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# Human Amnion as a Temporary Biologic Barrier after Hysteroscopic Lysis of Severe Intrauterine Adhesions: Pilot Study

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**ABSTRACT** **Study Objective:** To estimate the efficacy of fresh and dried amnion graft after hysteroscopic lysis of severe intrauterine adhesions in decreasing its recurrence and encouraging endometrial regeneration.

**Design:** Pilot prospective randomized comparative study (Canadian Task Force classification I).

**Setting:** Ain Shams Medical School, Cairo, Egypt.

**Patients:** Forty-five patients with severe intrauterine adhesions. Primary symptom was infertility with or without menstrual disorders such as amenorrhea or hypomenorrhea.

**Interventions:** Patients were randomized preoperatively using a computer-generated randomization sheet into 3 groups of 15 patients each. Allocation to any group was concealed in an opaque envelope, which was opened at the time of operation. Hysteroscopic lysis of intrauterine adhesions was followed by insertion of an intrauterine balloon only (group 1) or either fresh amnion graft (group 2) or dried amnion graft (group 3) for 2 weeks. Diagnostic hysteroscopy was performed at 2 to 4 months postoperatively.

**Measurements and Main Results:** Adhesion grade, menstruation, uterine length, complications, and reproductive outcome were determined. There was significant improvement in adhesion grade with amnion graft vs intrauterine balloon alone ($p = .003$). Improvement was greater with fresh amnion than with dried amnion ($p = .01$). Normal menstruation occurred in 4 patients (28.6%) in group 1, 5 (35.7%) in group 2, and 7 (46.7%) in group 3. Of 43 patients, 41 (95.3%) were treated in 2 endoscopic sessions (95.3%), and 2 patients (4.7%) were treated in 3 endoscopic sessions. Uterine perforations occurred in 2 patients (4.7%), and cervical tears in 3 (7.0%). Ten patients (23.3%) achieved pregnancy, 8 (80%) after amnion graft and 2 (20%) without amnion. Six of the 10 patients (60%) miscarried, and 4 (40%) were either still pregnant or delivered at term without complications.

**Conclusion:** Hysteroscopic lysis of severe intrauterine adhesions with grafting of either fresh or dried amnion is a promising adjunctive procedure for decreasing recurrence of adhesions and encouraging endometrial regeneration. Journal of Minimally Invasive Gynecology (2010) 17, 605–611 © 2010 AAGL. All rights reserved.

*Keywords:* Amnion graft; Hysteroscopy; Intrauterine adhesions; Intrauterine balloon; Reproductive outcome

Intrauterine adhesions (IUAs) are permanent adherents to the uterine walls with partial or complete obliteration of the uterine cavity after trauma to the basalis layer of the endometrium [1]. The syndrome occurs most frequently after incomplete abortion (50%), postpartum hemorrhage (24%), or elective abortion (17.5%) [2], and less commonly after myomectomy, hysterotomy, diagnostic curettage, cesarean section, tuberculosis, or uterine packing [2,3]. The prevalence of IUA ranges from 1.5% in women with infertility to 7% in women with secondary amenorrhea [4].

The pattern of obliteration of the uterine cavity differs according to the causative insult. In endometritis, it is lost entirely, including the cornual regions, which are spared after curettage [1]. In the most severe cases, the endometrial cavity can be entirely obliterated, without any evidence of viable endometrium. There is an individual constitutional element that causes certain patients to develop a severe form of IUA and others to be unaffected while undergoing the same traumatic procedures. This concept may also explain

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why some patients respond well to treatment, whereas others demonstrate recurrent adhesions [5].

Patients with IUAs may have amenorrhea, hypomenorrhea, dysmenorrhea, infertility, recurrent miscarriages, premature labor, and abnormal placentation [6]. Hysterosalpingography, hysteroscopy, ultrasound, and magnetic resonance imaging have been used to diagnose IUA, and treatment with operative hysteroscopy has replaced dilation and curettage or blind dissection [2,7].

It has been reported that either an intrauterine contraceptive device [8] or Foley catheter balloon [8,9] can be used to maintain separation of the raw (endometrium-free) areas of uterine walls separated after adhesiolysis. Auto-cross-linked hyaluronic acid gel has also been proposed as a means to prevent IUAs [10].

An ideal adjunctive therapy would be application of a biologically active mechanical separator that achieves 2 goals: suppression of adhesion formation and promotion of endometrial regeneration. Amnion seems to be promising in this respect [11]. Although fresh amnion has been used with some success as a dressing [12], its use has not gained much acceptance because of the risk of disease transmission and the inconvenience of retrieval. Lyophilized amnion (freeze-dried) is commercially available, and can be conveniently transported and stored, and careful screening in conjunction with processing and terminal sterilization ensures its safety [13]. The objective of the present study was to estimate the safety and efficacy of amnion grafts, both fresh and dried, as a temporary biologic barrier after hysteroscopic lysis of IUAs.

## Material and Methods

The study was approved by the institutional review board of Ain Shams Medical School and was conducted according to the guidelines on human experimentation of the 1975 Declaration of Helsinki. All patients provided signed informed consent.

This was a pilot, randomized, comparative study with blinded independent evaluation of changes in adhesion grade, menstruation, uterine length, number of operations needed to achieve a functional uterine cavity, reproductive outcome, and complications. The study was conducted from June 2004 to August 2009 in 45 women with severe IUAs diagnosed at office hysteroscopy [7]. Patients were randomized preoperatively using a computer-generated randomization sheet into 3 groups of 15 women each. Allocation to any group was concealed in an opaque envelope, which was opened at the time of operation. Group 1 received an intrauterine balloon without amnion graft, group 2 received fresh amnion, and group 3 received dried amnion. Infertility was the primary symptom, followed by hypomenorrhea or amenorrhea. A comprehensive infertility workup was performed, and patients having another cause of infertility and those with adhesions limited to the lower uterine segment or the upper cervical canal were excluded.

### *Amnion Graft Preparation*

Amnion and amniotic membranes are terms used variously in the literature. In this article, amnion is defined as the layer separated from the chorion, whereas amniotic membrane is defined as the membrane covering the amnion and chorion.

Preparation of fresh amnion graft has been described previously [14]. In brief, it was obtained 1 to 6 days preoperatively, under aseptic conditions, from healthy mothers who delivered via cesarean section and were proved seronegative for hepatitis B and C, human immunodeficiency virus, and syphilis using conventional serologic tests. Severely meconium-stained membranes were discarded. The amniotic membrane with the chorion was trimmed from the placenta and washed several times with sterile normal saline solution until grossly clean. Except for a small piece at the edge used to identify the chorionic side, the chorion was peeled off of the amnion using blunt dissection. The graft was stored at 4°C in sterile bags containing isotonic normal saline solution to which 50 000 IU of crystraline penicillin per 100 mL of saline solution was added [11].

Sterilized freeze-dried (lyophilized) amnion grafts were obtained from the Atomic Energy Organization of Egypt, and prepared according to the method of Nakamura et al [13].

## Surgical Technique

Two misopristol tablets, 200 μg, were inserted vaginally the night before the operation to facilitate cervical dilation. Operative hysteroscopy was performed with the patient under general anesthesia, in the follicular phase of the menstrual cycle; however, in women with amenorrhea, no special time was chosen. Simultaneous laparoscopy was performed in women with infertility if they did not undergo laparoscopy before or in those with previous complications of hysteroscopy such as uterine perforation or if uterine perforation occurred during the present procedure. Hystometry with uterine sounding was followed by lysis of IUAs using 5F pointed tips, semirigid scissors in a 5-mm rigid office hysteroscope, based on a 2.9-mm telescope (Karl Storz GmbH & Co. KB). In patients with thick fibrous adhesions, lysis was performed using a 9-mm working element along with a sheath and 4-mm 30-degree telescope (Karl Storz GmbH & Co. KB) equipped with a hysteroscopic monopolar knife (Collin operating knife) after cervical dilation to Hegar 9. The visualized adhesions were incised with 50- to100-W cutting current, adjusted according to visual tissue effects, from an isolated electrosurgical generator (Valleylab SSE2L; Valleylab, Inc., Boulder, CO). Glycine 1.5% (Glycocolle 1.5%; Aguettant Laboratory, Lyon, France) was used as distention medium, with intrauterine pressure between 120 and 150 mm Hg, automatically controlled using a Hamou Hysteromat (Karl Storz GmbH & Co KB) with termination of the procedure if fluid deficit exceeded 1 L.

In patients with a tubular uterine cavity, hysteroscopic metroplasty using the monopolar knife was performed, entailing 4 to 6 linear releasing incisions along the length of the uterine wall, from fundus to isthmus, perpendicular to the wall of the uterus, with decreasing depth of the incision as the section advanced toward the isthmus. The difficulty of the technique lies in the balance between the depth of the incisions and the risk of uterine perforation. To ensure safety, the depth of the incisions did not exceed 5 to 7 mm. A procedure was judged complete only when an adequate uterine cavity was obtained and, if possible, 1 or both tubal ostia were visualized.

### *Amnion Graft Application*

The freeze-dried amniotic membrane was hydrated using normal saline solution in a pan for 10 minutes before use [15]. The previously prepared fresh amnion graft was washed several times with sterile normal saline solution before application. Either amnion graft was cut to form a 5 × 5-cm piece. This was spread on the balloon end of an 8F pediatric Foley catheter so that the epithelial or basement membrane surface would be on top facing outward, where the inflated balloon acts as a mold for the amnion [11]. The catheter tip with the amnion on its surface was then introduced into the inside of the uterine cavity with the aid of a straight artery forceps. The balloon was inflated with 3 to 5 mL of saline solution. A loose knot was made in the catheter stem, which was then slipped upward to just below the inflated balloon, then tightened with the aid of the artery forceps, and the catheter stem was cut with scissors just below the knot after stretching the catheter stem so that the balloon with the graft on its surface was kept intrauterine. In patients with a patulous cervix that would not keep the inflated balloon inside the uterus, a cervical cerclage using braided polyester tape (Matrix Health Care SAE, Ameco, Egypt) was applied, and removed later with the balloon.

Postoperatively, ethinyl estradiol tablets (Laboratoires Cassenne, Puteaux, France), 50 μg/d, were administered for 50 days. All patients were discharged on the day of surgery, and were asked to report balloon expulsion or any sign or symptom of pelvic infection such as pelvic pain, fever, or abnormal vaginal discharge. Two weeks postoperatively, the balloon was removed transcervically using a crocodile forceps with the patient under paracervical anesthesia (lidocaine 2%, 6 mL, plus atropine, 0.5 mg in the same syringe), as an outpatient procedure without cervical dilation. In patients who had cervical cerclage, the tape was removed at the time of balloon extraction.

A second-look hysteroscopy was performed 2 to 4 months postoperative by an independent observer blinded to the method. The outcome measures assessed were improvement in adhesion grade, improvement in menstruation, increased uterine length at sounding, and complications. Subsequently, follow- up was via direct contact or telephone every 3 months for a mean (range) of 28 (6–60) months for menstrual pattern and fertility.

### *Statistical Analysis*

Data are given as count and percentage for categorical variables. Groups were compared using the $\chi^2$ test and Fisher exact test for categorized variables. For comparison of menstruation, uterine length, and adhesion score, the Kruskal-Wallis test was used. Data are given as median (interquartile range [IQR]; 25th–75th percentile). Pairwise comparison was performed using the Mann-Whitney test with Bonferroni correction. The critical level of significance was $<.02$.

Analyses were conducted using commercially available software (SPSS for Windows, release 15.0; SPSS, Inc., Chicago, IL). All p values refer to 2-tailed tests of significance, with $p < .05$ considered significant.

## Results

Of the 45 patients included in the study, 2 were lost to follow-up (1 each in groups 1 and 2), and were excluded from analysis. Median (range) patient age was 30.4 (26–40) years; parity was 1.2 (0–3); and the most probable cause of IUA was cesarean section (27 of 43 patients [62.8%]), followed by curettage after abortion (11 of 43; [25.6%]). The incidence of cesarean section was significantly higher in group 3 compared with either of the other 2 groups ($p = .003$). In 3 patients (7%), we did not find any obvious cause for IUA (Table1). There was no statistically significant difference between the 3 groups insofar as preoperative menstruation; amenorrhea was present in 8 of 14 patients (57.1%) in both groups 1 and 2, and in 9 of 15 patients (60%) in group 3 ($p = .99$). Uterine length was 5 cm or less in 12 of 14 patients in group 1 (85.7%), 6 of 14 in group 2 (42.9%), and 6 of 15 in group 3 (40%).

Reconstruction of a functional uterine cavity was achieved after 2 surgical sessions in 41 of 43 patients (95.3%), and after 3 sessions in 2 patients (4.7%), 1 each in groups 2 and 3. Cervical cerclage was performed in 4 patients (9.3%), 3 in group 2 and 1 in group 3. Fluid absorption was 305 (280–303) mL in group 1, 281 (220–500) mL in group 2, and 280 (247–340) mL in group 3 ($p = .99$). Blood loss was 30 (25–32) mL in group 1, 30 (25–33) mL in group 2, and 33 (30–35) mL in group 3 ($p = .06$). Procedure time including cervical dilation was 19 (17–23) min in group 1, 21(17–24) min in group 2, and 20 (17–23) min in group 3 ($p = .90$). No clinical evidence of infection was noted during the entire period of intrauterine stay of the graft or the balloon, and no difficulty was encountered in balloon extraction in any patient. Uterine perforations occurred in 2 patients (4.7%), 1 each in groups 1 and 3; both were treated conservatively. Cervical tears occurred in 3 patients (7%) during cervical dilation, 1 in each group, and spontaneous expulsion of the balloon occurred in 5 patients (11.6%), 2 in group 1, 2 in group 2, and 1 in group 3). In 8 of 29 patients (27.6%), 5 in group 2 and 3 in group 3, amniotic membrane was found attached to the uterine wall 2 months postoperatively, with new healthy endometrium underneath (Fig. 1).

Table 1
Characteristics of patients with grade III intrauterine adhesions[a]

| Variable | Group 1 (n = 14) | Group 2 (n = 14) | Group 3 (n = 15) | p Value |
|---|---|---|---|---|
| Age, yr | 30.5 (27–34) | 31.5 (29–35) | 30.5 (27–36) | .82 |
| Most probable cause (%) | | | | |
| Cesarean section | 7 (50)[b] | 6 (42.9)[c] | 14 (93.3)[d] | .009 |
| Dilation and curettage | 5 (35.7) | 5 (35.7) | 1 (6.7) | .11 |
| Pelvic inflammatory disease | 0 | 1 (7.1) | 0 | .98 |
| Genital tuberculosis | 0 | 1 (7.1) | 0 | .98 |
| Unknown | 2 (14.3) | 1 (7.1) | 0 | .14 |
| Menstruation, days | 2 (2–4) | 2 (2–4) | 2 (2–4) | .99 |
| Uterine length, cm | 5 (4.5–5) | 4.75 (4–5) | 5 (4.5–5) | .91 |

[a] Data are given as median (IQR) or No. (%). Bonferroni correction; critical level of significance, <.02.
[b] p = .02, b vs d.
[c] p = .007, c versus d.

There was an increase in uterine length and menstruation in all groups, but without statistical significance difference (p = .98 and p = .22, respectively). Adhesion grade was significantly reduced in the amnion graft groups vs the intrauterine balloon alone group (p = .003) (Table 2). Before surgery, in group 1, 8 women had amenorrhea, 4 had hypomenorrhea, and 2 had normal periods; in group 2, 8 women had amenorrhea, 5 had hypomenorrhea, and 1 had normal periods; and in group 3, 9 women had amenorrhea, 4 had hypomenorrhea, and 1 had normal periods. After surgery, in group 1, 3 women had amenorrhea, 7 had hypomenorrhea, and 4 had normal periods; in group 2, 2 women had amenorrhea, 4 had hypomenorrhea, and 8 had normal periods; and in group 3, 5 women had amenorrhea, 3 had hypomenorrhea, and 7 had normal periods. Improvement in adhesions grade, menstruation, and uterine length was greater with fresh amnion than with dried amnion but only statistically significant for the first (p = 0.01).

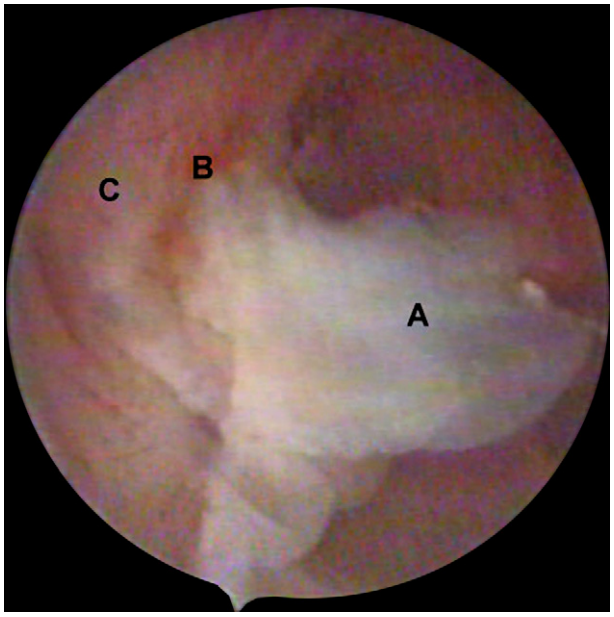


Fig. 1. Second-look hysteroscopy 2 months after surgery demonstrates amnion (A) attached to the uterine wall (B), with uncovered regenerating endometrium (C).

The pregnancy rate was 23.3% (10 of 43 patients). In the amnion graft groups, the rate was twice that in the intrauterine balloon alone group, but with no statistical significance (Table 3). All pregnancies were spontaneous except 3 that were achieved after in vitro fertilization (IVF). One pregnancy was terminated at 7 weeks' gestation because of a blighted ovum. Two patients underwent IVF treatment twice, but did not conceive. The other patients could not afford the cost of IVF. There were no obstetric complications in patients who are currently pregnant or who delivered during the study. All patients except 2 were followed up for at least 8 months.

## Discussion

Treatment of severe IUA is a great challenge in hysteroscopic surgery. Many studies fail to present their results according to the severity of the adhesions. In addition, lack of a universally accepted classification system makes comparison of treatment methods difficult [2,16]. The results of the present study are encouraging; adhesions grade was significantly reduced in the amnion graft groups vs the intrauterine balloon alone group (p = .003), with a nonsignificant increase in uterine length and menstrual flow. The improvement was greater with fresh amnion than with dried amnion, but was statistically significant only for adhesions grade (p = .01).

We acknowledge the shortcoming of relying on menstrual flow rather than fertility outcome as a sign of improvement after adhesiolysis. Although the presence of poor correlation between the diminution of menstrual bleeding and the surface of the endometrium involved with adhesions has been reported [17], none of our patients had pure cervicoisthmic adhesions that would infer good prognosis without amniotic grafting.

In the present study, cesarean section was the most common cause of IUA (62.8%) compared with other studies (2%–22%) [16,18]. This might be explained by either suboptimal surgical technique or increased incidence of infection [19] due to suboptimal antiseptic precautions, and the fact that most of the cesarean sections were unplanned. The incidence of IUA after abortion in this study was only

Table 2
Postoperative findings

| Variable | Group 1 (n = 14)[a] | Group 2 (n = 14)[a] | Group 3 (n = 15)[a] | p Value |
|---|---|---|---|---|
| Menstruation, days | 2 (1–3) | 3 (2–4) | 3 (2–4) | .27 |
| Menstruation improvement, days | 1 (1–2) | 2 (2–3) | 2 (1.0–2.5) | .22 |
| Uterine length, cm | 6 (5.5–7.0) | 6 (6.0–6.5) | 6 (5.25–7.0) | .93 |
| Uterine length increase, cm | 1 (1–2) | 1.5 (0.5–2.0) | 1 (1–2) | .98 |
| Adhesion score | 2 (2–2) | 1.5 (1–2) | 2 (1–2) | .27 |
| Adhesion score improvement | 1 (1–1) | 2 (1–3) | 2 (1.0–2.5) | .009 |

[a] Data are given as median (IQR). Group 2 had greater adhesion score improvement compared with group 1 (p = .006), and group 3 (p = .01). Bonferroni correction; critical level of significance, <.02.

25.6%, which is lower than in other studies [20], and could be explained by the lower incidence of induced abortion owing to religious constraints.

Adhesiolysis was performed using hysteroscopic scissors alone [2] or a monopolar knife (Collin knife). The monopolar knife has been used to divide thick fibrous adhesions and for linear myometrial incisions [21], in which it was found to be efficient, safe, and less expensive in a limited-resource setting. The possibility of thermal damage with adverse effects on development of the endometrium has been minimized by special consideration of mode, power output, duration of exposure to electrosurgical energy, and size of the electrode, along with cooling using continuous flow of irrigation fluid [22].

Some authors believe that hysteroscopic adhesiolysis under laparoscopic guidance to reduce the risk of uterine perforation is not necessary and not that preventive [20,23], whereas others are in favor of it [24]. In the present study, laparoscopy was not routine, but limited to certain indications as mentioned. Uterine perforation occurred in 2 patients (4.7%), an incidence similar to that reported by other investigators (2%–5%) [2,8]. Both were treated conservatively.

Recurrence of adhesions has been reported to occur in almost 50% of women with severe IUAs [2]; however, there was no mention of the number of patients with purely cervicoisthmic adhesions, which have a good prognosis, and only 1.6% of patients had IUAs after uterine surgery and not after cesarean section, and only 38.3% had total obliteration of the uterine cavity. In contrast, in the present study, 62.8% of IUAs developed after cesarean section which were thick and fibrous, with a poor prognosis [25], and all patients had complete obliteration of the cavity, tubular uterine cavity, or amalgamation of uterine walls.

Table 3
Reproductive outcome[a]

| Variable | Group 1 (n = 14) | Group 2 (n = 14) | Group 3 (n = 15) | p Value |
|---|---|---|---|---|
| No pregnancy | 12 | 10 | 11 | .62 |
| Pregnant | 2 (14.3) | 4 (28.6) | 4 (26.7) | |
| Ongoing or delivered at term | 1 (50) | 2 (50) | 1 (25) | .95 |
| Abortion in first trimester | 1 (50) | 2 (50) | 3 (75) | .32 |
| Total | 14 | 14 | 15 | |

[a] Data are given as No. (%).

We believe that linear myometrial incisions in the present study increased uterine perfusion by decreasing resistance exerted by contracture of fibrous tissues on the endometrial and subendometrial blood vessels, and that it increased the uterine cavity volume and exposed basal endometrium with possible stem cells remnants.

Several approaches have been described for prevention of adhesion recurrence [8,9]; however, there is no consensus on the postoperative regimen of choice. Significant improvement was noted in a previous study with the use of amnion graft [11], and use of amnion grafts in the present study holds hope, in view of contemporary opinion, because synechiae in such cases are invariably cohesive and tend to recur, and thus have a poor prognosis [17].

Several theories have been proposed regarding the source of cells for human endometrial regeneration; however, specific mechanisms have yet to be elucidated [26,27]. Stem cells exist in many, if not all, adult tissues as a small population of quiescent cells with the potential to regenerate the entire tissue in which they reside [28,29]. Human amnion epithelial cells (hAECs) produce factors or create a microenvironment for effective tissue repair and regeneration, possibly by stimulating endogenous stem cell populations. However, it is unclear whether the hAECs possess any inherent stem cell–like properties in vivo [30]. Indeed, banking of cord blood stem cells for future autologous use is now a well-established biotechnical industry in many countries [31], and more recently, the villous placenta [32], fetal membranes [33], and amniotic fluid [34] have been considered. Native hAECs express markers associated with pluripotent and multipotent stem cells. Another important aspect that makes hAECs and fetal membrane–derived stromal cells attractive for potential stem cell–based therapies is their low antigenicity. The hAECs also release several anti-inflammatory factors that lead to a significant reduction in HLA class II antigen-presenting cells found at sites of injury, prevent apoptosis [35], enhance wound healing and host cell proliferation [36], and suppress profibrotic transforming growth factor-beta isoforms and type II transforming growth factor-beta receptors in myofibroblast cells that deposit collagen, which leads to fibrosis [37]. Amniotic membrane has diverse properties against bacterial and viral infections [38], stores antibiotics and releases them over the course of a few days [39], and recently has been shown to

produce potent natural antimicrobials [40]. Clinical studies suggest that amniotic membrane as a biologic dressing has 2 primary favorable effects in addition to being an anatomical barrier. (1) It facilitates epithelialization by acting as a basement membrane substrate, facilitates migration of epithelial cells, reinforces adhesion of the basal epithelium, promotes cellular differentiation [41], and prevents cellular apoptosis [35], and (2) it inhibits inflammation and fibrosis.

Preservation of viability of fresh amniotic membrane has been demonstrated at 21 days after being placed in the pelvic cavity [42]. This is important because we and other researchers believe that raw surfaces should be kept apart for at least 7 days [8,43]. Although the recently suggested use of auto-cross-linked hyaluronic acid gel seems valuable in obviating cross-infection, it was able to keep uterine walls separated for only 3 days, and it lacks the putative advantages of a biological membrane [15].

In many parts of the world, ''fresh membrane'' is still used; however, in most Western countries, use of one or the other method of preservation is mandatory because of legislation requiring that the membrane be adequately screened for human immunodeficiency virus contamination. Freeze-dried (lyophilized) amnion addresses both of these issues because it is commercially available and can be conveniently transported and stored at room temperature for a long time without deterioration, and careful screening in conjunction with processing and terminal sterilization ensures its safety [13,44]. Clinically, we have observed that dried amnion is easier to handle and apply than fresh amnion, with less tendency for the balloon slipping during inflation, and because it is thin and pliable, it conforms closely to irregular surfaces of the uterine walls after adhesiolysis and myometrial scoring.

Data clearly show that viable amniotic epithelial cells have a beneficial effect on secretion of anti-inflammatory factors, and may be more effective in suppression of inflammation than the use of epithelium-deprived amniotic membrane. Available evidence, however, supports the notion that viability of the tissue components of the amniotic membrane is not essential for its biological effectiveness [45]. It has been reported that the amniotic membrane stroma contains growth factors [46], natural inhibitors of various proteases [47] and antiangiogenic substances [48], inhibitors of metalloproteinases and nitric oxide synthase, and potent anti-inflammatory proteins including interleukin (IL)–10 and IL-1 receptor antagonists [48], which makes dried amnion of great value in facilitation of epithelialization, tissue healing, and inhibition of inflammation and fibrosis. Lack of significant differences in efficacy of using fresh and dried amnion in the present study supports this. Most authorities agree that estrogen after synechiolysis is important to stimulate endometrial growth; however, little evidence exists to suggest the ideal dosing regimen or duration of therapy [6,49]. In the present study, estrogen was used for 50 days postoperatively to postpone shedding of the newly formed endometrium, to allow time for the amniotic membrane to act as a graft rather than a patch [46].

It has been reported that the incidence of intrauterine pregnancy after hysteroscopic lysis of severe IUAs ranges from 22.9% to 45.3%, and of live births from 28.7% to 32.1% [50,51]. However, in most of these studies, cases with IUAs after surgical trauma, known to have a poor prognosis because they are thick and fibrous, were excluded, whereas cases with purely cervicoisthmic adhesions, known to have good prognosis, were not. In the present study, 10 women (23.3%) conceived, and the rate in the amnion graft groups was double that of the intrauterine balloon alone group, but the difference was not statistically significant. This apparently low rate could be explained in that most adhesions in this series occurred after cesarean section delivery, which is known to be associated with myometrial fibrosis and decreased uterine perfusion [52]. In addition, most patients in this study did not undergo IVF because of financial constraints. Severe obstetric complications in subsequent pregnancies have been described by many authors [53,54]; however, in our series, no obstetric complications other than 4 miscarriages and 1 pregnancy terminated because of a blighted ovum after IVF.

## Conclusion

Hysteroscopic lysis of IUAs followed by amnion graft, either fresh or dried, is a promising adjunctive procedure for decreasing recurrence of adhesions and encouraging endometrial regeneration. Both grafts seem to be equally effective; however, dried amnion graft holds some advantages insofar as availability, prevention of cross-infection, and easier surgical application. Despite these promising findings, this preliminary report included a small number of patients, and valid conclusions cannot be drawn. Because of the paucity of such cases, a large multicenter study to confirm these findings seems warranted.

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