

A new method to repair recto-vaginal fistula: Use of human amniotic membrane in an animal model

Reza Roshanravan, Leila Ghahramani, Massood Hosseinzadeh, Mastoureh Mohammadipour, Sam Moslemi, Abbas Rezaianzadeh, Ali Reza Safarpour, Salar Rahimikazerooni, Seyed Vahid Hosseini

Colorectal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Recto-vaginal fistula is primarily one of the co-morbidities of vaginal delivery. These patients suffer from persistent malodor vaginal discharge. Various surgical techniques have been employed by surgeons in the course of time. This is the first trial of applying Human Amniotic Membrane (HAM) as a bio-prosthesis in repairing recto-vaginal fistula.

Materials and Methods: In a prospective animal study, 8 mixed-breed female dogs weighing 23-27 kg with the age of 12-18 months were selected. They were randomly divided into two groups for standard recto-vaginal fistula repair and fistula repair with human amniotic membrane. The Kruskal-Wallis and Mann Whitney tests were performed to indicate statistical differences.

Results: After 6 weeks, fistulas were evaluated both grossly and microscopically. In gross examination, there were no difference between the two groups and healing of fistula seemed to have been occurred in all dogs expect for one which had a persistent patent fistulous tract. Microscopic healing was scored according to epithelialization, collagenization inflammation, ulcer and necrosis of samples. Healing score was significantly higher in the HAM group than the standard group ($P = 0.029$).

Conclusion: Our findings revealed that using HAM as a bio-prosthesis to repair recto-vaginal fistula would result in better surgical and histological outcomes comparing to simple repair.

Key Words: Human amniotic membrane, recto-vaginal fistula, surgery, biomaterials

Address for correspondence:

Dr. Salar Rahimikazerooni, Colorectal Research Center Shiraz University of Medical Sciences, Shiraz, Iran.

E-mail: colorectal2@sums.ac.ir

Received: 25.05.2013, Accepted: 31.08.2013

INTRODUCTION

Recto-vaginal fistula is primarily one of the co-morbidities of vaginal delivery and sufferers

encounter occasional passage of flatus and stool from vagina.^[1] They also experience a persistent horrible smell of vaginal discharge.^[1,2] It goes without saying that such a condition has harmful effects on all aspects of patients' life particularly in less developed countries. This condition is so devastating that some researchers have likened women who endure the agonies of this disease to "dead women walking".^[3]

More than 99% of patients need surgical treatments. Various techniques have been employed by surgeons to address this disease in the course of time.^[4-7] However, two reasons have driven surgeons to apply

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.131033

Copyright: © 2014 Roshanravan. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

How to cite this article: Roshanravan R, Ghahramani L, Hosseinzadeh M, Mohammadipour M, Moslemi S, Rezaianzadeh A, et al. A new method to repair recto-vaginal fistula: Use of human amniotic membrane in an animal model. *Adv Biomed Res* 2014;3:114.

bio-prosthesis like Porcine Intestinal Sub-mucosa (PIS) in the last decade: Being technically straightforward and, low probability of sphincter injury.^[8]

On the other hand, some characteristics of human amniotic membrane (HAM) such as bio-compatibility, easy availability, elasticity and stability have encouraged researchers to consider HAM as a biologic dressing and appropriate bio-prosthesis for more than 100 years.^[9] Many studies have evaluated the application of HAM in different organs. For example, many surgeons examined the efficacy of HAM as a biologic dressing in burn wounds and desirable outcomes were reported.^[9,10] In addition, the hypothesis that some features of HAM show variation in different organs encouraged surgeons to utilize this bio-material in gastrointestinal tract surgeries.^[11,12]

This study was conducted to evaluate using HAM as a bio-prosthesis in repairing recto-vaginal fistula. To the best of our knowledge it is the first time that this bio-material has been examined for this purpose.

MATERIALS AND METHODS

In a prospective animal study, 8 mixed-breed female dogs weighing 23-27 kg with the age of 12-18 months were selected. Selection of animals, procedures and subsequent care were all adhered to guidelines and under supervision of Animal Care Committee of Iran Veterinary Organization. Dogs were initially evaluated for any probable underlying disease by a single veterinarian. All procedures were performed under aseptic conditions in the Department of Laboratory Animal Medicine of Shiraz University of Medical Sciences in accordance with the standards of laboratory bio-safety guidelines. HAM, used in this study, was provided by Shiraz Burn Research Center, preserved in glutaraldehyde and then frozen in -20°C . Protocol of anesthesia, surgical procedure, postoperative care, and sacrifice were identical for all animals. Anesthesia was induced by Intravenous Diazepam (10 mg) and sodium thiopental (0.5 mg) after endotracheal intubation. Then animals were maintained on controlled ventilation by halothane and 100% oxygen. Ringer's lactate was given intravenously throughout the operation at a rate of 8 mL/kg/h. Protocol of anesthesia was the same for all. Before induction a single dose of IV antibiotic (Penstrep 500 mg) was given to both groups.

First step

Under general anesthesia in lithotomy position, recto-vaginal septum was palpated bimanually. An iatrogenic recto-vaginal fistula was made with sharp and blunt dissection 4 cm above the anal verge. Then

a number 14 French catheter was passed through the induced fistulous tract to keep it open. Surgical diet was started 6 hours post operation then advanced. Dogs were observed for 4 weeks in standard cages. They were evaluated daily by a single veterinarian for abscess formation.

Second step

After 4 weeks, dogs were randomly put into 2 groups. We placed them on liquid diet two days before surgery. One episode of warm tap-water enema was given for clearance of rectum. Operation was performed under general anesthesia in sterile field in lithotomy position. The existence of recto-vaginal fistula was confirmed by a colorectal surgeon and a veterinarian with examination under anesthesia in all cases. In the first group (4 dogs), through a 3 cm transverse incision in perineal area, fistulectomy was done. The rectal side of fistula was repaired with Vicryl 3-0 in the form of interrupted stitches. In the second group (4 dogs), through a 3 cm transverse incision in perineal area fistula was identified and resected. Simple closure was done similar to the first group then a patch of HAM measuring 2×2 cm (which was protected from dehydration by being floated in normal saline for 5-10 min at 24°C) was applied on the site of primary closure and fixed with Vicryl 3-0. Postoperatively, 3 doses of IV antibiotic (Penstrep 500 mg) were given to both groups. Surgical diet was started 6 hours post-operation then advanced. Dogs were observed for 6 weeks.

All dogs survived up to the time of sacrifice. Finally they were killed by intravenous KCl infusion. Rectum and vagina were excised completely. The specimens were fixed in formaldehyde 10% solution (formalin). Samples were blindly labeled and sent to the Department of Pathology in Shiraz Faculty of Medicine for histo-pathological assessment. After gross examination and photography of fistulous tracts, histological sampling, tissue processing, slide preparation and H and E staining were performed. The slides were blindly studied by one pathologist. Histopathological findings were discussed for each sample on the basis of our modified scoring system. The scoring system was based on previous one which was suggested by Grenhalgh and Abramov *et al.*^[13] The major modified item used in this system was histological evidence of tissue necrosis.

RESULTS

In this experimental study, 8 mixed-breed female dogs weighing 23-27 kg underwent surgically

created (iatrogenic) recto-vaginal fistula. Then primary repair of fistula was performed by use of HAM in 4 dogs (case group) and without HAM in the other 4 (control group). After 6 weeks, fistulas were evaluated both grossly and microscopically.

In gross examination, there was no abscess formation, infection or pussy discharge. Healing of fistula seemed to have been occurred in all dogs, except for one which had a persistent patent fistulous tract.

Microscopic evaluation of harvested samples was performed by one pathologist. Modified scoring system for surgical wound healing^[1] was used to determine grade of healing in each sample. Healing was scored according to epithelialization, collagenization, inflammation, ulcer and necrosis of the samples [Table 1].

As shown in Table 2, in the treatment group, two samples (50%) were scored 3 and two (50%) were scored 4 [Figures 1 and 2]. In the control group, one sample (25%) was scored 1 and three samples (75%) were scored 2 [Figure 3]. The healing score was significantly higher in the HAM group ($P = 0.029$).

Statistical analysis

Data is presented with range for continuous variables and with count (percentage) for categorical variables.

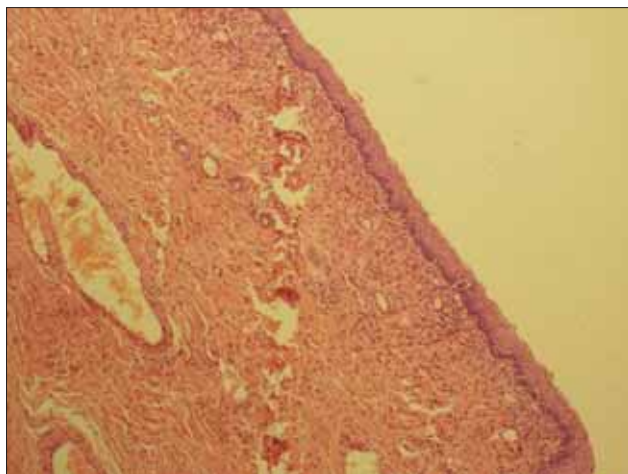


Figure 1: Complete epithelialization accompanied by sub-mucosal fibroblastic proliferation and neo-vascularization H and E, $\times 100$

The statistically significant difference in healing score between case and control groups was determined using Mann-Whitney u Test.

DISCUSSION

Our findings showed that repairing recto-vaginal fistula with amniotic membrane patch results in better outcome compared to simple repair in dogs. This result can be discussed from two different perspectives.

Firstly, the fact that our findings are generally in concordance with the results of other studies which reported the application of bio-prosthesis in repairing recto-vaginal fistulas is promising. For instance, Ellis *et al.*, applied porcine intestinal sub-mucosa for repair of recto-vaginal fistula on 34 patients and followed them for 10 months. They reported more than 80% success rate.^[1] Applying Surgisis for the same purpose in some other studies also led to positive outcomes.^[14] However, it should be noticed that bio-prosthesis has been put into practice for less than a decade. So, more studies is needed for better evaluation.^[15]

Secondly, the application of HAM as a bio-prosthesis should be taken into consideration. Many studies assessed the efficacy of HAM as a biologic dressing in skin ulcers and corneal problems and many of

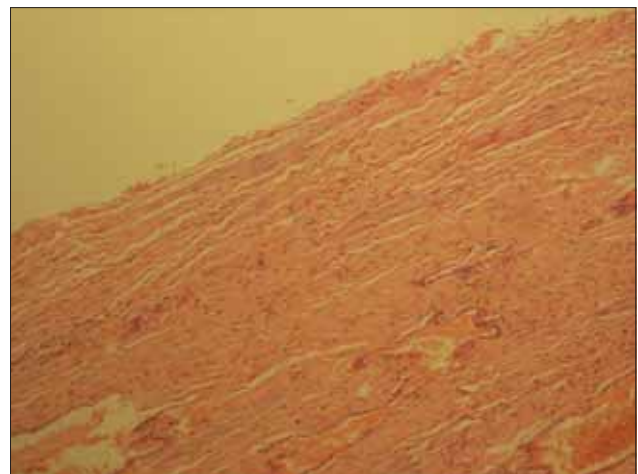


Figure 2: Repair by connective tissue, diffuse fibroblast proliferation with epithelialization H8E*100. H and E, $\times 100$

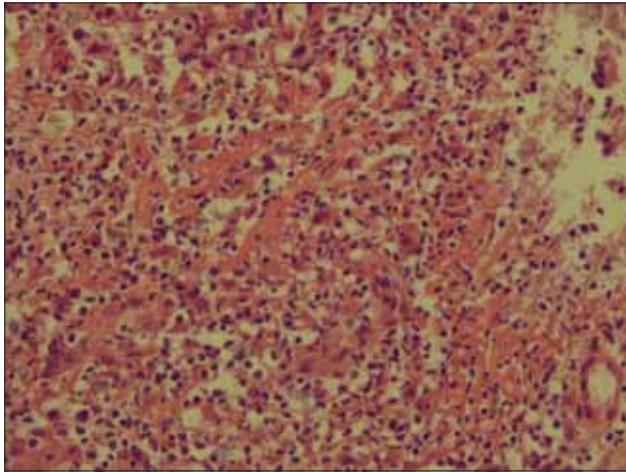
Table 1: Wound-healing histological scoring system

Score	Epithelialization	Collagenization	Inflammation	Neovascularization	Necrosis	Granulation tissue
1	None	None	Severe	None	Extensive	None
2	None	None	Moderate	None	Focal	Immature
3	partial	Partial	Mild	<5/HPF	None	Mild mature
4	Complete, immature	Complete, irregular	None	6-10/HPF	None	Mod mature
5	Complete, mature	Complete, regular	None	>10/HPF	None	Fully mature

HPF: High power field

Table 2: Healing score of samples in case and control groups

Group 1 (case)	Score	Group 2 (control)	Score
1	3 ⁺	1	2 ⁺
2	4 ⁺	2	1 ⁺
3	3 ⁺	3	2 ⁺
4	4 ⁺	4	2 ⁺

**Figure 3:** This figure shows exudation, focal necrosis, no epithelialization, incomplete fibroblast proliferation H and E, ×100

them reported better outcomes in comparison to some other methods.^[16,17] Moreover, in a few studies, the effectiveness of HAM has been examined in GI tract of animal models and the results have emphasized role of HAM in accelerating wound healing process.^[18]

As an illustration, Uludag *et al.*, applied HAM patch in colon anastomosis in rats in a series of researches. They examined different outcomes and finally concluded that using HAM decreases dehiscence rate, anastomotic leakage, intraabdominal abscesses, intestinal obstruction and adhesion formation.^[11,19,20] Similar results have been obtained from previous animal studies in rats and dogs using HAM for duodenal repair.^[18]

The application of HAM patch in repairing recto-vaginal fistula in dogs in our study clarified the advantages of HAM compared to PSIS (as a biologically active implant). Aungst *et al.* interposed PSIS grafts for recto-vaginal fistula repair in the New Zealand white rabbits and found no beneficial effect.^[21]

The small sample size, also the anatomical differences of perineal area in dogs compared to human beings, should be considered as the limitations of our study. On the other hand, recto-vaginal fistula in humans formed under special conditions, such as multiple trauma, Crohn's disease, abscess formation and radiotherapy. The difference between health condition

of women suffering from recto-vaginal fistula and cases of this study should be considered as the other limitation. In addition, the probable long-term adverse effects of HAM should be evaluated in further studies.

In spite of these limitations, to our knowledge this is the first time that clinical and histologic features of HAM applied in repairing recto-vaginal fistula have been examined. We used a modified scoring system in order to standardize histologic findings and provide a quantitative comparative context. Although quantitative assessment of wound healing process is challenging, we believe it would help researchers for more accurate comparison.^[13]

Finally, it can be concluded that using HAM as a bio-prosthesis for repairing recto-vaginal fistula would result in better surgical and histological outcomes comparing to simple repair.

ACKNOWLEDGEMENT

This article was extracted from the research proposal NO. 91-01-69-5180 approved by the research vice-chancellor of Shiraz University of Medical Sciences. Hereby, the authors would like to thank this vice-chancellery for financially supporting the study.

REFERENCES

1. Ellis CN. Outcomes after repair of rectovaginal fistulas using bioprosthesis. *Dis Colon Rectum* 2008;51:1084-8.
2. Rahman MS, Al-Suleiman SA, El-Yahia AR, Rahman J. Surgical treatment of rectovaginal fistula of obstetric origin: A review of 15 years' experience in a teaching hospital. *J Obstet Gynaecol* 2003;23:607-10.
3. Ahmed S, Genadry R, Stanton C, Lalonde AB. Dead women walking: Neglected millions with obstetric fistula. *Int J Gynaecol Obstet* 2007;99 Suppl 1:S1-3.
4. Mehrabani D, Ashraf MJ, Abbasi HR, Amini M, Tanideh N, Sabet B, et al. Comparison of simple closure and polytetrafluoroethylene patch methods for repair of rectovaginal fistula in dog. *J Appl Anim Res* 2009;35:73-6.
5. Li Destri G, Scilletta B, Tomaselli TG, Zarbo G. Rectovaginal fistula: A new approach by stapled transanal rectal resection. *J Gastrointest Surg* 2008;12:601-3.
6. González-Contreras QH, Castañeda-Argáiz R, Rodríguez-Zentner HA, Tapia-Cid de León H, Mejía-Ovalle RR, Espinosa-de Los Monteros A. Transposition of gracilis muscle for treatment of recurrent anal and rectovaginal fistula. *Cir Cir* 2009;77:319-21;297-9.
7. Cui L, Chen D, Chen W, Jiang H. Interposition of vital bulbocavernosus graft in the treatment of both simple and recurrent rectovaginal fistulas. *Int J Colorectal Dis* 2009;24:1255-9.
8. David E, Beck M, Patricia L, Roberts M, Anthony J, Senagore M, Michael J, Stamos M, Wexner SD. *The ASCRS textbook of colon and rectal surgery*. 2nd ed. New York: springer; 2011.
9. Kesting MR, Wolff KD, Hohlweg-Majert B, Steinstraesser L. The role of allogenic amniotic membrane in burn treatment. *J Burn Care Res* 2008;29:907-16.
10. Arai N, Tsuno H, Okabe M, Yoshida T, Koike C, Noguchi M, et al. Clinical application of a hyperdry amniotic membrane on surgical defects of the oral mucosa. *J Oral Maxillofac Surg* 2012;70:2221-8.

Roshanravan, *et al.*: Repairing recto-vaginal fistula with human amniotic membrane

11. Uludag M, Citgez B, Ozkaya O, Yetkin G, Ozcan O, Polat N, *et al.* Effects of amniotic membrane on the healing of primary colonic anastomoses in the cecal ligation and puncture model of secondary peritonitis in rats. *Int J Colorectal Dis* 2009;24:559-67.
12. Barlas M, Gokcora H, Erekul S, Dindar H, Yucesan S. Human amniotic membrane as an intestinal patch for neomucosal growth in the rabbit model. *J Pediatr Surg* 1992;27:597-601.
13. Abramov Y, Golden B, Sullivan M, Botros SM, Miller JJ, Alshahrour A, *et al.* Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model. *Wound Repair Regen* 2007;15:80-6.
14. Schwandner O, Fuerst A, Kunstreich K, Scherer R. Innovative technique for the closure of rectovaginal fistula using Surgisis mesh. *Tech Coloproctol* 2009;13:135-40.
15. Beck DE, Roberts PL, Senagore AJ, Stamos MJ, Wexner SD. *The ASCRS textbook of Colon and Rectal surgery* Second ed. New York: Spring; 2011.
16. Kobayashi N, Kabuyama Y, Sasaki S, Kato K, Homma Y. Suppression of corneal neovascularization by culture supernatant of human amniotic cells. *Cornea* 2002;21:62-7.
17. Mohammadi AA, Seyed Jafari SM, Kiasat M, Tavakkolian AR, Imani MT, Ayaz M, *et al.* Effect of fresh human amniotic membrane dressing on graft take in patients with chronic burn wounds compared with conventional methods. *Burns* 2013;39:349-53.
18. Schmidt LR, Cardoso EJ, Schmidt RR, Back LA, Schiazawa MB, d'Acampora AJ, *et al.* The use of amniotic membrane in the repair of duodenal wounds in Wistar rats. *Acta Cir Bras* 2010;25:18-23.
19. Uludag M, Ozdilli K, Citgez B, Yetkin G, Ipcioglu OM, Ozcan O, *et al.* Covering the colon anastomoses with amniotic membrane prevents the negative effects of early intraperitoneal 5-FU administration on anastomotic healing. *Int J Colorectal Dis* 2010;25:223-32.
20. Yetkin G, Uludag M, Citgez B, Karakoc S, Polat N, Kabukcuoglu F. Prevention of peritoneal adhesions by intraperitoneal administration of vitamin E and human amniotic membrane. *Int J Surg* 2009;7:561-5.
21. Aungst MJ, Bearss JJ, Lewis BS, Fischer JR, Bonhage MR, Wright J, Jr. Interposition grafts for rectovaginal fistula repair in the New Zealand white rabbit. *Int Urogynecol J* 2010;21:737-42.

Source of Support: The Research vice-chancellor of Shiraz University of Medical Sciences. **Conflict of Interest:** None declared.