimura R, et al. Trehalose may prevent postsurgical adhesions in a rabbit model of hysterotomy. J Vet Med Sci. 2011:1102030454-.
15. Sciarretta S, Yee D, Nagarajan N, Bianchi F, Saito T, Valenti V, et al. Trehalose-induced activation of autophagy improves cardiac remodeling after myocardial infarction. J Am Coll Cardiol. 2018; 71:1999-2010.
16. Koh S, Anandakumar C, Arulkumaran S, Chua S, Yuen W, Ng B, et al. Amniotic fluid plasminogen activators and inhibitors and TAT-complex levels during 2nd trimester pregnancy and labour. Fibrinolysis. 1995; 9:121-6.
17. Longaker MT, Adzick NS, Hall JL, Stair SE, Crombleholme TM, Duncan BW, et al. Studies in fetal wound healing, VII. Fetal wound healing may be modulated by hyaluronic acid stimulating activity in amniotic fluid. J Pediatr Surg. 1990; 25:430-3.
18. Tahmasebi S, Tahamtan M, Tahamtan Y. Prevention by rat amniotic fluid of adhesions after laparatomy in a rat model. International Journal of Surgery. 2012; 10:16-9.
19. Kerimoğlu S, Livaoğlu M, Sönmez B, Yuluğ E, Aynacı O, Topbas M, et al. Effects of human amniotic fluid on fracture healing in rat tibia. J Surg Res. 2009; 152:281-7.
20. Özgenel GY, Fílíz G. Combined application of human amniotic membrane wrapping and hyaluronic acid injection in epineurectomized rat sciatic nerve. J Reconstr Microsurg. 2004; 20:153-7.
21. Kaya C, Sever N, Cengiz H, Yıldız Ş, Ekin M, Yaşar L. A randomized controlled study of the efficacy of misoprostol and hyaluronic acid in preventing adhesion formation after gynecological surgery: a rat uterine horn model. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2014; 176:44-9.
22. Kelekci S, Uygur D, Yilmaz B, Sut N, Yesildaglar N. Comparison of human amniotic membrane and hyaluronate/carboxymethylcellulose membrane for prevention of adhesion formation in rats. Arch Gynecol Obstet. 2007; 276:355-9.
23. Güney G, Kaya C, Oto G, Yıldırım S, Özdemir H, Tokmak A. Effects of quercetin and surgicel for preventing adhesions after gynecological surgery: A rat uterine horn model. J Obstet Gynaecol Res. 2017; 43:179-84.
24. Esmaeili A, Abbasian B, Kazemini H, Adibi S. Effect of bovine amniotic fluid on intra-abdominal adhesion in male rats. International Journal of Surgery. 2010; 8:639-42.
25. Ohata A, Tamura N, Iwata K, Abe N, Doi K, Saito Y, et al. Trehalose solution protects mesothelium and reduces bowel adhesions. J Surg Res. 2014;191:224-30.
26. Leach RE, Burns JW, Dawe EJ, SmithBarbour MD, Diamond MP. Reduction of postsurgical adhesion formation in the rabbit uterine horn model with use of hyaluronate/carboxymethylcellulose gel. Fertil Steril. 1998;69:415-8.
27. Hooker GD, Taylor BM, Driman DK. Prevention of adhesion formation with use of sodium hyaluronate–based bioresorbable membrane in a rat model of ventral hernia repair with polypropylene mesh—A randomized, controlled study. Surgery. 1999;125:211-6.
28. Ray NF, Denton WG, Henderson SC, Seymour Perry M. Abdominal adhesiolysis: inpatient care and expenditures in the United States in 1994. J Am Coll Surg. 1998;186:1-9.
29. Takagi K, Araki M, Fukuoka H, Takeshita H, Hidaka S, Nanashima A, et al. Novel powdered anti-adhesion material: preventing postoperative intra-abdominal adhesions in a rat model. Int J Med Sci. 2013;10:467.
30. Tanaka K, Kawamura M, Otake K, Toiyama Y, Okugawa Y, Inoue Y, et al. Trehalose does not affect the functions of human neutrophils in vitro. Surg Today. 2014;44:332-9.
31. Lee SJ, Miwa, Y., Nishimura, R., Chung, U. I., Suzuki, S. and Sasaki, N. Effects of trehalose and sodium carboxymethyl cellulose on prevention of organ adhesion after laparotomy: A preliminary study. Jpn J Vet Anesth Surg. 2009;40:19-26.
32. Dahl L, Kimpton W, Cahill R, Brown T, Fraser R. The origin and fate of hyaluronan in amniotic fluid. J Dev Physiol. 1989;12:209-18.
33. Durmus AS, Han, M. C. . Effect of bovine amniotic fluid on intra abdominal adhesions. Indian Vet J. 2006;83:621-3.
34. Golan A, Stolik O, Wexler S, Niv D, Ber A, David M. The effect of amniotic fluid on intraperitoneal adhesion formation--an experimental study. Int J Fertil. 1991;36:317-20.

This paper has been checked for language accuracy by JOSAM editors.
The National Library of Medicine (NLM) citation style guide has been used in this paper.