A placental membrane contains the necessary “ingredients” for developing an extracellular matrix that can repair damaged tissue. The placental membrane contains a number of components that are imperative in the development of this foundational extracellular matrix, such as growth factors and collagen, which forms fibrils that provide structure for soft tissues. In addition to collagen, it is the growth factors that signal cells to regenerate and rebuild the body. The placental membrane captures the naturally rich growth factors in a sheet format that be easily applied during surgery. The placental membrane will resorb naturally in the body within 3 weeks.

The underlying premise of the placental membrane is that it provides a barrier layer with a robust source of tissue and vascular growth factors and provides a local anti-inflammatory environment, thus optimizing soft tissue healing of the surgical site (1,2,3). The broad number of properties found in a placental membrane, coupled with its immunologically privileged status presents it to be utilized in a number of future surgical applications.

References

1. Zelen CM, Serena TE, Denoziere G. A prospective randomised comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. Int Wound J 2013;10:502-7.

2. Patel VR, Samavedia S, Bates AS. Dehydrated Human Amnion/Chorion Membrane Allograft Nerve Wrap Around the Prostatic Neurovascular Bundle Accelerates Early Return to Continence and Potency Following Robot-assisted Radical Prostatectomy: Propensity Score-matched Analysis. Eur Urol 2015;67:977-80.

3. Fetterolf D, Savage R. Dehydrated human amniotic tissue improves healing time, cost of care. Today's Wound Clinic 2013:19-20.

The following pages contain the previously listed references and additional supporting studies.

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Human vital amniotic membrane reduces adhesions in experimental intraperitoneal onlay mesh repair

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Abstract
Background Various antiadhesive coatings have been proposed for intraperitoneal onlay meshes (IPOM). However, adhesions, mesh infections, and impaired integration remain clinically relevant problems. In this experiment, human vital amniotic membrane (AM) was tested as antiadhesive mesh coating. Vital AM complies with clinical standards of product safety.
Methods In this study, 24 rats were randomized to one control or two treatment groups (n = 8). An uncoated polypropylene mesh (Vitamesh) was implanted using open IPOM technique and fixed with four sutures. In the treatment groups, vital AM was attached to Vitamesh by fibrin sealant fixation. The observation period was 7 and 17 days. Vitamesh fixed by suture only served as the control condition (17 days). Adhesion formation, tissue integration, and neovascularization were assessed macroscopically and histologically.
Results All the meshes in the control group elicited severe adhesions. Vital AM was highly efficient in reducing adhesions to mesh and sutures. No foreign body reaction or unfavorable immunologic response to vital AM occurred. Tissue integration and neovascularization of coated meshes were good. Fibrin sealant yielded a reliable fixation.
Conclusion Human vital AM was highly effective in reducing adhesions to polypropylene mesh and sutures in experimental IPOM. No adverse effects were detected, and tissue integration of the mesh was good.

Keywords Antiadhesives · Hernia repair · Human amniotic membrane · Intraperitoneal mesh placement

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Intraabdominal peritoneal onlay mesh repair (IPOM) can be considered a well-established technique for selected patients [1, 2]. The laparoscopic view allows detection of multiple and occult hernia defects and offers undeniable advantages, especially for obese patients [3, 4].

For more than two decades, extended polytetrafluoroethylene (ePTFE) was the material of choice for antiadhesive coating of IPOM meshes [4, 5]. However, its undisputed efficacy for adhesion prevention is opposed by its susceptibility to infection [6]. The incidence of ePTFE mesh infection is as high as 10% in the literature and usually requires explantation [7, 8]. Shrinkage, wrinkling, and seroma formation are other frequent complications of ePTFE, leading to the development of new mesh coatings [9, 10].

Polylactic acid, methyl cellulose, and collagen matrices, currently used for adhesion prevention in modern IPOM meshes, are less susceptible to infection [11, 12]. However, adhesions remain a clinically relevant problem, which is not restricted to mesh technology. It becomes apparent that the formation of adhesions to perforating fixation devices (PFDs) is a frequent cause for small bowel obstruction [13–15].

Consequently, an optimized interaction of mesh and PFD seems desirable. This formed the rationale for testing

human vital amniotic membrane (AM) in an experimental model of IPOM and fixing it to the mesh with fibrin sealant [16, 17]. This biologic matrix has been used successfully for wound repair and regeneration [18–20].

A vital AM was used with mixed results in experimental hernia research almost a decade ago [16]. However, the irradiation and chemical processing required for devitalization of AM seemed a major disadvantage to us due to the inalterable deletion of adherent multi- and pluripotent stem cells [21–23]. Pluripotent stem cell marker-positive cells are scattered in the amniotic epithelium [24].

The primary outcome parameters in our study were adhesion formation and foreign body reaction. The secondary outcome parameters were tissue integration and dislocation of vital AM attached to the mesh by means of fibrin sealant only.

Methods

Male Sprague-Dawley rats weighing 400–450 g were obtained from the Institut fuer Labortierkunde und genetik der Medizinischen Fakultaet der Universitaet Wien, Himberg, Austria. The mesh used in this study was Vitamesh, a lightweight macroporpous polypropylene mesh manufactured by Proxy Medical (Galway, Ireland). The fibrin sealant was Artiss (four units of thrombin) manufactured by Baxter (Vienna, Austria).

The mesh and fibrin sealant were supplied by courtesy of their manufacturers. Vital AM was obtained from the Red Cross Blood Transfusion Service of Upper Austria, produced under "good manufacturing practice" (GMP) conditions. All reagents used were of analytical grade, and surgery was performed under sterile conditions at the Ludwig Boltzmann Institute in Vienna. The study protocol was approved by the authority of the Vienna city government.

Group randomization

The 23 rats in this study were randomized to one control and two treatment groups as follows:

- Control group ($n = 8$): Vitamesh was fixed by suture only, and the observation period was 17 days. Although an uncoated polypropylene mesh should not be used clinically in IPOM repair, it was chosen as a reliable and reproducible control group substance.
- Treatment group 1 (T1; $n = 8$): Vitamesh and PFD were covered with vital AM, the observation period was 7 days.
- Treatment group 2 (T2; $n = 8$): Vitamesh and PFD were covered with vital AM, and the observation period was 17 days.

The observation periods were chosen to detect early adhesion formation, dislocation of vital AM after full degradation of fibrin sealant, and possible foreign body reaction to the xenograft.

Processing of vital amnion

Placentas after cesarean section were collected with informed consent from the donors. Further processing was performed as described previously [17]. Briefly, AM was peeled off the placenta and washed extensively with phosphate-buffered saline (PBS). Subsequently, 3 × 3-cm AM transplants for grafting as well as microbiologic and viability testing were prepared. For microbiologic and initial viability testing, punch biopsies (diameter, 8 mm) were taken from a separate 3 × 3-cm transplant.

The AM grafts were attached to nitrocellulose disks (Millipore, Billerica, USA) and wrapped in 15-ml tubes with 10 ml of a cryoprotective medium containing RPMI 1640 (PAA, Pasching, Austria), FCS (PAA), DMSO (WAK Chemie, Steinbach, Germany), L-Glut (PAA), and an antibiotic–antimycotic solution (PAA). The transplants then were frozen with a controlled-rate freezer and cryopreserved at −80°C.

Before in vivo application, the AM grafts were thawed and washed several times with PBS. One cryopreserved transplant was taken for residual viability testing with the EZ4U cell proliferation and cytotoxicity assay (Biomedica, Vienna, Austria) as described by Hennerbichler et al. [17] (Fig. 1). All AM transplants were microbiologically negative and viable.

Implant size

The Vitamesh was precut to 2 × 2 cm^2 in the rats. The size of the implants was fitted to the anatomic and technical requirements (i.e., adequate distance to wound margins and explantation without mechanical damage to the samples). Vital AM was 3 × 3 cm^2 in T1 and T2.

Surgical model

The described procedure reproduces the open IPOM technique, placing the implant on the intact peritoneum and attaching it to the abdominal wall with a PFD [13].

Surgery in rats

The 23 rats were anesthetized with an intramuscular injection of Ketavet (ketamine-hydrochloride 100 mg/ml; Pharmacia, Erlangen, Germany) and Rompun (xylazine-hydrochloride; Bayer, Leverkusen, Germany).

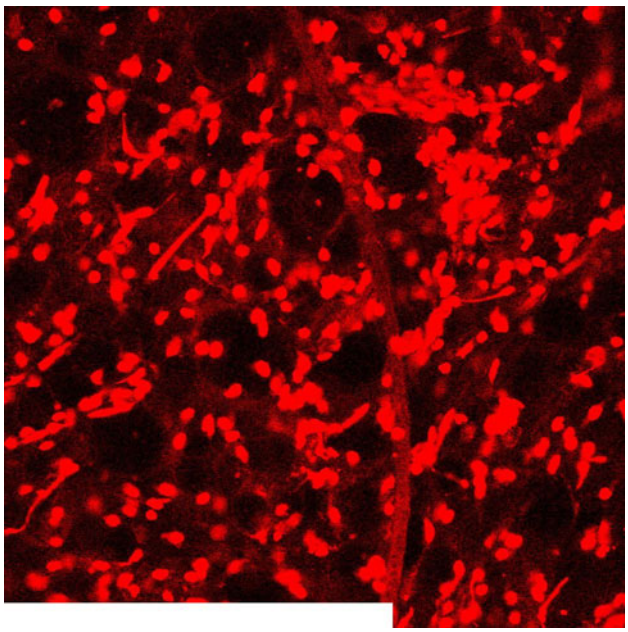


Fig. 1 Viable amniotic cells of in a sample which was implanted in this trial. The methodof EZ4U cell proliferation and cytotoxicity assay has beeen published previously. According toliterature, pluri potent stem cell marker positive cells are scattered in the amniotic epithelium

The abdomen was thoroughly shaved, and skin disinfection was performed. Subsequently, the skin was incised with a scalpel, and the subcutaneous fat tissue was bluntly detached from the abdominal muscles. A U-shaped laparotomy was made at the epigastral level from left to right, beginning and ending about 1.5 cm under the lateral rib cage. The abdominal wall was flipped caudally and the peritoneum exposed, allowing a direct view of the implant site. The mesh was placed on the peritoneum in a midline position with a distance of at least 1 cm between the implant margins and the incision. The mesh was sutured at all four corners of the Vitamesh (Synthofil 4/0; Ethicon, Norderstedt, Germany).

In the control group, the operation ended at this point. In the treatment groups (T1, T2), vital AM was attached to the Vitamesh with 0.2 ml of fibrin sealant. The mesh was spray-covered with the fibrin sealant (Easy Spray; Baxter, Vienna, Austria). The skin incision was closed in anatomic layers, and 1 ml of physiologic saline was administered subcutaneously to compensate for dehydration.

Postoperative care of the rats

The rats were kept in single cages during the remaining observation periods and checked daily for signs of infection, seroma formation, or abscess formation. Analgesic treatment (2 mg/kg bodyweight intramuscular application of Temgesic [buprenorphine], Merck, Vienna, Austria) was routinely applied once daily for 3 days postoperatively.

Autopsy of the rats

The rats were killed as scheduled in the randomization protocol under anesthesia by an intravenous injection of thiopental 1 ml (1 g; Sandoz, Kundl, Austria).

Macroscopy

Seroma formation, signs of local inflammation, and tissue integration were independently assessed by two investigators blinded to group assignment. The macroscopic score, described previously, is based on an A (no alteration) B (modest alteration) C (severe alteration) scale that already has been used reliably in studies on bio- and synthetic meshes [13].

Seroma formation

Absence of seroma was scored as A. A seroma (encapsulation with fluid) closely adjacent to the implant or containing less than 0.5 ml of fluid (verified by needle aspiration) was scored as B, and a seroma formation containing more than 0.5 ml of fluid was scored as C.

Local inflammation

No visible inflammation (defined as unfavorable inflammation with pus and debris) was scored as A. Small amounts of debris and pus were scored as B, and abscess formation was scored as C.

Tissue integration

Complete integration of the whole implant (tissue ingrowth and vascularization visible to the naked eye) was scored as A. An implant only partly integrated (<50% of surface area) was scored as B, whereas no detectable integration (e.g., no tissue ingrowth through the interstices of the mesh, edges of the implant not integrated) was scored as C.

Dislocation

Dislocation was defined as visible detachment of the mesh from the underlying abdominal wall. The implant found in its original position with all four edges adjacent to abdominal wall was scored as A. An implant with up to 30% of its surface area detached was scored as B, and any dislocation more severe (>30% free floating in the abdominal cavity) was scored as C. Failure of a suture knot led to a scoring of A in favor of the implant material. The dislocation of AM from Vitamesh was scored accordingly. A shrinkage of the mesh less than 10% of its original size was scored as A. Shrinkage of 10–30% was scored as B, and more than 30% shrinkage was scored as C.

Adhesions

Adhesions in the rats were scored according to the score first described by Vandendael (Table 1). This score offers the advantage of combining precise parameters describing strength, width, and amount of adhesion.

Histology

After macroscopic evaluation, all samples were fixed in 10% buffered formaldehyde solution (Merck) and embedded in paraffin. For the distinction of native and human (AM) collagen, 5-μm sections were stained with hematoxylin and eosin (H&E) and Picrosirius red. Blinded analysis and grading for the following parameters were performed [25]:

- Foreign body reaction (defined as prolonged neutrophil response, foreign body giant cells, and necrosis)
- Macrophages
- Lymphocytes and plasma cells
- Tissue integration based on neovascularization and fibroblast ingrowth.

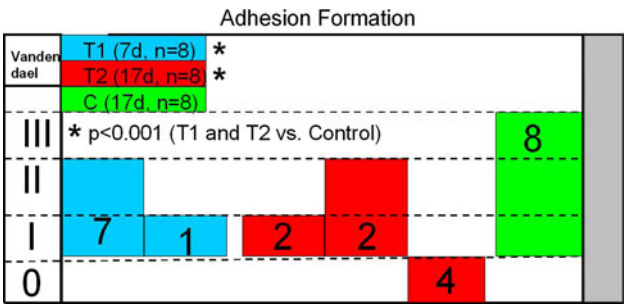
Histologic grading

The histologic grading scale consisted of 0 (no alteration), 1 (moderate alteration), 2 (strong alteration), and 3 (maximum alteration) compared with the tissue of native rats. This histologic grading system has been described previously [25].

Statistical analysis

Statistical analysis of the Vandendael adhesion scores between the treatment and the control groups was performed. The Kruskal-Wallis test and the Mann-Whitney *U* test were applied. A *P* value less than 0.005 was considered statistically significant.

Table 1 Adhesion formation: the results of the treatment groups and the control groups in terms of adhesion formation as assessed with the Vandendael score. There was a significant difference between treatment and control groups and adhesions to VITAL AM covered meshes were only mild and of low clinical relevance



Results

Intraoperative observations

The handling of vital AM was superb due to the clear distinction between the visceral and parietal sides. Vital AM easily slipped on mesh surface. The slow-clotting fibrin sealant allowed repositioning for 120 s.

Macroscopy

Excellent results were achieved in the treatment groups (T1 and T2) for the parameters "seroma formation," "local inflammation," "tissue integration," "dislocation," and "shrinkage," which, based on their absence, could all be scored A (Fig. 2). No macroscopic signs of unfavorable inflammation occurred in any sample, but neovascularization and an equal integration of the mesh were detectable. The meshes were firmly in place at autopsy, and no dislocation was found. The presence of vital AM amnion could not be verified macroscopically. Vitamesh showed no signs of shrinkage or contraction by surrounding scar tissue.

In the control group, the meshes were well integrated and free of seromas, but the macroscopic picture was expectedly dominated by massive adhesions and a pronounced local inflammation (score of C).

Adhesion formation

The results are illustrated in Table 1. Vital AM substantially reduced adhesions in T1 and T2. It was most remarkable how mesh and sutures were covered by a homogeneous, transparent cellular layer, interpreted as the ingrown residual vital AM. In T1 (observation period, 7 days) seven of eight meshes showed only mild adhesions (as measured by the Vandendael score) and translated to a clinically unproblematic finding (i.e., low risk of small bowel obstruction). One mesh in T1 showed moderate adhesions.

In T2 (observation period, 17 days), four meshes were free of adhesions, two meshes showed mild adhesions, and two meshes showed moderate adhesions. No mesh in T1 or T2 elicited severe adhesions, and the PFDs also were free of adhesions (Fig. 2).

In the control group, all the meshes were covered with severe adhesions, and it was apparent that the margins of the Vitamesh and sutures were the most critical areas.

The differences between the T1, T2, and control groups were highly significant according to both the Kruskal-Wallis and the Mann-Whitney *U* tests ($P < 0.001$). The differences between T1 and T2 were not significant ($P = 0.328$).

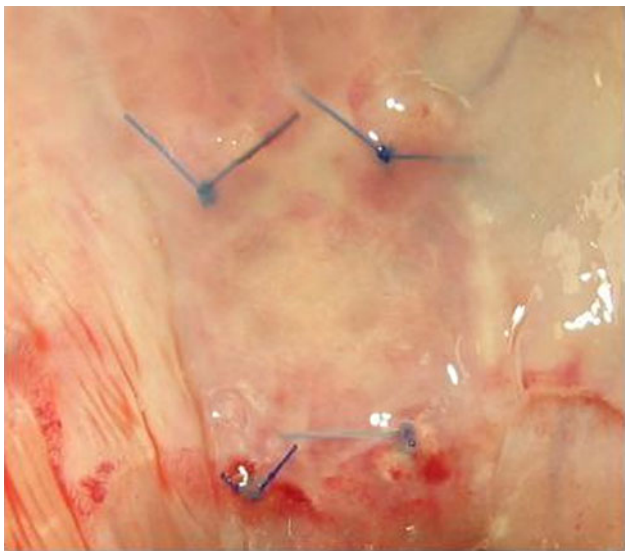


Fig. 2 17 days after implantation VITAL AM coated meshes appeared well integrated and were usuallyspared from adhesion formation. This Figure illustrates that even the sutures which have beencovered by VITAL AM are free of adhesions. The results obtained with VITAL AM were outstandingin terms of mesh integration and adhesion prevention

Histology

The H&E staining confirmed the absence of a foreign body reaction to vital AM. The scar formation around AM-coated meshes appeared physiologic, characterized by an equal deposition of collagen and ingrowth of vessels and leading to excellent implant integration. Macrophages, lymphocytes, and plasma cells were sparse, and no signs of an active inflammatory response were detected. These parameters were rated 1 and translate to an excellent performance of the xenogenic vital AM in rats.

Picrosirius red staining [26] was a useful tool for demonstrating the persistence of vital AM, which was already well integrated, providing protection from adhesions (Figs. 3, 4) at both time points of observation. No granulomatous cells were found in its vicinity. The clear distinction of vital AM verified the satisfying biocompatibility. The integration of uncoated meshes was comparable, but foreign body reaction was pronounced (histologic score, 2) due to the direct contact of the polypropylene with the viscerum.

Discussion

Vital AM provided excellent adhesion prevention and showed good biocompatibility, eliciting only a mild local inflammatory response (Fig. 2). The fibrin sealant served as safe fixation of vital AM and prevented its dislocation in the tested observation periods. Our findings with vital AM support the assumption that biologically active coatings could be specifically useful for adhesion prevention and tissue integration in hernia repair.

Voskerician et al. [27, 28] reported similar beneficial results achieved with human peritoneal membrane in experimental IPOM. Viability of AM seemed mandatory for its full benefits and potential to be realized. Vital AM contains pluripotent epithelial and mesenchymal stem cells, which otherwise would have been erased by irradiation or chemical processing [21–23, 29, 30].

As shown by our results, vital AM formed a functional layer that did not impair mesh integration but provided excellent adhesion prevention (Figs. 3, 4). Vital AM showed excellent handling characteristics, which definitely set it apart from many commercially available coatings that are difficult to insert through laparoscopic trocars and

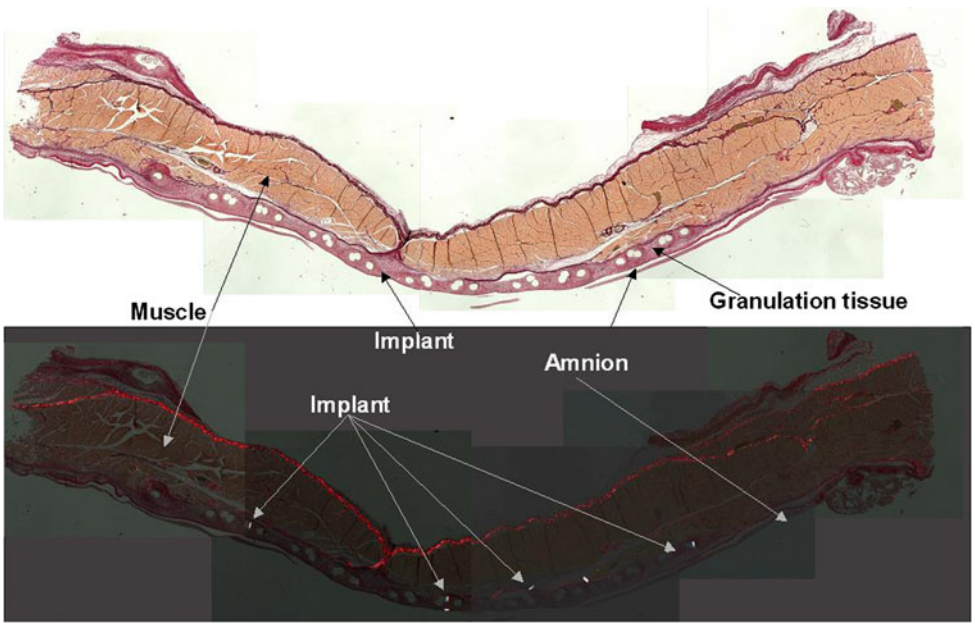


Fig. 3 The histology shows that the polypropylene mesh is already incorporated (after 17 days) in thesurrounding tissue and that the VITAL AM forms a tightly attached barrier preventing mesh andsutures from adhesions. The foreign body reaction (indicated by the granulamatous cells) is mildand limited to the close environment of the mesh fibers. The staining is Picosirius red which wasfound very suitable to discern the VITAL AM from the physiological scar tissue elicited by the mesh.The *upper image* is the view by back-light microscopy, the *lower image* is polarised

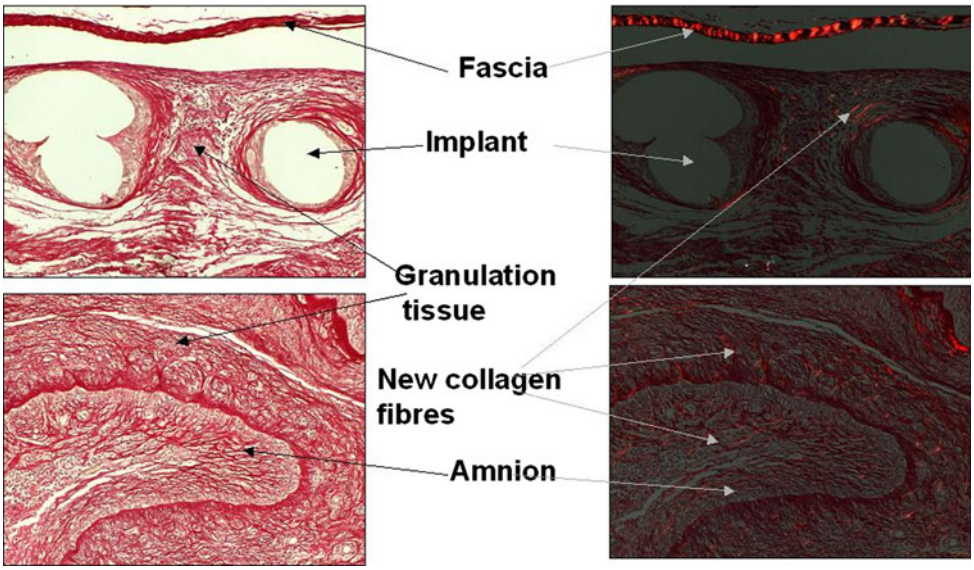


Fig. 4 The *upper images* show the good integration of the mesh and the excellent visualisation of collagenby the the Picosirius red staining (i.e., fascia). The *lower images* show persistence of the VITAL AMafter 17 days. *Left* are back light microscopy images, on the *right side* the polarised counterparts

sometimes can be altered easily by perforating fixation devices. As an alternative, slow-clotting fibrin sealant allowed manipulation of the vital AM for about 120 s and securely fixed vital AM [31].

Although vital AM was used as a xenograft in our animal model, no foreign body reaction or adverse effects associated with immunogenicity were observed. We are aware of this study's obvious limitations (e.g., the use of "pure" polypropylene in the control group). The control group was included because IPOM has no commonly accepted standard of care. Furthermore, the implant of uncoated Vitamesh guaranteed reproducible adhesion formation.

Concerning the translational issue, it must be emphasized that vital AM is manufactured by the blood bank of the Red Cross according to clinical GMP standards (Fig. 1) and already is available for clinical applications (i.e., wound dressing) [29, 32].

Conclusions

Vital human amniotic membrane provided excellent antiadhesion in experimental IPOM. Besides its superior handling characteristics, it showed good biocompatibility and rapid integration. As a consequence of our positive findings, we intend to continue the preclinical research in an IPOM trial using pigs and longer observation periods.

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Disclosures A. H. Petter-Puchner, R. H Fortelny, K. Mika, S. Hennerbichler, H. Redl, and C. Gabriel have no conflicts of interest or financial ties to disclose.

Appendix

See Table 2.

Table 2 Score by Vandendael

Scoring points	Parameter	Criteria
1	Width (mm)	<2
2		2–10
3		>10
1	Thickness (mm)	<1
2		1–3
3		>3
1	Strength	+
2		++
3		+++
1	Amount	0–2
2		3–4
3		>4
Grade 1 (mild)	1–4	
Grade 2 (moderate)	5–8	
Grade 3 (severe)	9–12	

This table describes the algorithm of the Vandendael score for scoring adhesion formation in animal trials. Its simple design and excellent reproducibility among observers are especially useful

References

1. Benhidjeb T, Benecke C, Strik MW (2008) Incisional hernia repair: sublay or intraperitoneal onlay mesh (IPOM)? Zentralbl Chir 133:458–463
2. Schug-Pass C, Sommerer F, Tannapfel A, Lippert H, Kockerling F (2009) The use of composite meshes in laparoscopic repair of abdominal wall hernias: are there differences in biocompatibily?

Experimental results obtained in a laparoscopic porcine model. Surg Endosc 23:487–495
3. Ching SS, Sarela AI, Dexter SP, Hayden JD, McMahon MJ (2008) Comparison of early outcomes for laparoscopic ventral hernia repair between nonobese and morbidly obese patient populations. Surg Endosc 22:2244–2250
4. Palanivelu C, Rangarajan M, Jategaonkar PA, Amar V, Gokul KS, Srikanth B (2009) Laparoscopic repair of diastasis recti using the "Venetian blinds" technique of plication with prosthetic reinforcement: a retrospective study. Hernia 13:287–292
5. Gananadha S, Samra JS, Smith GS, Smith RC, Leibman S, Hugh TJ (2008) Laparoscopic ePTFE mesh repair of incisional and ventral hernias. ANZ J Surg 78:907–913
6. Rauth TP, Poulose BK, Nanney LB, Holzman MD (2007) A comparative analysis of expanded polytetrafluoroethylene and small intestinal submucosa: implications for patch repair in ventral herniorrhaphy. J Surg Res 143:43–49
7. Trunzo JA, Ponsky JL, Jin J, Williams CP, Rosen MJ (2009) A novel approach for salvaging infected prosthetic mesh after ventral hernia repair. Hernia (in press)<AQ11>
8. Paton BL, Novitsky YW, Zerey M, Sing RF, Kercher KW, Heniford BT (2007) Management of infections of polytetrafluoroethylene-based mesh. Surg Infect Larchmt 8:337–341
9. Di Mugno M, Runfola M, Magalini S, Sermoneta D, Gui D (2006) Rippled mesh: a CT sign of abdominal wall ePTFE prosthesis infection. G Chir 27:384–387
10. Tsimoyiannis EC, Tsimogiannis KE, Pappas-Gogos G, Nikas K, Karfis E, Sioziou H (2008) Seroma and recurrence in laparoscopic ventral hernioplasty. JSLS 12:51–57
11. Burger JW, Halm JA, Wijsmuller AR, ten Raa S, Jeekel J (2006) Evaluation of new prosthetic meshes for ventral hernia repair. Surg Endosc 20:1320–1325
12. Olmi S, Scaini A, Erba L, Bertolini A, Croce E (2007) Laparoscopic repair of inguinal hernias using an intraperitoneal onlay mesh technique and a Parietex composite mesh fixed with fibrin glue (Tissucol): personal technique and preliminary results. Surg Endosc 21:1961–1964
13. Petter-Puchner AH, Walder N, Redl H, Schwab R, Ohlinger W, Gruber-Blum S et al (2008) Fibrin sealant (Tissucol) enhances tissue integration of condensed polytetrafluoroethylene meshes and reduces early adhesion formation in experimental intraabdominal peritoneal onlay mesh repair. J Surg Res 150:190–195
14. Withers L, Rogers A (2006) A spiral tack as a lead point for volvulus. JSLS 10:247–249
15. Karahasanoglu T, Onur E, Baca B, Hamzaoglu I, Pekmezci S, Boler DE et al (2004) Spiral tacks may contribute to intraabdominal adhesion formation. Surg Today 34:860–864
16. Szabo A, Haj M, Waxsman I, Eitan A (2000) Evaluation of seprafilm and amniotic membrane as adhesion prophylaxis in mesh repair of abdominal wall hernia in rats. Eur Surg Res 32:125–128
17. Hennerbichler S, Reichl B, Pleiner D, Gabriel C, Eibl J, Redl H (2007) The influence of various storage conditions on cell viability in amniotic membrane. Cell Tissue Bank 8:1–8
18. Said DG, Nubile M, Alomar T, Hopkinson A, Gray T, Lowe J et al (2009) Histologic features of transplanted amniotic membrane: implications for corneal wound healing. Ophthalmology 116:1287–1295
19. Ilancheran S, Moodley Y, Manuelpillai U (2009) Human fetal membranes: a source of stem cells for tissue regeneration and repair? Placenta 30:2–10
20. Kesting MR, Wolff KD, Hohlweg-Majert B, Steinstraesser L (2008) The role of allogenic amniotic membrane in burn treatment. J Burn Care Res 29:907–916
21. Toda A, Okabe M, Yoshida T, Nikaido T (2007) The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. J Pharmacol Sci 105:215–228
22. Yu SJ, Soncini M, Kaneko Y, Hess DC, Parolini O, Borlongan CV (2009) Amnion: a potent graft source for cell therapy in stroke. Cell Transplant 18:111–118
23. Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, Franchina M et al (2007) Term amniotic membrane is a high throughput source for multipotent mesenchymal stem cells with the ability to differentiate into endothelial cells in vitro. BMC Dev Biol 7:11
24. Miki T, Mitamura K, Ross MA, Stolz DB, Strom SC (2007) Identification of stem cell marker-positive cells by immunofluorescence in term human amnion. J Reprod Immunol 75:91–96
25. Petter-Puchner AH, Fortelny RH, Mittermayr R, Walder N, Ohlinger W, Redl H (2006) Adverse effects of porcine small intestine submucosa implants in experimental ventral hernia repair. Surg Endosc 20:942–946
26. Mateijsen MA, van der Wal AC, Hendriks PM, Zweers MM, Mulder J, Struijk DG et al (1999) Vascular and interstitial changes in the peritoneum of CAPD patients with peritoneal sclerosis. Perit Dial Int 19:517–525
27. Voskerician G, Jin J, Hunter SA, Williams CP, White M, Rosen MJ (2009) Human peritoneal membrane reduces the formation of intraabdominal adhesions in ventral hernia repair: experimental study in a chronic hernia rat model. J Surg Res 157:108–114
28. Jin J, Voskerician G, Hunter SA, McGee MF, Cavazzola LT, Schomisch S et al (2009) Human peritoneal membrane controls adhesion formation and host tissue response following intraabdominal placement in a porcine model. J Surg Res 156:297–304
29. Parolini O, Alviano F, Bagnara GP, Bilic G, Buhring HJ, Evangelista M et al (2008) Concise review: isolation and characterization of cells from human term placenta: outcome of the first international workshop on placenta derived stem cells. Stem Cells 26:300–311
30. Wolbank S, Hildner F, Redl H, Van GM, Gabriel C, Hennerbichler S (2009) Impact of human amniotic membrane preparation on release of angiogenic factors. J Tissue Eng Regen Med 3:651–654
31. Jain AK, Bansal R, Sukhija J (2008) Human amniotic membrane transplantation with fibrin glue in management of primary pterygia: a new tuck-in technique. Cornea 27:94–99
32. Stadler G, Hennerbichler S, Lindenmair A, Peterbauer A, Hofer K, Van GM et al (2008) Phenotypic shift of human amniotic epithelial cells in culture is associated with reduced osteogenic differentiation in vitro. Cytotherapy 10:743–752

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Original Article: Clinical Investigation

Early experience in reconstruction of long ureteral strictures with allogenic amniotic membrane

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Objective: To present our experience with the application of human amniotic membrane for the reconstruction of extensive ureteral wall defects.

Methods: Between 2003 and 2006, 11 patients underwent reconstructive surgery of the ureter. A human amniotic membrane allograft was used to supplement ureteral wall defects. Indications for the procedure included ureteral strictures of a 5.5 cm average (range, 3–8 cm) localized in different parts of the ureter: upper (5), middle (5) and lower (3). The etiology of ureteral loss was: postinflammatory after a complicated stone disease (5), iatrogenic (4) and idiopathic (2). Diagnosis of ureteral stricture was based on antegrade pyelography and excretory urography. Two patients had synchronous treatment for upper and middle ureteral stenosis. Treatment efficacy was assessed by excretory urography and ultrasound.

Results: The mean hospitalization time was 11.9 days, mean operation time 128 min and with an average follow up of 25.2 months. Complications included: stricture recurrence (1) and symptomatic urinary tract infections (2). Excretory urography showed lack of obstruction and normal width of ureters. In one patient, residual hydronephrosis was present on ultrasound.

Conclusions: The described method seems to be a promising tool in the reconstruction of extensive ureteral strictures.

Key words: amniotic membrane, graft, reconstruction, stricture, ureter.

Introduction

Minimally invasive management may represent a solution for short ureteral strictures of up to 2 cm.[1] Extensive ureteral strictures are not frequent but are difficult to manage and constitute a challenge to urologists. In most cases, the reconstruction and the supplementation of the damaged wall is necessary to achieve a satisfactory result. Ureteral replacement is usually performed when less invasive methods are not feasible or have failed. Ureteral stricture may have a varied etiology, including: iatrogenically-induced ureteral injuries (after gynecological and urological procedures in the pelvic area or radiotherapy), retroperitoneal fibrosis, complicated stone disease, or ureteral necrosis following kidney transplantation. Different procedures have been introduced to bridge ureteral defects including ureteral substitutions with intestinal segments, transuretero-ureterostomy, bladder flaps and even renal autotransplantation.[2] These interventions are complex, time-consuming and have a significant potential for complications.[3] The application of artificial biomaterials for ureteral replacement has also been reported; nevertheless, the results were sometimes far from those expected.[4] Furthermore, it was demonstrated that synthetic material might lead to encrustation on the artificially replaced ureter and could provoke foreign body reaction and subsequent rejection of the graft. This has brought about an increasing interest in the search for natural tissue with ureteral regenerative ability which could be used in reconstructive surgery. The material for the graft should be inexpensive, easily available and resistant to infection and rejection. Amniotic membrane, which has been recently used widely and successfully in ophthalmology for corneal and conjunctival reconstructions,[5,6] promised to be a valuable biomaterial. Amnion constitutes the inner part of the placental membrane. Amniotic membrane is composed of the connective tissue with a significant collagen and extracellular matrix composition. Its internal surface is covered with a single-layer cubical epithelium. It is avascular and has anti-scarring, anti-inflammatory and anti-angiogenetic properties. Moreover, it has been reported to possess the exclusive quality of preventing graft-versus-host disease[7] and to facilitate wound healing. Our aim was to assess the efficacy and suitability of human amniotic membrane as a scaffold for new tissue growth in the reconstruction of the extensive ureteral defects.

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Methods

A human amniotic allograft patch (10 cm × 16 cm) was prepared by the Transplantation Institute and Central Tissue Bank, Center of Biostructure, Institute of the Medical University of Warsaw. It was deeply frozen for preservation and radiation-sterilized with a dose of 35 kGy.

Written informed consent for the procedure was obtained from all of the study participants.

Eleven patients (four males and seven females) with a mean age of 51 years (range, 26–74) underwent reconstructive surgery of a ureteral obstruction between 19 February 2003 and 20 February 2006 (Table 1). The indications included extensive ureteral strictures of a 5.5 cm average (range, 3–8 cm) localized in various parts of the ureter: upper (5), middle (5) and lower (3). Two patients had synchronous treatment for upper and middle ureteral strictures. The etiology of ureteral loss was: postinflammatory after a complicated stone disease (5), iatrogenic (4) and idiopathic (2). Diagnosis of ureteral stricture was based on antegrade pyelography and excretory urography. Varied degrees of hydronephrosis and lack of contrast passage, or only a thin trace of this in the stenosed segment, were observed. Surgical action was performed under general anesthesia. A retroperitoneal approach to the ureter was

Table 1 Patients undergoing ureteral reconstruction

Patient age (years)	Follow up (months)	Stricture length (centimeters)	Etiology	Complications	Stricture location (part of the ureter)
51	5.1	8	Iatrogenic	–	Middle
26	19.4	6	Iatrogenic	–	Middle
26	20.3	4	Idiopathic	–	Lower
39	18.9	8 (3 + 5)	Iatrogenic	Stricture recurrence	Upper and middle
70	35.2	5	Postinflammatory/stone disease	Urinary tract infection	Lower
74	35.4	6.5 (3 + 3.5)	Postinflammatory/stone disease	–	Upper and middle
59	32.6	3	Postinflammatory/stone disease	–	Lower
49	28.5	5	Iatrogenic	–	Upper
64	25.5	5	Postinflammatory/stone disease	–	Upper
56	24.6	4	Idiopathic	–	Upper
48	32.2	6	Post inflammatory/stone disease	Urinary tract infection	Middle

Mean patient age, 51 years; mean follow up, 25.2 months; mean stricture length, 5.5 cm.

adopted in all cases. The approach in the upper and middle ureteral strictures was lumbar and, in the lower part of the ureter, ventral. A percutaneous nephrostomy was inserted before each procedure in order to derivate urine from hydronephrosis and to reduce the urine leak from the operated area. In all cases, human amniotic membrane was used to supplement the ureteral wall defect. Before implantation, the amniotic membrane was folded in order to obtain its carrier surface, and covered with cubical epithelium on both sides. Hence, there was no possibility of implanting the membrane improperly. The whole length of the stenosed part of the ureter was prepared and cleaned of adjacent tissues. Afterwards it was incised longitudinally without intersecting. The ureteral wall defect was then covered with human amniotic membrane in an on-lay fashion. The implant was sewed in without tension with interrupted suturing. Two pairs of proximal and distal single Dexon 5.0 sutures on each side were placed to fix the graft patch to the ureteral wall. Sutures were placed 1–2 mm from the ureteral edge with a minimum 3 mm of free margin left. A JJ-catheter was used as a ureteral stent to bridge the reconstructed segment for 3 weeks following the procedure. An excretory urography was done before removing the JJ. Suction was not used because of the danger of aspiration and damaging the implant. The wound was drained with a latex 1 cm diameter catheter with 3–4 holes and sawed with Dexon 1.0 sutures. A Foley catheter was put into the bladder. On the third to fourth day the drain was removed. Sutures were taken away on the seventh to eighth day. The Foley catheter was removed 5 days postoperatively. Nephrostomy was clamped on the seventh day and removed on the ninth. Follow up included excretory urography and ultrasound at 3, 9 and 24 months following intervention. A renal isotope scan was not routinely performed before and after the procedure.

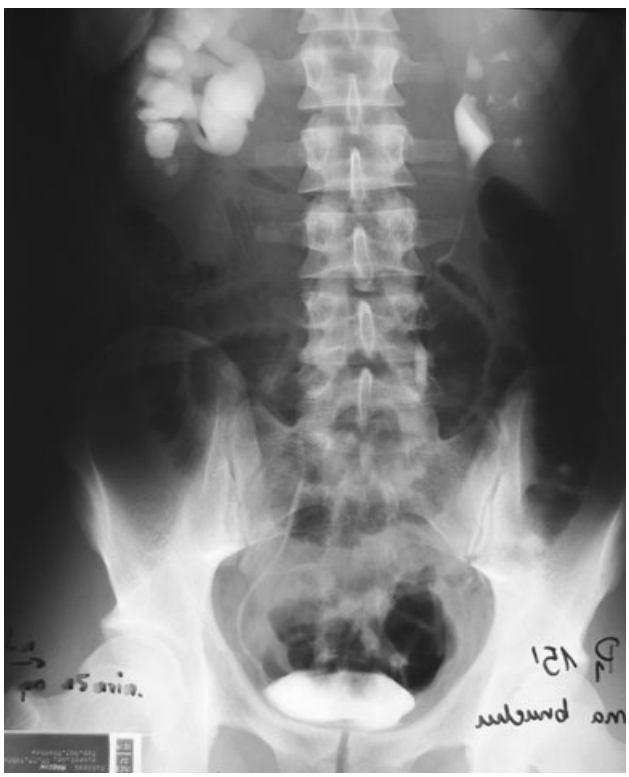

Fig. 1 Preoperative intravenous urography (IVU; 15 min after contrast injection) demonstrates extensive stricture of the upper part of the right ureter.

Results

The procedure had a successful outcome in all patients. There was no intraoperative bleeding. The mean hospitalization time was 11.9 days (range, 6–26 days), the mean operation time was 128 min (range, 70–270 min) and the average follow up was 25.2 months (range, 5–35 months). Complications included (Table 1): one stricture recurrence (in a patient harboring a double graft), and two cases of an isolated, symptomatic urinary tract infection. Excretory urography showed the unobstructed state and normal width of the operated ureter (Figs 1–3). In one patient, residual hydronephrosis was present on ultrasound, despite being non-obstructed. Up to now, we have not observed any significant alteration of patients' general condition.

Discussion

The most common use of an intestine or colon for ureteral reconstruction remains an invasive and complex procedure with a serious, up to 10.5%, potential for major complications including stricture of

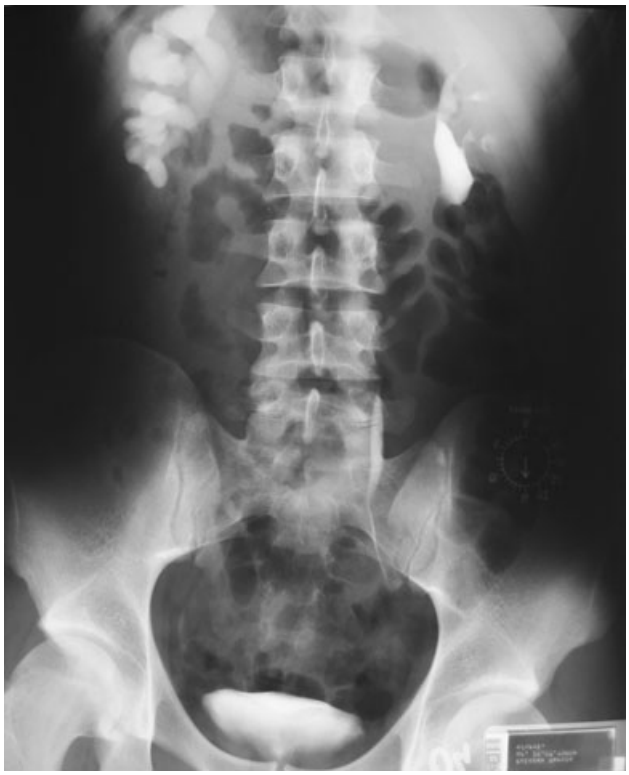

Fig. 2 Postoperative IVU (15 min after contrast injection). Passage of contrast appears to have improved (a piece of the upper part of the ureter begins to be visible).

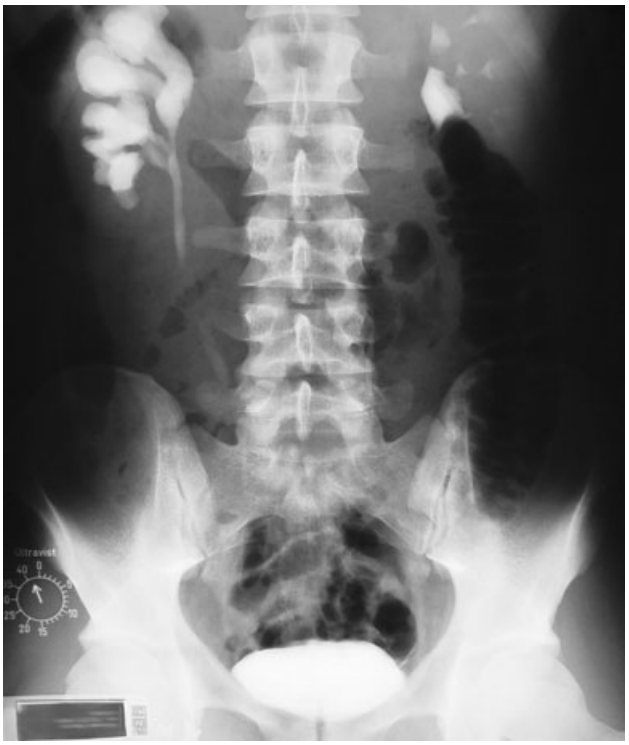

Fig. 3 Postoperative IVU (45 min after contrast injection). Lack of obstruction and regular width of the operated ureter.

anastomosis, graft obstruction, metabolic derangements and even chronic renal failure.[3] Because of the presence of the bowel in the urinary tract the risk of fistula formation, infection, stone disease or even cancer is elevated.[8] Moreover, the procedure is technically demanding, time-consuming and cannot be performed in patients with inflammatory bowel disease, azotemia or in those who lack enough small intestine.[3]

The Boari flap is considered to be a reasonable and safe option for treating extensive strictures of the lower part of the ureter.[9] However, the procedure might be complicated in patients with detrusor hypertrophy. Additionally, the intervention can restrict bladder capacity because it takes up a substantial proportion of bladder surface. Renal autotransplantation has been also reported in the management of ureteral strictures.[10] However, it is a complicated surgical procedure and tends to be accompanied by major and minor complications.

Some authors described the usefulness of small intestine submucosa (SIS) to supplement the ureteral loss in pigs.[11,12] Liatsikos *et al*. demonstrated the ability to bridge extensive ureteral strictures up to 7 cm.[11] SIS ability to stimulate the connective and the epithelial tissue growth allowed the regeneration of the ureteral defects. Greca *et al*. confirmed that the SIS graft behaves as a scaffold for ureteral reconstruction. Conversely, the study performed by El-Assmy[13] does not support the opinion that SIS is a suitable material for replacing extensive ureteral strictures above 4 cm. Obstruction of ureteral lumen and massive fibrosis were observed in all cases.

The application of the acellular matrix (AMX) for ureteral replacement has been reported in different studies, including our own work.[14,15] The results differed for each study. Those obtained by Osman *et al*. in a canine model were discouraging. Our results with AMX were, however, very promising, even though we had to stop performing this procedure because of the lack of material. In our opinion, the implantation method plays a key role in procedure efficiency. Our technique of AMX implantation was similar to that described with amniotic membrane. Osman *et al*. excised the ureteral stricture and afterwards replaced it with an AMX tube.

In our department, we obtained very promising early and late results in reconstructing the extensive ureteral strictures with human dura mater allograft.[16] However, because of the risk of Creutzfeldt–Jakob disease, dura mater was excluded from reconstructive surgery. This obliged us to search for another natural biomaterial with similar properties to continue our work with the reconstruction of the ureteral wall. Fishman *et al*. demonstrated that placental membrane is an acceptable biomaterial for bladder reconstruction. Moreover, they suggest its utility as a graft material for the urinary tract.[17] Amniotic membrane, as far as we know, has never been used for ureteral reconstruction. We used it as a scaffold for ureteral regeneration. Its potential for promoting the migration of host cells, attachment and spreading, as well as the absence of adverse immunological reaction, which is probably due to its anti-inflammatory properties and almost acellular composition, are the main advantages of amniotic membrane.[18] Furthermore, the graft is resistant to rejection and can be left in place if infection occurs. Avoiding the inclusion of intestinal segments, our technique can be used in all patients who have no contraindications to open surgery. It is a versatile, technically undemanding procedure for reconstructing all parts of the ureter. The ureteral stricture is only incised but not cut off. Its fragility is the major disadvantage of amniotic membrane. As it is easily damaged, it has to be carefully treated during the whole procedure and the use of suction is inadvisable. For the same reason, intraoperative bleeding should be controlled.

The treatment evaluation before and after procedure was based upon urography, ultrasound findings and the relief of symptoms.

Unfortunately, renal isotope scanning was not systematically performed and this did not allow us to compare kidney function.

Conclusions

Application of human amniotic membrane appears to be an encouraging method for managing extensive ureteral defects. The procedure is technically easy and short-term results are promising. Nevertheless, long-term follow up and analysis of more cases with precise renal function evaluation remains necessary. The cost-effectiveness of procedure and its durability should be assessed in consideration of this method as an alternative treatment option for ureteral reconstruction.

References

1 Hafez KS, Wolf JS Jr. Update on minimally invasive management of ureteral strictures. *J. Endourol.* 2003; **17**: 453–64.
2 Schoeneich G, Winter P, Albers P, Frohlich G, Muller SC. Management of complete ureteral replacement. Experiences and review of the literature. *Scand. J. Urol. Nephrol.* 1997; **31**: 383–8.
3 Chung BI, Hamawy KJ, Zinman LN, Libertino JA. The use of bowel for ureteral replacement for complex ureteral reconstruction: long-term results. *J. Urol.* 2006; **175**: 179–83.
4 Baltaci S, Ozer G, Ozer E, Soygur T, Besalti O, Anafarta K. Failure of ureteral replacement with Gore-Tex tube grafts. *Urology* 1998; **51**: 400–3.
5 Gris O, Lopez-Navidad A, Caballero F, del Campo Z, Adan A. Amniotic membrane transplantation for ocular surface pathology: long-term results. *Transplant. Proc.* 2003; **35**: 2031–5.
6 Chandra A, Maurya OP, Reddy B, Kumar G, Pandey K, Bhaduri G. Amniotic membrane transplantation in ocular surface disorders. *J. Indian Med. Assoc.* 2005; **103**: 364–6, 368.
7 Peris-Martinez C, Menezo JL, Diaz-Llopis M, Avino-Martinez JA, Navea-Tejerina A, Risueno-Reguillo P. Multilayer amniotic membrane transplantation in severe ocular graft versus host disease. *Eur. J. Ophthalmol.* 2001; **11**: 183–6.
8 Tanagho EA. A case against incorporation of bowel segments into the closed urinary system. *J. Urol.* 1975; **113**: 796–802.
9 del Pizzo JJ, Jacobs SC, Bartlett ST, Sklar GN. The use of bladder for total transplant ureteral reconstruction. *J. Urol.* 1998; **159**: 750–2.
10 Webster JC, Lemoine J, Seigne J, Lockhart J, Bowers V. Renal autotransplantation for managing a short upper ureter or after *ex vivo* complex renovascular reconstruction. *BJU Int.* 2005; **96**: 871–4.
11 Liatsikos EN, Dinlenc CZ, Kapoor R *et al.* Ureteral reconstruction: small intestine submucosa for the management of strictures and defects of the upper third of the ureter. *J. Urol.* 2001; **165**: 1719–23.
12 Greca FH, Noronha L, Bendhack M, Feres A, Soccol A, Duda JR. Use of small intestine submucosa as ureteral allograft in pigs. *Int. Braz. J. Urol.* 2004; **30**: 327–34.
13 El-Assmy A, Hafez AT, El-Sherbiny MT *et al.* Use of single layer small intestinal submucosa for long segment ureteral replacement: a pilot study. *J. Urol.* 2004; **171**: 1939–42.
14 Osman Y, Shokeir A, Gabr M, El-Tabey N, Mohsen T, El-Baz M. Canine ureteral replacement with long acellular matrix tube: is it clinically applicable? *J. Urol.* 2004; **172**: 1151–4.
15 Koziak A, Kania P, Marcheluk A, Dmowski T, Szczesniewski R, Dorobek A. Reconstruction of long ureteral obstructions using xenogenic acellular collagen membranes. *Ann. Transplant.* 2004; **9**: 18–20.
16 Koziak A, Kania P, Marcheluk A, Dmowski T, Szczesniewski R, Dorobek A. Reconstructive surgery of the extensive ureter strictures using human dura mater allografts. *Ann. Transplant.* 2004; **9**: 12–16.
17 Fishman IJ, Flores FN, Scott FB, Spjut HJ, Morrow B. Use of fresh placental membranes for bladder reconstruction. *J. Urol.* 1987; **138**: 1291–4.
18 Tyszkiewicz JT, Uhrynowska-Tyszkiewicz IA, Kaminski A, Dziedzic-Goclawska A. Amnion allografts prepared in the Central Tissue Bank in Warsaw. *Ann. Transplant.* 1999; **4**: 85–90.

Functional and anatomic results of amnion vaginoplasty in young women with Mayer-Rokitansky-Küster-Hauser syndrome

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Objective: To evaluate the surgical outcome and the long-term anatomic and functional results in young women with Mayer-Rokitansky-Küster-Hauser Syndrome (MRKH) undergoing neovaginal creation with amniotic membranes.
Design: Evaluation of surgical and functional outcome according to clinical records and validated questionnaires about sexuality (Female Sexual Function Index [FSFI]) over a 1.5-year follow-up period.
Setting: University hospital and referral center for pediatric and adolescent gynecology.
Patient(s): Seven patients with congenital vaginal aplasia with a mean age of 20.86 ± 3.56 years (range 17–26 years).
Intervention(s): McIndoe procedure modified by the application of human freeze-dried amniotic membranes.
Main Outcome Measure(s): Anatomic success was defined by a vaginal length ≥8 cm, and a width allowing the easy introduction of two fingers. FSFI scores were applied to define functional results.
Result(s): Mean neovaginal length was 9.3 cm (range 4–12 cm). The mean FSFI score was 30.0 ± 6.9. Major operative complications occurred in one patient. In six out of seven patients satisfactory anatomic and functional results were achieved.
Conclusion(s): The surgical dissection of the vesicorectal space and the application of human amnion over a vaginal mold to create a neovagina results in satisfying anatomic and functional outcome with low perioperative morbidity in MRKH patients. (Fertil Steril® 2010;94:317–23. ©2010 by American Society for Reproductive Medicine.)

Key Words: Mayer-Rokitansky-Küster-Hauser syndrome, vaginal reconstruction, amnion, vesicorectal space

Mayer-Rokitansky-Küster-Hauser Syndrome (MRKH) refers to a condition of mullerian agenesis where the mullerian ducts fail to develop resulting in the absence of a normal uterus and vagina in the presence of a normal 46,XX karyotype. A shallow vaginal pouch is present, and the fallopian tubes, ovaries, and secondary sex characteristics are normal (1, 2). The MRKH syndrome is the second most common cause of primary amenorrhea in young women. The estimated prevalence is 1 in 4,000–5,000 women (3, 4). It has been considered to be a sporadic anomaly, but recent reports suggest a genetic defect that is transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity. Nevertheless, the etiology of MRKH syndrome remains unclear (5). Associated congenital anomalies of the upper urinary tract, such as unilateral renal agenesis and pelvic or horseshoe kidneys, are reported to occur in 30%–40% of all cases of MRKH syndrome, and 10%–15% have skeletal anomalies involving the spine, ribs, and extremities (6).

Although numerous methods for creating a neovagina have been proposed, there is no consensus about which procedure should be considered to be the ideal standard. The major differences between the various procedures are access (i.e., laparoscopic, open transabdominal, or vaginal) and type of tissue used to cover the neovaginal cavity (amniotic membranes, peritoneal layers, recombinant artificial dermis, skin graft, intestinal tissue, etc.) (7).

The data regarding the anatomic and functional success of the technique based on the use of amniotic membranes to cover the vaginally dissected vesicorectal space in MRKH patients are very limited (8). The aim of the present study was to assess the perioperative, clinical, and functional course of all consecutive MRKH patients surgically treated in our institution by the creation of an "amnion" neovagina. Systematic anatomic, surgical, and functional data regarding sexuality are presented.

MATERIALS AND METHODS

From January 2005 to April 2008, we surgically treated seven MRKH patients. All of the patients presented to our

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Department of Pediatric and Adolescent Gynecology for further evaluation of primary amenorrhea. Diagnostic criteria for the MRKH syndrome were normal physical development according to the Tanner stages (9), blind-ending vagina, the finding of a fibrous remnant in place of the uterus at rectal examination and no signs of hematocolpos or hematometra due to hymenal atresia. Each patient underwent a transrectal or transabdominal sonography, which confirmed the absence of a normal developed uterus as well as ultrasonographic examination of the urinary system and karyotyping to exclude other differential diagnoses such as testicular feminization.

All patients underwent a vaginoplasty using chemically processed and sterilized freeze-dried human amniotic membranes. The human amnion was readily harvested from the amniotic sac of term infants delivered from healthy women. Split-thickness skin grafts are categorized as thin (0.005–0.012 in), intermediate (0.012–0.018 in), or thick (0.018–0.030 in), based on the thickness of the harvested graft. The thickness of the used amnion was similar to a classic thin split-thickness skin graft. The freeze-dried amniotic membranes we used, were specially processed, tested, and sterilized by the blood bank of our institution. Therefore, no additional testing was required to be performed on the donor amnion tissue before insertion into the neovaginal cavity.

A transverse incision was made at the vaginal dimple, and a vesicorectal cavity of approximately 12 cm was dissected to the level of the peritoneum. After thorough bipolar hemostasis a mold of glass covered by sofratyl and moisturized amniotic membranes was then inserted and the labia minora were secured around the stent to prevent expulsion. The freeze-dried amnion was soaked in sterile saline solution for approximately 10 minutes before insertion into the vaginal space, to form a soft and moisturized thin-layer tissue to easily cover the whole surface of a mold of glass covered by sofratyl. The different steps of the preparation of the vaginal mold are presented in Fig. 1. In that way, difficulties with the thin amnion shearing off the form were avoided.

Vaginal tamponade was then the method of filling the neovaginal cavity, by introducing a sterile gauze with estriol creme (1%) to promote epithelialization. The gauze was changed twice daily for the first 5 days. The patient remained on bedrest during this period, after which the mold was removed and replaced by a soft silicon vaginal dilator lubricated with estriol creme (1%) to induce epithelialization of the newly created surface. The patients were advised to continue using the dilator for the first 3–5 postoperative months to prevent contraction of the neovagina. The patients were also encouraged to engage in sexual activities to induce their motivation and comply with the required postoperative measures necessary to guarantee a successful procedure. Once the patients started engaging in sexual activities, the use of dilators was recommended for shorter periods of time.

All patients could be discharged within an average 10 days after surgery and were instructed how to use and apply the vaginal dilator and the estriol creme by adolescent gynecologists. All patients received a special vaginal dilator 12 cm long with an adjustable diameter, achieved by insufflation, to fit the individual proportions. Care of the dilators includes simple flush with water and soap after each use. Vaginal intercourse was allowed no earlier than 6–8 weeks after vaginoplasty, and this varied depending on the wound healing process and the grade of epithelialization of the neovagina. Patients were followed-up in the initial postoperative period once every 4 weeks for the first 3 months and then twice a year. The clinical evaluation included vaginal and rectal examinations as well as vaginoscopy. Functional results were additionally assessed by the Rosen Female Sexual Function Index (FSFI) questionnaire (10), validated for the German-speaking population (11). This short 19-item quiz is validated for assessment of female quality of life and detection of sexual dysfunction in adults. It has demonstrated its validity in earlier studies (11, 12), and its score is unbiased regarding age, education, and economic status of the patients. The 19 items are assigned to six separate domains of female sexual function; the first four relate to the four major categories of sexual dysfunction: desire, arousal, orgasmic, and sexual pain disorder. The fifth domain describes the quality of vaginal lubrication, and the sixth the global sexual and relationship satisfaction.

The vaginoplasty method using freeze- dried amniotic membranes has extensively been used in our institution inclusively in the field of pediatric surgery (i.e. burns), since more than 15 years, so no institutional review board approval was required. An informed written consent was obtained by every patient who was operated on this procedure prior to surgery.

RESULTS

Between July 2005 and August 2008, seven patients with a mean age of 20.86 $\pm$ 3.56 years (range 17–26 years) underwent vaginoplasty by human amniotic membranes. Median follow-up for the whole group of patients was 16.43 months (range 4–41 months).

Only one patient was identified to have a urinary tract abnormality, specifically horseshoe kidney. No skeletal anomalies were described. Mullerian remnants were seen in four of the seven patients (57%), presenting as a noncavitary streak of fibrous tissue. All patients presented initially to our clinic an average 1.2 years (range 0–3) before reconstructive surgery owing to primary amenorrhea carrying already the diagnosis of a MRKH syndrome. Two patients underwent at initial presentation a diagnostic laparoscopy to confirm the diagnosis. No routine laparoscopy was otherwise performed, because clinical examination and sonographic findings were considered to be sufficient to establish the diagnosis. All patients were counseled that the individual's readiness for vaginal manipulation and/or intercourse is critical for the timing of the surgery and so the operative procedure was planned when the young woman stated she was willing to become

sexually active. Three of the patients had a stable partner at the time of surgery.

The mean operative time was 24.7 ± 2.09 minutes (range 20–33 minutes) with only minimal blood loss in all cases. The mean length of hospital stay was 10.8 ± 0.97 days (range 8–14 days). Six of the seven patients had a rather uncomplicated hospital course; one patient developed a urinary tract infection within the first postoperative week, requiring oral antibiotic treatment. One patient developed extensive infection with ulceration of the rima ani and vulva 7 days after surgery. Speculum examination revealed a rectovaginal fistula, for which she required surgical repair with fistula excision and diverting loop ileostomy. The infection resulted in a stricture and shortening of the newly created vagina, so that the patient expressed the desire of a new neovagina 4 months after the initial procedure. She underwent neovaginal reconstruction using a sigmoid loop. This was further complicated by anastomotic leak and sepsis, and multiple reoperations were performed to control the peritonitis. Five weeks later she was discharged in very good condition and with satisfying functional and anatomic results of the neovagina.

In all of the other patients a satisfactory neovaginal length of at least 9 cm could be obtained (mean vaginal length 9.8 ± 1.7 cm, range 9–12 cm), and the neovaginal cavity was easily passable for two fingers already at the time of discharge. The follow-up examinations 1, 3, and 6 months after surgery revealed a well epithelialized neovaginal cavity, without stricture formation or shortening, because all of the patients successfully applied the vaginal phantom or had regular vaginal intercourse. The anatomic and functional results as well as the data regarding the operative complications are presented in Table 1. Six of the seven patients (85.7%) experienced regular or less regular vaginal intercourse in an average 5.28 ± 1.25 months after surgery; the range expanded from 2 to 7 months mainly depending on the psychologic ability of each woman. None of the patients stated having dyspareunia or contact bleeding during intercourse; however, five of the patients (71.4%) used lubricants, more in a prophylactic matter than owing to insufficient lubrification of the neovagina, according to their statements. The mean postoperative period the patients used almost permanently a vaginal phantom to prevent constriction of the neovaginal cavity was 5 ± 2.5 months (range 2–8 months).

When evaluating the functional success according to the FSFI, the mean full FSFI score of all five sexually active patients was 30.0 ± 6.9, thus corresponding to the equivalent score of 30.2 ± 6.1 reached by healthy women (10). Detailed values of the FSFI of our five sexually active MRKH patients after amnion vaginoplasty compared with the equivalent mean FSFI values of MRKH patients after sigmoid vaginoplasty and with healthy individuals are presented in Table 2.

The one patient with sigmoid neovagina after rectovaginal fistula formation was not included in the FSFI evaluation, because she no longer had an amnion neovagina.

DISCUSSION

Various studies have evaluated the functional and anatomic evaluation of several operative (7, 8, 12–30) and nonoperative (31, 32) vaginoplasty methods in patients with vaginal agenesis due to MRKH syndrome. However, no "ideal"

TABLE 1

Data of perioperative morbidity, anatomic, and functional characteristics related to vaginal reconstruction with human amniotic membranes in seven Rokitansky patients at least 4 months after vaginoplasty.

	Mean ± SD (range)	Patients, n (%)
Patient age (y)	20.86 ± 3.58 (17–26)	
Vaginal length (cm)	9.3 ± 2.3 (4–12)	
Operation time (min)	24.7 ± 2.09 (20–33)	
Length of hospital stay (d)	10.8 ± 0.97 (8–14)	
Time of vaginal phantom using after surgery (mo)	4.1 ± 1.07 (3–5)	
Start of regular vaginal sexual intercourse (mo after surgery)	5.28 ± 1.25 (2–7)	
Early operative complications:		
Infection and rectovaginal fistula formation		1 (14.3)
Urinary tract infection		1 (14.3)
Dyspareunia		0
Contact bleeding		0
Vaginal stenosis		0
Prophylactic use of lubricants during vaginal intercourse		5 (71.4)

Fotopoulou. Amnion neovagina in Rokitansky syndrome. Fertil Steril 2010.

TABLE 2

Female Sexual Function Index (FSFI) values of the five sexually active Mayer-Rokitansky-Küster-Hausen (MRKH) syndrome patients after amnion vaginoplasty compared with the equivalent mean FSFI values of MRKH syndrome patients after sigmoid vaginoplasty and with healthy individuals.

	MRKH syndrome patients		
Domain	After amnion vaginoplasty (n = 5)	After sigmoid vaginoplasty (n = 11; ref. 12)	Healthy women (n = 131; ref. 10)
Desire	4.9 ± 1.2	4.7 ± 0.9	4.1 ± 1.1
Arousability	5.0 ± 0.8	4.9 ± 0.6	5.0 ± 1.0
Lubrication	4.0 ± 0.7	5.0 ± 0.9	5.5 ± 0.9
Orgasm	5.5 ± 1.0	5.3 ± 0.8	5.0 ± 1.2
Satisfaction	5.3 ± 1.4	4.7 ± 1.6	5.1 ± 1.2
Pain	5.3 ± 1.8	3.5 ± 2	5.5 ± 1
Total score	30.0 ± 6.9	28.1 ± 6.8	30.2 ± 6.1

Note: The patient after rectovaginal fistula formation and subsequent sigmoid neovagina and the patient without sexual intercourse were not included in this FSFI evaluation. Full FSFI score is 36 points. Values are given as mean ± SD.

Fotopoulou. Amnion neovagina in Rokitansky syndrome. Fertil Steril 2010.

method is yet established which can be universally recommended. The choice of vaginoplasty depends on numerous factors, including patient preparedness, preference, and expectations and certainly the surgeon's experience. Regardless of the surgeon's personal preference, the patient should be thoroughly informed about treatment alternatives, including description of potential complications, long-term durability, and results on long-term sexual function. Thomas and Brock (33), in a review of nonoperative and operative alternatives for vaginal replacement, concluded that the most popular

TABLE 3

Relevant series and case reports in the literature concerning the treatment of Rokitansky (MRKH) syndrome by human amniotic membranes.

Author, y (ref.)	No. of MRKH patients	Anatomic success	Complete epithelialization/ metaplasia of the amnion into squamous cells	No. of major complications
Tancer et al., 1979 (36)	1	100%	yes	0
Karjalainen et al., 1980 (38)	3	100%	yes	0
Morton and Dewhurst, 1986 (30)	27	92.6%	yes	1 (rectal injury)
Ashworth et al., 1986 (21)	15	100%	NR	NR
Mac, 1988 (37)	9	100%	yes	0
Nisolle and Donnez, 1992 (35)	—	"good"	NR	NR
Bleggi-Torres et al., 1997 (16)	10	100%	yes	0
Sharma et al., 2008 (14)	17	88.23%	yes	2 (1 rectal injury and 1 rectovaginal fistula)
Tözüm, 1976 (20)	1	100%	yes	0

Note: NR = not reported.

Fotopoulou. Amnion neovagina in Rokitansky syndrome. Fertil Steril 2010.

methods of vaginal substitution include passive dilation, inlay skin grafts, rotational myocutaneous flaps, and bowel substitute vaginoplasty. The American College of Obstetricians and Gynecologists committee opinion on adolescent health care (34) published that the condition can usually be successfully managed in a conservative way by the use of vaginal dilators as long as the patient is sufficiently motivated. If a surgical option is considered, a number of approaches are available; with the Abbè-McIndoe operation being the most common.

The Abbè-McIndoe technique (29) is indeed considered to be a valid treatment option for vaginoplasty, but no consensus has been reached on what material should be used for the neovagina canal wall lining (27). Various authors have reported on the use of several artificial or biologic materials to cover the neovaginal cavity and induce epithelialization. Autologous human amniotic membranes (14, 16, 21, 30), homologous amniotic membranes (20), peritoneal layers of the pouch of Douglas (13), artificial dermis and recombinant basic fibroblast growth factor (7), split-thickness skin graft from the buttocks (29), oxidized cellulose (18), acellular human dermal allograft (26) and autologous in vitro–cultured vaginal tissue (27) are some of the applied tissues that have been named.

Limited and rather older reports are available on the anatomic and functional success of vaginoplasty with human amnion (14, 16, 20, 21, 30, 35, 36). The most relevant series are presented in Table 3. One of the first attempts of vaginal reconstruction from amniotic membranes was in 1934 in the French-language literature, where Brindeau used amnion to construct a neovagina for a patient with mullerian agenesis (39).

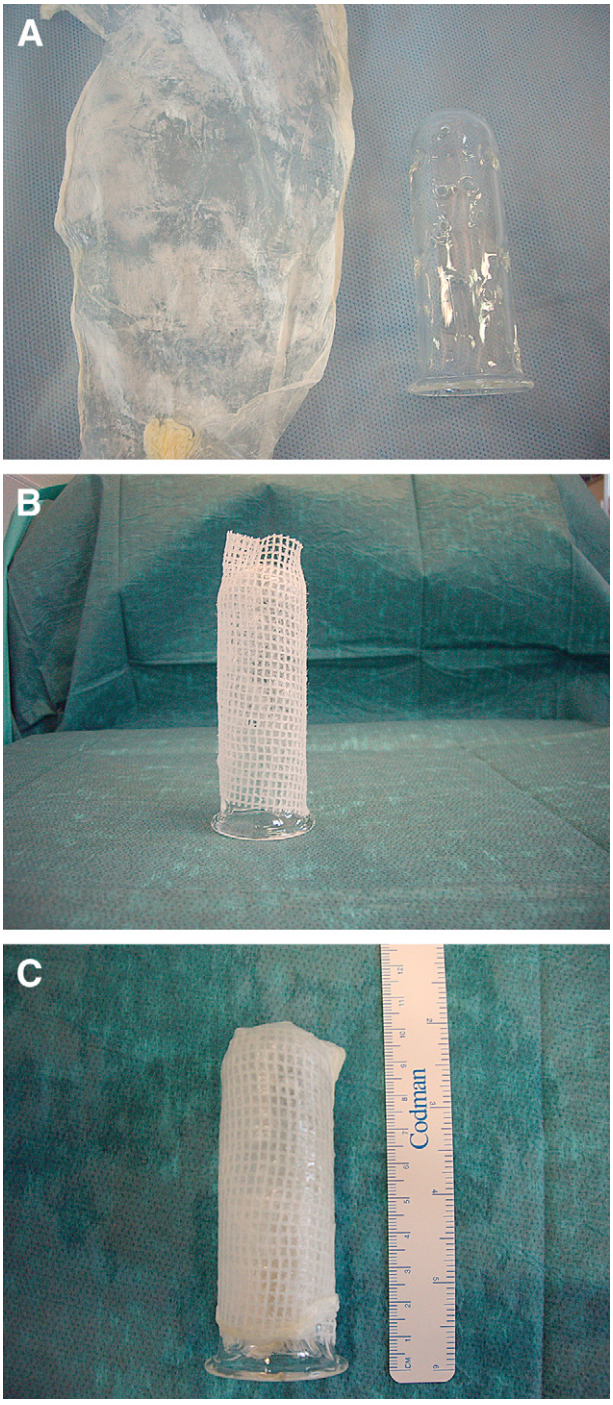
In the present analysis, we report very good anatomic and functional results of amnion vaginoplasty similar to results from previous reports. These findings confirm those obtained by many previous authors applying the same method (Table 3). In a total of 68 reported patients (14, 16, 20, 30, 36, 38), all major complications were related to rectal injury (3 cases, 4.4%) with otherwise very satisfying anatomic results.

Of great importance is the fact that following diagnosing of vaginal agenesis in adolescent girls, it is important to wait until the patient is ready to engage in sexual activity before initiating any kind of treatment. For those patients who have a 2–3-cm hymenal fossa, Frank's method of progressive vaginal dilation should be always offered (8), but the patient should be informed that success has proven to be variable and unpredictable and that the patient needs to be highly motivated and willing to continue long-term dilations (21, 41). Effective management should, moreover, include a careful comprehensive psychologic preparation and support of the patient.

We believe that vaginal construction from amniotic membranes carries the following advantages over other operative reconstructive methods. The human amnion appears to have

FIGURE 1

Pictures of the freeze-dried human amniotic membranes and the vaginal mold that is inserted for vaginoplasty in Mayer-Rokitansky-Küster-Hauser syndrome patients. (**A**) Freeze-dried amnion and vaginal mold. (**B**) Vaginal mold covered with sofratyl. (**C**) Vaginal mold wrapped in sofratyl and moisturized amniotic membranes just before insertion into the neovaginal cavity.



Fotopoulou. Amnion neovagina in Rokitansky syndrome. Fertil Steril 2010.

great advantages over the split skin graft previously used in the original Abbè-McIndoe technique (29). Amnion is readily available, and there is no need for additional incisions as at the donor site in the split skin graft technique. Furthermore, there have been no problems with immune rejection, because human amniotic epithelial cells do not express on their surfaces histocompatibility antigens such as HLA-A, -B, -C, and -DR or ®2- microglobulin. Akle et al. (40) found no evidence of tissue rejection after implanting amnion subcutaneously in volunteers.

Compared with the laparoscopic approach of the Vecchietti method, where the creation of the neovagina is achieved by invagination using an acrylic "olive" that is laparoscopically placed against the vaginal dimple (8), the present method requires no abdominal incision. Furthermore, the Vecchietti straight thread-bearing cutting needle, which subperitoneally reaches the space between the bladder and rectum, can also cause injuries of the neighboring organs, such as the bladder (8). Rectal injuries and local infections are the major risk factors predisposing rectovaginal fistulas. During dissection of the vesicorectal space, the surgeon should always avoid any contamination of the amnion graft with bacteria from the rectal flora. He/she can, for additional help, insert an index finger into the rectum, during rectovesical dissection, so that the anatomic borders are more easily recognized and sharp injury of the rectal mucosa is avoided. If, nevertheless, a rectal injury occurs, a temporary diverting loop ileostomy could be considered after rectal repair, to promote local wound healing and prevent future fistula formation.

Despite the lack of evidence from randomized trials comparing the various vaginoplasty operative methods, we conclude, based on our results, that the neovaginal reconstruction using freeze-dried amniotic membranes is a safe and easy technique, without induction of scar at the donor site, and should be the preferred surgical technique, as a modification of the McIndoe procedure, for patients with Rokitansky syndrome.

Still, in the absence of an "ideal" treatment strategy, patients should be objectively and professionaly informed also about the nonsurgical alternatives, so that an individual ideal method, addressed to each patient́s preference, is then selected.

REFERENCES

1. Schmid-Tannwald I, Hauser G. Deutung der "atypischen" Formen des Mayer-Rokitanski-Küster-Hauser syndroms. Geburtshilfe Frauenheilkd 1977;37:386–92.
2. Griffin J, Edwards C, Madden J, Harrod M, Wilson J. Congenital absence of the vagina, the Mayer-Rokitanski-Küster-Hauser syndrome. Ann Intern Med 1976;85:224–36.
3. Capraro V, Gallego M. Vaginal agenesis. Am J Obstet Gynecol 1976;124:96–107.
4. Chervenak F, Stangel J, Nemec M, Amin H. Mayer-Rokitanski-Küster-Hauser syndrome. N Y State J Med 1982;82:23–7.
5. Morcel K, Camborieux L, Guerrier D, Programme de Recherches sur les Aplasies Müllériennes. Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome. Orphanet J Rare Dis 2007;2:13.
6. Basile C, De Michele V. Renal abnormalities in Mayer-Rokitanski-Küster-Hauser syndrome. J Nephrol 2001;14:316–8.
7. Noguchi S, Nakatsuka M, Sugiyama Y, Chekir C, Kamada Y, Hiramatsu Y. Use of artificial dermis and recombinant basic fibroblast growth factor for creating a neovagina in a patient with Mayer-Rokitansky-Küster-Hauser syndrome. Hum Reprod 2004;19:1629–32.
8. Fedele Luigi, Bianchi Stefano, Zanconato Giovanni, Raffaelli Ricciarda. Laparoscopic creation of a neovagina in patients with Rokitansky syndrome: analysis of 52 cases. Fertil Steril 2000;74:384–9.
9. Tanner JM. Growth of adolescents. Oxford: Blackwell Scientific, 1962.
10. Rosen R, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, Ferguson D, D'Agostino R Jr. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther 2000;26:191–208.
11. Berner MM, Kriston L, Zahradnik HP, Härter M, Rohde A. Validity and reliability of the German Female Sexual Function Index (FSFI-d). Geburtsh Frauenheilk 2004;64:293–303.
12. Communal PH, Chevret-Measson M, Golfier F, Raudrant D. Sexuality after sigmoid colpopoiesis in patients with Mayer-Rokitansky-Küster-Hauser Syndrome. Fertil Steril 2003;80:600–6.
13. de Marques HS, dos Santos FL, Lopes-Costa PV, dos Santos AR, da Silva BB. Creation of a neovagina in patients with Rokitansky syndrome using peritoneum from the pouch of Douglas: an analysis of 48 cases. Fertil Steril 2008;90:827–32.
14. Sharma D, Dahiya K, Chechi K, Sirohiwal S. Vaginoplasty with amnion grafting: an experience. J Gynecol Surg 2008;24:61–5.
15. Rajimwale A, Furness PD 3rd, Brant WO, Koyle MA. Vaginal construction using sigmoid colon in children and young adults. BJU Int 2004;94:115–9.
16. Bleggi-Torres LF, Werner B, Piazza MJ. Ultrastructural study of the neovagina following the utilization of human amniotic membrane for treatment of congenital absence of the vagina. Braz J Med Biol Res 1997;30:861–4.
17. Hensle TW, Shabsigh A, Shabsigh R, Reiley EA, Meyer-Bahlburg HF. Sexual function following bowel vaginoplasty. J Urol 2006;175:2283–6.
18. Sharma JB, Gupta N, Mittal S. Creation of neovagina using oxidized cellulose (surgicel) as a surgical treatment of vaginal agenesis. Arch Gynecol Obstet 2007;275:231–5.
19. Creatsas G, Deligeoroglou E, Makrakis E, Kontoravdis A, Papadimitriou L. Creation of a neovagina following Williams vaginoplasty and the Creatsas modification in 111 patients with Mayer-Rokitansky-Küster-Hauser syndrome. Fertil Steril 2001;76:1036–40.
20. Tözüm R. Homotransplantation of the amniotic membrane for the treatment of congenital absence of the vagina. Int J Gynaecol Obstet 1976;14:553–6.
21. Ashworth MF, Morton KE, Dewhurst J, Lilford RJ, Bates RG. Vaginoplasty using amnion. Obstet Gynecol 1986;67:443–6.
22. Vesanović S, Panajotović L, Garalejić E. [Sensibility of vagina reconstructed by McIndoe method in Mayer-Rokitansky-Küster-Hauser syndrome]. Vojnosanit Pregl 2008;65:449–55. Serbian.
23. Borkowski A, Czaplicki M, Dobronski P. Twenty years of experience with Krzeski's cystovaginoplasty for vaginal agenesis in Mayer-Rokitansky-Küster-Hauser syndrome: anatomical, histological, cytological and functional results. BJU Int 2008;101:1433–40.
24. Cai B, Zhang JR, Xi XW, Yan Q, Wan XP. Laparoscopically assisted sigmoid colon vaginoplasty in women with Mayer-Rokitansky-Küster-Hauser syndrome: feasibility and short-term results. BJOG 2007;114:1486–92.
25. Darwish AM. A simplified novel laparoscopic formation of neovagina for cases of Mayer-Rokitansky-Küster-Hauser syndrome. Fertil Steril 2007;88:1427–30.
26. Kuohung W, Thompson SR, Laufer MR. Use of acellular human dermal allograft for vaginoplasty in Mayer-Rokitansky-Küster-Hauser syndrome: a case report. J Reprod Med 2007;52:864–7.
27. Panici PB, Bellati F, Boni T, Francescangeli F, Frati L, Marchese C. Vaginoplasty using autologous in vitro cultured vaginal tissue in a patient with Mayer-von-Rokitansky-Küster-Hauser syndrome. Hum Reprod 2007;22:2025–8.
28. Creatsas G, Deligeoroglou E. Expert opinion: vaginal aplasia: creation of a neovagina following the Creatsas vaginoplasty. Eur J Obstet Gynecol Reprod Biol 2007;131:248–52.
29. McIndoe A. The treatment of congenital absence and obliterative conditions of the vagina. Br J Plast Surg 1950;2:254.

30. Morton KE, Dewhurst CJ. Human amnion in the treatment of vaginal malformations. Br J Obstet Gynaecol 1986;93:50–4.
31. Lee MH. Non-surgical treatment of vaginal agenesis using a simplified version of Ingram's method. Yonsei Med J 2006;47:892–5.
32. Williams B. Formation of an artificial vagina by Frank's method. J Obstet Gynaecol Br Emp 1957;64:241–2.
33. Thomas JC, Brock JW 3rd. Vaginal substitution: attempts to create the ideal replacement. J Urol 2007;178:1855–9.
34. American College of Obstetricians and Gynecologists Committee on Adolescent Health Care. ACOG committee opinion no. 355: Vaginal agenesis: diagnosis, management, and routine care. Obstet Gynecol 2006;108:1605–9.
35. Nisolle M, Donnez J. Vaginoplasty using amniotic membranes in cases of vaginal agenesis or after vaginectomy. J Gynecol Surg 1992;8:25–30.
36. Tancer ML, Katz M, Veridiano NP. Vaginal epithelialization with human amnion. Obstet Gynecol 1979;54:345–9.
37. Mac ND. [A technique of vaginoplasty by inferior approach in the treatment of Rokitansky-Küster-Hauser syndrome. Mac's method]. Rev Fr Gynecol Obstet 1988;83:91–6. French.
38. Karjalainen O, Myllynen L, Kajanoja P, Tenhunen A, Purola E, Timonen S. Management of vaginal agenesis. Ann Chir Gynaecol 1980;69:37–41.
39. Brindeau A. Creation d́un vagin artificiel a Í aide des membranes ovulaires d́un ouef a terme. Gynecol Obstet (Paris) 1934;29:385.
40. Akle CA, Adinolfi M, Welsh KI, Leibowitz S, McColl I. Immunogenicity of human amniotic epithelial cells after transplantation into volunteers. Lancet 1981;7;2(8254):1003–5.
41. Saraf S, Saraf P. The McIndoe vaginoplasty: revisited. Internet J Gynecol Obstet 2007;6(2).

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Amniotic membrane and amniotic cells: Potential therapeutic tools to combat tissue inflammation and fibrosis?

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ABSTRACT

In addition to the placenta, umbilical cord and amniotic fluid, the amniotic membrane is emerging as an immensely valuable and easily accessible source of stem and progenitor cells. This concise review will focus on the stem/progenitor cell properties of human amniotic epithelial and mesenchymal stromal cells and evaluate the effects exerted by these cells and the amniotic membrane on tissue inflammation and fibrosis.

1. Introduction

1.1. Human amniotic epithelial and mesenchymal stromal cells

The human amniotic membrane (AM) contains two distinct cell populations. Human amniotic epithelial cells (hAEC) are cuboidal to columnar cells that form a monolayer lining the membrane and are in direct contact with the amniotic fluid. hAEC arise from the embryonic epiblast and are amongst the first cells to differentiate from the conceptus [1,2]. In contrast, human amniotic mesenchymal stromal cells (hAMSC) are dispersed in an extra-cellular matrix largely composed of collagen and laminin, and are derived from the extraembryonic mesoderm [1]. Both cell types originate during the pre-gastrulation stages of embryogenesis before the delineation of the three primary germ layers [1,2]. Embryonal carcinoma cells that are formed prior to gastrulation have been shown to retain stem cell-like properties. Thus, the early origin of the AM cells was a major reason that led to investigations into the plasticity and stemness of these cells.

Efficient protocols have been established for hAEC and hAMSC isolation from term placenta and are generally based on the separation of the AM from the chorionic membrane and subsequent enzymatic digestion [2–5]. A typical term AM yields between $150–200 \times 10^6$ hAEC and $20–50 \times 10^6$ hAMSC [6]. In culture, hAMSC exhibit plastic adherence and fibroblast-like morphology, while hAEC display a typical cobblestone epithelial phenotype. Many of the surface and intracellular stem/progenitor markers expressed by AM cells are listed in Tables 1 and 2. However, there is considerable variation in the percentages of AM cells expressing these markers reported by different investigators. The levels and pattern of marker expression appear to depend on the isolation protocol used and vary with expansion [7]. Gestational age dependant changes in marker expression have also been found. Surface markers such as CD44, CD49e and CD13 were significantly lower in hAEC derived from third trimester compared to first trimester [8] and hAEC expressing the pluripotency associated Nanog, Sox-2, Tra-1-60 and Tra-1-80 genes were higher in second trimester AM compared to term [9]. Further, cells with pluripotency associated markers have been found to be randomly distributed in the epithelial layer of term delivered AM [10]. This heterogeneity in distribution and gestational age dependant changes is also likely to contribute to different sub-populations being analyzed [2,5,8,11–14] and impact on investigations into stem cell properties and possibly pregnancy related studies using AM cells.

Interestingly, hAEC and hAMSC also express a repertoire of lineage associated genes (Tables 1 and 2), suggesting that they could act as progenitors and differentiate into various cell types. Indeed, hAEC and hAMSC have the ability to differentiate *in vitro* into cells from each of the three germ layers (Tables 1 and 2). After stimulating cells in media supplemented with growth factors, hormones and/or other additives, differentiation was monitored by evaluating the

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Table 1
Phenotype of undifferentiated hAEC and hAEC induced to differentiate *in vitro*.

Undifferentiated hAEC			
Marker groups	Markers	Detection methods	References
Mesenchymal and hematopoietic markers	CD10+, CD13+, CD29+, CD44+, CD49e+, CD73+, CD90+, CD105+, CD117+, CD166+, STRO-1+, CD14−, CD34−, CD45−, CD49d−, HLA-ABC +, HLA-DQ+/−, HLA-DR−	FACS	[2,7,11–15]
Stem cell markers	POU5F1 (OCT-4)+, Sox-2+	RT-PCR, immunocytochemistry	[2,11,14,15]
	FGF-4+, Rex-1+, CFC1+, Nanog+, DPPA3+, PROM1+, PAX6+, FOXD3−, GDF3−, TERT−	RT-PCR	[2,11,14,15]
	SSEA-3+, SSEA-4+, Tra 1-60+, Tra 1-81+, SSEA-1-	FACS, immunocytochemistry	[2,7,13–15].
	GCTM2+	immunocytochemistry	[11,15]
Neural lineage associated markers	Nestin, GAD, MBP, NF-M, NSE, CNPase, PLP, DM-20	RT-PCR	[2,14]
	Nestin, MAP2, GFAP	immunocytochemistry	[15]
	Neurofilament proteins, MAP2, MAP2 kinase	Immunocytochemistry	[39]
Lung associated markers	Nkx2.1, mucin, occludin, aquaporin-5, caveolin-1	RT-PCR, FACS	[11]
Hepatic lineage associated markers	Albumin, α-FP	RT-PCR, western blot, immunocytochemistry	[40]
	Albumin, α-1AT, CK18, GS, CPS-1, PEPCK, CYP2D6, CYP3A4, α-FP, TTR, TAT, CYP2C9, HNF3-γ, C/EBP-α	RT-PCR	[41]
Cardiomyogenic lineage associated markers	GATA-4, Nkx 2.5, MLC-2A, MLC-2V, MYL-7, ANP, CACNA1C, KCND3	RT-PCR	[14,15]
Pancreatic lineage associated markers	PDX-1	RT-PCR	[14]
Others	CD31-, CD324+ (E-cadherin), ABCG2/BCRP+, vimentin+, PanCK+,	FACS, immunocytochemistry	[2,11]

Differentiated hAEC			
Lineages	Characterization of differentiation: markers/cell morphology/tissue specific functions	Detection methods	References
Adipogenic	PPARγ; LPL; cells enlarge three/four times and are multinucleated; cells contain lipid deposits	RT-PCR, Oil Red O stain	[7,15]
Osteogenic	OSC, OSN; cells enlarge two/three times ad are binucleated; cells contain calcium deposits	RT-PCR, Von Kossa stain, Alzarin Red stain	[7,15]
Chondrogenic	Expression of collagen I and II; synthesis of proteoglycans	immunohistochemistry, Toluidine Blue Stain	[7]
Myogenic	ATCA2; MyoD1, skeletal muscle myosin heavy chain; elongated cells, multinucleated cells	immunocytochemistry	[8,15]
Neural	Increased expression of nestin, GAD, MBP, NF-M, NSE; expression of GFAP, CNP; elongated cells with neuronal- or astrocyte-like morphology	RT-PCR, immunocytochemistry	[14]
	Few nestin+ and MAP-2+ cells with neuronal morphology; numerous GFA + cells with astrocyte-like morphology	immunocytochemistry	[15]
Lung	Production of SPs A–D, SP-D secretion; epithelial phenotype with lamellar body formation	electron microscopy, immunohistochemistry, ELISA	[11]
Hepatic	Albumin; α-1AT, HNF-4α; CYP1A activity	RT-PCR, immunohystochemistry, EROD assay	[14]
	Albumin, HGF; features of hepatocytes	immunocytochemistry, FACS, transmission electron microscopy	[15]
Cardiomyogenic	GATA-4, MYL-7, ANP, CACNA1C, KCND3, TTNT; features of relatively mature cardiomyocytes	RT-PCR, FACS, transmission electron microscopy	[15]
	Increased expression of GATA-4, Nkx 2.5, MLC-2A, MLC-2V; expression ofα-actinin	RT-PCR, immunocytochemistry	[14]
Pancreatic	Increased expression of Pdx-1; expression of Pax-6, Insulin, NKx 2.2, glucagon	RT-PCR, immunocytochemistry	[14]
	AMY2B, glucagon; features of exocrine acinar beta cells	RT-PCR, immunocitochemistry, FACS, transmission electron microscopy	[15]

morphological changes (e.g. changes into cells with neuron, hepatocyte and cardiomyocyte-like features), expression of various lineage-specific genes, as well as assessing acquired abilities to exert tissue-specific functions (Tables 1 and 2). However, the level of maturation achieved *in vitro* may be variable [2,15], and could be due to the inability to express genes present in the terminally differentiated cells, shortcomings in the induction media, extra-cellular matrices, oxygen tensions and culture conditions used.

Clonal colony formation is an important feature of adult tissue derived stem cells. There are conflicting reports on the clonogenicity of AM cells. hAEC and hAMSC were found to be clonogenic with hAMSC forming colonies that could be expanded for at least 15 passages [5,15], whereas others report the absence of clonal colony formation by hAEC and hAMSC [13]. Generally, at much higher seeding densities, hAEC and hAMSC can be kept in culture for 5-10 passages [5,12,13]. Interestingly, after a few passages hAEC change from the cuboidal epithelial shape into elongated stromal-like cells that express markers associated with mesenchymal and fibroblast cells and show reduced differentiation potential [12,13]. The reason/s for these changes remains uncertain, but may be due to senescence, epigenetic modifications and to the autocrine/paracrine effects of growth factors such as EGF and TGFβ that are known to induce an epithelial to mesenchymal transition. Although the phenotypic changes are not as marked compared to hAEC, the morphology of hAMSC and differentiation potential also declines with expansion [5,12].

There is some evidence that AM cells can differentiate into cardiomyocytes, neural, alveolar epithelium and pancreatic β-islet cells following xenotransplantation and secrete proteins produced by hepatocytes [11,16]. Further, trophic factors secreted by hAEC and hAMSC could exert angiogenic, growth promoting, anti-inflammatory and anti-fibrotic effects following transplantation [16]. Thus, with a view to potential therapeutic applications, researchers are also developing isolation protocols in accordance with current guidelines for clinical use [16,17]. Culture of hAEC in

Table 2
Phenotype of undifferentiated hAMSC and of hAMSC induced to differentiated *in vitro*.

Undifferentiated hAMSC			
Marker groups	Markers	Detection methods	References
Mesenchymal and hematopoietic markers	CD3-, CD13+, CD14-, CD29+, CD34-, CD44+, CD45-, CD49e+, CD54+, CD73+, CD90+, CD105+, CD117 (weak), CD166+, $CD27^{low}$+, STRO-1+, HLA-A-B-C+, HLA-DR−	FACS	[1,2,7,8,13,42]
Stem cell markers	SSEA-3+, SSEA-4+	FACS, immunocytochemistry	[1,13]
	POU5F1 (OCT-4)+, Rex-1+, BMP-4+	RT-PCR	[1,13]
Endothelial marker	CD31-, VEGF receptor 1 and 2: FLT-1+ and KDR+	FACS	[1]
Hepatic lineage associated markers	Albumin, CK18, α-FP, α1-AT, HNF4α	RT-PCR	[43]
Pancreatic lineage associated markers	PDX-1	RT-PCR	[44]
Cardiomyogenic lineage associated markers	GATA-4, MLC-2A, MLC-2V, MLC-2v, cTnI, and cTnT, α-subunits of the cardiac-specific L-type calcium channel, Kv4.3	RT-PCR	[45]
Neural lineage associated markers	Nestin and musashi1, Tuj1 and NF-M, GFAP	RT-PCR, immunocytochemistry	[46]
Others	CD349+, CD140b+, CD324 (E-cadherin)-, vimentin+	immunocytochemistry	[2]

Differentiated hAMSC			
Lineages	Characterization of differentiation: markers/cell morphology/tissue specific functions	Detection methods	References
Adipogenic	LPL; accumulation of lipid deposits	RT-PCR, Oil-red O stain	[5]
Chondrogenic	Collagen-II; cartilage-specific metachromasia	RT-PCR, Toluidine Blue stain	[5]
Osteogenic	OPN; induction of calcium deposition	RT-PCR, Alizarin red stain	[5]
Myogenic	myoD, myogenin,desmin	RT-PCR, immunocytochemistry	[42]
	myoD1, skeletal muscle myosin heavy chain; features of myotubes	immunocytochemistry	[8]
Hepatic	Increased expression of albumin, CK18, α-FP, α1-AT, HNF4α; storage of glycogen	RT-PCR, immunocytochemistry; PAS staining	[43]
Pancreatic	Increased expression of Pdx-1, Isl-1, Pax-4, Pax-6, expression of insulin, glucagon, somatostatin; appearance of islet-like cell clusters	RT-PCR, immunocytochemistry	[44]
Cardiomyogenic	GATA-4, MLC-2A, MLC-2V, cTnI, and cTnT, α-subunits of the cardiac-specific L-type calcium channel, Kv4.3, induction of Nkx2.5, ANP and cardiac-specific gene -myosin heavy chain; integrate in cardiac tissues in co-culture experiments	RT-PCR, immunocytochemistry	[45]
Angiogenic	Increased expression of FLT-1, KDR, ICAM-1, appearance of CD34 positive cells, expression of vWF; features of endothelial cells	FACS	[42]
Neurogenic	Increased expression of nestin, musashi1, Tuj1 and NF-M, GFAP	RT-PCR, immunocytochemistry	[46]

serum free medium appears to lead to the expression of hematopoietic and monocytic markers, high telomerase activity and long telomere lengths [17], whereas hAEC that are routinely cultured in fetal calf serum (FCS) supplemented media lack these markers and telomerase activity [12–14]. Comparisons of cells grown in FCS with serum free media or media containing acceptable alternatives for clinical use such as platelet lysate and human serum may be warranted, as for example, a high level of telomerase activity is linked to teratoma and tumor formation. Injection of primary or passaged hAEC and hAMSC that have been cultured in FCS into mice, rodents and swine has so far not led to tumour formation [14,15,18–20], but the fusion of amniotic cells with host cells to form dysplastic precursors cannot be ruled out. Further, under serum free conditions a selection of surface markers were differentially expressed by primary and passage 5 hAEC [17]. This reinforces the possibility that culture conditions may select different cell populations, thereby altering the phenotype of the naïve population. Thus, further investigation would be beneficial as results reported on cell replacement, inflammation and fibrosis following xenotransplantion have been reported using primary hAEC and hAMSC prepared using FCS.

1.2. Pre-clinical studies investigating amniotic cells

Lung and liver fibrosis, myocardial infarct and stroke are leading causes of mortality and together with their long term morbidity places major burdens on health care systems worldwide. In pre-clinical animal disease models, human amniotic cells were found to make a modest contribution toward replacing damaged alveolar epithelium, endothelium and heart muscle [11,21,22], whereas a more significant contribution is likely to be their anti-inflammatory and anti-scarring effects.

Pooled MSC from amniotic and chorionic membranes and hAMSC alone have been injected directly into infarcted rat hearts following arterial ligation. Treated rats showed increased capillary density, improved left ventricular function and fractional shortening and a reduction in fibrotic scar tissue [21,22].

hAEC and hAMSC have also been evaluated as a treatment for liver and lung fibrosis. Hereditary, pathogenic, environmental and lifestyle factors can induce inflammation in liver and lungs and lead to collagen deposition in response to wound healing. The repeated insults lead to apoptosis and necrosis of cells, immune cell infiltration, release of pro-inflammatory cytokines, activation of resident cells into collagen depositing myofibroblast cells, altered tissue architecture and compromised organ function. Administration of Bleomycin, is widely used to mimic the phases of lung inflammation and fibrosis observed in patients with generic pulmonary fibrosis and acute respiratory distress syndrome. A 1:1 mixture of hAEC and hAMSC/human chorionic MSC was administered intra-tracheally or intra-peritoneally into Bleomycin-treated, immunocompetent C57/Bl6 mice [19]. Irrespective of the route of administration, human DNA and cytokeratin-19 positive cells were localized over the two week test period in lungs of mice receiving xenotransplants. Importantly, treated mice showed reduced neutrophil infiltration and fibrosis area whereas macrophage and lymphocyte numbers did not show significant changes [19]. Another study using Bleomycin injured SCID mice tested hAEC [11]. The data showed that following intravenous delivery, some hAEC persisted in the lungs over the four week test period, reduced IL-1, IL-6, TNFα protein levels and collagen in lungs and augmented regeneration leading to improved lung architecture [11].

The toxin carbon tetra chloride (CCl_4) is used to provoke liver fibrosis in mice and rodents. A study investigating the effects of hAEC in CCl_4 injured C57/Bl6 mice, found that following intravenous delivery, cells engrafted and persisted for several weeks in the liver [18]. Similar to effects observed in Bleomycin injured lungs, IL-6, TNFα protein and collagen content declined in the liver. Furthermore, the number of hepatocytes undergoing apoptosis and

number of activated collagen depositing hepatic stellate cells declined significantly in hAEC treated mice [18].

The therapeutic potential of AM derived cells has also been examined in neurological disorders. In particular, stroke has been a major target disease for testing the efficacy of transplantation of AM derived cells. Stroke remains a serious unmet medical condition worldwide and in the US stroke is the primary cause of disability and the third leading cause of mortality. Following the initial stroke episode, inflammation is a major cause of secondary cell death. Although the anti-inflammatory effects of AM derived cells could be beneficial in reducing stroke progression the optimal time and mode of cell delivery need careful assessment. That inflammation may represent a double-edged sword is exemplified in stroke, in that a dynamic modulation of the many inflammatory components in response to ischemic injury is necessary in order to facilitate the functional benefits of cell therapy. For example, the chemokine stromal cell-derived factor-1 or SDF-1, an early pathological inflammatory factor secreted soon after the stroke facilitates the migration of transplanted cells and therefore the early mitigation of SDF-1 may be detrimental. In parallel, stroke may lend a non-conducive brain microenvironment, requiring control of inflammation to some extent to enhance graft survival. To this end, following middle artery occlusion in rats, hAEC and hAMSC were transplanted into the presumed ischemic penumbra (instead of the necrotic core) 2 days after stroke and found to significantly improve motor and neurological deficits by 7 and 14 days and increase the number of healthy host cells within the penumbra [16]. hAEC injection into the penumbra can also lead to reduced infarct size [23]. While direct cell transplantation into the ischemic penumbra is feasible, non-invasive peripheral cell injection may allow a larger patient population to benefit from cell therapy in view of stroke's abrupt onset and rapid progression of debilitating disease symptoms.

In addition to the amniotic cells, the AM membrane itself can exert ameliorative effects and are summarized below.

1.3. Ongoing clinical applications using amniotic membranes

Human AM have a long history in clinical utility. The first application was reported a century ago where the membranes were used as biological dressings to heal skin wounds; a practice that continues to the present day. Currently, AM are also used for treating dermal burns and for open non-healing ulcers and surgical, infected and traumatic wounds [6,24,25]. Since the 1940s, AM have been used in the management of ocular surface disorders. The membrane is used as a graft (with the amniotic epithelium facing outwards) or as a patch (epithelium facing inwards) to cover and repair corneal, conjunctival and limbal defects and surgical incisions made during corrective surgery [26–28]. Further, hAEC and frozen stored AM intact or denuded of the epithelium are being used as feeder layers for the expansion of limbal and corneal stem cells for subsequent transplantation [29]. These ongoing applications led to pre-clinical studies testing the effects of AM on inflammation and fibrosis in lungs and liver.

1.4. Potential innovative applications of amniotic membranes

Recently, small pieces of the entire AM were found to be effective against cardiac ischemia [30] and liver fibrosis [31]. Cardiac ischemia was induced in rats by coronary artery ligation and fragments of AM from human term placenta were applied as patches onto the infarcted myocardium. During the two month follow-up period, treated rats showed improved cardiac dimensions and contractile functions including higher left ventricular ejection fraction, fractional shortening and wall thickening compared to non-treated rats [30].

Liver fibrosis was induced in rats through bile duct ligation (BDL) and AM fragments patched onto the surface of the injured liver [31]. While fibrosis progressed rapidly in controls leading to cirrhosis within 6 weeks of BDL, fibrosis was confined to the portal/periportal regions of the liver in AM-treated rats, without any evidence of cirrhosis and a nearly 50% reduction in collagen deposition [31]. Furthermore, the application of AM significantly delayed the gradual progression of the ductular reaction and reduced the area occupied by activated hepatic myofibroblast cells that deposit collagen [31].

While these initial pre-clinical findings suggests that AM and its' cells may have potential uses in these disease settings, stringent evaluation in larger animal models and comparisons against other cells such as adult bone marrow MSC and hematopoietic stem cells that have also shown to exert beneficial effects and currently being evaluated in clinical trials would be useful. Furthermore, very little is known about the factors that enable survival following xenotransplantation and mechanisms that trigger and lead to the ameliorative effects; some of the potential mechanisms are described below.

1.5. Potential mechanisms involved

A notable feature in the pre-clinical studies outlined above is amniotic cell survival in the absence of overt host responses after xenotransplantation into immunocompetent animals that had not been previously treated with immunosuppressants. Cells that could be transplanted across MHC barriers, without immunosuppression, offer immense scope for wide allogeneic therapeutic applications. hAMSC and hAEC express low levels of HLA Class IA and lack HLA-DR, co-stimulatory molecules CD40, CD80 and CD86 that engage T-cell receptors or are presented indirectly via antigen-presenting cells (APC), to fuel T-cell expansion [1,2,8,13]. One-way lymphocyte reactions have also demonstrated that hAEC and hAMSC fail to induce human T-cell proliferation [32]. Indeed, hAEC have been transplanted into allogeneic volunteers and during trials for lysosomal storage diseases without adverse sequelae attributed to the hAEC [1]. While these studies support the notion that amniotic cells can be transplanted across MHC barriers, generation of antibodies, effects of repeated cell injection as opposed to the possibility of tolerance induction need careful evaluation.

Further, hAEC and hAMSC can modulate immune cell activities. Amniotic cells suppress T-cell proliferation [32,33] with cell–cell contact and trophic factors being likely contributors. Although little is known about the effector molecules responsible, PGE2, TNF-Ra, IL-10, TGFβ and soluble HLA-G from hAEC and/or hAMSC are likely to play a role. hAMSC also inhibit the generation and maturation of APC. In transwell experiments, that only allow passage of soluble factors, hAMSC blocked differentiation and maturation of peripheral blood monocytes into dendritic cells (DC) preventing expression of the DC marker CD1a and reducing HLA-DR, CD80 and CD83 expression [34]. Furthermore, the blockade of monocyte maturation impaired their stimulatory activities on allogeneic T-cells [34]. Investigation into possible mechanism/s showed that hAMSC arrest monocytes in the G0 phase of the cell cycle, abolish TNFα and chemokines CXCL10, CXCL9 and CCL5 whilst greatly elevating the secretion of Th2-related cytokines CCL2, CXCL8 and IL-6 [34]. Effect of hAEC on APC is not known, but hAEC may restrain monocyte migration and activation via MIF-1.

The beneficial effects exerted by AM in the pre-clinical models of myocardial infarction and liver fibrosis, cannot be attributed to cell replacement in the injured tissue. Indeed, no cells derived from transplanted AM were found to have migrated and engrafted into the myocardium or liver. Most likely, the effects observed were associated with the release of soluble factors by cells and molecules

bound to the collagenous stromal matrix of the AM patch that exert paracrine mechanisms to support survival, differentiation and proliferation of host cells. The mechanisms are still undefined, however it has been reported that AM release potent immunomodulatory and anti-inflammatory cytokines (IL-10, IL-6) [35], growth factors associated with wound healing, including angiogenic factors (VEGF, PDGF angiogenin) [36], inducing proliferation (epidermal-, keratinocyte-, hepatocyte- and basic fibroblast growth factors) [37] and differentiation (TGFβ) [36].

The AM was also found to have reduced scarring in the myocardial infarct and livers of animals receiving membrane patches. In ophthalmic investigations it has been shown that hyaluronic acid present in the matrix of the AM can suppress TGFβ and inhibit the differentiation of conjunctival and limbal fibroblasts into myofibroblasts [38]. As TGFβ is a potent pro-fibrogenic cytokine its' reduction can inhibit collagen synthesis. Potentially, similar mechanisms may partly account for the reduction in scarring following the patching of AM. hAEC and hAMSC transplantation was also shown to elicit potent anti-fibrotic effects. Lowering of TGFβ protein was noted in Bleomycin and CCl_4 injured lungs and livers respectively, of mice receiving hAEC, coupled with an induction of collagen degrading matrix metalloproteinases and a reduction of their inhibitors, the TIMP proteins [11,18]. Again paracrine mechanisms induced by factors secreted by the hAEC may be involved. However, while studies show that hAEC are retained for several weeks, cell numbers engrafting are low and decline over time. Further, there is mounting evidence of transdifferentiation of hAEC *in vivo*. Early studies reported differentiation into neural cells, while recent studies report the presence of surfactant protein producing cells in lungs and albumin and α-antitrypsin secreting cells characteristic of hepatocytes in the liver. Whether the growth factor and cytokine milieu of the differentiated cells contribute to inflammation and fibrosis reduction is unknown. A recent study by Tsuji provides insights suggesting that hAMSC differentiating into cardiomyocytes may indeed play such a role [22].

In summary, hAEC and hAMSC have the capacity to differentiate into multiple cell lineages. In addition, the anti-inflammatory and anti-fibrotic effects of these cells and the AM have been demonstrated following transplantation into animal disease models. Ongoing studies relating to safety and efficacy of the transplanted hAEC, hAMSC and AM and mechanisms leading to reparative effects in diseased organs would make a valuable contribution in assessing the true potential of these cells for clinical applications.

Conflict of Interest

The authors state they have no conflict of interest.

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References

[1] Ilancheran S, Moodley Y, Manuelpillai U. Human fetal membranes: a source of stem cells for tissue regeneration and repair? Placenta 2009;30:2–10.
[2] Parolini O, Alviano F, Bagnara GP, Bilic G, Buhring HJ, Evangelista M, et al. Concise review: isolation and characterization of cells from human term placenta: outcome of the first international workshop on placenta derived stem cells. Stem Cells 2008;26:300–11.
[3] Miki T, Marongiu F, Dorko K, Ellis EC, Strom SC. Isolation of amniotic epithelial stem cells. Curr Protoc Stem Cell Biol; 2010 [Chapter 1]:Unit 1E 3.
[4] Marongiu F, Gramignoli R, Sun Q, Tahan V, Miki T, Dorko K, et al. Isolation of amniotic mesenchymal stem cells. Curr Protoc Stem Cell Biol; 2010 [Chapter 1]:Unit 1E 5.
[5] Soncini M, Vertua E, Gibelli L, Zorzi F, Denegri M, Albertini A, et al. Isolation and characterization of mesenchymal cells from human fetal membranes. J Tissue Eng Regen Med. 2007;1:296–305.
[6] Toda A, Okabe M, Yoshida T, Nikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. J Pharmacol Sci. 2007;105:215–28.
[7] Diaz-Prado S, Muinos-Lopez E, Hermida-Gomez T, Rendal-Vazquez ME, Fuentes-Boquete I, de Toro FJ, et al. Multilineage differentiation potential of cells isolated from the human amniotic membrane. J Cell Biochem 2010;111:846–57.
[8] Portmann-Lanz CB, Schoeberlein A, Huber A, Sager R, Malek A, Holzgreve W, et al. Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. Am J Obstet Gynecol 2006;194:664–73.
[9] Izumi M, Pazin BJ, Minervini CF, Gerlach J, Ross MA, Stolz DB, et al. Quantitative comparison of stem cell marker-positive cells in fetal and term human amnion. J Reprod Immunol 2009;81:39–43.
[10] Miki T, Mitamura K, Ross MA, Stolz DB, Strom SC. Identification of stem cell marker-positive cells by immunofluorescence in term human amnion. J Reprod Immunol 2007;75:91–6.
[11] Moodley Y, Ilancheran S, Atienza D, Samuels C, Wallace EM, Jenkin G, et al. Human amnion epithelial stem cell transplantation abrogates lung fibrosis and augments repair. Am J Respir Crit Care Med 2010;182:643–51.
[12] Stadler G, Hennerbichler S, Lindenmair A, Peterbauer A, Hofer K, van Griensven M, et al. Phenotypic shift of human amniotic epithelial cells in culture is associated with reduced osteogenic differentiation in vitro. Cytotherapy 2008;10:743–52.
[13] Bilic G, Zeisberger SM, Mallik AS, Zimmermann R, Zisch AH. Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy. Cell Transplant 2008;17:955–68.
[14] Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells 2005;23:1549–59.
[15] Ilancheran S, Michalska A, Peh G, Wallace EM, Pera M, Manuelpillai U. Stem cells derived from human fetal membranes display multilineage differentiation potential. Biol Reprod 2007;77:577–88.
[16] Parolini O, Alviano F, Bergwerf I, Boraschi D, De Bari C, De Waele P, et al. Toward cell therapy using placenta-derived cells: disease mechanisms, cell biology, preclinical studies, and regulatory aspects at the round table. Stem Cells Dev 2010;19:143–54.
[17] Murphy S, Rosli S, Acharya R, Mathias L, Lim R, Wallace E, et al. Amnion epithelial cell isolation and characterization for clinical use. Curr Protoc Stem Cell Biol.; 2010 [Chapter 1]:Unit 1E 6.
[18] Manuelpillai U, Tchongue J, Lourensz D, Vaghjiani V, Samuel CS, Liu A, et al. Transplantation of human amnion epithelial cells reduces hepatic fibrosis in immunocompetent CCl(4)-Treated mice. Cell Transplant 2010;19:1157–68.
[19] Cargnoni A, Gibelli L, Tosini A, Signoroni PB, Nassuato C, Arienti D, et al. Transplantation of allogeneic and xenogeneic placenta-derived cells reduces bleomycin-induced lung fibrosis. Cell Transplant 2009;18:405–22.
[20] Bailo M, Soncini M, Vertua E, Signoroni PB, Sanzone S, Lombardi G, et al. Engraftment potential of human amnion and chorion cells derived from term placenta. Transplantation 2004;78:1439–48.
[21] Ventura C, Cantoni S, Bianchi F, Lionetti V, Cavallini C, Scarlata I, et al. Hyaluronan mixed esters of butyric and retinoic acid drive cardiac and endothelial fate in term placenta human mesenchymal stem cells and enhance cardiac repair in infarcted rat hearts. J Biol Chem 2007;282:14243–52.
[22] Tsuji H, Miyoshi S, Ikegami Y, Hida N, Asada H, Togashi I, et al. Xenografted human amniotic membrane-derived mesenchymal stem cells are immunologically tolerated and transdifferentiated into cardiomyocytes. Circ Res 2010;106:1613–23.
[23] Liu T, Wu J, Huang Q, Hou Y, Jiang Z, Zang S, et al. Human amniotic epithelial cells ameliorate behavioral dysfunction and reduce infarct size in the rat middle cerebral artery occlusion model. Shock 2008;29:603–11.
[24] 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.
[25] Singh R, Chouhan US, Purohit S, Gupta P, Kumar P, Kumar A, et al. Radiation processed amniotic membranes in the treatment of non-healing ulcers of different etiologies. Cell Tissue Bank 2004;5:129–34.
[26] Kruse FE, Cursiefen C. Surgery of the cornea: corneal, limbal stem cell and amniotic membrane transplantation. Dev Ophthalmol 2008;41:159–70.
[27] Sangwan VS, Burman S, Tejwani S, Mahesh SP, Murthy R. Amniotic membrane transplantation: a review of current indications in the management of ophthalmic disorders. Indian J Ophthalmol 2007;55:251–60.
[28] Burman S, Tejwani S, Vemuganti GK, Gopinathan U, Sangwan VS. Ophthalmic applications of preserved human amniotic membrane: a review of current indications. Cell Tissue Bank 2004;5:161–75.
[29] Chen YT, Li W, Hayashida Y, He H, Chen SY, Tseng DY, et al. Human amniotic epithelial cells as novel feeder layers for promoting ex vivo expansion of limbal epithelial progenitor cells. Stem Cells 2007;25:1995–2005.
[30] Cargnoni A, Di Marcello M, Campagnol M, Nassuato C, Albertini A, Parolini O. Amniotic membrane patching promotes ischemic rat heart repair. Cell Transplant 2009;18:1147–59.

[31] Sant`Anna LB CA, Ressel L, Vanosi G, Parolini O. Amniotic Membrane application reduces liver fibrosis in a bile duct ligation rat model. Cell Transplant (in press).
[32] Wolbank S, Peterbauer A, Fahrner M, Hennerbichler S, van Griensven M, Stadler G, et al. Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue. Tissue Eng. 2007;13:1173–83.
[33] Magatti M, De Munari S, Vertua E, Gibelli L, Wengler GS, Parolini O. Human amnion mesenchyme harbors cells with allogeneic T-cell suppression and stimulation capabilities. Stem Cells 2008;26:182–92.
[34] Magatti M, De Munari S, Vertua E, Nassauto C, Albertini A, Wengler GS, et al. Amniotic mesenchymal tissue cells inhibit dendritic cell differentiation of peripheral blood and amnion resident monocytes. Cell Transplant 2009;18: 899–914.
[35] Paradowska E, Blach-Olszewska Z, Gejdel E. Constitutive and induced cytokine production by human placenta and amniotic membrane at term. Placenta 1997;18:441–6.
[36] Steed DL, Trumpower C, Duffy D, Smith C, Marshall V, Rupp R, et al. Amnion-derived cellular cytokine solution: a physiological combination of cytokines for wound healing. Eplasty 2008;8:e18.
[37] Tahara M, Tasaka K, Masumoto N, Adachi K, Adachi H, Ikebuchi Y, et al. Expression of messenger ribonucleic acid for epidermal growth factor (EGF), transforming growth factor-alpha (TGF alpha), and EGF receptor in human amnion cells: possible role of TGF alpha in prostaglandin E2 synthesis and cell proliferation. J Clin Endocrinol Metab 1995;80:138–46.
[38] Tseng SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. J Cell Physiol 1999;179:325–35.
[39] Sakuragawa N, Thangavel R, Mizuguchi M, Hirasawa M, Kamo I. Expression of markers for both neuronal and glial cells in human amniotic epithelial cells. Neurosci Lett. 1996;209:9–12.
[40] Sakuragawa N, Enosawa S, Ishii T, Thangavel R, Tashiro T, Okuyama T, et al. Human amniotic epithelial cells are promising transgene carriers for allogeneic cell transplantation into liver. J Hum Genet 2000;45:171–6.
[41] Takashima S, Ise H, Zhao P, Akaike T, Nikaido T. Human amniotic epithelial cells possess hepatocyte-like characteristics and functions. Cell Struct Funct. 2004;29:73–84.
[42] Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, Franchina M, et al. Term amniotic membrane is a high throughput source for multipotent mesenchymal stem cells with the ability to differentiate into endothelial cells in vitro. BMC Dev Biol. 2007;7:11.
[43] Tamagawa T, Oi S, Ishiwata I, Ishikawa H, Nakamura Y. Differentiation of mesenchymal cells derived from human amniotic membranes into hepatocyte-like cells in vitro. Hum Cell 2007;20:77–84.
[44] Tamagawa T, Ishiwata I, Sato K, Nakamura Y. Induced in vitro differentiation of pancreatic-like cells from human amnion-derived fibroblast-like cells. Hum Cell 2009;22:55–63.
[45] Zhao P, Ise H, Hongo M, Ota M, Konishi I, Nikaido T. Human amniotic mesenchymal cells have some characteristics of cardiomyocytes. Transplantation 2005;79:528–35.
[46] Sakuragawa N, Kakinuma K, Kikuchi A, Okano H, Uchida S, Kamo I, et al. Human amnion mesenchyme cells express phenotypes of neuroglial progenitor cells. J Neurosci Res. 2004;78:208–14.

Review Article

Amniotic therapeutic biomaterials in urology: current and future applications

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Abstract: To examine the rationale and applications of amniotic tissue augmentation in urological surgery. Published literature in English-language was reviewed for basic science and clinical use of amniotic or amnion-chorionic tissue in genitourinary tissues. Basic science and animal studies support the likely benefit of clinical applications of amnion-derived tissues in a variety of urologic interventions. The broad number of properties found in amniotic membrane, coupled with its immunologically privileged status presents a number of future applications in the urological surgical realm. These applications are in their clinical infancy and suggest that further studies are warranted to investigate the use of these products in a systematic fashion.

Keywords: Amnion; chorion; dehydrated membranes; hypospadias; urethral reconstruction

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Historical medical uses of amniotic membrane

The medical uses of the human placenta probably date back centuries, with the first description of its use in a treatise in 1593 by a Chinese clinician Li Shi-Zhen (1). The first mention of the medical use of amniotic membrane in the Western literature appeared in 1910 by Davis, who reported on a series of cases where it was used as a skin graft in a large case series at Johns Hopkins Hospital (2). This was shortly followed by Drs. Stern and Sabella, collaborators who separately published studies using this material in wounds and burns (3,4). Through the rest of the 20th century the medical use of the placenta, and more specifically the amniotic membrane, was described in a number of medical indications including; but not limited to, the following types of cases: (I) skin grafting; (II) lower extremity diabetic ulcers (5); (III) lower extremity venous leg ulcers (6); (IV) general wounds (7); (V) conjunctival surgery and repair (8); (VI) burns (9,10); (VII) periodontal disease and dentistry (11,12); (VIII) vaginal reconstruction and OB/GYN applications (13); (IX) neurosurgical applications including spine surgery (14,15); (X) orthopedic surgery applications (16).

Properties and function of amniotic tissues

While amniotic membrane was originally used because of its recognized ability to substitute as a skin like tissue with healing properties, the underlying physiologic, biochemical and cytological properties of the tissue are reported to confer a number of additional properties, in addition to simply performing as a skin substitute. These properties include: (I) contains essential growth factors (17); (II) modulation of inflammation (18); (III) reduction of scar tissue formation (18); (IV) barrier properties (14); (V) immunologically privileged tissue (19); (VI) enhancement of wound healing (20); (VII) reduction of pain in burns and wounds; (VIII) innate antibiotic properties (21). There

are varying degrees of documentation of each of the above properties, but these attributes appear regularly in the literature.

The underlying bimolecular mechanisms responsible for the above properties are becoming increasingly well characterized, in a growing, robust literature (1,17,22-24) (*Table 1*). Briefly, active participation of mesenchymal and circulating stem cells, activated by a wide array of growth factors and cytokines, and the provision of a collagen based architecture present a unique structure that promotes healing and regeneration of tissues in which the amniotic membrane and its components are applied (22).

Preclinical urologic applications of amniotic tissue products

The general history of the use of amniotic membrane products and the more recent understanding of the underlying mechanism of action behind their properties suggested a number of urologic applications. Preclinical work has explored a number of urogenital applications.

The use of amniotic membrane as a potential material for bladder repair extends back to the early 1980s (25). More recently, Iigma and others demonstrated that transplantation of preserved human amniotic membrane could successfully be used for bladder augmentation in rats, with resulting regeneration of a number of tissues in the bladder being demonstrated as early as 3 months postoperatively (26).

Salehipour *et al.* have evaluated the use of human amniotic membrane in the reconstruction of long ureteral defects in dogs (27). In this study, the use of human amniotic membrane for the reconstruction of ureteral defects in a canine model was studied. The authors used chorion prepared and properly treated for surgical insertion into dogs with circumferentially cut defects, and while they did not believe the approach to be useful for long (3 cm) defects, they speculated that use of the amniotic membrane might be studied for shorter defects or as a patch graft.

Shakeri *et al.* looked at the use of amniotic membrane as a xenograft for urethroplasty in rabbits. The authors concluded that amniotic membrane technology was an inexpensive, easy, and biodegradable graft yielding very little antigen effect and a viable option in surgical urethroplasty approaches (28).

Wang *et al.* looked at a variation of this idea, namely using the collagen scaffolding of amniotic membrane as a potential regenerative material in urethroplasty, and obtained preliminary success in that approach (29). The author's concluded that tissue-engineered denuded human amniotic scaffold (dHAS) created by separating the basement membrane layer of amniotic membrane minimizes potential rejection and maximizes the biocompatibility of amniotic membrane, making it a potential ideal xenograft for urethral reconstruction. This concept was also explored by Gunes and others, who compared the use of buccal mucosa and amniotic membrane for urethroplasty in a rabbit model (30). The group examined whether buccal mucosa, amniotic membrane, or both might be useful in urethroplasty using epithelial transformation as the experimental endpoint, noting highest efficacy in the combined tissue application. After 8 weeks, the best epithelial transformations were observed in the combined group.

In another study, Shakeri also noted that amniotic membrane maybe a substitute for transitional epithelium of the bladder in dogs. The authors concluded that grafts remained in place in all cases, except in one of the dogs in the augmentation group that developed patch perforation, urine leakage and finally peritonitis. In others, histological examinations revealed evidence of regeneration of normal-appearing urothelium, lamina propria, neovascularization, retracting placental patch, and reconstitution of a normal-appearing and functioning bladder. This suggests that placental membranes, because of their low antigenic properties, easy availability and tolerability by the host urinary tract, could provide an excellent graft material for urinary tract reconstructions (31).

Comparison of amniotic membrane with other materials in preclinical work was conducted by Sharifiaghdas *et al.* (32). They examined the use of poly lactic-co-glycolic acid (PLGA), PLGA/collagen and human amniotic membrane (hAM) for human urothelial and smooth muscle cell engineering. The authors demonstrated significant improvement of cell attachment and growth achieved by collagen coating (PLGA/collagen) compared to PLGA and hAM. hAM was a weaker matrix for bladder engineering purposes.

Human amniotic fluid and isolates prepared from human amniotic membrane derived mesenchymal stem cells have also had some preliminary scientific work performed on their inherent biological properties. Human amniotic membrane mesenchymal stem cells were interestingly noted to have a suppressive effect on prostate cancer cells (33). Sedrakyan *et al.* found that amniotic fluid stem cells seemed to reduce the formation of renal fibrosis in a mouse model

Table 1 Regulators of wound healing and inflammation found in dHACM (17,22)

Regulators of wound healing and inflammation found in dHACM
Regulators of soft tissue healing in dHACM
Cytokines
Angiogenin (Ang)
Angiopoietin-2 (ANG-2)
Basic fibroblast growth factor (bFGF)
Beta nerve growth factor (β-NGF)
Bone morphogenetic protein 5 (BMP-5)
Brain-derived neurotrophic factor (BDNF)
Endocrine gland-derived vascular endothelial growth factor (EG-VEGF)
Epidermal growth factor (EGF)
Fibroblast growth factor 4 (FGF-4)
Growth hormone (GH)
Heparin binding egf-like growth factor (HB-EGF)
Hepatocyte growth factor (HGF)
Insulin-like growth factor 1 (IGF-I)
Insulin-like growth factor binding protein 1 (IGFBP-1)
Insulin-like growth factor binding protein 2 (IGFBP-2)
Insulin-like growth factor binding protein 3 (IGFBP-3)
Insulin-like growth factor binding protein 4 (IGFBP-4)
Insulin-like growth factor binding protein 6 (IGFBP-6)
Keratinocyte growth factor (KGF/FGF-7)
Placental growth factor (PlGF)
Platelet-derived growth factor AA (PDGF-AA)
Platelet-derived growth factor BB (PDGF-BB)
Transforming growth factor alpha (TGF-α)
Transforming growth factor beta 1 (TGF-β1)
Vascular endothelial growth factor (VEGF)
Vascular endothelial growth factor D (VEGF-D)
Matrix metalloproteinases
Matrix metalloproteinase 1 (MMP-1)
Matrix metalloproteinase 2 (MMP-2)
Matrix metalloproteinase 3 (MMP-3)
Matrix metalloproteinase 8 (MMP-8)
Matrix metalloproteinase 9 (MMP-9)
Matrix metalloproteinase 10 (MMP-10)
Matrix metalloproteinase 13 (MMP-13)
Protease inhibitors
Alpha 1 antitrypsin (α1AT)
Alpha 2 macroglobulin (α2M)
Tissue inhibitor of metalloproteinase 1 (TIMP-1)
Tissue inhibitor of metalloproteinase 2 (TIMP-2)
Tissue inhibitor of metalloproteinase 4 (TIMP-4)
Regulators of inflammation in dHACM
Cytokines
Granulocyte colony-stimulating factor (GCSF)
Granulocyte macrophage colony-stimulating factor (GM-CSF)
Growth differentiation factor 15 (GDF-15)
Interferon gamma (IFNγ)
Interleukin 1 alpha (IL-1α)
Interleukin 1 beta (IL-1β)
Interleukin 1 receptor antagonist (IL-1RA)
Interleukin 4 (IL-4)
Interleukin 5 (IL-5)
Interleukin 6 (IL-6)
Interleukin 7 (IL-7)
Interleukin 10 (IL-10)
Interleukin 12 p40 (IL-12p40)
Interleukin 12 p70 (IL-12p70)
Interleukin 15 (IL-15)
Interleukin 17 (IL-17)
Macrophage colony-stimulating factor (MCSF)
Osteoprotegerin (OPG)
Prostaglandin E2 (PGE2)
Chemokines
B lymphocyte chemoattractant (BLC/CXCL13)
Chemokine ligand 1 (I-309/CCL1)
Eotaxin 2
Interleukin 8 (IL-8)
Interleukin 16 (IL-16)
Macrophage inflammatory protein 1 alpha (MIP-1α/CCL3)
Macrophage inflammatory protein 1 beta (MIP-β1/CCL4)
Macrophage inflammatory protein 1 delta (MIP-1δ/MIP-5/CCL15)
Monocyte chemotactic protein 1 (MCP-1/CCL2)
Monokine induced by gamma interferon (MIG/CXCL9)
Regulated on activation, normal t-cell expressed and secreted (RANTES/CCL5)

dHACM, dehydrated human amnion/chorion membrane.

of acute tubular necrosis (34).

Amniotic membrane has also been used as a supportive scaffold for other procedures. For example, Burgers *et al.* used nerve grafts, nerve growth factor and a supportive scaffold made from amniotic membrane to repair surgically induced erectile dysfunction in rats. In this study, the use of fetal amniotic membrane as an alternative growth factor matrix was used to improve the regeneration of ablated cavernous nerves in rats as a model to study surgically damaged nerves. The use of membrane as an alternative nerve growth matrix improved electrically stimulated erections and mating behavior in these mice (35).

The underlying logic in many of these preclinical studies focuses on both the structural and regenerative properties of amniotic membrane. In the first case, the underlying structure of the membrane, created by various collagen types, forms an architecture or scaffold that assists in the re-creation of normal tissue. In the second case, the biologically active growth factors, cytokines and other biomolecules initiate and modulate the regenerative process that involves the recruitment and activation of stem cells and fibroblasts in the area under consideration.

Clinical urological applications of amniotic tissues in humans

The broadly recognized ability of amniotic tissues to help in healing and regenerative repair of tissues suggested a number of other direct clinical applications. Preclinical work and initial evaluation of these tissues have been attempted in a number of urogenital indications. Koziak *et al.* built on the previous preclinical work described above and investigated the use of amniotic membrane in the reconstruction of long ureteral strictures in 11 patients (36,37). Several reports of the use of amniotic membrane to repair vesicovaginal fistulas have also been reported. In each case, successful use of the material has permitted a less aggressive operative or non-operative approach to this problem (38).

Most recently, the use of an amniotic membrane protective layer in the surgical field of patients undergoing robotic assisted laparoscopic prostatectomy as a means of protecting adjacent nerve bundles from scarring has been advanced by a number of clinicians (39,40). The notion that amniotic membrane might be useful in preventing adhesions at the surgical site in DaVinci robot prostatectomies was initially developed and evolved across a number of sites. Patel *et al.* published a retrospective series of these patients demonstrating improvement in both urinary incontinence and erectile function in the short-term post-operative period (41). For completeness, it is worth noting that amniotic membrane has found numerous applications in OB/GYN surgery as well, with applications in the repair of various abnormalities of the vagina, uterus and related structures in patients (42-49).

At our institutions, we have applied amniotic membrane technology in the following four realms of adult and pediatric urology: (I) proximal and redo-hypospadias repairs; (II) complex penile reconstruction in Peyronie's disease; (III) microsurgical cord denervation procedures; (IV) posterior urethroplasty in the male with a history of pelvic radiation.

In the field of hypospadias surgery, proximal hypospadias comprises most of the severe cases and results in higher surgical complication rates (50). Between 6–20% of hypospadias patients are diagnosed with proximal hypospadias (51,52). Hypospadias is corrected surgically with the goal of improving cosmetic appearance as well as normalizing erectile function and voiding. Surgery creates a straight phallus, with the meatus residing at the tip of the glans, with a proper and symmetrical appearance of both the glans and penile shaft. For more severe hypospadias, specifically for proximal hypospadias, a variety of surgical techniques can be employed. Unfortunately, even the most skilled surgeons cannot guarantee a positive outcome. Complications from proximal hypospadias repair range from 6–30% depending on the severity of the defect, the surgical technique utilized, and the experience of the surgeon (53). Surgical results are also poorer in re-operative cases. Common complications include urethrocutaneous fistulas (UCF), urethral stricture, urethral diverticulum, and persistent ventral curvature (54). UCF, or a reopening of the surgical site, can occur in 13–33% of patients, depending on the different surgical techniques utilized (50-52,55-58). Current data suggests that an experienced pediatric urologist successfully can close fistulas in 71%, 72%, 77%, 100%, and 100% of patients after fistula repairs 1 to 5, respectively (59). With these high reoperation rates, there is a significant need to investigate innovative approaches to reduce complication rates. One such approach is the potential application of dehydrated human amnion/chorion membrane (dHACM). The underlying premise is to provide a barrier layer with a robust source of tissue and vascular growth factors and provide a local anti-inflammatory environment, thus optimizing soft tissue healing of the surgical site (20,41,60-62).

In the field of Peyronie's disease, surgical correction of curvature with either permanent plication sutures or graft material usually requires full or partial mobilization of the deep dorsal neurovascular bundle to allow for proper placement of surgical material as well as to avoid potentially devastating complications such as glans numbness and ischemia. Typically, the neurovascular bundle is placed back in its anatomical position and in most cases, it will overly the site of surgical curvature correction. The inflammatory response associated with healing and the subsequent formation of fibrotic tissue and neuroma has been theorized as the cause for post-operative pain with the associated graft material or suture knots. The use of dHACM has been postulated to be used as an interposition graft in between the plication knots or graft material and the neurovascular bundle in an effort to reduce fibrosis and neuroma formation and therefore improve pain outcomes. Anecdotally, we have noticed diminished postoperative pain and more rapid recovery, and are currently studying, in a more formal manner, the utility of using this interposition graft in these cases.

In the field of chronic orchialgia, management strategies are aimed at identifying specific etiologies of the pain and managing those directly (i.e., varicocelectomy, vasectomy reversal, epididymectomy). When a specific etiology cannot be identified, the individual has failed conservative management, or has failed surgical management and spermatic cord denervation may be discussed. Methods that have been employed to improve outcomes of spermatic cord denervation include the use of a provocative pre-operative spermatic cord block and the intraoperative use of the operating room microscope. Even with these improvements in pre-operative screening and surgical techniques the success rate of the procedure is still not 100% (current studies success rates range from 70–90%) and there is a reported orchiectomy rate of 10–20% after the surgery due to persistent pain (63,64). The utilization of dHACM as a wrap at the site of denervation has been theorized to reduce the formation of fibrosis and neuroma and therefore reduce persistent pain post-operatively (63).

In the field of posterior urethral contracture in the male with a history of pelvic radiation, surgical management is aimed at resecting the affected scarred or infected tissue, achieving a watertight tension free anastomosis, and providing a healthy bed of tissue to allow for good wound healing. However, even with these surgical tenants, necrosis and reformation of scar tissue still may occur even in the most skilled hands. The use of dHACM as a wrap at the site of urethral anastomosis has been theorized to recruit healthy tissue ingrowth and improve surgical outcomes. Clinical outcomes are currently being evaluated.

Potential future applications of human amniotic tissues

The broad number of properties found in amniotic membrane, coupled with its immunologically privileged status presents a number of future applications, particularly given the historical preclinical and clinical uses described above. New applications continue to be proposed, and the potential for combining amniotic membrane allografts with other biomaterials expands this horizon further.

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Footnote

Conflicts of Interest: William O. Brant: proctor, consultant, and grant recipient, Boston Scientific; Siam Oottamasathien: scientific advisory consultant, GlycoMira Therapeutics Inc. The other authors have no conflicts of interest to declare.

References

1. Silini AR, Cargnoni A, Magatti M. The Long Path of Human Placenta, and Its Derivatives, in Regenerative Medicine. Front Bioeng Biotechnol 2015;3:162.
2. Davis JW. Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. Johns Hopkins Medical Journal 1910;15:307-96.
3. Stern M. The grafting of preserved amniotic membrane to burned and ulcerated surfaces, substituting skin grafts. JAMA 1913;60:973-4.
4. Sabella N. Use of fetal membranes in skin grafting. Med Records NY 1913;83:478-80.
5. Zelen CM, Serena TE, Snyder RJ. A prospective, randomised comparative study of weekly versus biweekly application of dehydrated human amnion/chorion membrane allograft in the management of diabetic foot ulcers. Int Wound J 2014;11:122-8.
6. Serena TE, Carter MJ, Le LT. A multicenter, randomized, controlled clinical trial evaluating the use of dehydrated

human amnion/chorion membrane allografts and multilayer compression therapy vs. multilayer compression therapy alone in the treatment of venous leg ulcers. Wound Repair Regen 2014;22:688-93.
7. Forbes J, Fetterolf DE. Dehydrated amniotic membrane allografts for the treatment of chronic wounds: a case series. J Wound Care 2012;21:290,292,294-6.
8. Asoklis RS, Damijonaityte A, Butkiene L. Ocular surface reconstruction using amniotic membrane following excision of conjunctival and limbal tumors. Eur J Ophthalmol 2011;21:552-8.
9. Adly OA, Moghazy AM, Abbas AH. Assessment of amniotic and polyurethane membrane dressings in the treatment of burns. Burns 2010;36:703-10.
10. Kesting MR, Wolff KD, Hohlweg-Majert B. The role of allogenic amniotic membrane in burn treatment. J Burn Care Res 2008;29:907-16.
11. Chen E, Tofe A. A literature review of the safety and biocompatibility of amnion tissue. J Imp Adv Clin Dent 2010;2:67-75.
12. Holtzclaw D, Toscano N. Amnion chorion allograft barrier: indications and techniques update. J Imp Adv Clin Dent 2012 4:25-38.
13. Sarwar I, Sultana R, Nisa RU. Vaginoplasty by using amnion graft in patients of vaginal agenesis associated with Mayor-Rokitansky-Kuster-Hauser syndrome. J Ayub Med Coll Abbottabad 2010;22:7-10.
14. Tao H, Fan H. Implantation of amniotic membrane to reduce postlaminectomy epidural adhesions. Eur Spine J 2009;18:1202-12.
15. Subach BR, Copay AG. The use of a dehydrated amnion/chorion membrane allograft in patients who subsequently undergo reexploration after posterior lumbar instrumentation. Adv Orthop 2015;2015:501202.
16. Demirkan F, Colakoglu N, Herek O. The use of amniotic membrane in flexor tendon repair: an experimental model. Arch Orthop Trauma Surg 2002;122:396-9.
17. Koob TJ, Rennert R, Zabek N. Biological properties of dehydrated human amnion/chorion composite graft: implications for chronic wound healing. Int Wound J 2013;10:493-500.
18. Tseng SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. J Cell Physiol 1999;179:325-35.
19. Hao Y, Ma DH, Hwang DG. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea 2000;19:348-52.
20. Zelen CM, Serena TE, Denoziere G. A prospective randomised comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. Int Wound J 2013;10:502-7.
21. Stock SJ, Kelly RW, Riley SC. Natural antimicrobial production by the amnion. Am J Obstet Gynecol 2007;196:255.e1-6.
22. Lim JJ. A Primer on Amniotic Membrane Regenerative Healing. Grand Rapids: MiMedx Group 2015.
23. Parolini O, Caruso M. Review: Preclinical studies on placenta-derived cells and amniotic membrane: an update. Placenta 2011;32 Suppl 2:S186-95.
24. Parolini O, Solomon A, Evangelista M. Human Term Placenta as a Therapeutic Agent: From the first clinical applications to future perspectives. In: Berven E, ed. Human Placenta: Structure and Development. Hauppauge, NY: Nova Science 2010:1-48.
25. Norris MA, Cohen MS, Warren MM. Bladder reconstruction in rabbits with glutaraldehyde-stabilized amniotic membranes. Urology 1982;19:631-5.
26. Iijima K, Igawa Y, Imamura T. Transplantation of preserved human amniotic membrane for bladder augmentation in rats. Tissue Eng 2007;13:513-24.
27. Salehipour M, Mohammadian R, Jahanbini S. Is amniotic membrane a suitable biomaterial for reconstruction of long ureteral defects? Saudi J Kidney Dis Transpl 2013;24:135-8.
28. Shakeri S, Haghpanah A, Khezri A. Application of amniotic membrane as xenograft for urethroplasty in rabbit. Int Urol Nephrol 2009;41:895-901.
29. Wang F, Liu T, Yang L. Urethral reconstruction with tissue-engineered human amniotic scaffold in rabbit urethral injury models. Med Sci Monit 2014;20:2430-8.
30. Güneş M, Altok M, Güneş A. A Novel Approach to Penile Augmentation Urethroplasty Using Buccal Mucosa and Amniotic Membrane: A Pilot Study in a Rabbit Model. Urology 2016;87:210-5.
31. Shakeri S, Masoudi P, Yazdani M. Evaluation of human amniotic membrane as a substitute for transitional epithelium of bladder in dog. J Appl Anim Res 2008;33:55-59.
32. Sharifiaghdas F, Naji M, Sarhangnejad R. Comparing supportive properties of poly lactic-co-glycolic acid (PLGA), PLGA/collagen and human amniotic membrane for human urothelial and smooth muscle cells engineering. Urol J 2014;11:1620-8.
33. Rolfo A, Giuffrida D, Giuffrida MC. New perspectives

for prostate cancer treatment: in vitro inhibition of LNCaP and PC3 cell proliferation by amnion-derived mesenchymal stromal cells conditioned media. Aging Male 2014;17:94-101.
34. Sedrakyan S, Da Sacco S, Milanesi A. Injection of amniotic fluid stem cells delays progression of renal fibrosis. J Am Soc Nephrol 2012;23:661-73.
35. Burgers JK, Nelson RJ, Quinlan DM. Nerve growth factor, nerve grafts and amniotic membrane grafts restore erectile function in rats. J Urol 1991;146:463-8.
36. Koziak A, Marcheluk A, Dmowski T. Reconstructive surgery of male urethra using human amnion membranes (grafts)--first announcement. Ann Transplant 2004;9:21-4.
37. Koziak A, Salagierski M, Marcheluk A. Early experience in reconstruction of long ureteral strictures with allogenic amniotic membrane. Int J Urol 2007;14:607-10.
38. Barski D, Gerullis H, Ecke T. Repair of a vesico-vaginal fistula with amniotic membrane - Step 1 of the IDEAL recommendations of surgical innovation. Cent European J Urol 2015;68:459-61.
39. Razdan S. Protective patch linked to erectile function recovery: 90% of patients receiving membrane during robotic RP regain function at 3 months. Available online: http://urologytimes.modernmedicine.com/urology-times/news/protective-patch-linked-erectile-function-recovery?page=full
40. Razdan S. First in the world use of amniotic membrane in robotic prostatectomy for early return of erections. Available online: https://www.youtube.com/watch?v=osfAIT6QTws
41. Patel VR, Samavedia S, Bates AS. Dehydrated Human Amnion/Chorion Membrane Allograft Nerve Wrap Around the Prostatic Neurovascular Bundle Accelerates Early Return to Continence and Potency Following Robot-assisted Radical Prostatectomy: Propensity Score-matched Analysis. Eur Urol 2015;67:977-80.
42. Amer MI, Abd-El-Maeboud KH, Abdelfatah I. Human amnion as a temporary biologic barrier after hysteroscopic lysis of severe intrauterine adhesions: pilot study. J Minim Invasive Gynecol 2010;17:605-11.
43. Amer MI, Abd-El-Maeboud KH. Amnion graft following hysteroscopic lysis of intrauterine adhesions. J Obstet Gynaecol Res 2006;32:559-66.
44. Ashworth MF, Morton KE, Dewhurst J. Vaginoplasty using amnion. Obstet Gynecol 1986;67:443-6.
45. Chou CT, Lee C, Hwang JL. Amniotic membrane used for vulvar adhesion treatment. Arch Gynecol Obstet 2001;265:223-4.
46. Fotopoulou C, Sehouli J, Gehrmann N, et al. Functional and anatomic results of amnion vaginoplasty in young women with Mayer-Rokitansky-Küster-Hauser syndrome. Fertil Steril 2010;94:317-23.
47. Mhaskar R. Amniotic membrane for cervical reconstruction. Int J Gynaecol Obstet 2005;90:123-7.
48. Zafar M, Saeed S, Kant B. Use of amnion in vaginoplasty for vaginal atresia. J Coll Physicians Surg Pak 2007;17:107-9.
49. Price DT, Price TC. Robotic repair of a vesicovaginal fistula in an irradiated field using a dehydrated amniotic allograft as an interposition patch. J Robot Surg 2016;10:77-80.
50. Steven L, Cherian A, Yankovic F. Current practice in paediatric hypospadias surgery; A specialist survey. J Pediatr Urol 2013;9:1126-30.
51. Duckett JW, Baskin L. Hypospadias In: Adult and Pediatric Urology. 3rd. St. Louis: Mosby 1996.
52. Schneuer FJ, Holland AJ, Pereira G. Prevalence, repairs and complications of hypospadias: an Australia population-based study. Arch Dis Child 2015;100:1038-43.
53. Bhat A, Mandal AK. Acute postoperative complications of hypospadias repair. Indian J Urol 2008;24:241-8.
54. Retik AB, Atala A. Complications of hypospadias repair. Urol Clin North Am 2002;29:329-39.
55. Huang LQ, Ge Z, Tian J. Retrospective analysis of individual risk factors for urethrocutaneous fistula after onlay hypospadias repair in pediatric patients. Ital J Pediatr 2015;41:35.
56. Pippi Salle JL, Sayed S, Salle A. Proximal hypospadias: A persistent challenge. Single institution outcome analysis of three surgical techniques over a 10-year period. J Pediatr Urol 2016;12:28.e1-7.
57. Snodgrass W, Bush N. Tubularized incised plate proximal hypospadias repair: Continued evolution and extended applications. J Pediatr Urol 2011;7:2-9.
58. Snodgrass WT, Granberg C, Bush NC. Urethral strictures following urethral plate and proximal urethral elevation during proximal TIP hypospadias repair. J Pediatr Urol 2013;9:990-4.
59. Waterman BJ, Renschler T, Cartwright PC. Variables in successful repair of urethrocutaneous fistula after hypospadias surgery. J Urol 2002;168:726-30.
60. Fetterolf D, Savage R. Dehydrated human amniotic tissue improves healing time, cost of care. Today's Wound Clinic 2013:19-20.
61. Group M. Products: AmnioFix 2016. Available online: http://www.mimedx.com

62. Sheikh ES, Sheikh ES, Fetterolf DE. Use of dehydrated human amniotic membrane allografts to promote healing in patients with refractor non-healing wounds. Int Wound J 2014;11:711-7.
63. Craig JR, Hotaling JM, Brant WO. Use of Dehydrated Amniotic Membrane Allograft at the Time of Microscopic Denervation of the Spermatic Cord for Chronic Orchialgia Improves Outcomes 2016 Annual Fall Scientific Meeting of SMSNA. Scottsdale, AZ: Sexual Medicine Society of North America, 2016.
64. Strom KH, Levine LA. Microsurgical denervation of the spermatic cord for chronic orchialgia: long-term results from a single center. J Urol 2008;180:949-53.

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The use of an amniotic membrane graft to prevent postoperative adhesions*

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Grafts of trypsin-treated, gamma-irradiated human amniotic membranes were used to cover injured uterine horns of nulliparous female rabbits to prevent adhesions. In this study, the gradual integration of the membranes into the serosal layer of the uterus, together with marked neovascularization, was observed. By the 30th postoperative day, the grafts had been completely integrated, with little evidence of rejection and no evidence of infection at the graft sites. Of 30 uterine horns treated with membrane grafts, only 4 (13.4%) showed any adhesion formation at or among the graft sites. All of the 24 untreated controls showed adhesion formation at the site of injury. Furthermore, whatever adhesions were found in membrane-treated horns could be graded as thin and filmy, accounting for <10% of the surface area of the graft, whereas the controls showed dense, thick adhesions covering 50% to 100% of the injured areas. We conclude that these specially prepared amniotic membranes are safe and effective in dramatically reducing postoperative adhesion formation in this animal model. Fertil Steril 55:624, 1991

The presence of pelvic or abdominal adhesions is known to be a major cause of infertility in the human female. Adhesions may result from a great number of medical conditions or from surgical intervention.[1,2] Illness leading to adhesion formation includes pelvic inflammatory or other pelvic or abdominal inflammatory processes, resulting either from infection or endometriosis. The surgical procedures required by these and other pathological conditions, e.g., cysts, tumors, may also result in adhesion formation. Adhesions may, in turn, be associated with infertility by causing occlusion of the fallopian tubes or by interfering with tubal-ovarian function, inhibition of ovum pick-up being the best example. It is postulated that the formation of adhesions evolves from trauma to serosal surfaces followed by release of a fibrinogen-rich exudate and subsequent deposits of fibrin. If the fibrin fails to lyse and becomes organized, adhesion formation may result. This leads either to thick or filmy adhesive bands that may bridge the pelvic organs or tissues or to the dense fixation of these structures to each other.

Through the years, a great number of natural and synthetic graft materials have been employed in an effort to reduce adhesion formation on traumatized surfaces but with only marginally successful results. Natural materials have included peritoneum, omentum, fat, and amnion, as well as amnion plus chorion.[3–10] Synthetic materials, including polyvinyl alcohol film and tantalum foil, were used in the past and, more recently, barriers consisting of Gelfilm and Gelfoam paste (Upjohn Co., Kalamazoo, MI); Surgicel (Johnson and Johnson, New Brunswick, NJ); and Silastic (Dow-Corning, Midland, MI); as well as meshes of Gore-Tex (Gore-Tex, Gore, TX) and Interceed (Johnson and Johnson) have been employed.[6,11–17] The newer materials have led to more promising results. In the present study, macroscopic and microscopic peri-

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toneal adhesions to an amniotic membrane graft were studied in a rabbit model to determine if these specially processed membranes could reduce adhesion formation when placed over experimentally damaged tissues.

MATERIALS AND METHODS

Amniotic Membrane Grafts

Amniotic membranes were harvested from freshly delivered human placentas taken at the time of cesarean section. The amniotic membranes were manually separated from the chorion and washed in distilled water. The clean membranes were first treated by soaking for 3 hours in a 10% solution of trypsin. Subsequently, they were irradiated with gamma radiation to sterilize them in the following manner. First, the entire membrane underwent an 8-hour 20-minute irradiation at 60,000 rads/h for a total dose of 500,000 rads. The membranes were then cut into smaller squares of approximately 2 × 2 cm and reradiated at 60,000 rads/h for a total of 33 hours and 20 minutes, equivalent to a two-million rad dose. The small squares thus prepared were frozen at −70°C in distilled water to maintain them until they were used (within 4 weeks). Just before use, the membranes were thawed at 22°C. No antibiotics were used during this process.

Surgical Procedure

Study animals consisted of nulliparous female New Zealand rabbits, each weighing at least 3.5 kg to ensure adequate size of the pelvic organs. Six rabbits were assigned to groups A through C and 18 to group D. At the time of surgery, the rabbits were anesthetized with a mixture consisting of ketamine (10 mg/kg), promazine (1 mg/kg), and xylozine (6 mg/kg). The abdomen was shaved, subjected to sterile prep, and draped. Sterile microsurgical techniques under the operating microscope were employed, as previously described by Badaway et al.[3] Experimental injuries consisted of a series of incisions through the serosal and muscularis layers of the uterine horn extending into the endometrial cavity, with frequent avulsion of the mucosa. The cuts, 1 cm long and spaced 5 mm apart, were created with microscissors. Gross hemostasis was obtained with bipolar electrocautery. Membrane grafts, approximately 1 × 2 cm in size, were sutured into place over the lesion in a single layer

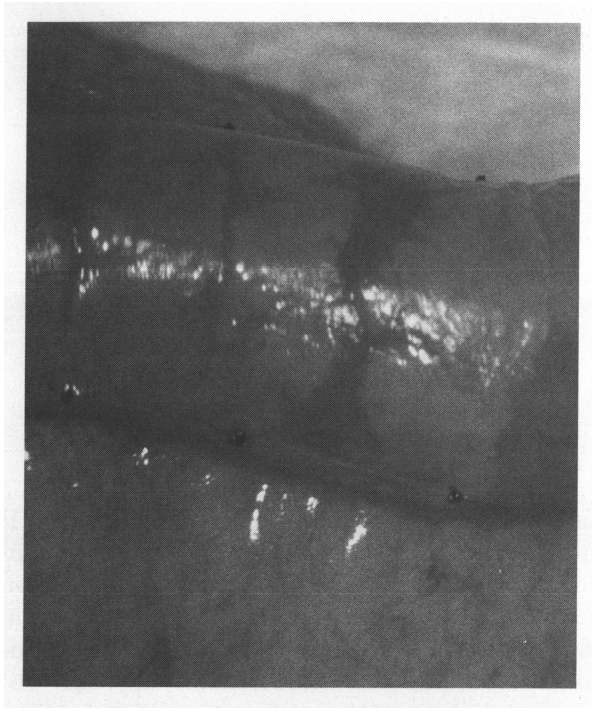

Figure 1 Uterine horn showing incision sites covered with amniotic membrane sutured into place.

using multiple interrupted sutures of 7-0 polyglactin (Fig. 1). The maternal side of the membrane was placed against the injury, and the fetal side faced into the abdominal cavity. The glistening fetal surface can easily be distinguished from the matte maternal side, even after freezing and thawing. This orientation was chosen in the expectation that the rougher side of the membrane would incorporate itself better to the injury site, and the smoother surface would afford better protection against adhesion formation. After surgery, the abdomen was closed in three layers and a sterile dressing left in place for 72 hours. All animals received procaine penicillin at a dosage of 50 mL/kg intramuscularly four times daily × five doses postoperatively and were maintained in a vivarium at 27°C with 40% to 70% humidity and given food pellets and water ad libitum.

Description of Experimental Groups

Animals were randomly assigned to three groups of 6 animals each and a fourth group of 18 animals. Each rabbit was subjected to two surgical procedures, the initial laparotomy including the designated operative procedure and a second-look laparotomy to evaluate the effects of the experimental intervention. The groups were as follows: group A (n = 6) was the background control group. The ab-

Table 1 Comparison of Results in Membrane-treated Versus Untreated Uterine Hours After Experimental Injury

Group	Horn	Surgical procedure	No. with adhesions	Types of adhesions	Amount of surface affected
					%
A (n = 6)	Right	None	0		
	Left	None	0		
B (n = 6)	Right	Incisions	6 (100)[b]	Dense, thick	75 (50 to 100)
	Left	None	0		
C (n = 6)	Right	Incisions + membrane	0		
	Left	Membrane only	1 (17)[a]	Filmy, thin	10
D (n = 18)	Right	Incisons + membrane	3 (17)[a]	Filmy, thin	10 (0 to 20)
	Left	Incisions + sutures	18 (100)[a]	Dense, thick	75 (50 to 100)

[a] Values in parentheses are percents. Right versus left horn: Fisher's exact test, in each case $P < 0.05$.

domen in this group was opened, exposed to no specific injury or treatment, and closed. Group B (n = 6) was the model control group in which controlled injuries, as described above, were made on one uterine horn of each animal. The contralateral horn was not injured, and no therapeutic interventions were made on either horn. Group C (n = 6) was the first treatment group in which injuries were carried out on one uterine horn as in group B, and then both injured and noninjured horns were covered with membrane grafts held in place with microsutures. Group D (n = 18) formed the second treatment group in which both uterine horns were experimentally injured in a similar manner. One horn was then treated by suturing a membrane into place, and the contralateral horn was treated with interrupted, hemostatic microsutures of 7-0 maxon.

Thirty days after the initial laparotomy and surgical intervention, each animal was reoperated on. Adhesions were photographed and evaluated relative to their presence or absence and percentage of surface included and graded as to adhesion quality (thin, thick, filmy, or dense). All initial surgery was completed by one team of surgeons (R.L.Y., J.M.C.), and subsequent evaluation of all results by the second team (B.A.M., G.Z.) in blinded fashion. Additionally, all specimens underwent standard histologic examination. Permanent sections were created from paraffin blocks, and hematoxylin and eosin staining was used on the sections. Statistical analysis was performed using Fisher's exact test, with significance accepted at $P < 0.05$.

RESULTS

All results are summarized in Table 1. The background control group plus model control group B confirmed the validity of the model by demonstrating that there were no background adhesions from laparotomy alone and that the experimental injury was sufficient to cause dense adhesions in 100% of the cases if untreated. These included surface adhesions as well as loop-to-loop adhesions leading to severe tortuosity of the involved horn (Fig. 2). It was further noted that there was no crossover of these adhesions to the uninjured contralateral horn ($P = 0.002$).

Experimental group C showed no significant difference ($P = 0.5$) in adhesion formation on sites of membrane grafts placed over injured versus noninjured uterine horns. Thin, filmy adhesions were found in only one case on the noninjured horn and

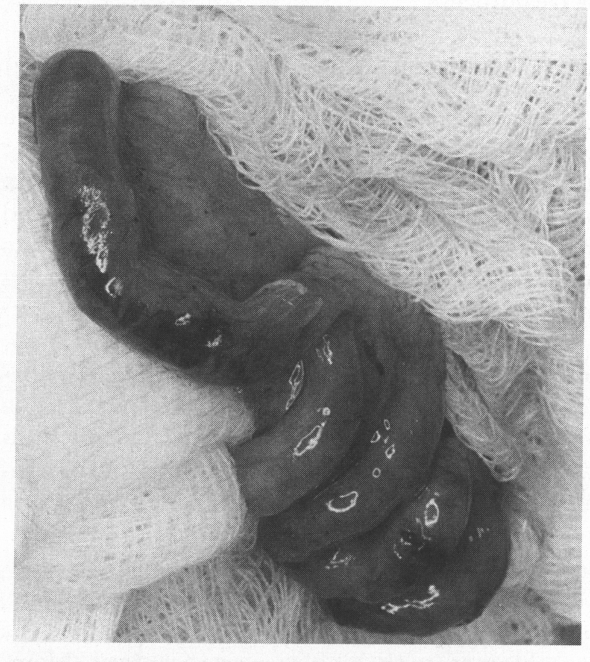

Figure 2 Right and left horns demonstrating tortuosity secondary to dense adhesions on the right horn (*below*) and absence of adhesions on the membrane-treated left horn (*above*).

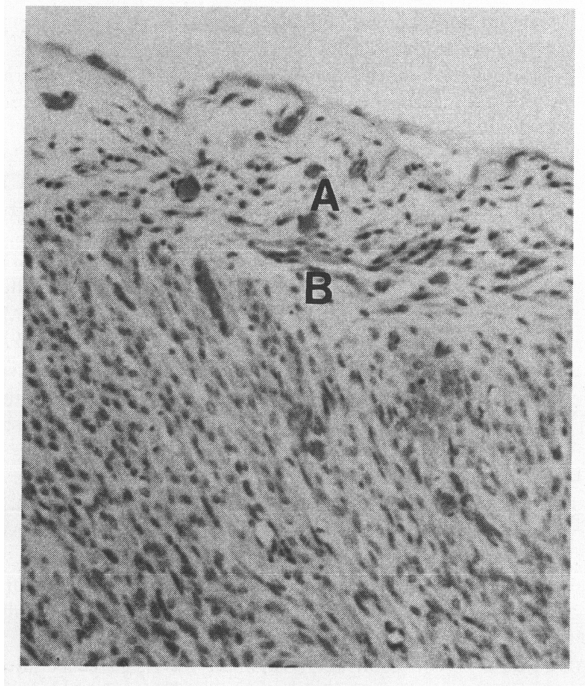

Figure 3 Photomicrograph of serosal surface of uterine horn covered by amniotic membrane (**A**) demonstrating both neovascularization of the membrane and a lack of significant inflammatory response in the serosa at the interface (**B**).

in no cases on the injured side. Finally, in experimental group D, membrane grafts significantly reduced the formation of adhesions as compared with those found at the site of hemostatic microsutures. Dense, thick adhesions over an average of 75% of the surface area of the sites of injury were noted on the horns treated with the sutures in 100% of the cases. In contrast, only 17% of the injured horns ($P = 0.0000003$) that had been covered with membrane grafts showed any adhesions, and these were thin, filmy, and covered only about 10% of the injured/grafted area. Histologic studies showed that the membranes were integrated with the serosal layer and showed neovascularization at the site of the graft. Minimal polymorphonuclear infiltration of the serosal surfaces was present, suggesting no significant immunological response (Fig. 3).

DISCUSSION

The use of human amnion as a surgical adjunct has a long history. An excellent review has been published by Trelford and Trelford-Sauder.[5] Amnion has been used to prevent pelvic and abdominal adhesions in a number of experimental animal models, as well as in human patients.[8–10] The natural membrane has been used in tubal surgery,[4] and there exists an extensive experience with its use in vaginal reconstructive surgery in women.[18,19] Other applications include repair of conjunctival defects,[20] reconstruction of the bile duct,[21] and prevention of meningocerebral adhesions after craniotomy.[6,7] Ongoing research at our own institution involves the use of amniotic membranes in plastic surgery, peripheral neurosurgery, as well as extensive use in urologic surgery. Its primary role in humans, however, has remained in the areas of burns, ulcers, other skin trauma, and in wound healing.[22–25]

No substantial literature exists describing its potential in preventing intra-abdominal adhesions in humans, although some progress has been made elsewhere in this area through the use of a number of synthetic agents. Badaway et al.[3] recently reported on the intra-abdominal application of amniotic membranes to prevent adhesions in the rat model. They noted little effect inhibiting adhesion formation on serosal surfaces but observed somewhat better results on the parietal peritoneum. The explanations that they offered for the lack of success involved problems with postoperative organ immobility and blood pooling, both of which may play a role in adhesion formation after human surgery.

Our own success with the rabbit model may be, in part, explained by the novel preparation of the membranes as described above. A study of the literature reveals almost as many different methods of preparing and storing the membranes as there are case or experimental reports. Our own previous poor results with glutaraldehyde-treated membranes (unpublished data), as well as equally unsatisfactory experience elsewhere with alcohol pretreatment and oven drying[7] or simple freezing in saline,[3] seem to indicate that the radiation retreatment after trypsin washing may have some advantage. The membranes thus prepared underwent adequate neovascularization and caused no significant inflammatory infiltration. This also seems to support a conclusion of no significant immunological reaction induced by the membranes, as also observed in the Badaway et al.[3] study.

The thinness and the exceptional compliant quality of the membranes made them extremely facile to use. Single layer application is mandatory in the pelvis and abdomen to prevent fibrosis formation within the membrane itself, a phenomenon we had observed previously in unreported experiments. Although synthetic grafts are applied without suturing, in our procedures, the membranes

were fixed in place using microsutures. In the human, this adds the potential for concomitant use of liquid antiadhesive adjuvants, such as dextran. An added technical advantage to the use of amniotic membranes, as opposed, apparently, to the synthetic meshes, is the fact that they can be applied and sutured over surfaces not perfectly dry.

It now remains to test the membranes against the formation of adhesions involving the parietal peritoneum. The aim would be to improve the outcome of procedures involving extensive endometriosis or pelvic sidewall adhesions of the adnexal structures. This model has been difficult to establish,[2] but earlier results in the rat with more simply prepared membranes have already shown some promise.[3]

REFERENCES

1. Ryan GB, Grobety J, Majno G: Postoperative peritoneal adhesions: a study of the mechanisms. Am J Pathol 65:117, 1971
2. Diamond MP, DeCherney AH: Pathogenesis of adhesion formation/reformation: application to reproductive surgery. Microsurgery 8:103, 1987
3. Badaway SZA, Baggish MS, ElBakry MM, Baltoyannis P: Evaluation of tissue healing and adhesion formation after an intra-abdominal amniotic membrane graft in the rat. J Reprod Med 34:198, 1989
4. Ten Berge BS, Lok TT: Plastic surgery of closed tubes with chorion-amnion. Fertil Steril 5:339, 1954
5. Trelford JD, Trelford-Sauder M: The amnion in surgery, past and present. Am J Obstet Gynecol 134:833, 1979
6. Pudenz RH, Odom GL: Meningocerebral adhesions: an experimental study of the effect of human amniotic membrane, amnioplastin, beef allantoic membrane, cargile membrane, tantalum foil, polyvinyl alcohol films. Surgery 12:318, 1942
7. Chao Y-C, Humphreys S, Penfield W: A new method of preventing adhesions. The use of amnioplastin after craniotomy. Br Med J 1:517, 1940
8. Massee JS, Symmonds RE, Dockery MB, Gollenback GA: Use of fetal membranes as replacement for pelvic peritoneum after pelvic exenteration in the dog. Surg Forum 13: 407, 1962
9. Trelford-Sauder M, Trelford JD: Replacement of the peritoneum with amnion following pelvic exenteration. Surg Gynecol Obstet 145:699, 1977
10. Trelford-Sauder M, Dawe EJ, Trelford JD: Use of allograft amniotic membranes for control of intra-abdominal adhesions. J Med 9:273, 1978
11. INTERCEED (TC7) Adhesion Barrier Study Group: Prevention of postsurgical adhesions by INTERCEED (TC7), an absorbable adhesion barrier: a prospective, randomized multicenter clinical study. Fertil Steril 51:933, 1989
12. Diamond MP, Linsky CB, Cunningham T, Constantine B, deZerega GS, DeCherney AH: A model for sidewall adhesions in the rabbit: reduction by an absorbable barrier. Microsurgery 8:197, 1987
13. Soules MR, Dennis L, Bosarge A, Moore DE: The prevention of postoperative pelvic adhesions: an animal study comparing barrier methods with dextran 70. Am J Obstet Gynecol 143:829, 1982
14. Goldberg JM, Toledo AA, Mitchell DE: An evaluation of the Gore-Tex surgical membrane for the prevention of postoperative peritoneal adhesions. Obstet Gynecol 70:846, 1987
15. Galan N, Leader A, Malkinson T, Taylor P: Adhesion prophylaxis in rabbits with SURGICEL and two absorbable microsurgical sutures. J Reprod Med 28:662, 1983
16. Hixson C, Swanson LA, Friedman CI: Oxidized cellulose for preventing adnexal adhesions. J Reprod Med 31:58, 1986
17. Larsson B, Nisell H, Granberg I: Surgicel: an absorbable hemostatic material in prevention of peritoneal adhesions in rats. Acta Chir Scand 144:375, 1978
18. Tracer ML, Katz M, Veridiano NP: Vaginal epithelialization with human amnion. Obstet Gynecol 54:345, 1979
19. Burger K: Artificial vaginal reconstruction with the help of amnion. Zentralbl Gynakol 61:2437, 1937
20. deRoetth A: Plastic repair of conjunctival defects with fetal membranes. Arch Ophthalmol 23:522, 1940
21. Scudamore CH, Becker CD, Fache JS, Bianco R, Shackleton CR, Burhenne HJ, Owen DA, Schechter MT, Seccombe D: Human amnion as a bioprosthesis for bile duct reconstruction in the pig. Am J Surg 155:635, 1988
22. Faulk WP, Matthews R, Stevens PJ, Bennett JP, Burgos H, Hsi BL: Human amnion as an adjunct in wound healing. Lancet 1:1156, 1980
23. Robson MC, Krizek TJ, Koss N, Samburg JL: Amniotic membranes as a temporary wound dressing. Surg Gynecol Obstet 136:904, 1973
24. Bennett JP, Matthews R, Faulk WP: Treatment of chronic ulceration of the legs with human amnion. Lancet 1:1153, 1980
25. Robson MC, Samburg JL, Krizek TJ: Quantitative comparison of biological dressings. Surg Forum 23:503, 1972

Evaluation of processed human amnion membrane to prevent post-operative adhesions in an ovine laminectomy model

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INTRODUCTION: Postoperative fibrosis after surgery is a consequence of wound healing. After a spinal laminectomy, peridural adhesion results in tethering, traction and compression of the thecal sac and nerve roots causing radicular pain and lower extremity weakness.

One approach to prevent adhesion formation is to place a mechanical barrier between the dura mater and the overlying tissue. Several materials have been utilized, although none have been widely accepted. Amnion Tissue (AT) has been used in a variety of surgical procedures and has a distinct orientation with an epithelial surface that acts as a natural barrier to prevent adhesions and a stromal surface that is readily adherent. Biologically, AT has been demonstrated to reduce inflammation and inhibit vascularization.

PURPOSE: The aim of this study was to evaluate the ability of processed human amnion (AM) to act as a physical and biological barrier to limit the formation of epidural fibrosis and scar adhesions between the paraspinal muscles and the dura mater and to evaluate the biologic response of implanted human AM in an ovine laminectomy model.

METHODS: Human AT was obtained from consenting cesarean sections. The AT was separated from the chorion and the spongy layer removed. The resulting tissue (35-70 um thickness) was rinsed with sterile saline and treated with 1% glutaraldehyde (v/v) for 15 minutes at room temperature. The residual glutaraldehyde was rinsed off with sterile 0.9% saline then placed between two layers of sterile backing material and cut into patches. AM were terminally sterilized by electron beam.

With IACUC approval, a two level lumbar dorsal laminectomy procedure was performed in seven skeletally mature female sheep (Ovis aries) at the L2/L3 and L4/L5 levels. AM samples were placed over the laminectomy site in five sheep with either the epithelial surface away from the dura mater (Group EA, n=5) or the epithelial surface towards the dura mater (Group ET, n=5). All AM treatments were assigned randomly to laminectomy sites. Two sheep (Group C, n=4) underwent the laminectomy procedure without any membrane barrier implantation to serve as a control group.

At eight weeks, sites were evaluated histologically for fibrosis, inflammation (granulomatous and lymphocytic), and the presence, severity and location of adhesion in three areas of the graft.

RESULTS: All AM implants were an effective anti-adhesion barrier, were largely intact and no substantial adverse effects were observed. In comparison, all control treatment sites had extensive adhesions to the dura mater (Figure 1). All AM implants with the epithelial surface of the AM facing away from the dura mater prevented adhesion between the dura mater and the surrounding fibrous tissue (Figure 2). The amount of reactive fibrosis in the implant area was reduced in AM implant sites as compared to control treatment sites (Table 1), although there were no significant differences among treatment groups ($p = 0.10$). When the AM epithelial side was placed against the dura mater, adhesion was significantly ($p < 0.05$) reduced as compared to the control group. Lymphocytic inflammation ranged from none to mild for all treatments (AM treated or control) and there were no significant differences among groups.

DISCUSSION: The current study demonstrated that processed human AM serves as an effective biological and physical barrier to prevent peridural adhesions and to reduce fibrosis after dorsal laminectomy. Compared to the control sites that lacked a physical barrier, AM significantly decreased adhesions ($p<0.05$) and trended towards reduced fibrosis. AM also appeared to be immune-privileged, as evidenced by a mild lymphocytic inflammation with no significant differences as compared to control treatments.

AM was evaluated in two orientations: with the epithelial side towards (ET) the dura and with the epithelial side away (EA) from the dura. When the epithelial layer of the AM was oriented away from the dura, there was a clear gap and prevention of adhesion between the AM and the filling fibrosis. No such gap existed when the stromal surface of the AM was towards the dura mater and histologic analysis revealed tissue continuity between the dura and the AM. Exposing the stromal side of AM to fibrotic infill (ET) led to moderate granulomatous inflammation as compared to Controls and EA. Although moderate, previous studies demonstrated that suture alone produced a greater granulomatous inflammatory response than the AM treatment.

When surgical revision is required, an important consideration is to have a dissection plane that is easily identifiable at sites where the membrane was implanted. The AM orientations present different scenarios upon revision. When the epithelial side is towards the dura, adhesions were prevented between the AM and the dura. In contrast, when the epithelial side is facing away from the dura, the dissection plane would be between the defect scar and the AM.

Fig. 1 No Implant, Control

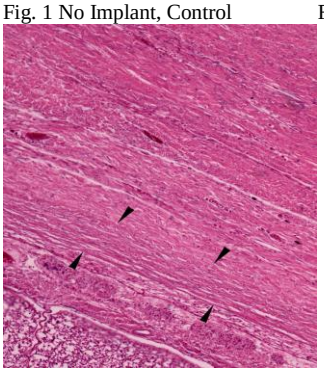

Fig. 2 AM Epithelial away (EA)

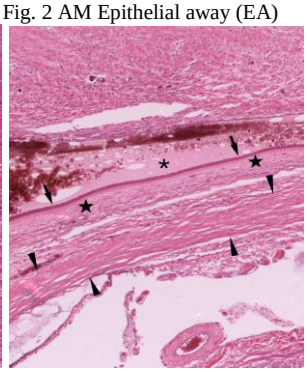

Fig.1 Histophotomicrograph showing a control sheep without implant that had extensive reactive fibrosis that fills the laminectomy site with complete and extensive adhesion to the dura (arrowheads).

Fig.2 Histophotomicrograph showing laminectomy site with the AM implant having the epithelial surface (large arrows) away from the dura. Image is oriented with filling fibrosis at the top, dura and spinal cord towards the bottom. There is tissue continuity of the stroma surface of the AM to the dura (arrowheads) with minimal reactive fibrosis (stars) forming a thickened dural membrane. This is separated from the overlying fibrosis filling the vertebral defect by a complete cleared cleft (asterick). There is minimal inflammation associated with the AM implant. Large arrow= epithelial surface of AM implant. Small arrow= multinucleated macrophages. Arrowhead= original dura. Star= AM associated reactive fibrosis. Asterick= cleft. HE, 100X.

Table 1: Histologic Scoring

	C	EA	ET
Fibrosis	2.6 +- 0.7	0.9 +- 0.3	1.8 +- 0.9
Adhesion of Fill Fibrosis to Implant	(2.0 +/- 0)	0.0 +- 0.0	2.0 +- 0.0
Adhesion Between Dura and Implant	No Implant	1.9 +- 0.3	0.5 +- 0.5
Lymphocytic Inflammation	0.2 +- 0.4	0.7 +- 0.6	0.9 +- 1.0
Granulomatous Inflammation	0.08 +- 0.3	0.3 +- 0.5	1.9 +- 0.7

The parenthetical value for the control reflects the adhesion score to the dura.

CONCLUSIONS: The AM implant reduced epidural fibrosis and adhesion to the dura mater without undue inflammation and thus appears to be a promising candidate for clinical evaluation.

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Postoperative Adhesion Development Following Cesarean and Open Intra-Abdominal Gynecological Operations: A Review

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Abstract

In this review, we discuss the pathophysiology of adhesion development, the impact of physiological changes associated with pregnancy on markers of adhesion development, and the clinical implications of adhesion development following cesarean delivery (CD). Although peritoneal adhesions develop after the overwhelming majority of intra-abdominal and pelvic surgery, there is evidence in the literature that suggests that patients having CD may develop adhesions less frequently. However, adhesions continue to be a concern after CD, and are likely significant, albeit on average less than after gynecological operations, but with potential to cause significant delay in the delivery of the baby with serious, lifelong consequences. Appreciation of the pathophysiology of adhesion development described herein should allow a more informed approach to the rapidly evolving field of intra-abdominal adhesions and should serve as a reference for an evidence-based approach to consideration for the prevention and treatment of adhesions.

Keywords

adhesions, cesarean, gynecological operations

Introduction

Adhesions are an enigmatic condition with protean clinical manifestations; they are defined as abnormal fibrous connection between 2 anatomically different surfaces. The principles of microsurgery, initially described by Swolin in 1967[1] and popularized in the 1980s,[2] are now accepted as the basis for good surgical practice. Although such principles are sensible, the extent to which microsurgical techniques decrease adhesion development remains unclear. This is compounded by the lack of prospective, randomized, blinded clinical trials in humans on this topic, with most recommendations being based on animal studies, and opinions of recognized authorities in our profession. This is understandable, in view of financial requirements of funding such a study.

Adhesions remain a scourge after abdominal and pelvic surgery. Notable among its potential sequelae are infertility[3] with increased risk of ectopic pregnancy, should the patient subsequently conceive,[4] abdominal and pelvic pain,[5] bowel obstruction,[6] and difficult repeat surgical procedures.[7] In addition, abdominopelvic adhesions may interfere with the disbursement of intraperitoneal chemotherapy in patients with abdominal or pelvic cancer.[8] After gynecologic surgery, intraperitoneal adhesions form in 55% to 100% of patients[9-11]; however, rates of adhesion development recorded at a second cesarean delivery (CD) are lower and ranged from 24% to 46%, although they increase from 43% to 75% at the third, and up to 83% at the fourth CD.[12-14] The lower rates of adhesion reported at the second CD compared to laparotomy for nonobstetric indications would suggest that patients having CD may develop fewer adhesions. In addition, evidence in the literature suggests that the consequences of postoperative adhesions as it relates to bowel obstruction,[15] infertility,[16,17] ectopic pregnancy,[18] and chronic pain[19] may be less following CD compared with gynecological surgery. In part, the reduction in these consequences may be a function of where adhesions develop after CD compared with gynecological procedures on the posterior uterus, with the anterior cul-de-sac being most common following CD.

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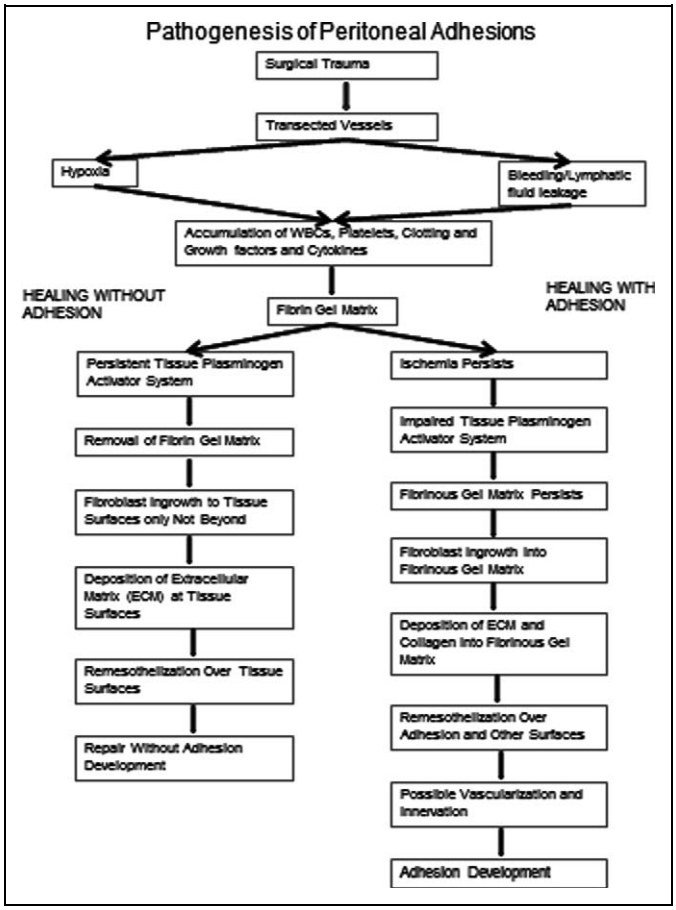

Figure 1. Proposed scheme for the pathogenesis of peritoneal adhesion development following injury.WBCs, white blood cells.

Adhesiogenesis is a culmination of increased extracellular matrix (ECM) production associated with diminished matrix degradation, combined with decreased fibrinolytic activity.[20,21] Physiological changes in pregnancy favor decreased fibrinolysis,[22] with an increased propensity for adhesion development. Despite a general understanding of some of the precise molecular and cellular mechanism underlying the development of adhesions, the reason/reasons why adhesion development is less prevalent following CD remains elusive.

For the purpose of this review, a PubMed search up to October 2010 using MeSH terms cesarean/cesarean delivery/section, laparotomy, gynecological operations, open myomectomy, and adhesion development was undertaken, and relevant studies reviewed whether they addressed adhesion markers and adhesion development following CD and gynecological uterine or adnexal operations. Studies were included only if data on the outcome variable (adhesion development) were provided, and it was possible to construct a 2×2 table. Odds ratios (ORs) and 95% confidence intervals (CIs) were computed using SPSS version 17.

This review will discuss the pathophysiology of adhesion development, the impact of the physiological changes associated with pregnancy on adhesion markers and adhesion development, and the clinical implications of adhesion development following CD. We will also present evidence from the published literature supporting a decrease in the propensity for adhesion development following CD compared with gynecological operations, as well as propose possible etiological consideration for such differences.

Pathophysiology of Adhesion Development

Our laboratory has hypothesized that adhesions develop as a response to hypoxia, whereby the body tries to reestablish oxygen and nutrient supply to tissues that have been injured by surgery or previous pathology.[21] Tissue injury results in bleeding and leakage of lymphatic fluid from transected vessels, a process that is accentuated by concomitant histamine release (Figure 1). These result in the accumulation of red and white blood cells, platelets, clotting and growth factors, and cytokines which coagulate to form a fibrin clot overlying the injured tissue. As normal healing is accomplished, the tissue plasminogen activator (tPA) system present in the peritoneal mesothelium and its underlying fibroblasts functions to remove the fibrinous gel matrix,[20] and consequently halt the potential for subsequent cellular migration into the fibrinous clot. Therefore, during normal healing without adhesions, the fibrinous mass is removed by fibrinolysis, before fibroblast ingrowth and deposition of ECM between injured tissues has been achieved, and thus allowing tissue to heal without inappropriate attachments to other tissues. Alternatively, if fibrinolytic activity is reduced (as with reduction in tPA in association with tissue hypoxia), and the fibrinous mass persists, fibroblast ingrowth occurs with deposition of ECM material including collagen, which forms abnormal connections between tissue surfaces (which possibly become vascularized and innervated) to form adhesions (Figure 1).[20,21]

Several molecular biologic observations have been made in recent years comparing normal peritoneum and adhesion fibroblasts, with the characterization of an ''adhesion fibroblast phenotype.''[21] These adhesion fibroblasts express adhesiogenic factors produced in less quantity or in some cases almost not at all, by normal fibroblasts (Figure 2). Such adhesion phenotype can be induced when normal human peritoneal fibroblasts are cultured in vitro under hypoxic conditions. Work in our laboratory and those of others show that compared with normal peritoneal fibroblasts, adhesion fibroblasts produce elevated basal levels of transforming growth factor beta1 (TGF-β1),[23-25] vascular endothelial growth factor (VEGF),[26] α-smooth muscle actin (α-SMA),[27] and components of the ECM such as type I collagen and fibronectin,[28] decreased ratios of plasminogen activator/plasminogen activator inhibitor 1 (tPA/PAI-1),[29] and matrix metalloproteinase 1/tissue inhibitor of metalloproteinase (MMP-1/TIMP-1).[21,30] In addition, the expression of cyclooxgenase 2 (COX-2) messenger RNA (mRNA) and protein in adhesion fibroblasts, and the induction of COX-2 in peritoneal fibroblasts in response to hypoxia indicate a possible inflammatory response[31] (Figure 2). This fact was

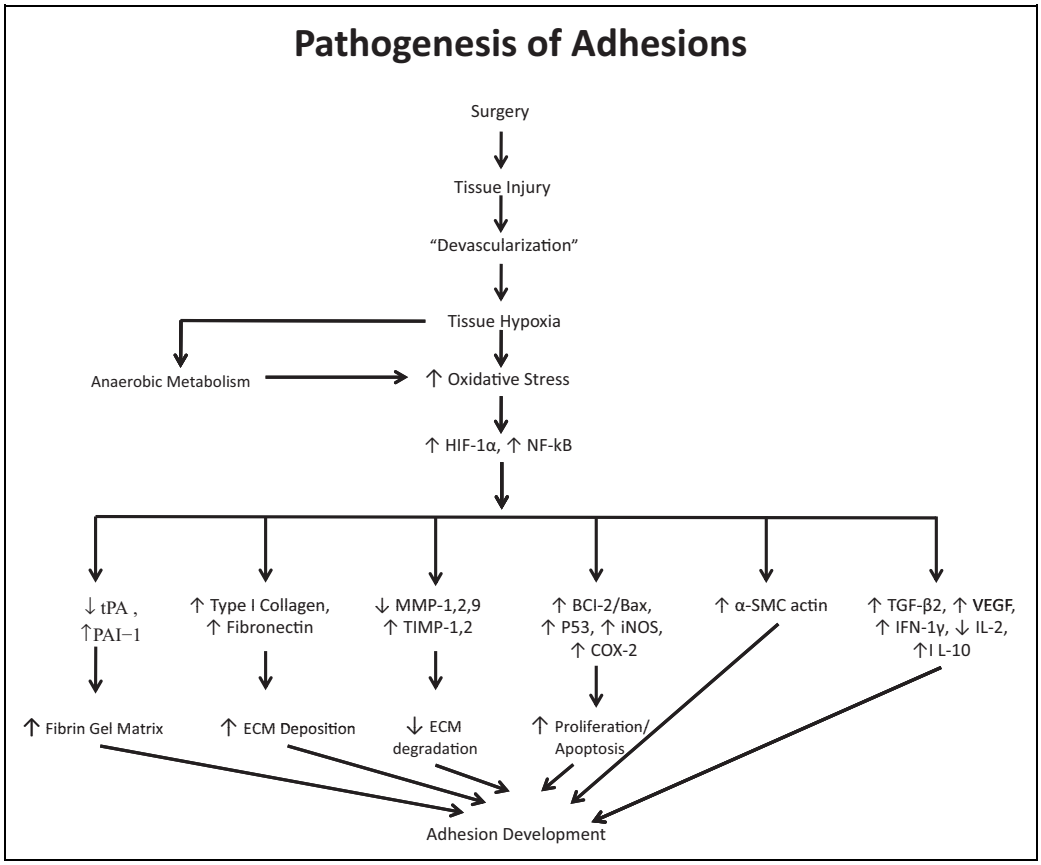

Figure 2. Proposed scheme for the pathogenesis of adhesion development following injury and induction of gene expression. ↑, an increase; ↓, a decrease; BCl-2, B-cell CLL/lymphoma 2; BAX, BCl2-associated X; COX-2, cyclooxygenase 2; ECM, extracellular matrix; HIF, hypoxia-induced factor; IFNy, Interferon-γ; IL, interleukin; iNOS, inducible nitrous oxide synthase; MMP, matrix metalloproteinases; NADP, nicotine adenine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; P53, tumor protein 53; PAI-1, plasminogen activator inhibitor; TGF-β1, transforming growth factor-beta; TIMP, tissue inhibitor of matrix metalloproteinases; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor.

buttressed by work from Ivarsson and colleagues[32] who show that treatment with the proinflammatory mediators such as lipopolysaccharide (LPS) and tumor necrotic factor-α (TNF-α) results in an overall decreased fibrinolytic capacity, as manifested by a decrease in the expression of tPA and an increase in PAI-1 and PAI-2. Finally, there is evidence to suggest that adhesion formation may be mediated, at least in part by hypoxia-inducible factors[33] and the nuclear factor κB (NF-κB) family of proteins.[34]

There is increasing evidence to suggest that reactive nitrogen and oxygen species such as nitric oxide (NO), superoxide ($O_2^{\bullet -}$), and lipid peroxidation (LPO) produced under oxidative stress may contribute to the development of postoperative adhesions[21,35-37] (Figure 3). Hypoxia has also been shown to play a role in the production of these free radicals both in vivo and in vitro. Reactive nitrogen and oxygen radicals are produced after oxygen supply interruption and or restoration and have been implicated in a number of signal transduction pathways.[38,39] Nitric oxide is produced during conversion of arginine to citrulline; molecular oxygen and nicontinamide adenine dinucleotide dihydrophosphate (NADPH) are required at this level, with tetrahydrobiopterin (H_4B) acting as a cofactor (Figure 3). Bioregulatory NO is generated by enzymes collectively termed nitric oxide synthetases (NOSs)[40,41] of which there are 3 isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). The synthesis of NO can be inhibited by endogenously produced methylated analogues of arginine which are competitive inhibitors of NOS namely asymmetric dimethyl arginine (ADMA) and monomethyl arginine (l-NMMA).

In biological systems, superoxide dismutase (SOD) protects against the deleterious actions of the $O_2^{\bullet -}$ by catalyzing its dismutation to hydrogen peroxide (H_2O_2), which is utilized in combination with chloride ions by myeloperoxidase (MPO), a highly cationic heme protein, to generate cytotoxic hypochlorous acid (HOCI) and diffusible radical species[42-44] (Figure 3).

Adhesion development depends on a disturbance in the tightly controlled balance between ROS production and elimination, either via augmentation of ROS generation or defective/deficient antioxidant defenses for their elimination. This results in a buildup of intracellular ROS which may lead to persistent changes in signal transduction and gene expression, thereby giving rise to oxidative stress-related pathological states.

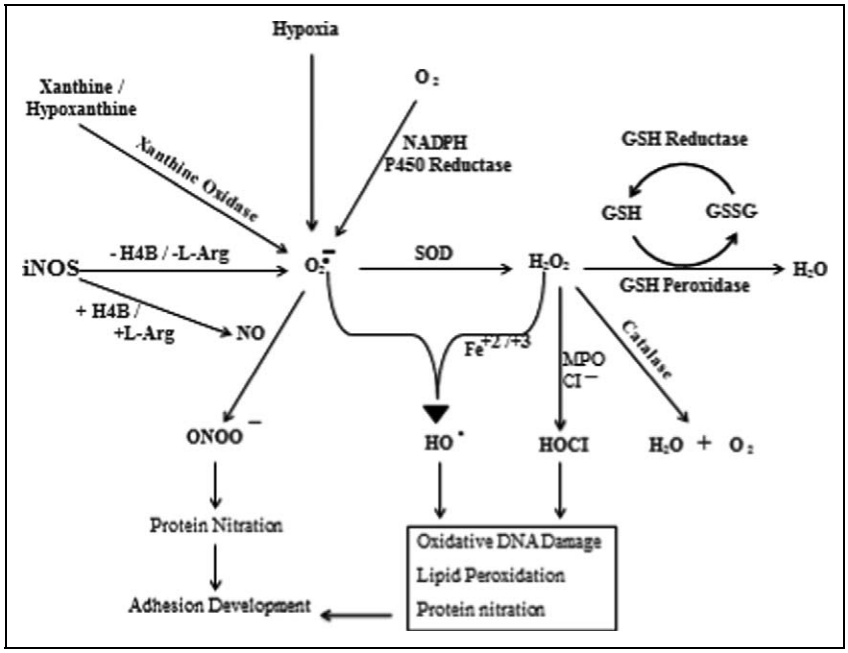

Figure 3. Proposed scheme for the interaction of operative oxidative metabolic reaction and free radicals associated adhesion development.Cl^-, chloride ion; Fe^{2+} and Fe^{3+}, elemental iron; GSH, glutathione; GSSG, glutathione disulfide; H_2O, wáter; H4B, tetrohydrobiopterin; HOCl, hypochlorous acid; MPO, myeloperoxidase; O_2, molecular oxygen; $O_2^{\bullet-}$, superoxide anion; NADP, nicotine adenine dinucleotide phosphate; NO, nitric oxide; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen specie; SOD, superoxide dismutase.

Intracellular ROS levels are kept under tight control by the enzymatic activities of antioxidant proteins, such as SOD, catalase, glutathione (GSH), and peroxidases, as well as by nonenzymatic compounds such as tocopherol, β-carotene, vitamin E, and ascorbate,[45,46] and by the action of low-efficiency ROS scavengers such as free amino acids, peptides, and proteins.[47] There is evidence to suggest that postoperative oxidative stress may be linked to neutrophil recruitment[48] and decreased fibrinolytic activity[49] and, subsequently, the development of intra-abdominal adhesions. Therefore, antioxidants, by reducing levels of oxidative stress and increasing fibrinolytic and MMP activities postoperatively, may contribute to reduction in adhesion development.[50] These experiments have been carried out in the rat model, where antioxidants such as methylene blue,[49,51] indigo carmine,[52] and neurokin 1 receptor (NK-1R) CJ-12-255 (Pfizer, Groton, CT, USA)[48] antagonist have been shown to inhibit postoperative adhesion development. In addition, work in our laboratory shows that adhesion fibroblasts produce less NO than normal fibroblast[53] and hypoxia, through the production of $O_2^{\bullet-}$, causes normal peritoneal fibroblasts to irreversibly acquire the adhesion phenotype.[54] Scavenging $O_2^{\bullet-}$ with SOD, even in the presence of hypoxia, prevented the development of the adhesion phenotype in vitro.[54] Thus, scavenging oxygen-free radicals may be beneficial for the prevention and or reduction of postoperative adhesions.

We have also shown that adhesion fibroblasts exhibit lower apoptosis and higher protein nitration compared to normal peritoneal fibroblasts.[21,55] This mechanism involves caspase 3 *S*-nitrosylation and is found to be significantly higher in adhesion fibroblasts compared to normal peritoneal fibroblasts.[55] This observed increase in *S*-nitrosylation resulted in a 30% decrease in caspase 3 activity in adhesion fibroblasts, while treatment with peroxynitrite resulted in a dose-response increase in caspase 3 *S*-nitrosylation, leading to a decrease in caspase 3 activity and apoptosis in normal peritoneal fibroblasts.[55]

Clinical Evidence of a Lower Prevalence of Adhesions Following CD Compared With Adhesions Following Intra-Abdominal Operations

Although peritoneal adhesions develop in the overwhelming majority of intra-abdominal and pelvic surgery,[56] there is evidence in the literature that suggests that patients having CD may develop fewer adhesions. The clinical consequences of adhesions resulting from gynecological surgery are well known[3-7] compared with those that develop following CD.[57] The type of surgical approach (laparoscopy or laparotomy) and the role of closure of peritoneum in gynecologic surgeries and CD have long been debated as important factors that influence the development and extent of postoperative adhesions.

Although causation is often difficult to prove, some of the complications discussed herein are likely associated with adhesions resulting from surgical trauma. Tulandi and coworkers[58] performed a second-look laparoscopy on 26 infertile women 6 weeks after undergoing abdominal myomectomy for large

uterine fibroids. In all, 94% of women with posterior uterine wall incisions and 56% of women with fundal or anterior incisions developed adnexal adhesions.[58] In a small case–control study involving 14 patients in each group, Bulletti[59] and his group compared the development of adhesions postmyomectomy performed laparoscopically or abdominally. On second-look laparoscopy, they documented adhesion formation in 64% of patients in the laparotomy group; a figure similar to that reported by Tulandi and colleagues for fundal anterior uterine wall myomectomy.[58]

Brill and collaborators[60] performed a second-look laparoscopy on 360 women undergoing operative laparoscopy after a previous laparotomy to assess for adhesions between the abdominal wall and the underlying omentum and bowel. Overall, patients with prior midline incisions had significantly more adhesions than those with pfannenstiel incisions (OR, 2.10; CI, 1.38-3.18). Patients with midline incisions performed for gynecologic indications had significantly more adhesions than all types of incisions performed for obstetric indications (OR, 1.65; CI, 0.97-2.83, $P = .054$). The presence of adhesions in patients with previous obstetric surgery was not affected by the type of incision in this study. Similarly, Ashrafinia and Colleagues[61] performed a second-look laparoscopy on 50 women who had undergone a previous laparotomy for obstetrics and gynecologic surgery to determine the extent of adhesion formation and found that women with midline or pfannenstiel incision for gynecologic surgery had more adhesions than those with incisions for obstetric surgery.

One reason against classical uterine incisions and the acceptance of low transverse uterine incisions is the formation of adhesions between the uterine scar and the anterior abdominal wall. Recent literature on this subject is lacking as classical CD are rarely performed in modern obstetrics. Most of the literature on this subject dates back to many decades, and such reports may be due to the technique, the type of suture materials available, and infection. Leuwen[62] reported such adhesions in 76 out of 117 repeated CD, while they were present in all but 2 of 39 cases of repeat CD at the Boston Lying-In Hospital in a report by Mason[63] in 1911. However, adhesions continue to occur despite lower uterine incisions, albeit less to the anterior abdominal wall compared to classical incisions. As stated previously, the incidence of adhesion development increases with the number of CDs performed.[12,13] Similar finding was reported by Juntunen and colleagues[64] who reported a significantly higher risk of intraperitoneal adhesions in patients undergoing their 4th to 10th CD compared to those having their 1st, 2nd, or 3rd CD (OR, 8.1; CI, 2.7-23.8).

Adhesions Causing Small Bowel Obstruction and Bowel Injury, in Intra-Abdominal Surgery Versus CD

Reproductive tract surgery carries a risk of injury to the gastrointestinal (GI) tract. This is due to several factors including close surgical proximity of these organs, disease processes that can distort anatomy such as adhesions and endometriosis, delayed mechanical and energy effects, and the inability to directly visualize organ surfaces. Adhesions are indeed believed to be the most common cause of small bowel obstruction (SBO)[15,65-68] which may occur in the immediate postoperative period after abdominal surgery with obstruction occurring or recurring in as much as 29% of women reported up to 25 years later.[69] One systematic review of the published literature on the risk of postsurgical gynecological SBO[6] found that the overall incidence of adhesion related readmission was 11.1%. A reanalysis of their data confirmed their conclusion that the lowest incidence of bowel obstruction was after previous CD. Bowel obstruction was significantly less likely to occur following previous CD (0.1%) compared with after open; appendectomy (1.37%), total abdominal hysterectomy ([TAH] 15.6%), and adnexal surgery (23.9%; Table 1). Also, Al-Took and collaborators[15] evaluated the relationship between adhesion-related SBO following CD and gynecologic operations and found that the incidence of SBO after CD was significantly less. Reanalyses of their data showed a significantly decreased incidence of SBO following CD (0.05%) compared with TAH (1.64%) and adnexal surgery (0.87%), but not compared with myomectomy (0.41%; Table 1). The interval between the initial laparotomy and the bowel obstruction in this study varies from 1 month to more than 20 years with a median interval of 5.3 years. Furthermore, adhesions that involved the site of closure of the pelvic peritoneum after hysterectomy or that was attached to the anterior abdominal wall were responsible for SBO in 85% and 15% cases, respectively.[15] Similar findings were observed in a relatively small case series by Stricker and colleagues[68] who noted that hysterectomy was the most common previously performed operation linked to bowel obstruction, with CD being less likely than myomectomy to cause subsequent intestinal obstruction. However, it should be noted that the follow-up in these studies varied considerably and may have influenced the rate of SBO reported. Nevertheless, the low incidence of SBO reported that following CDs may be attributed to the location of the incision in the lower uterine segment where the incision is covered by the bladder and protected by the enlarged uterus, and the nonuse of self-retaining retractors that may cause abrasion of the pelvic and abdominal peritoneum.[15,68]

The incidence of bowel injury and inadvertent enterotomy during reoperation may be as high as 19% with laparotomy and 10% when adhesiolysis is performed with the laparoscope.[72] Although such risks are low after the first repeat CD,[70,71,73] they significantly increase with increasing number of CDs even when performed electively,[71] especially when a midline rather than a pfannenstiel skin incision was used as route of entry into the abdomen[70] (Table 1).

Adhesions Causing Urinary Tract Injury in Intra-Abdominal Pelvic Surgery Versus CD

Lower urinary tract injury at the time of CD is an uncommon complication. Such injuries are usually caused by endometriosis on the sidewall and adhesions from previous CD,[70,71,74-76] which occur while developing the bladder flap over the lower

Table 1. Adhesion-Related Small Bowel Obstruction (ARSBO) and Bowel Injury Following Gynecological Surgery and Cesarean Delivery

Authors	Study Design	Country	No. of Patients	Previous Laparotomy/ARSBO		OR (95% CI)
Barmparas et al.[6 a]	Systemic Review	United States	304 673	Proportion of SBO mostly due adhesions		Unadjusted
				Cesarean delivery	10/12 980 (0.1%)	1
				Hysterectomy	3182/20 377 (15.6%)	0.004 (0.002-0.008)
				Adnexal surgery	1105/4621 (23.9%)	0.002 (0.001-0.005)
				Appendectomy	3663/266 695 (1.4%)	0.06 (0.03-0.10)
Al-Took et al.[15 a]	Cohort	Canada	9789	Proportion of ARSBO		Unadjusted
				Cesarean delivery	3/6480 (0.1%)	1
				Hysterectomy	35/2140 (1.6%)	0.03 (0.01-0.09)
				Adnexal Surgery	8/924 (0.9%)	0.05 (0.01-0.20)
				Myomectomy	1/245 (0.4%)	0.11 (0.01-1.09)
Makoha et al.[70]	Cohort	Saudi Arabia	3164 underwent 1-8 CDs	Inadvertent bowel injury		Unadjusted
				Abdominal incision at CD		1
				Pfannenstiel	1/2713 (0.04%)	6.03 (0.38-96.52)
				Midline	1/451 (0.22%)	
Silver et al.[71 a]	Cohort	United States	30 132 underwent elective CDs	Inadvertent bowel injury		
				Number of CD		Unadjusted
				1st to 3rd CD	41/28 333 (0.1%)	1
				4th to ≥6th CD	26/1799 (1.4%)	10.12 (6.18-16.58)
				Inadvertent ureteric injury		Unadjusted
				Number of CD		1
				1st to 3rd CD	5/28 333 (0.02%)	9.46 (2.26-39.63)
				4th to ≥6th CD	3/1799 (0.2%)	

Abbrevications: 1, reference group; No., number; CD, cesarean delivery.
[a] Odds ratio (OR) and confidence interval (CI) calculated from data provided in the manuscripts by the authors, using SPSS version 17.

uterine segment,[74,76] and increase with the number of previous CD.[70,71,74,75] Most adhesion-related urinary tract injuries following hysterectomy occur during adhesiolysis performed at laparoscopy and hence are not comparable to laparotomy for repeat CD. Repeat myomectomies are rarely performed to the degree with which repeat CDs are performed. A literature search revealed a case series of 3, all from 3 sisters with 2 to 4 recurrent uterine myomas, who underwent between 1 and 3 repeat myomectomies before undergoing TAH. All but the third sister suffered significant bowel or bladder injury.[77]

Injury to the bladder during CD may be related to adhesion of that organ high up on the lower uterine segment. In the Finish study[64] mentioned above, patients undergoing their 4th to 10th CD had a significantly higher proportion of "cranial" bladder attachment compared with those undergoing their 1st, 2nd, or 3rd CD (OR, 9.9; CI, 5.0-19.9). The incidence[70,74,76,78] of bladder injury in women having repeat CD varies from 0.31% to 0.81%. In a case–control study from Canada, Phipps et al[74] reported that women with a bladder injury (cases) during CD were more likely to have had a prior CD and prior pelvic surgery compared with those with no bladder injury (control group), with an adjusted OR (AOR) associated with prior CD of 3.82 (Table 2). In a recent cohort study from Sydney, Australia, involving 574 women who underwent laparoscopic hysterectomy, the odds of inadvertent cystotomy among women with a history of ≥3 prior CD was significantly higher compared with those with no prior CD[79] (Table 2). In addition, adhesions encountered during the procedure were greater in the bladder injury group than in controls (60% vs 10%; $P < .01$). In the study by Rahman and collaborators[76] mentioned above, the incidence of bladder injury was 3 times higher among women who underwent repeat compared with primary CD. Previous pelvic surgery and presence of adhesions were responsible for all the cystotomies in the repeat CD group compared with 35.7% in the primary CD group (Table 2). Furthermore, the site of bladder injury following repeat CD overlies the dome in over 90% of cases.[74,81] In addition, the most common time for bladder injury to occur during CD was during the creation of the bladder flap (43%-60%) followed by during entry into the peritoneal cavity (30%-33%), and finally during the uterine incision or delivery (10%-24%).[74,81] These studies support the assertion that adhesions to the lower uterine segment are responsible for most of the occurrences of bladder injury. Undoubtedly, however, other factors such as operator experience and circumstances under which CD is performed (emergent, urgent, and elective) also play a part and were not always controlled for in most of the studies.

In the study from Jeddah, Saudi Arabia, mentioned above,[70] the incidence of bladder injury (0.6%) was increased with increasing CD number, more so when a midline compared with a pfannenstiel incision was used for CD (Table 2). Furthermore, the authors found that adhesions were almost universally present in all women who had bladder injury and after multivariate analysis for the effect of confounders (operator experience, abdominal incision type, adhesions, elective vs emergency CD, anterior placenta previa, and CD number), abdominal incision type maintained a significant association with risk of bladder injury (Table 2). However, Khashoggi,[80] and Rashid and Rashid[81] both

Table 2. Injury to the Bladder and Ureter at Cesarean Delivery

Authors	Study Design	Country	Study Population	Outcome		OR (95% CI)
Phipps et al.[74] [a]	Case Control	United States	42/14 757 (0.28%), 42 cases of bladder injury at CD compared with a randomly selected cases of CD (n = 84) with no bladder injury	Proportion of patients with bladder injury at CD		Adjusted
				Primary CD	14/42 (32%)	1
				Prior CD	28/42 (67%)	3.82 (1.62-8.97)
				Previous pelvic surgery[a]		
				Bladder injury		Unadjusted
				No	5/84 (6%)	1
				Yes	8/42 (19%)	3.72 (1.13-12.19)
				Presence of adhesions[a]		
				Bladder injury		Unadjusted
				No	8/84 (10%)	1
				Yes	25/42 (60%)	13.97 (5.38-36.27)
Wang et al.[79] [a]	Cohort	Australia	Patients who underwent laparoscopic hysterectomy with history of ≥1 CD (n = 141) compared with no previous CD (n = 433)	Inadvertent cystotomy[a]		
				Previous CD		
				No	5/433 (1.2%)	Unadjusted
				Yes	7/141 (5.0%)	4.47 (1.40-14.32)
				Inadvertent cystotomy		
				Previous CD		Adjusted
				No	5/38 (13.2%)	1
				1 or 2	3/14 (2.1%)	2.17 (0.51-9.35)
				≥3	4/6 (66.7%)	18.44 (5.15-66.0)
				Ureteric injury[a]		
				Previous CD		Unadjusted
				No	0/433 (0.0%)	1
				Yes	2/141 (1.4%)	0.99 (0.97-1.01)
				Conversion to laparotomy		
				Previous CD[a]		Unadjusted
				No	24/433 (5.5%)	1
				Yes	15/141 (10.6%)	2.03 (1.03-3.99)
Rehman et al.[76]	Cohort	Saudi Arabia	Patients who underwent CD (n = 7708)	Inadvertent cystotomy[a]		Unadjusted
				Previous CD		
				Primary CD	14/5241 (0.3%)	1
				Repeat CD	20/2467 (0.8%)	3.05 (1.54-6.05)
				Previous pelvic surgery and presence of adhesions		1
				Primary CD	5/14 (35.7%)	2.80 (1.39-
				Repeat CD	20/20 (100%)	5.65)
Makoha et al.[70]	Cohort	Saudi Arabia	Patients who underwent 1-8 CD (n = 3164)	Inadvertent cystotomy		
				Abdominal incision at CD		Adjusted
				Pfannenstiel	9/2713 (0.3%)	1
				Midline	10/451 (2.2%)	3.89 (1.40-8.90)
Silver et al.[71] [a]	Cohort	United States	30 132 underwent elective CDs	Inadvertent cystotomy		
				Number of CD		Unadjusted
				1st to 3rd CD	41/28 333 (0.1%)	1
				4th to ≥6th CD	26/1799 (0.3%)	3.95 (1.61-9.67)
Khashoggi et al.[80] [a]	Case–control	Saudi Arabia	Patients who underwent 2-8 CD (n = 290)	Inadvertent cystotomy		
				Number of CD		Unadjusted
				2nd and 3rd CD	1/140 (0.7%)	1
				4th-8th CD	2/150 (1.3%)	1.89 (0.17-20.95)
Rashid and Rashid[81] [a]	Case–control	Saudi Arabia	Patients who underwent 3-9 CD (n = 614)	Inadvertent cystotomy[a]		
				Number of CD		Unadjusted
				3rd and 4th CD	2/306 (0.7%)	1
				5th-9th CD	4/308 (1.3%)	2.80 (0.36-11.00)

Abbreviation: CD, cesarean delivery; 1, reference group.
[a] Odds ratio (OR) and confidence interval (CI) calculated from data provided in the manuscripts by the authors, using SPSS version 17.

from Saudi Arabia did not find increased bowel or bladder injury in association with previous high-order CDs. These authors evaluated women who underwent their 4th to 8th and 5th to 9th CDs, respectively, and compared them with a control group of patients undergoing 2nd to 3rd and 3rd to 4th CD and found that despite the presence of adhesions higher-order repeat CD carry no

specific additional risk for the mother or the baby when compared with the lower order repeat CD (Table 2). However, these later 2 studies[80,81] were case–control, not cohort studies, and the incidence of bladder injury was not analyzed in relation to the type of skin incision made at CD.

Intuitively, one would expect that a midline sub-umbilical incision (MLSI) would be safer than a pfannenstiel incision in repeat CD; hence, surgeons may place too much confidence in the safety of MLSI incisions, and therefore act with less caution than would be exercised with a pfannenstiel. However, the inferior end of such midline incisions may be carried over the bladder dome if plastered high up over the lower uterine segment, making trauma more likely. In the studies by Ashrafinia and Colleagues[61] and Brill et al,[60] mentioned previously, patients with midline incisions had more adhesions than those with pfannenstiel incisions which may involve bowel or bladder, supporting the findings by Makoha and collaborators.[70] In the study by Al-Took et al,[15] also mentioned above, excluding adhesions between the small bowel and the pelvis, in the other 33 women (70.2%), the adhesions were found between the previous abdominal incision and the intestine. These suggest that a MLSI is less safe than pfannenstiel for peritoneal access in women undergoing multiple CDs.

Ureteral injury following repeat CD on the other hand is rarely a result of previous adhesions, being attributable most often to ureteral transection or ligation associated with uterine incision extensions in the lower uterine segment or to attempts to achieve hemostasis during cesarean hysterectomy (CH).[82] Eisenkop and Colleagues[78] found that during a 5-year period, the incidence of ureter injuries during CD at the Los Angeles County/University of Southern California Medical Center was 0.09%. However, a recalculation of data in the study by Silver and collaborators[71] showed that the rate of ureteral injury following repeat CD may increase dramatically after more than 3 repeat CD (Table 2).

Closure and Nonclosure of the Visceral and Parietal Peritoneum

One of the highly debated and contentious issues regarding adhesion development following lower segment CD is the closure or nonclosure of the visceral and parietal peritoneum. General surgeons have long abandoned the closure of visceral and parietal peritoneum based on the studies mainly in oncology patients that suggested more adhesion development following closure.[83-85] Respected authorities such as The United Kingdom Royal College of Obstetricians and Gynaecologists suggested that nonclosure of the peritoneum is associated with fewer postsurgical complications and can be used in many gynecological procedures.[86] However, studies on this subject have concluded that insufficient data are available to make a pronouncement on the issue and that adequately powered and appropriately designed trials are needed.[87,88] A recent study by Malvasi and colleagues[89] supports nonclosure of the visceral peritoneum for CD. These authors performed light microscopy and scanning electron microscopy on specimens obtained from patients having a repeat CD following nonclosure and closure of the peritoneum in their first CD. Light microscopy revealed significant ($P < .05$) reactive mesothelial hyperplasia (51.8% vs 13.7%), submesothelial fibrosis (48.1% vs 6.8%), and neoangiogenesis of mesothelial stroma (44.4% vs 12%), while scanning electron microscopy showed more patients with pericytes on the surface of microvessels (26.3 $\pm$ 1.4 vs 11.5 $\pm$ 1.1) in the closure compared with the nonclosure group. The authors concluded that closure enhances inflammatory reactions, based on reactive and regenerative mesothelial hyperplasia and submesothelial fibrosis.

For other reports, adhesions found at the time of repeat CD have confirmed previous clinical and animal studies that suggest that peritoneal nonclosure does not promote, and might even decrease, adhesion development.[90,91] In a small randomized study from Iran[92] involving 45 patients randomized to closure (24) and nonclosure of both visceral and parietal peritoneum (21) in which only 31 returned for repeat CD, intra-abdominal adhesions were significantly less in the nonclosure group (Table 3). However, another randomized study from Thailand,[93] in which only 18% (65 of 360) of the patients randomized returned for a repeat CD, found no statistical significant difference between patients who underwent nonclosure of both visceral and parietal peritoneum, nonclosure of only visceral peritoneum, and closure of both visceral and parietal peritoneum regarding postoperative complications or number of adhesion formation (Table 3). Nonetheless, this was a small study and their results could be biased due to a type 2 error. However, in contrast, one prospective cohort study[94] of women undergoing their first repeat CD, irrespective of whether the visceral peritoneum was closed or not, found that after controlling for potential confounding variables, parietal peritoneal closure at primary CD was 5-fold protective against all adhesions and 3-fold protective against dense adhesions (Table 3). The authors concluded that the practice of nonclosure of the parietal peritoneum at CDs should be questioned.

The effects of peritoneal closure with chromic catgut suture after reproductive surgery by pfannenstiel incisions have also been studied clinically and by second-look laparoscopy.[95] These authors found no statistically significant difference in the rate of adhesion to the anterior abdominal wall between the group with peritoneal closure (22.2%) and the group without peritoneal closure (15.8%; Table 3).

Aside from peritoneal closure, the techniques used to close the hysterotomy incision in the lower uterine segment, and propensities for bladder adhesions have also been studied. Blumenfeld and colleagues[96] from Stanford University, in a secondary analysis from a prospective cohort study of women undergoing their first repeat CD, found that single compared with double-layer closure was associated with a 7-fold increase in the odds of developing bladder adhesions (OR, 6.96; CI, 1.72-28.1). However, bladder adhesions were not influenced by visceral (OR, 2.70; CI, 0.33-22.2), or parietal (OR, 0.73; CI, 0.15-3.45) peritoneal closure or use of chromic catgut (OR, 0.93; CI, 0.18-4.92]. Thus, there is still debate, regarding the role of closure or nonclosure of the peritoneum in adhesion development. Larger,

Table 3. Adhesion Development Following Peritoneal and Nonperitoneal Closure After Gynecological Surgery and Cesarean Delivery

Authors	Study Design	Country	Population	Treatment Groups		RR (95% CI)
Zareian and Zareian[92]	Randomized trial	Iran	45 CDs of which only 31 returned for second CD	Parietal and visceral peritoneum		
				Adhesion development		
				Non closure	3/18 (15%)	1
				Closure	7/13 (54%)	3.2 (1.0-10.2)
Weerawetwat et al.[93]	Randomized trial	Thailand	360 CDs of which only 65 returned for second CD	Parietal (a) and visceral peritoneum (b)		
				Moderate-to-severe adhesions		
				Nonclosure of a and b	3/20 (15%)	
				Closure of a only	2/20 (10%)	NS
				Closure of a and b	3/25 (12%)	
Lyell et al.[94]	Prospective cohort	United States	173 patients who underwent their 1st repeat CD	Parietal peritoneum		
				All adhesions		Adjusted OR
				Left open at 1st CD	77/106 (73%)	1
				Closed at 1st CD	35/67 (52%)	0.20 (0.08-0.49)
				Dense adhesions only		
				Left open at 1st CD	48/106 (45%)	1
				Closed at 1st CD	20/67 (30%)	0.32 (0.13-0.79)
Tulandi et al.[95]	Cohort	Canada	120 of 333 women who underwent reproductive surgery by laparotomy via a pfannenstiel incision	Assessment by second-look laparoscopy after closure or nonclosure of parietal peritoneum; adhesions to anterior abdominal wall		OR
				Non Closure	9/57 (15.8%)	1
				Closure	14/63 (22.2%)	1.52 (0.60-3.85)

Abbreviations: CD, cesarean delivery; CI, confidence Intervals; RR, relative Risk; OR, odds Ratio.

adequately powered, well-designed trials will be needed to further assess this issue and may vary with the clinical circumstances.

Operating Time at Repeat CD Versus Repeat Abdominal Surgery

Dissecting adhesions before executing the planned operation takes time at subsequent abdominal surgery,[97,98] increases hospital stay and readmissions, and predisposes patients to complications as enumerated above.[65,99,100] There is some evidence to suggest that postoperative morbidity and mortality of patients who need adhesiolysis is higher than that of patients with a virgin abdomen.[101,102]

In one colorectal surgery study,[97] previous surgery prolonged the median incision time (defined as time taken from skin incision to complete opening of the peritoneal cavity, including division of adhesions immediately related to the incision) from 5 (range, 3-10) to 8 (range, 4-39) minutes ($P < .0001$) and the median division of adhesion time (defined as time taken to divide any relevant intra-abdominal adhesions for adequate access to carry out the procedure) from 0 (range, 0-30) to 15 (range, 0-240) minutes. In yet another colorectal surgery study[98] of 198 patients who underwent abdominal operations, 55% had previous abdominal procedures. In total, 83% of patients with prior surgery had adhesions, whereas only 7% of patients had adhesions on their initial operation. Patients with prior surgery also had higher-grade adhesions ($P < .001$). Patients with prior surgery required a mean of 21 minutes to open their abdomens (defined as time from skin incision to when the surgeon's usual abdominal retractor was placed), whereas patients without prior surgery required a mean of 6 minutes ($P < .01$).

Cesarean delivery is not immune in this regard; Greenberg and colleagues,[103] in a secondary analysis of a prospective cohort study of 145 women who underwent their first repeat CD found that adhesion severity predicted delayed delivery of the newborn. The authors reported that the mean incision to delivery time in women with a summed weighted adhesion scores >3 was significantly higher, compared to those with scores ≤ 3 (19.8 minutes vs 15.6 minutes, respectively; $P = .04$). More importantly, by 30 minutes after skin incision was made, 17.9% of women with adhesion scores >3 remained undelivered, versus 5.1% of those with scores ≤ 3 ($P = .04$). Delivery times have also been reported to increase with increase in the number of previous CDs. Tulandi and colleagues[13] found that compared with a first CD (7.7 ± 0.3 minutes), the delivery time was significantly longer at subsequent CDs (second CD, 9.4 ± 0.1 minutes; 95% CI, 1-2; third CD, 10.6 ± 0.3 minutes; 95% CI, 2-4; ≥ 4 CD, 10.4 ± 0.1 minutes; 95% CI, 1-2). Similar findings were reported by Morales et al[12] who in a cohort study found that compared with primary CD, delivery of the infant was delayed 5.6 minutes (52%) with 1 previous CD, 8.5 minutes (79%) after 2 CDs, and 18.1 minutes (169%) during the fourth ($P < .001$ for all comparisons). These authors[12,13] also found that delay in delivery correlated with adhesion severity. Such delay in the delivery of the newborn may have serious lifelong consequences for the baby and their family.

Whether extensive adhesiolysis before delivery increase the blood loss and need for transfusion during CD is also debatable.

Although some have suggested that significant blood loss is associated with higher-order CDs[71,73,81] others disagree.[64] In addition, while some have reported that the risk of blood transfusion increased significantly with increase in the number of prior CDs,[71,73] others have either found no difference overall[81,104] or no difference in those undergoing CD without labor irrespective of the number of prior CDs.[71] These would suggest that other variables aside from adhesions may be responsible for the amount of blood loss and need for transfusion in patients undergoing repeat CDs.

Adhesion-Associated Infertility Following Previous CD Versus Previous Abdominal Surgery

There is evidence in the published literature that suggests that pelvic adhesion can cause infertility[3,105-107] with an increased risk of ectopic pregnancy,[4] should the patient conceive. In fact, it has been shown that adhesions may contribute to infertility in about 40% of infertile couples[100] and represent the sole infertility factor in up to 15% of cases.[106] Postsurgical complications affecting the fallopian tubes seem to be an important cause. Lalos[108] examined data from 120 women with tubal infertility and 26 pregnant women and found that previous abdominal surgery, especially pelvic surgery, was the most frequent risk factor present in 59% of the infertile women followed by pelvic inflammation (42%) and endometriosis (10%). The proportions of patients with previous CD in the 2 groups were no different (2.5% vs 2.4%).

Risk of infertility or subfertility following CD[109,110] is more contentious. There has been speculation that postoperative endomyometritis, pelvic adhesions, and uterine cavity damage following CD may predispose to subsequent infertility, and women who deliver by CD have been shown to be less likely to have a subsequent pregnancy. Hemminki,[111] from Helsinki, Finland, reviewed 8 existing cohort-type studies before 1994 and compared their subsequent reproduction after CD with a comparable control group and suggested that a CD was a risk factor for lowered fertility. A similar finding was reported by Mollison and collaborators,[112] and LaSala and Berkeley[113] (Table 4). In the latter study, the 17 patients with infertility did not have a higher incidence of postpartum endomyometritis, prolonged rupture of membranes, or placental abnormalities than controls. Only 4 of the 17 study patients with infertility in this study had verified tubal or intrauterine disease as the sole cause of their infertility. The other 13 women had a cause that either was not clearly related to CD or was unknown.

It has also been reported that patients with prior CDs may take longer to conceive compared to women with no prior CD[112,114] (Table 1). Whether this is due to a direct effect of the procedure on future fertility or due to deliberate avoidance of a future pregnancy is unclear. Most studies, however, lack information about the desire of women to conceive. Nonetheless, several studies have suggested that the reduced fertility following CD was to a large degree voluntary and not related to the indication, nor to any physical consequence, of the CD[16,17,109] (Table 1). One case–control study from Aberdeen, United Kingdom,[115] found that after adjusting for confounding factors, prior CD did not appear to be significantly associated with tubal infertility as the AOR (95% CI) for previous CD for infertile and fertile controls were 1.06 (0.73-1.52) and 1.2 (0.9-1.7), respectively. In addition, a population-based case–control study of 61 married women diagnosed with secondary infertility due to tubal problems who had a previous viable pregnancy were compared with 343 married women who had a previous viable pregnancy and then had a live birth that was conceived at the same time the infertile women began trying to conceive. The risk of tubal infertility was not substantially elevated in women who had a previous CD in the most recent viable pregnancy compared to women with vaginal delivery[116] (Table 4). To date, all the studies on CD and subsequent subfertility are either case–control or cohort-type studies. Despite methodological flaws associated with these studies, evidence is lacking that patients with previous CD have a higher incidence of subsequent tubal disease than controls; additionally, while the apparent reduced fertility following CD may in part be voluntary.

Adhesion-Associated Risk of Ectopic Pregnancy Following Previous CD Versus Previous Abdominal Surgery

It is well known that peritubal and periovarian adhesions resulting from previous pelvic infection,[117] previous pelvic surgery,[118] and endometriosis[119] are risk factors for ectopic pregnancy. Whether pelvic adhesions secondary to previous CD is another risk factor is debatable. An earlier report of an increased risk of ectopic pregnancy related to previous CD after adjusting for age and parity (AOR, 8.0; CI, 2.0-32.7)[120] was confirmed by Mollison and colleague[112] who found that women who delivered by CD were 67% more likely to have an ectopic pregnancy in their next pregnancy compared with women who delivered by spontaneous vaginal delivery (OR, 1.67; CI, 1.03-2.66). Also, a case–control study from Ankara, Turkey[121] found that the relationship observed in the univariate analysis with CD (crude OR, 2.0; CI, 1.2-3.1) did not change after adjustment for main risk factors (AOR, 2.1; CI, 1.2-3.6). However, after adjusting for age, parity, marital status, history of pelvic inflammatory disease, infertility, douching, and smoking, Kendrick and colleagues[18] found no evidence of such an increase (AOR, 0.6; CI 0.4-1.1). At the present time, it is unclear whether previous CD predisposes to subsequent ectopic pregnancy. Larger studies are required to clarify the role of previous CD in the pathogenesis of ectopic pregnancy.

Adhesion-Associated Chronic Pelvic Pain Following Previous CD Versus Previous Abdominal Surgery

Although pain evaluation for the most part is subjective and associated with several potential confounders, one review[5] concluded that adhesions can cause pelvic pain, and adhesiolysis relieves pain in up to 60% to 90% of cases. However, a randomized clinical trial found significant less pain after adhesiolysis

Table 4. Subsequent Fertility After Cesarean Delivery

Authors	Study Design	Country	Number	Study Population	Odds Ratio (95% CI)
LaSala and Berkeley[113]	Cohort	United States	570	[b]Previous primary CD/VD	
				Risk of subfertility	Adjusted
				Overall	3.40 (1.24-9.35)
				Controlled for contraception use or sterilization	3.67 (1.33-10.12)
				Excluding patients with previous history of infertility	2.98 (1.04-8.52)
Collin et al.[109a]	Cross-sectional survey	Sub-Saharan Africa	35 398	Previous CD/VD	Adjusted
				Overall	0.83 (0.73-0.96)
				>1 year to conceive, parity = 1	1.0 (0.80-1.20)
				>1 year to conceive, parity ≥2	1.9 (1.10-3.10)
				Odds of pregnancy in 5 years	0.75 (0.62-0.89)
				Desire for further children	0.67 (0.54-0.84)
Mollison et al.[112]	Population-based cohort	Scotland	25 371	Previous CD/SVD/IVD	Adjusted
				Previous CD vs SVD	0.89 (0.82-0.96)
				Previous CD vs IVD	1.01 (0.94-1.08)
Murphy et al.[114a]	Population-based cohort	England	14 541	Parous women	Adjusted
				Previous CD/VD >1 year to conceive	
				Overall	1.53 [1.09-2.14]
				Parity = 1	1.05 (0.66-1.69)
				Parity ≥2	2.97 (1.72-5.10)
Saraswat et al.[115]	Case-control	Scotland	19 840	Secondary infertility	Adjusted
				TD (Gp1) vs No TD (Gp2)	1.06 (0.73-1.52)
				Gp1 vs no infertility (Gp3)	1.20 (0.90-1.70)
Wolf et al.[116]	Case–control	United States	404	CD and subsequent tubal infertility	Adjusted
				Previous CD vs VD	1.2 (0.40-3.70)
Jolly et al.[110]	Cohort, posted questionnaire 64% response rate	England	170	[b]Previous CD/SVD/IVD	Unadjusted
				CD vs vaginal delivery after 5 years followup	1.44 (0.72-2.87)
				Tried not pregnant	
Bhattacharya et al.[17a]	Cohort, posted questionnaire 60% response rate	Scotland	1675	[b]Previous CD/SVD/IVD	Unadjusted
				CD vs vaginal delivery after a mean of 12-14 years	
				No further viable pregnancy	1.08 (0.82-1.42)
				Desire for further children	1.78 (1.32-2.29)

Abbreviations: CD, cesarean delivery, Gp, group, IVD, instrumental vaginal delivery; No, number of subjects in the study; SVD, spontaneous vaginal delivery; TD, tubal disease; vs, versus.

[a] Provided information on the desire of women to conceive.

[b] OR (CI) calculated from data provided by the authors using SPSS version 17.

in only the subgroup of women with severe, vascularized, and dense adhesions involving bowel (stage IV) but not between the 2 groups overall.[122] The authors and others[123] have concluded that adhesiolysis for the treatment of pelvic pain has not been shown to be effective in achieving pain control.

Specific to CD, Almeida and collaborators,[124] conducted a retrospective case–control study of 116 women with previous CD submitted to laparoscopy for the diagnosis of chronic pelvic pain and 83 asymptomatic patients submitted to tubal ligation by laparoscopy and found that after logistic regression analysis chronic pelvic pain was associated with a history of CD (OR, 3.7; CI, 1.7-7.7), as well as with endometriosis (OR, 8.5; CI, 3.4-21.4), and sequelae of pelvic inflammatory disease (OR, 10.5; CI 3.2-34). However, the latter study did not observe an association between pelvic pain and pelvic adhesions in patients with previous CD and controls (OR, 1.7; CI, 0.8-3.5). In a Finish study[64] mentioned above, patients in the third trimester before undergoing their 4th to 10th CD reported lower abdominal pains significantly more often than patients undergoing their 1st, 2nd, or 3rd CD (OR, 44.1; CI, 5.9-327.3); however, the 2 groups were not equal in all respects. In another study by Stark et al,[19] no correlation between the prior clinical symptoms and the operative findings at repeat CD was found regarding abdominal pains, urinary symptoms, dyspareunia, or dysmenorrhea. Surprisingly, although nonsignificant, these authors also found that women with adhesions reported fewer postoperative GI symptoms than the women with no adhesions. The preponderance of evidence does not support adhesion-associated chronic pelvic pain following previous CD. A reason for this might be the location of adhesion mainly in the lower pole of the uterus and anterior cul-de-sac away from bowel. At the present time, it is unclear whether CD-related adhesions cause chronic pelvic pain. Further studies are

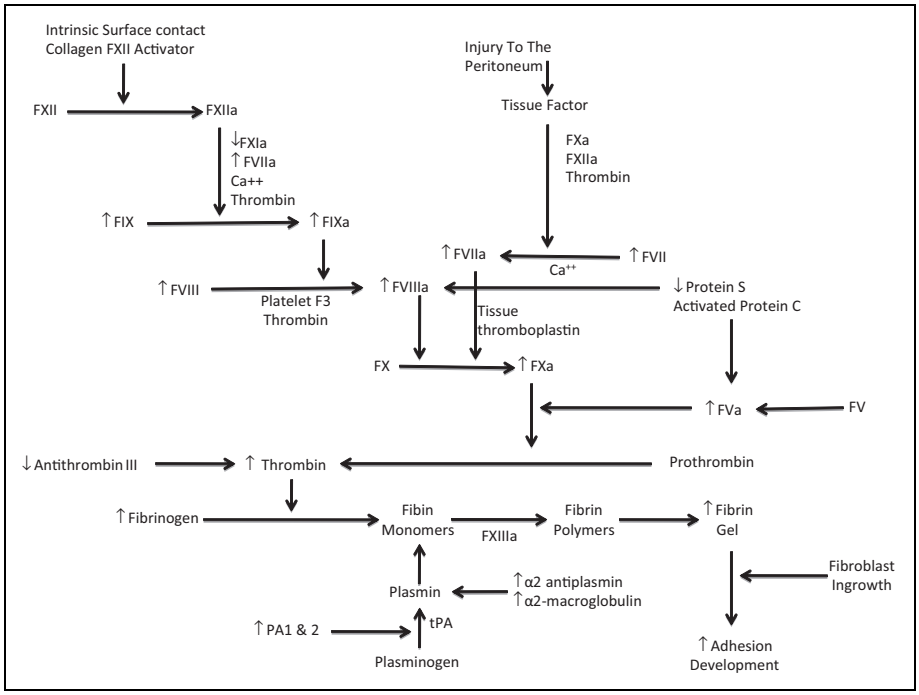

Figure 4. Proposed scheme for the interaction of the coagulation cascade and adhesion development in pregnancy. ↑, denote an increase; ↓, a decrease; a, activated; Ca+, elemental calcium; F, coagulation factors; PAI, plasminogen activator inhibitor; tPA, tissue plasminogen.

needed to clarify this issue before the performance of adhesiolysis can be recommended for the treatment of pelvic pain after CD.

Other Adhesion-Related Issues During Repeat CD

Emergent CH is often performed for life-threatening obstetric complications during CD or within 24 hours postpartum. Cesarean delivery rate has increased over the past several decades.[125] This increase in primary CD and lately a decrease in the vaginal birth after CD (VBAC) rate will naturally lead to an increase in the number of repeat CD.[126] With this increase in repeat CD comes associated risk factors for CH such as placenta previa, placenta accrete, and uterine rupture.[127] As mentioned previously, the number and severity of abdominopelvic adhesions and associated intra-abdominal organ damage increase with the number of prior CD.[64,70,74,76] In the study by Silver and colleagues[71] mentioned previously, approximately 9% of women with a history of ≥5 prior CDs required a peripartum hysterectomy. Nevertheless, the presence of pelvic adhesion per se is unlikely to be the sole indication for CH. However, the location, extent, and severity of pelvic adhesion may influence the CH approach, and sway the surgeon to opt for a supracervical (SH) rather than a total hysterectomy (TH). However, in our own study published recently,[127] the numbers of patients with prior CD were equally matched between those who underwent peripartum TH and SH (72.5% vs 81.4%), the injury rate to bowel (8.8% vs 10%), and the bladder (15.0% vs 15.7%) were no different. Finally, adhesions discovered at CD may limit assess and prevent the surgeon from carrying out concurrently planned procedure such as tubal ligation.[129] In one cohort study, 1.61% of patients scheduled for tubal ligation at repeat CD could not have the operation performed solely due to adhesions from previous CD.[130]

Physiological Changes in Pregnancy and How it Might Impact Adhesion Markers and Adhesion Development After CD

Pregnancy is associated with adaptation of maternal physiology aimed at accepting the fetal allograft, aside from satisfying the fetus's nutritional, metabolic, and physical needs. Such physiological changes affect virtually all the organs of the body including the human uterus which undergoes profound tissue remodeling during pregnancy. The effect of pregnancy on the myometrium is due to interplay of increasing levels of estrogens and progesterone initially produced by the corpus luteum of pregnancy and later by the placenta.

The question at hand is whether pregnancy alters the adhesion development process, and whether this could account for an apparent decrease in the likelihood of adhesion development following CD compared with gynecological operations. It appears that physiological changes in pregnancy favor adhesiogenesis and thus cannot account for the decrease in adhesion development associated with CD. In normal pregnancy, there is a marked increase in the procoagulant activity in maternal blood characterized by elevation of procoagulation factors such as factors VII, VIII, IX, fibrinogen, and von Willebrand factor, which are maximal near term (Figure 4).[22] There is also a decrease in physiological anticoagulants manifested by a significant reduction in protein S activity and by acquired activated protein C (APC) resistance. Proteins C and S are 2

vitamin K-dependent plasma proteins that work in concert as a natural anticoagulant system. Activated protein C is the proteolytic component of the complex and protein S serves as an APC-binding protein that is essential for assembly of the anticoagulant complex on cell surfaces. The anticoagulant activity is expressed through the selective inactivation of FV(activated)a and FVIIIa[131] (Figure 4). In addition, estrogen-induced increase in α2-antiplasmin and α2-macroglobulin[132,133] has been observed during pregnancy. Thus, the overall fibrinolytic activity is impaired during pregnancy and may not return completely to normal for 6 to 8 weeks after delivery.[134]

As mentioned previously,[29] work in our laboratory has shown that tPA activity of the peritoneum exists in the mesothelial cells, as well as within fibroblasts and that compared with normal peritoneal fibroblasts, adhesion fibroblasts produce reduced basal levels of tPA/PAI-1. Rehman and collaborators[135] evaluated specimens obtained from the superior margin of the lower uterine segment incision at the time of elective (prior to onset of labor) CD. These authors found that PAI-1 was upregulated 7.5-fold, while ER-α was downregulated 2.9-fold in the myometrium of term pregnant compared to nonpregnant women, suggesting that pregnancy may be an adhesiogenic state with increasing propensity to healing by secondary intention and adhesion development after CD. Prochazkova et al[136] examined venous blood samples in normal pregnant women and noted that while the level of PAI-1 increased during the entire course of pregnancy, the level of tPA did not change significantly leading to a decreased tPA/PAI-1 ratio as pregnancy progresses, thus also consistent with pregnancy having an enhanced propensity for adhesion development. In addition, Hahn and Korsan-Bengtsen[137] studied coagulation parameters, fibrinolysis, and hormonal levels in peripheral, and uterine venous blood before elective CD in 10 women at term and found lower levels of fibrinolytic inhibitors in uterine blood than in peripheral blood. In addition, during the course of the operation, the authors reported a shortening of the activated partial thromboplastin time and an increase in the number of platelets and FVIII activity in peripheral and uterine blood. These changes favor a tendency to clot formation within the myometrium during CD. However, whether the decrease in fibrinolytic inhibitors within the uterine vasculature is due to decreased synthesis or secretion or increased extraction is unknown, which undoubtedly may lead to different interpretations.

While Prochazkova and colleagues[136] found the venous levels of MMP-9 (first trimester average level 8371, second and third trimester 8290 and 7470, respectively) and TIMP-2 (first trimester average level 92.5 ng/mL, second and third trimester 98.5 and 96.5 ng/mL, respectively) did not change significantly throughout pregnancy, others[138,139] reported that during labor at term, the myometrium is associated with increased expression of MMP-9. Further studies are needed to assess the role of MPP and TIMP in the pathogenesis of adhesion development following CD.

Oxidative stress is a feature of normal pregnancy; it induces vascular endothelial cell dysfunction and, in excess, contributes to the pathophysiology of abnormal placentation and preeclampsia,[140,141] and has also been demonstrated in parturient term and preterm myometrial samples.[142] It is unknown whether oxidative stress in these women alters adhesion development following CD and whether there is an increase in adhesion development in women with preeclampsia compared to those without the disease. Verification of this possibility requires further study and is now underway in our institution.

Smooth muscle cell actin (α-SMCA) isoforms are a major component of the myometrial contractile apparatus and cytoskeleton, which is modified during pregnancy. We have shown that when normal fibroblasts develop the adhesion phenotype, they are characterized in part by an overexpression of α-SMCA.[27] Using the rat model, Shynlova and colleagues[143] showed that both α-SMCA (vascular-specific actin isoform) and γ-actin (predominant in visceral smooth muscle) were detected in the rat myometrium, and the expression of both their mRNA and protein was high throughout pregnancy. Further studies are required to determine whether α-SMCA expression in the peritoneum is further increased during adhesion development following CD.

Although great details are known about the physiological changes in each system, in most cases, the relative contributions and the interactions between dysregulation of the coagulation system, oxidative stress, and tissue hypoxia on adhesion development in pregnancy are still incompletely understood and require further studies.

Proposed Mechanisms to Explain Why Adhesion Development is Less Following CD

Despite the physiologic changes associated with pregnancy just described which would tend to promote adhesion development following CDs, uterine adhesions after CDs are less than those reported after myomectomies. The reasons why adhesion development is less following CD remains largely a mystery. Five basic hypotheses may be proposed to explain the reason why adhesion development is less following CD.

In the first, adhesions may be less after CD because of less tissue hypoxia due to greater tissue perfusion associated with physiological changes in pregnancy. In pregnancy, there are physiological changes that could theoretically protect against tissue hypoxia compared to the nonpregnant state. These include increased cardiac output,[144] increased red cell mass,[145] increased uterine blood flow,[146] and alteration in the shape of the oxyhemoglobin dissociation curve which is shifted to the right in pregnancy (produced by an increase in the 2,3-diphosphoglycerate level in red blood cells), such that oxygen is delivered to tissues more efficiently compared to the nonpregnant state.[147] Given that adhesions develop in response to hypoxia, less hypoxia associated with pregnancy may ameliorate adhesion development.

The second hypothesis relates to 1 of the basic principles of good healing, which is that the injured site be at rest. The lower segment transverse incision is made along the distribution of muscle fibers in the lower uterine segment, which is more

fibrous than muscular, and is subjected to fewer movements than the upper segment in the puerperium. Thus, the low transverse incision is relatively at rest during the puerperium, and by virtue of its fibrous nature responds less to oxytocin stimulation compared with the upper segment.

The third hypothesis relates to the location of the lower segment incision. By virtue of its location, the lower uterine segment incision is covered by the bladder which is constantly being filled and emptied during the healing process. Although unproven, the constant filling and emptying of the bladder in the puerperium is likely to disrupt any fibrinous strands between the uterus and the bladder, and between the lower uterine segment and the anterior abdominal wall, thus decreasing adhesion development at this location. Classical uterine incisions, in contrast, transect the muscle fibers of the muscular upper uterine segment, which despite suturing, is subjected to great movements during the puerperium, a process that is accentuated by breast feeding. It is therefore not surprising that the classical cesarean scar has been proven to have a greater propensity to rupture before and during labor.[148] Although uterine rupture is rare (<1%) with one previous low transverse scar, uterine rupture rates in women with previous classical scar and T-shaped scar ranged between 4% and 9%.[128] Such incisions have few indications for their performance and have largely been abandoned for the low-transverse and low-vertical incisions, except in special circumstances.

A fourth hypothesis is that, although CD entails 1 single incision in the lower uterine segment, the number of uterine incisions at myomectomy has varied from an average[149,150] of 3 ± 2 to 5 ± 1. An increased number of uterine incisions by inference will be associated with more tissue handling; therefore, adhesion development to the uterus will be more likely to follow myomectomy compared with CD. Furthermore, uterine adhesions after myomectomy have been associated with an increasing number of uterine incisions.[11] Although preoperative treatment with gonadotropin-releasing hormone agonist (GnRH-a) for 3 months before open abdominal myomectomy was used in 1 study to decrease adhesion development, this strategy did not decrease adhesion formation compared with placebo.[150] This latter study also reported that for every additional centimeter of incision length at myomectomy, the total adhesion area over the uterine serosal surface increased by 0.55 cm, while the number of myomas removed and the number of incisions were each positively correlated with total adhesion area.

Finally, hematoma within the low transverse CD incision must be rare, as no recorded case was found in a PubMed search up to January 2011. However, hematoma in the myomectomy bed was observed postoperatively by ultrasonography in 40 (24%), 28 (17%), and 12 (7%) patients on day 2, day 7, and 1 month, respectively, in one study.[151] In the latter study, a preoperative myoma volume >110 cm^3 measured by transvaginal ultrasound, the use of a tourniquet, and the experience of the surgeon were significantly correlated with the formation of uterine scar hematomas. Such hematomas increase the amount of exudate that had to be removed by the fibrinolytic system during healing, which may increase adhesion development, especially if such hematoma were to reach the serosa.

Despite the advantages associated with the lower segment CD scar, such scars are still relatively associated with poor healing. Juntunen and colleagues[64] reported a significantly higher percentage of thin (<2 mm) lower uterine segment in patients undergoing their 4th to 10th CD (study group) compared to those having their 1st, 2nd, or 3rd CD (control; OR, 60.4; CI, 18.4-198.3), while 10.1% of study group had membranous, transparent, or "lacerated" lower segment, none in the control group did. A recent systematic review of 12 eligible studies[152] which included 1834 women in whom ultrasound was used to evaluate the CD scar, reported a 6.6% rate of scar defect. Addition of sonohysterogram to such evaluation in another study found that a much higher percentage (20%) had large defects.[153] Therefore, incomplete healing of the low transverse uterine incision as determined by transvaginal ultrasound may occur more frequently than earlier thought.

Prevention of Adhesions Following CD

The burden of adhesion-related complications has enormous personal, litigious, and economic costs to patients, physicians, health care facilities, and the society. In 1994 alone, adhesiolysis procedures were performed during 303 836 hospitalizations, with the total costs of abdominal adhesion-related problems in the United States estimated at over $1.3 billion dollars annually.[154] Such costs are likely to increase with increasing CD rates; hence efforts should be geared toward measures that will decrease postoperative adhesion development.

Hypoxia and increased oxidative stress appear to be a common contributory factor in the pathogenesis of adhesions. Therapies directed at more specific aspects of the pathophysiologic mechanism of the disease including MMP inhibitors, GnRH-a and antagonists, immune modulators, antioxidants, and free radical scavengers may help as they have shown promise in animals.[155-158]

Two antiadhesion barriers approved for use following gynecologic surgical procedure in the United States have been tried in CD. Modified sodium hyaluronate/carboxymethylcellulose (Seprafilm; Genzyme Corporation, Cambridge, Massachusetts) reduces adhesions by mechanical separation of injured tissue surfaces during peritoneal repair[159,160] and have been studied extensively in gynecologic[11] and general surgery.[105,161] More recently, Seprafilm has been studied in CDs.[14] Fushiki and colleagues[14] performed a prospective cohort study of Seprafilm placement at the time of primary CD with a view to reducing adhesive disease. Reanalysis of their data showed that at repeat CD, the incidence and severity of adhesions were significantly reduced in the Seprafilm group compared with the control group (OR, 11.54; CI, 2.24-59.49); as were an adhesion score of 0.07 vs 1.32, respectively; $P = .001$.

Oxidized-regenerated cellulose (Interceed; Johnson and Johnson Medical, Arlington, Texas) is the second adhesion barrier available, although this product is not approved for use in

CD in the United States. While its primary mode of action is considered a barrier separating injured tissue surfaces, oxidized-regenerated cellulose inhibits hydrogen peroxide production by macrophages and competes with LPS for the scavenger receptors on macrophages, thus potentially reducing the release of inflammatory mediators, cellular growth factors, and secretion of matrix components that are promoters of the adhesion fibroblast.[162] In a small Korean study available only in abstract form, Kim and collaborators evaluated 8 patients who underwent CD and who received Interceed at the vesicouterine fold, and 37 patients who underwent standard closure without Interceed. No adhesion developed in the 8 patients in the Interceed group, while all patients in the non-Interceed group had adhesions ranging from mild to severe.[163] However, the need for meticulous hemostasis[57] may limit the use of Interceed for adhesion prevention following CD.

Larger, well-designed, randomized studies are needed to corroborate these findings and to assess the place of these adhesion barriers in the prevention of adhesion development following CD. In the meantime, only meticulous hemostasis and the use of appropriate surgical techniques are available to the obstetrician to minimize post-CD adhesion development.

Conclusions

Attempts to summarize the interactions and changes between complex coagulation factors, growth factors, cytokines, and immune systems in pregnancy are predictably complex. Although great details are known about each system, in most cases, the link between dysregulation of the coagulation system, growth factors, and cytokines is still incompletely understood. These uncertainties have delayed the formulation of standard preventive measures for the prevention of adhesion development following CD, although some have shown promise. The stage is now set to pursue our hypothesis in greater depth and ascertain why despite an increased propensity to adhesions associated with pregnancy, adhesion development is less prevalent after CD.

Declaration of Conflicting Interests

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References

1. Swolin K.50 fertility operations. I. Literature and methods. *Acta Obstet Gynecol Scand*. 1967;46(2):234-250.
2. Winston RM. Microsurgery of the fallopian tube: from fantasy to reality. *Fertil Steril*. 1980;34(6):521-530.
3. Vrijland WW, Jeekel J, van Geldorp HJ, Swank DJ, Bonjer HJ. Abdominal adhesions: intestinal obstruction, pain, and infertility. *Surg Endosc*. 2003;17(7):1017-1022.
4. Marana R, Catalano GF, Muzii L. Salpingoscopy. *Curr Opin Obstet Gynecol*. 2003;15(4):333-336.
5. Duffy DM, diZerega GS. Adhesion controversies: pelvic pain as a cause of adhesions, crystalloids in preventing them. *J Reprod Med*. 1996;41(1):19-26.
6. Barmparas G, Branco BC, Schnuriger B, Lam L, Inaba K, Demetriades D. The incidence and risk factors of post-laparotomy adhesive small bowel obstruction. *J Gastrointest Surg*. 2010;14(10):1619-1628.
7. Holmdahl L, Risberg B. Adhesions: prevention and complications in general surgery. *Eur J Surg*. 1997;163(3):169-174.
8. Potter KL, Held-Warmkessel J. Intraperitoneal chemotherapy for women with ovarian cancer: nursing care and considerations. *Clin J Oncol Nurs*. 2008;12(2):265-271.
9. Diamond MP. Incidence of postsurgical adhesions. In: GS diZerega, ed. *Peritoneal Surgery*. New York, NY: Spriger-Verlag; 2000:217-220.
10. Diamond MP. Surgical aspects of infertility. In: J S, ed. *Gynecology and obstetrics*. Vol 2. Philadelphia, PA: Harper & Row; 1988:1-23.
11. Diamond MP. Reduction of adhesions after uterine myomectomy by Seprafilm membrane (HAL-F): a blinded, prospective, randomized, multicenter clinical study. Seprafilm Adhesion Study Group. *Fertil Steril.* 1996;66(6):904-910.
12. Morales KJ, Gordon MC, Bates GW, Jr. Postcesarean delivery adhesions associated with delayed delivery of infant. *Am J Obstet Gynecol*. 2007;196(5):461.e461-e466.
13. Tulandi T, Agdi M, Zarei A, Miner L, Sikirica V. Adhesion development and morbidity after repeat cesarean delivery. *Am J Obstet Gynecol*. 2009;201(1):56.e51-e56.
14. Fushiki HIT, Kobayashi H, Yoshimoto H. Efficacy of Seprafilm as an adhesion prevention barrier in cesarean sections. *Obstet Gynecol Treat*. 2005;91:557-561.
15. Al-Took S, Platt R, Tulandi T. Adhesion-related small-bowel obstruction after gynecologic operations. *Am J Obstet Gynecol*. 1999;180(2 Pt 1):313-315.
16. Tollanes MC, Melve KK, Irgens LM, Skjaerven R. Reduced fertility after cesarean delivery: a maternal choice. *Obstet Gynecol*. 2007;110(6):1256-1263.
17. Bhattacharya S, Porter M, Harrild K, et al. Absence of conception after caesarean section: voluntary or involuntary? *BJOG*. 2006;113(3):268-275.
18. Kendrick JS, Tierney EF, Lawson HW, Strauss LT, Klein L, Atrash HK. Previous cesarean delivery and the risk of ectopic pregnancy. *Obstet Gynecol*. 1996;87(2):297-301.
19. Stark M, Hoyme UB, Stubert B, Kieback D, di Renzo GC. Postcesarean adhesions—are they a unique entity? *J Matern Fetal Neonatal Med*. 2008;21(8):513-516.
20. Dunn RC, Buttram VC, Jr. Tissue-type plasminogen activator as an adjuvant for post surgical adhesions. *Prog Clin Biol Res*. 1990;358:113-118.

21. Saed GM, Diamond MP. Molecular characterization of postoperative adhesions: the adhesion phenotype. *J Am Assoc Gynecol Laparosc*. 2004;11(3):307-314.
22. Szecsi PB, Jorgensen M, Klajnbard A, Andersen MR, Colov NP, Stender S. Haemostatic reference intervals in pregnancy. *Thromb Haemost*. 2010;103(4):718-727.
23. Saed GM, Diamond MP. Hypoxia-induced irreversible up-regulation of type I collagen and transforming growth factor-beta1 in human peritoneal fibroblasts. *Fertil Steril*. 2002;78(1): 144-147.
24. Chegini N. The role of growth factors in peritoneal healing: transforming growth factor beta (TGF-beta). *Eur J Surg Suppl*. 1997(577):17-23.
25. Idell S, Zwieb C, Boggaram J, Holiday D, Johnson AR, Raghu G. Mechanisms of fibrin formation and lysis by human lung fibroblasts: influence of TGF-beta and TNF-alpha. *Am J Physiol*. 1992;263(4 Pt 1):L487-L494.
26. Diamond MP, El-Hammady E, Munkarah A, Bieber EJ, Saed G. Modulation of the expression of vascular endothelial growth factor in human fibroblasts. *Fertil Steril*. 2005;83(2):405-409.
27. Saed GM, Diamond MP. Differential expression of alpha smooth muscle cell actin in human fibroblasts isolated from intraperitoneal adhesions and normal peritoneal tissues. *Fertil Steril*. 2004;(suppl 3):1188-1192.
28. Saed GM, Zhang W, Chegini N, Holmdahl L, Diamond MP. Alteration of type I and III collagen expression in human peritoneal mesothelial cells in response to hypoxia and transforming growth factor-beta1. *Wound Repair Regen*. 1999;7(6):504-510.
29. Saed GM, Diamond MP. Modulation of the expression of tissue plasminogen activator and its inhibitor by hypoxia in human peritoneal and adhesion fibroblasts. *Fertil Steril*. 2003;79(1): 164-168.
30. Saed GM, Zhang W, Diamond MP. Molecular characterization of fibroblasts isolated from human peritoneum and adhesions. *Fertil Steril*. 2001;75(4):763-768.
31. Saed GM, Munkarah AR, Diamond MP. Cyclooxygenase-2 is expressed in human fibroblasts isolated from intraperitoneal adhesions but not from normal peritoneal tissues. *Fertil Steril*. 2003;79(6):1404-1408.
32. Ivarsson ML, Holmdahl L, Falk P, Molne J, Risberg B. Characterization and fibrinolytic properties of mesothelial cells isolated from peritoneal lavage. *Scand J Clin Lab Invest*. 1998;58(3):195-203.
33. Shavell VI, Saed GM, Diamond MP. Review: cellular metabolism: contribution to postoperative adhesion development. *Reprod Sci*. 2009;16(7):627-634.
34. Cookson VJ, Chapman NR. NF-kappaB function in the human myometrium during pregnancy and parturition. *Histol Histopathol*. 2010;25(7):945-956.
35. Shukla A, Rasik AM, Shankar R. Nitric oxide inhibits wounds collagen synthesis. *Mol Cell Biochem*. 1999;200(1-2):27-33.
36. Ferrini MG, Vernet D, Magee TR, et al. Antifibrotic role of inducible nitric oxide synthase. *Nitric Oxide*. 2002;6(3):283-294.
37. Jiang ZL, Zhu X, Diamond MP, Abu-Soud HM, Saed GM. Nitric oxide synthase isoforms expression in fibroblasts isolated from human normal peritoneum and adhesion tissues. *Fertil Steril*. 2008;90(3):769-774.
38. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44-84.
39. Terada LS, Guidot DM, Leff JA, et al. Hypoxia injures endothelial cells by increasing endogenous xanthine oxidase activity. *Proc Natl Acad Sci U S A*. 1992;89(8):3362-3366.
40. Yamamoto Y, Konig P, Henrich M, Dedio J, Kummer W. Hypoxia induces production of nitric oxide and reactive oxygen species in glomus cells of rat carotid body. *Cell Tissue Res*. 2006;325(1):3-11.
41. Zhu H, Bunn HF. Oxygen sensing and signaling: impact on the regulation of physiologically important genes. *Respir Physiol*. 1999;115(2):239-247.
42. Kettle AJ, van Dalen CJ, Winterbourn CC. Peroxynitrite and myeloperoxidase leave the same footprint in protein nitration. *Redox Rep*. 1997;3(5-6):257-258.
43. Weiss SJ, Klein R, Slivka A, Wei M. Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. *J Clin Invest*. 1982;70(3):598-607.
44. Tahboub YR, Galijasevic S, Diamond MP, Abu-Soud HM. Thiocyanate modulates the catalytic activity of mammalian peroxidases. *J Biol Chem*. 2005;280(28):26129-26136.
45. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev*. 2002;82(1):47-95.
46. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundam Appl Toxicol*. 1993;21(4):433-441.
47. Stadtman ER. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. *Ann Rev Biochem*. 1993;62:797-821.
48. Reed KL, Heydrick SJ, Aarons CB, et al. A neurokinin-1 receptor antagonist that reduces intra-abdominal adhesion formation decreases oxidative stress in the peritoneum. *Am J Physiol Gastrointest Liver Physiol*. 2007;293(3):G544-G551.
49. Heydrick SJ, Reed KL, Cohen PA, et al. Intraperitoneal administration of methylene blue attenuates oxidative stress, increases peritoneal fibrinolysis, and inhibits intraabdominal adhesion formation. *J Surg Res*. 2007;143(2):311-319.
50. Reed KL, Stucchi AF, Leeman SE, Becker JM. Inhibitory effects of a neurokinin-1 receptor antagonist on postoperative peritoneal adhesion formation. *Ann NY Acad Sci*. 2008;1144:116-126.
51. Kluger Y, Weinbroum A, Ben-Avraham R, Galili Y, Klausner J, Rabau M. Reduction in formation of peritoneal adhesions by methylene blue in rats: a dose response study. *Eur J Surg*. 2000;166(7):568-571.
52. Gul A, Kotan C, Dilek I, Gul T, Tas A, Berktas M. Effects of methylene blue, indigo carmine solution and autologous erythrocyte suspension on formation of adhesions after injection into rats. *J Reprod Fertil*. 2000;120(2):225-229.
53. Saed GM, Abu-Soud HM, Diamond MP. Role of nitric oxide in apoptosis of human peritoneal and adhesion fibroblasts after hypoxia. *Fertil Steril*. 2004;82(suppl 3):1198-1205.
54. Fletcher NM, Jiang ZL, Diamond MP, Abu-Soud HM, Saed GM. Hypoxia-generated superoxide induces the development of the adhesion phenotype. *Free Radic Biol Med*. 2008;45(4):530-536.

55. Jiang ZL, Fletcher NM, Diamond MP, Abu-Soud HM, Saed GM. S-nitrosylation of caspase-3 is the mechanism by which adhesion fibroblasts manifest lower apoptosis. *Wound Repair Regen*. 2009; 17(2):224-229.
56. Ellis H, Moran BJ, Thompson JN, et al. Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. *Lancet*. 1999;353(9163):1476-1480.
57. Gonzalez-Quintero VH, Cruz-Pachano FE. Preventing adhesions in obstetric and gynecologic surgical procedures. *Rev Obstet Gynecol*. 2009;2(1):38-45.
58. Tulandi T, Murray C, Guralnick M. Adhesion formation and reproductive outcome after myomectomy and second-look laparoscopy. *Obstet Gynecol*. 1993;82(2):213-215.
59. Bulletti C, Polli V, Negrini V, Giacomucci E, Flamigni C. Adhesion formation after laparoscopic myomectomy. *J Am Assoc Gynecol Laparosc*. 1996;3(4):533-536.
60. Brill AI, Nezhat F, Nezhat CH, Nezhat C. The incidence of adhesions after prior laparotomy: a laparoscopic appraisal. *Obstet Gynecol*. 1995;85(2):269-272.
61. Ashrafinia M, Vazirichimeh Z, Dastjerdi MV, Moiini A. Adhesion Formation in Patients with Previous Laparotomies. *J Am Assoc Gynecol Laparosc*. 1996;3(4 suppl):S2.
62. Leuwen V. *Ann de. Gynec*. 1904;2.s., i,:577-580.
63. Mason NR. The end results of caesarean section. *Boston Med Surg J*. 1911;CLXIV:889-891.
64. Juntunen K, Makarainen L, Kirkinen P. Outcome after a high number (4-10) of repeated caesarean sections. *BJOG*. 2004;111(6):561-563.
65. Diamond MP, Hershlag A. Adhesion formation/reformation. *Prog Clin Biol Res*. 1990;358:23-33.
66. Miller G, Boman J, Shrier I, Gordon PH. Etiology of small bowel obstruction. *Am J Surg*. 2000;180(1):33-36.
67. Monk BJ, Berman ML, Montz FJ. Adhesions after extensive gynecologic surgery: clinical significance, etiology, and prevention. *Am J Obstet Gynecol*. 1994;170(5 Pt 1):1396-1403.
68. Stricker B, Blanco J, Fox HE. The gynecologic contribution to intestinal obstruction in females. *J Am Coll Surg*. 1994;178(6):617-620.
69. Fevang BT, Fevang J, Lie SA, Soreide O, Svanes K, Viste A. Long-term prognosis after operation for adhesive small bowel obstruction. *Ann Surg*. 2004;240(2):193-201.
70. Makoha FW, Fathuddien MA, Felimban HM. Choice of abdominal incision and risk of trauma to the urinary bladder and bowel in multiple cesarean sections. *Eur J Obstet Gynecol Reprod Biol*. 2006;125(1):50-53.
71. Silver RM, Landon MB, Rouse DJ, et al. Maternal morbidity associated with multiple repeat cesarean deliveries. *Obstet Gynecol*. 2006;107(6):1226-1232.
72. Swank DJ, Van Erp WF, Repelaer Van Driel OJ, Hop WC, Bonjer HJ, Jeekel H. A prospective analysis of predictive factors on the results of laparoscopic adhesiolysis in patients with chronic abdominal pain. *Surg Laparosc Endosc Percutan Tech*. 2003;13(2):88-94.
73. Nisenblat V, Barak S, Griness OB, Degani S, Ohel G, Gonen R. Maternal complications associated with multiple cesarean deliveries. *Obstet Gynecol*. 2006;108(1):21-26.
74. Phipps MG, Watabe B, Clemons JL, Weitzen S, Myers DL. Risk factors for bladder injury during cesarean delivery. *Obstet Gynecol*. 2005;105(1):156-160.
75. Sobande A, Eskandar M. Multiple repeat caesarean sections: complications and outcomes. *J Obstet Gynaecol Can*. 2006; 28(3):193-197.
76. Rahman MS, Gasem T, Al Suleiman SA, Al Jama FE, Burshaid S, Rahman J. Bladder injuries during cesarean section in a University Hospital: a 25-year review. *Arch Gynecol Obstet*. 2009; 279(3):349-352.
77. Kulenthran A, Sivanesaratnam V. Recurrent uterine myomata in three sisters—an uncommon occurrence. *Int J Gynaecol Obstet*. 1988;27(2):289-291.
78. Eisenkop SM, Richman R, Platt LD, Paul RH. Urinary tract injury during cesarean section. *Obstet Gynecol*. 1982;60(5):591-596.
79. Wang L, Merkur H, Hardas G, Soo S, Lujic S. Laparoscopic hysterectomy in the presence of previous caesarean section: a review of one hundred forty-one cases in the Sydney West Advanced Pelvic Surgery Unit. *J Minim Invasive Gynecol*. 2010;17(2):186-191.
80. Khashoggi TY. Higher order multiple repeat cesarean sections: maternal and fetal outcome. *Ann Saudi Med*. 2003;23(5):278-282.
81. Rashid M, Rashid RS. Higher order repeat caesarean sections: how safe are five or more? *BJOG*. 2004;111(10):1090-1094.
82. Shellhaas CS, Gilbert S, Landon MB, et al. The frequency and complication rates of hysterectomy accompanying cesarean delivery. *Obstet Gynecol*. 2009;114(2 Pt 1):224-229.
83. Kadanali S, Erten O, Kucukozkan T. Pelvic and periaortic pertioneal closure or non-closure at lymphadenectomy in ovarian cancer: effects on morbidity and adhesion formation. *Eur J Surg Oncol*. 1996;22(3):282-285.
84. Hugh TB, Nankivell C, Meagher AP, Li B. Is closure of the peritoneal layer necessary in the repair of midline surgical abdominal wounds? *World J Surg*. 1990;14(2):231-233; discussion 233-234.
85. Pearl ML, Rayburn WF. Choosing abdominal incision and closure techniques: a review. *J Reprod Med*. 2004;49(8):662-670.
86. Kingdom. RCoOaGU. Peritoneal closure. *Guideline*. 2002;15:1-7.
87. Bamigboye AA, Hofmeyr GJ. Closure versus non-closure of the peritoneum at caesarean section. *Cochrane Database Syst Rev*. 2003(4):CD000163.
88. Berghella V, Baxter JK, Chauhan SP. Evidence-based surgery for cesarean delivery. *Am J Obstet Gynecol*. 2005;193(5):1607-1617.
89. Malvasi A, Tinelli A, Farine D, et al. Effects of visceral peritoneal closure on scar formation at cesarean delivery. *Int J Gynaecol Obstet*. 2009;105(2):131-135.
90. Stark M, Chavkin Y, Kupfersztain C, Guedj P, Finkel AR. Evaluation of combinations of procedures in cesarean section. *Int J Gynaecol Obstet*. 1995;48(3):273-276.
91. Joura EA, Nather A, Hohlagschwandtner M, Husslein P. Peritoneal closure and adhesions. *Hum Reprod*. 2002;17(1):249-250.
92. Zareian Z, Zareian P. Non-closure versus closure of peritoneum during cesarean section: a randomized study. *Eur J Obstet Gynecol Reprod Biol*. 2006;128(1-2):267-269.
93. Weerawetwat W, Buranawanich S, Kanawong M. Closure vs nonclosure of the visceral and parietal peritoneum at cesarean delivery: 16 year study. *J Med Assoc Thai*. 2004;87(9):1007-1011.
94. Lyell DJ, Caughey AB, Hu E, Daniels K. Peritoneal closure at primary cesarean delivery and adhesions. *Obstet Gynecol*. 2005; 106(2):275-280.

95. Tulandi T, Hum HS, Gelfand MM. Closure of laparotomy incisions with or without peritoneal suturing and second-look laparoscopy. *Am J Obstet Gynecol*. 1988;158(3 Pt 1):536-537.
96. Blumenfeld YJ, Caughey AB, El-Sayed YY, Daniels K, Lyell DJ. Single- versus double-layer hysterotomy closure at primary caesarean delivery and bladder adhesions. *BJOG*. 2010;117(6):690-694.
97. Coleman MG, McLain AD, Moran BJ. Impact of previous surgery on time taken for incision and division of adhesions during laparotomy. *Dis Colon Rectum*. 2000;43(9):1297-1299.
98. Beck DE, Ferguson MA, Opelka FG, Fleshman JW, Gervaz P, Wexner SD. Effect of previous surgery on abdominal opening time. *Dis Colon Rectum*. 2000;43(12):1749-1753.
99. Diamond MP, Daniell JF, Feste J, et al. Adhesion reformation and de novo adhesion formation after reproductive pelvic surgery. *Fertil Steril*. 1987;47(5):864-866.
100. Diamond MP, Decherney AH. Pathogenesis of adhesion formation/reformation: application to reproductive pelvic surgery. *Microsurgery*. 1987;8(2):103-107.
101. Lower AM, Hawthorn RJ, Ellis H, O'Brien F, Buchan S, Crowe AM. The impact of adhesions on hospital readmissions over ten years after 8849 open gynaecological operations: an assessment from the Surgical and Clinical Adhesions Research Study. *BJOG*. 2000;107(7):855-862.
102. Lower AM, Hawthorn RJ, Clark D, et al. Adhesion-related readmissions following gynaecological laparoscopy or laparotomy in Scotland: an epidemiological study of 24 046 patients. *Hum Reprod*. 2004;19(8):1877-1885.
103. Greenberg M DK, Blumenfeld Y, Caughey A, Lyell D. Do adhesions at repeat cesarean delay delivery of the newborn? *Am J Obstet Gynecol*. 2011;204(1):S267-S268.
104. Rouse DJ, MacPherson C, Landon M, et al. Blood transfusion and cesarean delivery. *Obstet Gynecol*. 2006;108(4):891-897.
105. Becker JM, Dayton MT, Fazio VW, et al. Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective, randomized, double-blind multicenter study. *J Am Coll Surg*. 1996;183(4):297-306.
106. Milingos S, Kallipolitis G, Loutradis D, et al. Adhesions: laparoscopic surgery versus laparotomy. *Ann N Y Acad Sci*. 2000;900:272-285.
107. Diamond MP, Freeman ML. Clinical implications of postsurgical adhesions. *Hum Reprod Update*. 2001;7(6):567-576.
108. Lalos O. Risk factors for tubal infertility among infertile and fertile women. *Eur J Obstet Gynecol Reprod Biol*. 1988;29(2):129-136.
109. Collin SM, Marshall T, Filippi V. Caesarean section and subsequent fertility in sub-Saharan Africa. *BJOG*. 2006;113(3):276-283.
110. Jolly J, Walker J, Bhabra K. Subsequent obstetric performance related to primary mode of delivery. *Br J Obstet Gynaecol*. 1999;106(3):227-232.
111. Hemminki E. Impact of caesarean section on future pregnancy—a review of cohort studies. *Paediatr Perinat Epidemiol*. 1996;10(4):366-379.
112. Mollison J, Porter M, Campbell D, Bhattacharya S. Primary mode of delivery and subsequent pregnancy. *BJOG*. 2005;112(8):1061-1065.
113. LaSala AP, Berkeley AS. Primary cesarean section and subsequent fertility. *Am J Obstet Gynecol*. 1987;157(2):379-383.
114. Murphy DJ, Stirrat GM, Heron J. The relationship between Caesarean section and subfertility in a population-based sample of 14 541 pregnancies. *Hum Reprod*. 2002;17(7):1914-1917.
115. Saraswat L, Porter M, Bhattacharya S. Caesarean section and tubal infertility: is there an association? *Reprod Biomed Online*. 2008;17(2):259-264.
116. Wolf ME, Daling JR, Voigt LF. Prior cesarean delivery in women with secondary tubal infertility. *Am J Public Health*. 1990;80(11):1382-1383.
117. Mol F, van Mello NM, Mol BW, van der Veen F, Ankum WM, Hajenius PJ. Ectopic pregnancy and pelvic inflammatory disease: a renewed epidemic? *Eur J Obstet Gynecol Reprod Biol*. 2010;151(2):163-167.
118. Ankum WM, Mol BW, Van der Veen F, Bossuyt PM. Risk factors for ectopic pregnancy: a meta-analysis. *Fertil Steril*. 1996;65(6):1093-1099.
119. Clayton HB, Schieve LA, Peterson HB, Jamieson DJ, Reynolds MA, Wright VC. Ectopic pregnancy risk with assisted reproductive technology procedures. *Obstet Gynecol*. 2006;107(3):595-604.
120. Parazzini F, Tozzi L, Ferraroni M, Bocciolone L, La Vecchia C, Fedele L. Risk factors for ectopic pregnancy: an Italian case-control study. *Obstet Gynecol*. 1992;80(5):821-826.
121. Karaer A, Avsar FA, Batioglu S. Risk factors for ectopic pregnancy: a case-control study. *Aust N Z J Obstet Gynaecol*. 2006;46(6):521-527.
122. Peters AA, Trimbos-Kemper GC, Admiraal C, Trimbos JB, Hermans J. A randomized clinical trial on the benefit of adhesiolysis in patients with intraperitoneal adhesions and chronic pelvic pain. *Br J Obstet Gynaecol*. 1992;99(1):59-62.
123. Hammoud A, Gago LA, Diamond MP. Adhesions in patients with chronic pelvic pain: a role for adhesiolysis? *Fertil Steril*. 2004;82(6):1483-1491.
124. Almeida EC, Nogueira AA, Candido dos Reis FJ, Rosa e Silva JC. Cesarean section as a cause of chronic pelvic pain. *Int J Gynaecol Obstet*. 2002;79(2):101-104.
125. Deneux-Tharaux C, Carmona E, Bouvier-Colle MH, Breart G. Postpartum maternal mortality and cesarean delivery. *Obstet Gynecol*. 2006;108(3 Pt 1):541-548.
126. Menacker F, Declercq E, Macdorman MF. Cesarean delivery: background, trends, and epidemiology. *Semin Perinatol*. 2006;30(5):235-241.
127. Imudia AN, Hobson DT, Awonuga AO, Diamond MP, Bahado-Singh RO. Determinants and complications of emergent cesarean hysterectomy: supracervical vs total hysterectomy. *Am J Obstet Gynecol*. 2010;203(3):e221-e225.
128. Scott JR. Avoiding labor problems during vaginal birth after cesarean delivery. *Clin Obstet Gynecol.* 1997;40(3):533-541
129. Sbarra M, Boyd M, Dardarian TS. Complications due to adhesion formation following cesarean sections: a review of deliveries in three cases. *Fertil Steril*. 2009;92(1):394.e313-e396.
130. Uygur D, Gun O, Kelekci S, Ozturk A, Ugur M, Mungan T. Multiple repeat caesarean section: is it safe? *Eur J Obstet Gynecol Reprod Biol*. 2005;119(2):171-175.
131. Esmon CT, Vigano-D'Angelo S, D'Angelo A, Comp PC. Anticoagulation proteins C and S. *Adv Exp Med Biol*. 1987;214:47-54.

132. Petersen CM. Alpha 2-macroglobulin and pregnancy zone protein. Serum levels, alpha 2-macroglobulin receptors, cellular synthesis and aspects of function in relation to immunology. *Dan Med Bull*. 1993;40(4):409-446.
133. Wallmo L, Gyzander E, Karlsson K, Lindstedt G, Radberg T, Teger-Nilsson AC. alpha 2-Antiplasmin and alpha 2-macroglobulin—the main inhibitors of fibrinolysis—during the menstrual cycle, pregnancy, delivery, and treatment with oral contraceptives. *Acta Obstet Gynecol Scand*. 1982;61(5):417-422.
134. Hellgren M. Hemostasis during normal pregnancy and puerperium. *Semin Thromb Hemost*. 2003;29(2):125-130.
135. Rehman KS, Yin S, Mayhew BA, Word RA, Rainey WE. Human myometrial adaptation to pregnancy: cDNA microarray gene expression profiling of myometrium from non-pregnant and pregnant women. *Mol Hum Reprod*. 2003;9(11): 681-700.
136. Prochazkova J, Dhaifallah I, Mechurova A, et al. [Monitoring of endothelial activation markers during physiological pregnancy]. *Ceska Gynekol*. 2010;75(2):92-100.
137. Hahn L, Korsan-Bengtsen K. The coagulation system during caerean section. Coagulation, fibrinolysis and hormonal levels in peripheral and uterine venous blood during caesarean section. *Acta Obstet Gynecol Scand*. 1975;54(1):49-55.
138. Roh CR, Oh WJ, Yoon BK, Lee JH. Up-regulation of matrix metalloproteinase-9 in human myometrium during labour: a cytokine-mediated process in uterine smooth muscle cells. *Mol Hum Reprod*. 2000;6(1):96-102.
139. Choi SJ, Oh S, Kim JH, Roh CR. Changes of nuclear factor kappa B (NF-kappaB), cyclooxygenase-2 (COX-2) and matrix metalloproteinase-9 (MMP-9) in human myometrium before and during term labor. *Eur J Obstet Gynecol Reprod Biol*. 2007; 132(2):182-188.
140. Myatt L. Review: reactive oxygen and nitrogen species and functional adaptation of the placenta. *Placenta*. 2010;31(suppl): S66-S69.
141. Aris A, Benali S, Ouellet A, Moutquin JM, Leblanc S. Potential biomarkers of preeclampsia: inverse correlation between hydrogen peroxide and nitric oxide early in maternal circulation and at term in placenta of women with preeclampsia. *Placenta*. 2009; 30(4):342-347.
142. Khan RN, Matharoo-Ball B, Shaw RW. Antioxidant enzyme expression, lipid peroxidation, and protein oxidation in human myometrium with parturition. *Reprod Sci*. 2010;17(1): 78-84.
143. Shynlova O, Tsui P, Dorogin A, Chow M, Lye SJ. Expression and localization of alpha-smooth muscle and gamma-actins in the pregnant rat myometrium. *Biol Reprod*. 2005;73(4):773-780.
144. Mesa A, Jessurun C, Hernandez A, et al. Left ventricular diastolic function in normal human pregnancy. *Circulation*. 1999; 99(4):511-517.
145. Taylor DJ, Lind T. Red cell mass during and after normal pregnancy. *Br J Obstet Gynaecol*. 1979;86(5):364-370.
146. Palmer SK, Zamudio S, Coffin C, Parker S, Stamm E, Moore LG. Quantitative estimation of human uterine artery blood flow and pelvic blood flow redistribution in pregnancy. *Obstet Gynecol*. 1992;80(6):1000-1006.
147. Leibson RG, Likhnitzky II, Sax MG. Oxygen transport of the foetal and maternal blood during pregnancy. *J Physiol*. 1936; 87(2):97-112.
148. Schrinsky DC, Benson RC. Rupture of the pregnant uterus: a review. *Obstet Gynecol Surv*. 1978;33(4):217-232.
149. Vercellini P, Trespidi L, Zaina B, Vicentini S, Stellato G, Crosignani PG. Gonadotropin-releasing hormone agonist treatment before abdominal myomectomy: a controlled trial. *Fertil Steril*. 2003;79(6):1390-1395.
150. Coddington CC, Grow DR, Ahmed MS, Toner JP, Cook E, Diamond MP. Gonadotropin-releasing hormone agonist pretreatment did not decrease postoperative adhesion formation after abdominal myomectomy in a randomized control trial. *Fertil Steril*. 2009; 91(5):1909-1913.
151. Darwish AM, Nasr AM, El-Nashar DA. Evaluation of post-myomectomy uterine scar. *J Clin Ultrasound*. 2005;33(4): 181-186.
152. Jastrow N, Chaillet N, Roberge S, Morency AM, Lacasse Y, Bujold E. Sonographic lower uterine segment thickness and risk of uterine scar defect: a systematic review. *J Obstet Gynaecol Can*. 2010;32(4):321-327.
153. Vikhareva Osser O, Valentin L. Risk factors for incomplete healing of the uterine incision after caesarean section. *BJOG*. 2010; 117(9):1119-1126.
154. Ray NF, Denton WG, Thamer M, Henderson SC, Perry S. Abdominal adhesiolysis: inpatient care and expenditures in the United States in 1994. *J Am Coll Surg*. 1998;186(1):1-9.
155. Portz DM, Elkins TE, White R, Warren J, Adadevoh S, Randolph J. Oxygen free radicals and pelvic adhesion formation: I. Blocking oxygen free radical toxicity to prevent adhesion formation in an endometriosis model. *Int J Fertil.* 1991; 36(1):39-42.
156. Salaris SC, Babbs CF, Voorhees WD, 3rd. Methylene blue as an inhibitor of superoxide generation by xanthine oxidase. A potential new drug for the attenuation of ischemia/reperfusion injury. *Biochem Pharmacol*. 1991;42(3):499-506.
157. Ozcelik B, Serin IS, Basbug M, Uludag S, Narin F, Tayyar M. Effect of melatonin in the prevention of post-operative adhesion formation in a rat uterine horn adhesion model. *Hum Reprod*. 2003;18(8):1703-1706.
158. Sharpe-Timms KL, Zimmer RL, Jolliff WJ, Wright JA, Nothnick WB, Curry TE. Gonadotropin-releasing hormone agonist (GnRH-a) therapy alters activity of plasminogen activators, matrix metalloproteinases, and their inhibitors in rat models for adhesion formation and endometriosis: potential GnRH-a-regulated mechanisms reducing adhesion formation. *Fertil Steril*. 1998;69(5):916-923.
159. Gago LA, Saed GM, Wang RX, Kruger M, Diamond MP. Effects of oxidized regenerated cellulose on the expression of extracellular matrix and transforming growth factor-beta1 in human peritoneal fibroblasts and mesothelial cells. *Am J Obstet Gynecol*. 2003;189(6):1620-1625; discussion 1625-1626.
160. Tarhan OR, Eroglu A, Cetin R, A YN, Bulbul M, Altuntas YR. Effects of seprafilm on peritoneal fibrinolytic system. *ANZ J Surg*. 2005;75(8):690-692.

161. Vrijland WW, Tseng LN, Eijkman HJ, et al. Fewer intraperitoneal adhesions with use of hyaluronic acid-carboxymethylcellulose membrane: a randomized clinical trial. *Ann Surg*. 2002;235(2): 193-199.
162. Reddy S, Santanam N, Reddy PP, Rock JA, Murphy AA, Parthasarathy S. Interaction of Interceed oxidized regenerated cellulose with macrophages: a potential mechanism by which Interceed may prevent adhesions. *Am J Obstet Gynecol*. 1997; 177(6):1315-1320; discussion 1320-1311.
163. Kim TH, Kim JS, Lee HH, Nam KH, Lee KH, Lee JJ. Prevention of vesicouterine adhesions after cesarean with Interceed [abstract]. *Korean Soc Fetal Med*. 2006;2:194.