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EXPERIMENTAL STUDY

Effect of allograft amniotic membrane use on adhesion formation after Cesarean section in pregnant rat

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Abstract: *Objective:* We aimed to investigate the effect of allograft amniotic membrane utilization to prevent the post-operative adhesion formation.
Background: In 24 pregnant inbreed Wistar-Albino rats, pregnancy was terminated by forming bilateral uterine horn defect via cesarean section at 20th gestational day. Rats were assigned in three groups randomly.
Methods: In the first group, abdomen closure was achieved without administration of any intra-peritoneal material following standard surgical intervention. In the second group Seprafilm was used to cover the defect at anterior horn of uterus; whereas amniotic membrane of the rat itself was used in the third group. After 3 weeks, all rats were sacrificed and re-laparotomy was performed to determine adhesions scores.
Results: No significant difference was found in adhesion scores between the group 1 and group 3 and also between group 2 and group 3, supporting the previous findings in the literature.
Conclusion: We observed that direct application of allograft amniotic membrane, which is an adhesion barrier used after cesarean section, to injured surface had no effect in the prevention of adhesions *(Tab. 3, Fig. 5, Ref. 28)*. Full Text in PDF *www.elis.sk.*
Key words: adhesion, amniotic membrane, rat, uterine horn, cesarean section.

Peritoneal adhesion formation after abdominal and pelvic operations is common and this can be a source of considerable morbidity. The incidence of intraperitoneal adhesions ranges from 67 to 93 % after general surgical abdominal operations and up to 97% following open gynecological pelvic procedures (1). The necessity to reduce the development of postoperative surgical adhesions is pronounced. More than 440,000 procedures for abdominopelvic peritoneal adhesiolysis are performed each year in the United States, creating a serious health problem to patients at a cost of more than 1.2 billion annually (2, 3).

The main approaches to prevent adhesion formation include the adjustment of surgical techniques, avoidance of foreign material exposure, and the applications of adjuvant treatment (1, 4, 5). Other effective measures include careful tissue handling, keeping tissues moist and the use of micro- and atraumatic instruments to reduce serosal injury (6). Adjuvant therapy falls into two main categories; administering drugs that alter the adhesion-producing inflammatory cascade and separating serosal surfaces during the early stages of wound repair with barriers (7).

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Human amnion has been used successfully in various surgical conditions, either as a surface covering (leg ulcers and wounds, lining of the cavity following radical mastectomy, traumatic ulcers, treatment of burns) in order to encourage epithelization, or to prevent adhesions in the abdominal cavity or edema and adhesions following craniotomy and brain surgery (8). The amniotic membrane is a tissue of fetal origin and composed of three major layers: a single epithelial layer, a thick basement membrane and an avascular mesenchyme. It contains basement membrane components, growth factors and proteinase inhibitors (9, 10). Studies indicate that this membrane possesses antibacterial properties and low immunogenicity, can promote epithelization and wound healing, inhibit inflammation and scarring, and regulate angiogenesis (11–13).

Antiadhesion barriers basically fall under two main categories: macromolecular solutions and mechanical devices. In recent years both kinds of barriers have demonstrated real progress in adhesion prevention (6, 14). Seprafilm (Genzyme Corporation, Cambridge, MA, USA) is a bioresorbable, nonimmunogenic membrane which turns to gel within 24 hours of application while remaining in place to separate adhesiogenic tissues during the first few days when adhesions are likely to develop (15). HA-CMC reduced the incidence of postoperative adhesions to the incision line by greater than 50 %, and the mean adhesion rate was 40% less when compared to controls undergoing laparotomy (16).

The aim of this study, in which a standard experimental model of adhesion was used, was to prevent adhesion formation, resulted from tissue injury, by an barrier method consisting of using the amniotic membrane as an allograft, which is an ease and safe applicable material.

Methods

In the present study, 30 female and 5 male inbreed Wistar-Albino rats (3 months old, 250–300 g) were used. Before and during experiment, each rat was maintained in an individual cage at a mean temperature of 22 °C with a light-dark schedule of 12 + 12 h, with free access to standard rat chow and tap water. After acclimatization period, 3 female rats and 1 male rat were maintained in same cage for reproduction by polygamy method. Next day, vaginal smears were evaluated by a microscope on x10 magnification. Female rats with spermatozoa on vaginal smear were recorded as pregnant and caged individually.

On the day 21 of gestation, rats were anesthetized by subcutaneous ketamine (90 mg/kg) and xylazine (3 mg/kg) injections, then surgical preparation was achieved in a sterile manner by using povidone iodine. During surgery, rats were allowed for spontaneous respiration and a table lamp was used to maintain body temperature of 37 °C. After covering surgical site with sterile clothing, 3 cm median incision was done on abdominal skin. Then a 3x3 cm template, which was prepared in a standard size from a radiograph, was subcutaneously placed after detachment of skin, muscle and fascia. Abdomen was exposed by a vertical full-thickness incision of abdominal wall, muscle, fascia and peritoneum. After abdominal exposure, uterine horn containing fetus were taken out from abdomen and 1 cm incision was performed on the anti-mesenteric surface at the middle region of both uterine horns. Placenta was washed out by sterile saline after removal of fetus and placenta. Blood clots and tissue residues were cleansed (Fig. 1).

Amniotic membrane, which was separated from chorionic layer, was cut into pieces of 2x1 cm (2 cm^2); these pieces were awaited in sterile saline containing 1 million IU penicillin and 1 g Streptomycin per liter. 1 cm incision at each horn of uterine, which became bicornuate, was primarily repaired by using 5/0 Vicryl. After primary repair, abdominal closure was achieved without any additional intervention in Group 1, whereas it was performed after covering incision line with 2 cm^2 Seprafilm adhesion barrier in the Group 2. No additional intervention was needed, as Seprafilm adapted to shape of tissue by adhering to it. In the Group 3, abdominal closure was done after placing 2 cm^2 amniotic membrane over incision line which was prepared from rat itself. After amniotic membrane was covered over surgical site, as rough surface being faced to uterine incision and smooth surface being faced to outer side, it was handled from edges by a thin forceps and cauterized to mesenteric area under uterus; thus, uterine fixation was achieved. 3 rats died due to post-operative dehydration and 3 other died during experiment, thus they were excluded from analysis.

Tab. 1. Modified Nair's macroscopic adhesion classification.

Grade 0	No adhesion; Indefinite adhesion
Grade 1	A single adhesive band between organs or organ and abdominal wall
Grade 2	Two adhesive bands between organs or organ and abdominal wall
Grade 3	More than two adhesive bands between organs or organ and abdominal wall, or adhesion to intestinal loops without adhesion to abdominal wall Marked adhesion
Grade 4	Thick and complex adhesive band between organs or organ and abdominal wall or direct adhesion of viscera to abdominal wall

Tab. 2. Modified Zühlke's microscopic adhesion classification.

Grade 0	No adhesion, no reaction between tissues
Grade 1	Weak connective tissue, scarce cell, novel and former fibrin, thin reticullin fibrils
Grade 2	Connective tissue with scarce cell and capillaries
Grade 3	Thicker connective tissue, dense cells, more dense vessels with thicker walls, scarce elastic fibrils and smooth muscle fibrils, scarce collagen fibrils.
Grade 4	Thick or nodular granulation tissue, dense collagen fibrils and smooth muscle fibrils

Rats began to receive standard rat chow and water 4 hours after operation. At the post-operative week 3, rats were sacrificed by intra-cardiac thiopentalin (100 mg/kg) injection. In order to prevent injury of incision line, skin was opened over fascia and U-shaped, full-thickness, bilateral subcostal incision was performed to open anterior abdominal wall. In all rats, adhesions between intra-abdominal viscera and anterior region of uterine horn was scored macroscopically by using modified Nair's adhesion scoring system in a double-blind manner (Tab. 1).

After macroscopic assessment, adhesion band with involved viscera were excised in rats with adhesion formation, while an excision involving all layers of uterine horn was performed to obtain pathological samples in those without adhesion formation. Then pathological specimens were fixed in plates containing 10 % buffered formalin. Tissue sections were stained by H&E and evaluated under light microscope. Pathologist was blind to group of the specimen. After histopathological evaluation, specimens were scored according to modified scoring system of microscopic evaluation, defined by Zühlke. A total score was calculated for each animal during statistical analysis (Tab. 2).

Statistical analysis was performed by using SPSS (Statistical Package for Social Sciences) for Windows version 16.0. Descriptive methods, frequency and percentage, were used to assess data.

Tab. 3. Statistical comparisons of macroscopic and microscopic adhesion scores of groups.

	Groups	n	Mean	Standard Deviation	Median	Result
Microscopic adhesion	Cesarean	8	2.37	0.74	2.5	
	Seprafilm	8	1.75	0.70	2.0	KW= 4.63
	Amnion	8	1.37	1.06	1.5	p=0.098
Macroscopic adhesion	Cesarean	8	2.62	0.74	2.5	
	Seprafilm	8	2.00	0.92	2.0	KW=3.94
	Amnion	8	1.50	1.19	1.5	p=0.139

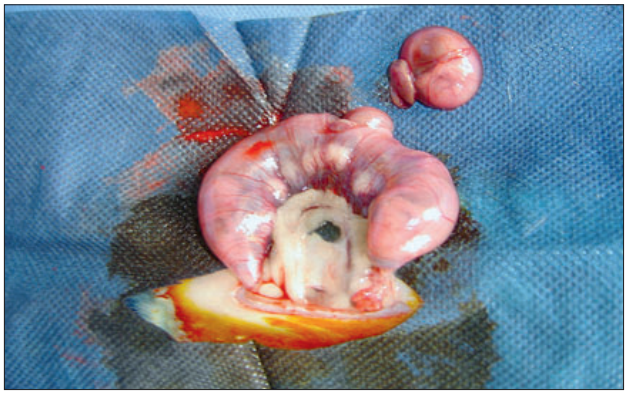

Fig. 1. Removing gestational products from uterus and fetus.

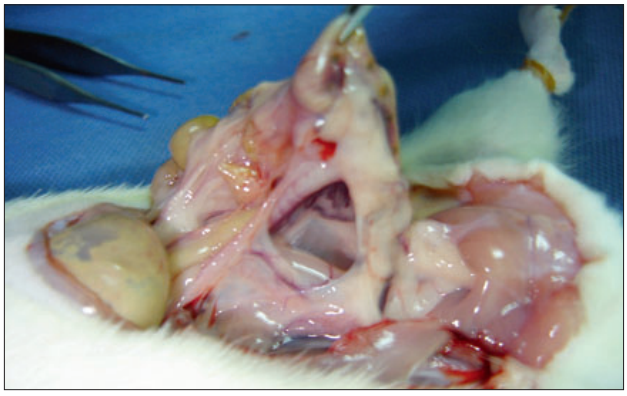

Fig. 2. A subject with Grade 4 adhesion (Group 1: Cesarean control group).

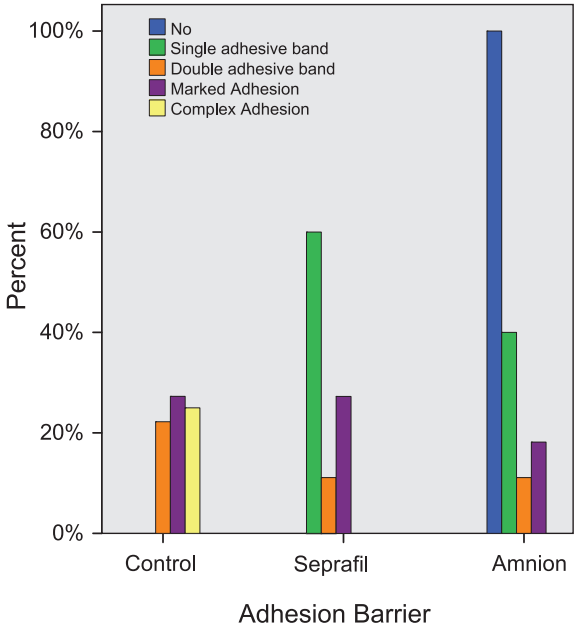

Fig. 3. Distributional percentage of adhesion barriers according to groups regarding to macroscopic results.

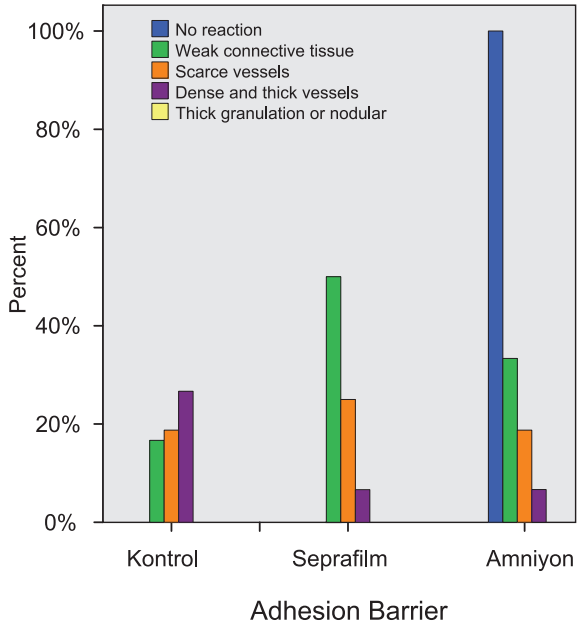

Fig. 4. Distributional percentage of adhesion barriers according to groups regarding to microscopic results.

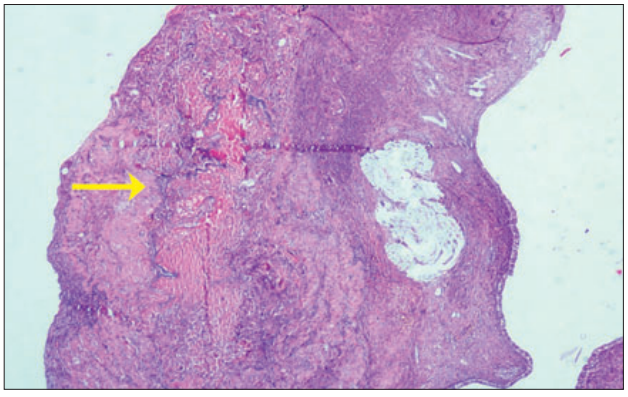

Fig. 5. Histopathological appearance in the amniotic membrane group: Grade 0; no adhesion, no reaction between tissues (HE x40).

Kruskall-Wallis test was used in the assessment of macroscopic and microscopic results.

Results

In the control group in which subjects underwent ceaserean section alone, 4 subjects (50.0 %) scored 2 points, whereas 3 subjects (37.5 %) scored 3 points and 1 subject (12.5 %) scored 4 points in the macroscopic assessments. This result indicated that selected model was effective in adhesion formation and substantially appropriate for demonstrating the positive and negative contribution of evaluated material (Fig. 2).

We found no significant difference between control group and group 2 in terms of macroscopic adhesion scores ($p>0.05$). Graphic

1 shows statistical comparisons of groups regarding macroscopic adhesion scores (Fig. 3).

We found no significant difference between control group and group 2 in terms of macroscopic adhesion scores; also we found no significant difference between Seprafilm group and amniotic membrane group in the microscopic adhesion evaluation (Fig. 4).

When macroscopic and microscopic adhesion scores of the groups were compared, no statistically significant difference was found (Tab. 3). When median values were considered, adhesion scores were found highest in cesarean section group, but not significant.

Discussion

Intra-abdominal adhesion formation is initiated by the increase in vascular permeability and secretion of fibrin-rich exudate which are triggered by peritoneal injury. This injury results in an increase in the levels of plasminogen-activator inhibitor-1 and 2, which are secreted from mesothelial, endothelial, and inflammatory cells. This facilitates adhesion formation by further decreasing plasminogen-activator activity (1, 17). Studies that aim to prevent adhesions have focused on the prevention of various steps of this physiopathologic process. Antiinflammatory agents, antioxidants, anticoagulants, fibrinolytics and bioreabsorbable physical barriers have been used in this regard (18–22).

The amniotic membrane has many characteristics which make it potentially suitable in the prevention of peritoneal adhesions. Physical barriers have been used in an attempt to prevent adhesion formation by limiting tissue opposition during the critical period of peritoneal healing. This has been shown to take approximately 7 days in the rat model (17).

In the present study, subjects were sacrificed on the 21st day. In the group 3, that involved amniotic membrane, histological evaluation showed that amniotic membrane was incorporated to serosal surfaces and neo-vascularization was initiated at the graft side. We think that stability of amniotic membrane on the injured surface without displacement is effective in obtaining improved results (Fig. 5). Kelekci et al., who investigated the effect of the amniotic membrane on adhesions stated that in animals, in which the membrane slipped off the damaged surface, the adhesion scores increased and better results could have been obtained by stabilizing the amniotic membrane on the damaged serosal surface (23).

Similarly Young et al reported that the amniotic membrane had a slippery structure and recommended fixing the membrane on the injured surface with multiple 7/0 polyglactin sutures. They also reported that the maternal side needs to be placed against injury and the fetal side facing the abdominal cavity while placing the membrane (24). After amniotic membrane was covered over surgical site, as rough surface being faced to uterine incision and smooth surface being faced to outer side, it was handled from edges by a thin forceps and cauterized to mesenteric area under uterus; so uterine fixation was achieved. We also believe that placing the maternal side of the membrane against the injury is very important; this facilitates healing of the serosal injury and neovascularization contributes to this healing (25).

In our study, amniotic membranes collected from pregnant rats were used as an allograft adhesion barrier in order to cover uterine horn. Our finding, that states no statistically significant difference between control group and study group, made us to think of failure in fixation of amniotic membrane because higher success rate was observed in amniotic membranes which were successfully fixed and survived. Furthermore, it was also interesting to observe that adhesion occurred at mesenteric part of the posterior region where cauterization performed, rather than uterine surface, in rats in which amniotic membrane was used and adhesion occurred. We observed that allograft tissue use caused somewhat improvement in the mechanism of adhesion formation, but failed to prevent antigenic response to foreign body.

Hyaluronic acid/carboxymethylcellulose (Seprafilm) is the most extensively tested adhesion prevention agent in general surgery. It is absorbed within 7 days and excreted from the body within 28 days (26). Its safety with regard to systemic or specific complications, such as abdominal abscess, wound sepsis, anastomotic leak, and prolonged ileus, has been established in many studies, including a safety study of 1,791 patients with abdominal or pelvic surgery (27). In our experimental adhesion model, we found that no significant difference was achieved in terms of microscopic and macroscopic adhesion formation by Seprafilm applied anterior side of uterine horn. The experience with Seprafilm in gynecologic surgeries is fairly limited. Diamond, in a prospective, randomized, blinded multicenter study of 127 women undergoing myomectomy, compared Seprafilm with no treatment. The incidence, severity, and extent of adhesions were assessed laparoscopically at a mean of 23 days after the initial procedure. The incidence, measured as the mean number of sites adherent to the uterine surface, was significantly less in treated patients than in untreated patients (mean ± standard error of the mean, 4.98±0.52 vs 7.88±0.48 sites; $p<0.05$), severity and extent of adhesions (mean ± standard error of the mean, 13.2±1.67 vs 18.7±1.66 cm^2; $p<0.05$) were significantly less in the treated group (28).

To date, several adhesion preventing materials have been investigated for adhesion formation after abdominal surgery. Adhesion barriers might be used in videoendoscopic surgical procedure as well as open surgeries.

In our study, we aimed to prevent adhesions by covering placental amniotic membrane over primary and additional surgical areas after cesarean section. We observed that incision side at lower uterine segment was augmented with fibrin and collagen tissue in addition to scar tissue at area covered by amniotic membrane. We thought that desire for normal vaginal delivery should be enhanced and intra-partum and post-partum complications should be lowered in vaginal delivery induction in patients with previous cesarean section.

We observed that direct application of amniotic membrane to injured surface had no effect on the prevention of adhesion. Thus; we suggested that further studies are necessary for this safe, easy applicable material.

In conclusion, application of amniotic membrane has a crucial, technical importance, which leading difficulties in the treatment. Amniotic membrane application is a technically hard procedure.

So bioabsorbable mechanic barrier application should be primarly considered in procedures involving high risk.

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