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The Value of Use of Freeze-Dried Amniotic Membrane Graft Placement after Operative Hysteroscopy in Decreasing Intrauterine Adhesions

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Abstract

Objective: The aim of the current study was to test the efficacy of the use of freeze-dried amniotic membrane graft in decreasing occurrence and recurrence of intrauterine adhesions after operative hysteroscopy.

Methods: This clinical trial was conducted at the Early Cancer Detection and Endoscopy Unit at Ain Shams University Maternity Hospital. A total of 30 women who underwent operative hysteroscopy were included. After hysteroscopic procedure, freeze-dried AM graft was applied over pediatric Foley's catheter (8 F), which was inserted deflated then inflated according to available uterine cavity. The catheter and graft were kept for 2 weeks and then removed. All included women were reviewed and follow-up diagnostic hysteroscopy was performed one month postoperatively.

Results: The mean age of included women was 30.1 ± 6.3 years (range: 20-45 years). Of the included women, 23 (76.7%) had intrauterine adhesions [Asherman's syndrome], while 7 (23.3%) had other pathologies [submucous myoma, endometrial polyp or uterine septum]. Among women with Asherman's syndrome, the postoperative rates of amenorrhea and postoperative rates of moderate/severe intrauterine adhesions were significantly lower when compared to the preoperative rates.

Conclusion: Placement of intrauterine freeze-dried AM graft following hysteroscopic adhesiolysis seems to be significantly associated with improved uterine length, reduced rates of amenorrhea and reduced rates of recurrent adhesions.

Keywords: *Freeze-dried amnion graft; Operative hysteroscopy; Intrauterine adhesions; Asherman's syndrome*

Introduction

Intrauterine adhesions (IUA) or Asherman's syndrome is one of the most challenging causes of infertility and recurrent miscarriage [1]. IUA may complicate severe or chronic inflammatory uterine conditions (e.g. genital tuberculosis, postabortive or postpartum sepsis) or surgical interventions (e.g. curettage, Cesarean section or hysteroscopic surgery) [2]. The current gold-standard tool for diagnosis and treatment of IUA is hysteroscopy [3].

Despite the advances in use of diagnostic and operative hysteroscopy, management of IUA remains challenging [4]. The standard management of IUA is physical removal of adhesions and prevention of the formation of new adhesions. In order to prevent the formation of new adhesions, no opposing surface epithelial defects should exist postoperatively [5]. For this, several approaches were suggested, including pharmacological building-up of normal endometrium (through intensive sequential estrogen and progestin treatment), postoperative placement of intrauterine balloon or intrauterine device, and the placement of intrauterine amnion graft [3,6-10]. Application of a biologically-active mechanical separator, rather than placement of a 'physical barrier' is advantaged by achieving two goals; suppression of adhesion formation and promotion of epithelial healing [10].

The use of whole human fetal membranes or amniotic membrane (AM) alone in surgery has been primarily developed to aid the repair of surface epithelial defects in various tissues, like the skin, eye, abdominal wall, and peritoneum [11]. Although the field of obstetrics and gynecology is more concerned with fetal membranes, the use of amnion has not been that popular, being restricted to its use as a graft in forming an artificial vagina or a barrier to prevent postoperative

intra-abdominal adhesion formation [12]. The use AM graft following hysteroscopic lysis of IUA was shown to be associated with significant improvement of outcomes (restoration of menses and successful pregnancy) and significant reduction in the risk of recurrence of adhesions [3,10]. The main concern with AM graft placement is the possible risk of transmission of infection. Antibiotic disinfection is applied to harvested grafts. Safety against transmission of viruses is effected by donor selection and testing for serological markers of presently known transmissible viruses at the time of donation and again 3-4 months later. In addition, appropriate selection of the AM graft is important. AM grafts should be harvested from women undergoing planned elective prelabor Cesarean section with intact fetal membranes [10]. Long-term methods for preservation of AM grafts are needed. Perfect graft disinfection should be ensured. In addition, strict election criteria make ideal AM grafts not always readily available. Moreover, maintenance of the integrity of the basement membrane and stromal matrix appears to be central to promoting rapid re-epithelialization, and, therefore, should be considered in selecting the preservation method of AM grafts. Various methods have been used to preserve AM grafts including "fresh" (or more appropriately hypothermic) storage, freezing, and freeze-drying. Freeze-drying of AM grafts appears to fulfill all these targets: perfect disinfection, long-term preservation, and maintenance of graft structural integrity. The aim of the current study was to test the efficacy of the use of freeze-dried amnion graft in decreasing occurrence and recurrence of intrauterine adhesions after operative hysteroscopy.

Methods

This clinical trial was conducted at the Early Cancer Detection and Endoscopy Unit at Ain Shams University Maternity Hospital during the period between January and June 2016. The study protocol was in agreement to the Helsinki Declaration of the Principles of Ethical Medical Research [last updated in Brazil, 2013] and was approved by the Ethical Medical Committee of Obstetrics and Gynecology Department, Ain Shams University. All participating women and amnion graft donors signed informed written consents after thorough explanation of the purpose and procedures of the study. A total of 30 women planned to undergo operative hysteroscopy were recruited in the trial.

Amniotic Graft

Universal ethical approval: The process is a patent pending process that safely and gently separates amniotic membranes, cleans and reassembles various layers, and then dehydrates these membranes in a way that preserves the key elements associated with healing. Processed dehydrated amniotic tissue is regulated under Section 361 of the Public Health Service Act by the United States Food and Drug Administration (FDA) [13]. Tissue stabilization was accomplished by a dehydration step; finally, an inner and outer peel pouch system was used for packaging which is then sterilized with Electron-beam irradiation. The final implantable amnion allografts can be stored at room temperature for up to five years and is rehydrated prior to use [14].

Amniotic graft donors: Amniotic grafts were obtained from pregnant women who underwent elective caesarean section for a term viable fetus. Women had to be seronegative for hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and human immune-deficiency virus (HIV). Women with prelabor rupture of membranes (PROM), meconium-stained membranes, women who had any signs of systemic or local infection, women with autoimmune disease, and, women who had uncontrolled diabetes mellitus, and those on chronic steroid therapy were not included as amniotic graft donors.

Amnion graft preparation: Amniotic membranes were obtained from placental tissues of pregnant patients who underwent elective caesarean section after screening for HBV, HCV, HIV, and syphilis. Amnion samples were washed separately with tap water to remove blood debris. The washed samples were washed in sterile isotonic saline 3 times (ten minutes each), then washed with 0.05% sodium hypochlorite for 10 min., and finally washed 3 times with sterile water (10 min each). The membranes were kept in plastic containers with sterile physiological saline, and preserved temporarily in a 4°C. Samples were placed in a cool box and transported immediately to the Amniotic Membrane Tissue Preparation Lab, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo.

In a laminar air flow, the membranes (glossy, translucent and thinner membrane) were separated from the chorion (opaque and thicker) under aseptic condition. The membranes were cut into approx. 5x5-cm pieces by surgical scissor and flattened on gauze dressing; so that their epithelial surface was upward. The membranes were kept overnight in freezer and then lyophilized in freeze dryer and packaged in polyethylene bag until use.

Operative hysteroscopy procedure: Hysteroscopy procedures were performed during the early proliferative phase of the menstrual cycle. Normal saline was used as a distention medium. Hysteroscopy is performed at the Early

Cancer Detection Unit at Ain Shams University Maternity Hospital, using the following equipment (Karl Storz®, Tuttlingen, Germany): rigid, 30º Hamou II telescope [model 26157 BT] with a Hopkins II lens system, with the sheath has a 4-mm outer diameter [model 26163 V with 2.9 mm rollens]; Karl Storz endoskope Camera, telecom DXpal model 20 23 20 20 by Storz. Dissection of IUA was performed using hysteroscopic-guided scissors (details). Uterine septum, endometrial polyps and submucous myomas were resected using hysteroscopic resectoscope (details). After procedure, freeze-dried AM graft was applied over pediatric Foley's catheter (8 F), which was inserted deflated then inflated according to available uterine cavity. The catheter and graft were kept for 2 weeks and then removed. All included women were reviewed and follow-up diagnostic hysteroscopy was performed one month postoperatively.

Outcomes: The primary outcome was the rate and grade of intrauterine adhesions one month postoperatively. Adhesions were classified into mild adhesions (filmy adhesions causing partial uterine cavity occlusion), moderate adhesions (fibromuscular adhesions that may bleed upon division that partially or totally occlude the uterine cavity), and severe adhesions (adhesions composed of connective tissues only and not likely to bleed upon division; these adhesions may partially or totally occlude the uterine cavity).

Secondary outcomes included improvement in the uterine length on uterine sounding and the rates of amenorrhea and restoration of normal menstrual flow.

Statistical methods: Statistical analysis was performed using SPSS for Windows version 20.0. Difference between two related groups was analyzed using paired student's t-test with the mean paired difference (MPD) and its 95% confidence interval (95% CI); or McNemar's test with the risk ratio (RR) and its 95% CI. Significance level was set at 0.05.

Results

A total of 30 women were recruited in the current trial. The mean age of included women was 30.1 ± 6.3 years (range: 20-45 years). Of the included women, 8 (26.7%) were nulliparous, 22 (73.3%) were parous; 23 (76.7%) had intrauterine adhesions [Asherman's syndrome], while 7 (23.3%) had other pathologies [submucous myoma, endometrial polyp or uterine septum].

The postoperative mean values of uterine length were significantly higher when compared to the preoperative values in included women (those who had and those who did not have Asherman's syndrome) (Table 1).

Uterine Length (cm)	Preoperative	Postoperative	MPD (95% CI)	*P*[1]
All Women (n=30)	3.4 ± 0.5	6.1 ± 0.8	2.8 (2.6 to 2.9)	<0.001
Women with Asherman's Syndrome (n=23)	3.3 ± 0.5	6.1 ± 0.8	2.7 (2.5 to 3.0)	<0.001
Women without Asherman's Syndrome (n=7)	3.5 ± 0.4	6.3 ± 0.8	2.8 (2.4 to 3.3)	<0.001

Data presented as mean ± SD
MPD (95% CI) mean paired difference and its 95% confidence interval
[1]Analysis using paired student's t-test
Table 1: Difference between Pre- and Postoperative Uterine Lengths in Included Women

The postoperative mean values of duration of menses were significantly higher when compared to the preoperative values in included women (those who had and those who did not have Asherman's syndrome) (Table 2).

Duration of Menses (days)	Preoperative	Postoperative	MPD (95% CI)	*P*[1]
All Women (n=30)	1.8 ± 1.5	3.1 ± 1.4	1.2 (0.9 to 1.6)	<0.001
Women with Asherman's Syndrome (n=23)	1.4 ± 1.4	2.7 ± 1.4	1.3 (0.8 to 1.8)	<0.001
Women without Asherman's Syndrome (n=7)	3.3 ± 1.0	4.3 ± 0.8	1.0 (0.5 to 1.5)	<0.001

Data presented as mean ± SD
MPD (95% CI) mean paired difference and its 95% confidence interval
[1]Analysis using paired student's t-test
Table 2: Difference between Pre- and Postoperative Durations of Menses in Included Women

Among women with Asherman's syndrome, the postoperative rates of amenorrhea and postoperative rates of moderate/severe intrauterine adhesions were significantly lower when compared to the preoperative rates. Placement of freeze-dried amnion graft after operative hysteroscopy was significantly associated with almost 9-fold and 3-fold reduction in the rates of amenorrhea and moderate/severe adhesions, respectively (Table 3, Figure 1).

Women with Asherman's Syndrome (n=23)	Preoperative	Postoperative	*P*[1]	RR (95% CI)	NNT
Amenorrhea	9 (39.1%)	1 (4.3%)	<0.001	9.0 (1.24 to 65.42)	3
Moderate/Severe Intrauterine Adhesions	22 (95.7%)	7 (30.4%)	<0.001	3.14 (1.68 to 5.87)	2

Data presented as number (percentage)
RR (95% CI) risk ratio and its 95% confidence interval
[1]Analysis using McNemar's test
NNT number needed to treat
Table 3: Difference between Pre- and Postoperative Rates of Amenorrhea and Moderate/Severe Intrauterine Adhesions in Included Women with Asherman's Syndrome

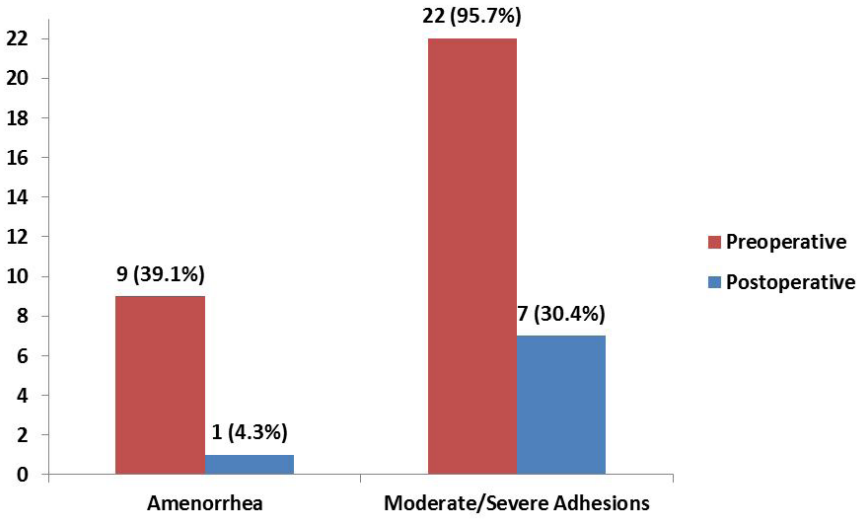

Figure 1: Bar-Chart showing Difference between Pre- and Postoperative Rates of Amenorrhea and Moderate/Severe Intrauterine Adhesions in Included Women with Asherman's Syndrome

Discussion

The current study showed that placement of intrauterine freeze-dried AM graft following operative hysteroscopic procedure was associated with significant improvement regarding uterine length, duration of menses and risk of recurrence of moderate/severe IUA.

In a previous two published studies, Amer et al. used fresh AM grafts after hysteroscopic adhesiolysis. In the first non-randomized pilot study, Amer and Abd-El-Maeboud compared postoperative placement of intrauterine AM graft (n=12) versus placement of intrauterine inflated balloon of a Foley's catheter (n=13) [3]. The rates of amenorrhea 4 months postoperatively were 16.7% and 23.1%, respectively. The rates of recurrent adhesions (though minimal in most cases) were 0% and 100% in both groups, respectively [3].

In the second randomized controlled study, Amer et al. compared intrauterine placement of inflated balloon of a Foley's catheter (n=15) versus placement of fresh AM graft (n=15) versus placement of dried AM graft (n=15). The rates of restoration of normal menses were 26.8%, 35.7% and 46.7%, respectively. The rates of pregnancy were 80% and 20% in women who had AM graft and those who did not, respectively [10].

Freeze-drying of AM graft offers three advantages: proper disinfection, long-term preservation and maintenance of structural integrity of the AM graft, which allows for adequate re-epithelialization and restoration of normal endometrial lining.

When compared to other alternatives, placement AM graft after hysteroscopic adhesiolysis seems to be associated with better outcomes. In a study conducted by Lin et al., placement of intrauterine heart-shaped balloon (n=82) was compared to placement of intrauterine device (n=80) following hysteroscopic adhesiolysis, the rates of adhesion reformation after 1-2 months were 30% and 35%, respectively [8].

In a study conducted on 90 women who underwent hysteroscopic resection of uterine septum, postoperative estrogen treatment was not found to prevent intrauterine adhesion or improve reproductive outcomes [6].

In conclusion, placement of intrauterine freeze-dried AM graft following hysteroscopic adhesiolysis seems to be

significantly associated with improved uterine length, reduced rates of amenorrhea and reduced rates of recurrent adhesions.

References

1. Yu D, Li TC, Xia E, Huang X, Liu Y, et al. (2008) Factors affecting reproductive outcome of hysteroscopic adhesiolysis for Asherman's syndrome. Fertil Steril 89: 715-22.

2. Nappi C, Di Spiezio Sardo A, Greco E, Guida M, Bettocchi S, et al. (2007) Prevention of adhesions in gynaecological endoscopy. Human Reproduction Update 13: 1-16.

3. Amer MI, Abd-El-Maeboud KH (2006) Amnion graft following hysteroscopic lysis of intra uterine adhesions. J Obstet Gynecol Research 32: 559-66.

4. Salma U, Xue M, Md Sayed AS, Xu D (2014) Efficacy of intrauterine device in the treatment of intrauterine adhesions. Biomed Res Int 589-96.

5. Chen FP, Soong YK, Hui Y (1997) Successful treatment of severe uterine synechiae with transcervical resectoscopy combined with laminaria tent. Hum Reprod 12: 943-7.

6. Roy KK, Negi N, Subbaiah M, Kumar S, Sharma JB, et al. (2014) Effectiveness of estrogen in the prevention of intrauterine adhesions after hysteroscopic septal resection: a prospective, randomized study. J Obstet Gynaecol Res 40: 1085-8.

7. Lin X, Wei ML, Li TC, Zhou F, Zhang SY (2014) A prospective randomized control trial to compare the efficacy of intrauterine balloon and intrauterine contraceptive device in the prevention of adhesion reformation following hysteroscopic adhesiolysis. Gynecol Surg 11: 89-90.

8. Lin XN, Zhou F, Wei ML, Yang Y, Li Y, et al. (2015) Prospective, randomized, controlled trial comparing the efficacy of intrauterine balloon and intrauterine contraceptive device in the prevention of adhesion reformation after hysteroscopic adhesiolysis. Ferti Steril 104: 235-40.

9. Di Spiezio Sardo A, Calagna G, Scognamiglio M, O'Donovan P, Campo R, et al. (2016) Prevention of intrauterine post-surgical adhesions in hysteroscopy. A systematic review. Eur J Obstet Gynecol Reprod Biol 203: 182-92.

10. Amer MI, Abd-El-Maeboud KHI, Abdelfatah I, Salama FA, Abdallah AS (2010) Human amnion as a temporary biologic barrier after hysteroscopic lysis of severe intrauterine adhesions: pilot study. J Min Invas Gynecol 17: 605-11.

11. Arora M, Jaroudi KA, Hamilton CJ, Dayel F (1994) Controlled comparison of Interceed and amniotic membrane graft in the prevention of postoperative adhesions in the rabbit uterine horn model. Euro J Obstet Gynecol Reprod Biol 55: 179-82.

12. Morton KKE, Dewhurst C (1986) Human amnion in the treatment of vaginal malformations. Br J Obstet Gynaecol 93: 50-4.

13. Niknejad H, Deihim T, Solati-Hashjin M, Peirovi H (2011) The effects of preservation procedures on amniotic membrane's ability to serve as a substrate for cultivation of endothelial cells. Cryobiology 63: 145-51.

14. Malhotra C, Jain AK (2014) Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. World J Transplant 4: 111-21.