

**THE REPUBLIC OF TURKEY
FIRAT UNIVERSITY
THE INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF SURGERY**



**INVESTIGATION OF EFFECTIVENESS
POLYPROPYLENE MESH COATED BOVINE
AMNIOTIC MEMBRANE WITH ADHESION
BARRIER (POLYETHYLENE GLYCOL) IN
REPAIR OF ABDOMINAL WALL HERNIAS IN
RATS**

MASTER THESIS

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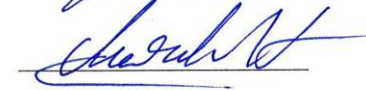
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ETHICAL DECLARATION

I declare that I have performed this thesis study with my own studies, that it is not contrary to the ethics at all stages from the planning of the works to the obtaining of the findings and to the writing phase, that I have obtained all information and data in this thesis under academic and ethical rules, that I have referred to data, information and interpretations that were included in this thesis but which was not obtained in the findings of this thesis.

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21.06.2017

A handwritten signature in blue ink, consisting of a stylized 'H' and 'Q' followed by a horizontal line.

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1. ABSTRACT

The purpose of this experimental work was to investigate the effectiveness of polypropylene mesh coated bovine amniotic membrane with 5% Polyethylene glycol 4000 as adhesion barrier in the repair of experimental 2 x 2 cm of abdominal hernias in rats.

Thirty-two rats were divided into four groups. A 2 cm x 2 cm defect was created in the full thickness of abdominal muscle on the anterior abdominal wall at a distance of 1 cm from the xiphoid process. Polypropylene mesh was implanted in the abdominal cavity with 0/2 vicryl as inlay simple interrupted sutures (Group I,II,III,IV). The bovine amniotic membrane was cover the abdominal face of the graft (Group III and Group IV). It was given before the abdominal closure 5 ml of 5% Polyethylene glycol 4000 (Group II and Group IV) and 5 ml of 0.9% NaCl (Group I and Group III).

After 21 days following the operations, a total of 32 rats were euthanized. Macroscopic evaluation was performed according to the scoring system. Grafts were excised along with abdominal wall for histopathological evaluation and were evaluated under light microscope with respect to fibrosis and inflammation.

SPPS 22 program was used for statistical analysis. The differences between the groups was evaluated by Kruskal Wallis analysis of variance and Mann-Whitney U test.

Comparison of the groups in terms of macroscopic adhesion severity grade; Group IV (Polypropylene mesh, bovine amniotic membrane and 5 % Polyethylene glycol 4000) was significantly different from Group I (Control group) ($p < 0.05$). Group II (Polypropylene mesh and 5 % Polyethylene glycol

4000) was not significantly different from Group III (Polypropylene mesh, bovine amniotic membrane and 0.9 % NaCl) ($p > 0.05$). Group II and Group III were not significantly different from Group I (Control group) ($p > 0.05$). Similar results were obtained in the comparison of groups according to fibrosis and inflammation.

According to the results of this experimental study, the combined use of bovine amniotic membrane and 5% Polyethylene glycol 4000 were helpful to prevent the complications of polypropylene mesh.

Key Words: Polypropylene mesh, Polyethylene glycol, amniotic membrane, hernia, adhesion.

2. ÖZET

Ratlarda Karın Duvarı Fıtıklarının Onarımında Adezyon Bariyerli (Polietilen Glikol) Sığır Amnion Membrani ile Örtülmüş Polipropilen Mesh'in Etkinliğinin Araştırılması

Bu çalışmanın amacı ratlarda deneysel olarak oluşturulan 2 x 2 cm ebadında karın fıtıklarının onarımında sığır amnion membranı ile örtülmüş Polipropilen mesh ve adezyon bariyeri olarak % 5 Polietilen glikol 4000'nin etkinliğini araştırmaktır.

Otuz iki rat dört gruba ayrıldı. Anterior karın duvarında ksifoid çıkıntıya 1 cm uzaklıkta tam katlı karın kası 2 x 2 cm olacak şekilde defect oluşturuldu. Polipropilen mesh inlay olarak karın boşluğuna basit ayrı dikişlerle 0/2 vicryl kullanılarak implante edildi (Grup I,II,III,IV). Sığır amnion membranı greftin karın içine bakan yüzüne örtüldü (Grup III ve Grup IV). Karın kapatılmadan önce 5 ml %5 Polietilen glikol 4000 (Grup II ve Grup IV) ve 5 ml %0.9 NaCl (Grup I ve Grup III) karın içine verildi.

Operasyonları takiben 21 gün sonra, toplam 32 rata ötenazi yapıldı. Makroskopik değerlendirme, puanlama sistemine göre yapıldı. Histopatolojik değerlendirme için greftler abdominal duvar ile birlikte eksize edilerek fibrosis ve inflamasyon açısından ışık mikroskobu altında değerlendirildi.

İstatistiksel analiz için SPSS 22 programı kullanıldı. Gruplar arasındaki farklılıklar Kruskal Wallis Varyans Analizi ve Mann-Whitney U testleri ile değerlendirildi.

Makroskopik adezyon şiddet derecesi açısından grupların karşılaştırılması sonucunda; Grup IV (Polipropilen mesh, sığır amnion membranı ve %5 Polietilen

glikol 4000) Grup I' den (Kontrol grubu) anlamlı olarak farklıydı ($p < 0.05$). Grup II (Polipropilen mesh ve %5 Polietilen glikol 4000) ve Grup III (Polipropilen mesh, sığır amnion membranı ve %0.9 NaCl) grupları arasında anlamlı farklılık gözlenmedi ($p > 0.05$). Grup II ve Grup III istatistiksel olarak Grup I ile karşılaştırıldığında anlamlı bir farklılık saptanmadı ($p > 0.05$). Grupların fibrozis ve inflamasyona göre yapılan karşılaştırılmasında benzer sonuçlar alındı.

Bu deneysel çalışmanın sonuçlarına göre, sığır amnion membranı ve %5 Polietilen glikol 4000 kombinasyonu polipropilen mesh'in komplikasyonlarını önlemede yararlı olduğu kanısına varıldı.

Anahtar kelimeler: Polipropilen mesh, polietilen glikol, amnion membrane, fitik, adezyon.

3. INTRODUCTION

3.1. Definition of Hernia

Hernia is described as an abnormal bulge of an organ or tissue through a defect or muscles weakness of abdominal wall or from the wall of the cavity that normally contain it (1-4). Hernia is characterized as the relocation of an organ through an ordinary gap or a pathological gap (5). Abdominal hernia is defined as the abnormal protruding or bulging of organ or tissue through deformities of abdominal wall, steady structure or from fasciae are not covered by strait muscle fiber. The abdominal wall of animal is hard strong wall that protecting the internal organs from outer damage and their herniation, the most natural hernias is real hernia, in which the displaced organs are surrounded to a peritoneal sac. External abdominal hernias are imperfections in outside mass of the abdomen permit projection of abdominal substance may include the abdominal wall anywhere other than umbilicus, inguinal ring, femoral canal, or scrotum. Internal abdominal hernias are those that happen through a ring or tissue within the abdomen or thorax for example (diaphragmatic and hiatal hernia) (1,4,6,7).

3.1.1. Causes of Hernia

- 1- Wall defects (1,8,9).
- 2- Trauma (4, 8-12).
- 3- Debridement of necrotizing infections (1).
- 4- Laparotomy (9,10,13,14).
- 5- After abdominal surgical interventions (15,16).

- 6- Infections (17).
- 7- Herniation or surgical resection (18-20).
- 8- Loss of abdominal wall substance (21).
- 9- Muscles weakness and strain (3).
- 10- Falling or casting on uneven ground (7).
- 11- Automobile accident (7).
- 12- Deep wounds (7).
- 13- Abscess and physiological disturbances (7).
- 14- Multiple birth (7).
- 15- After midline incision (10,22-24).
- 16- Anything that results in an increases in abdominal pressure can causes a hernia such as diarrhea, constipation or obesity (3,17).

3.1.2. History of Hernia and Synthetic Meshes

The abdominal hernia was first diagnosed in 1804 (25). A traumatic abdominal hernia was first reported in 1906 (26). The material was first used for repairing hernias in 1900 (15). In 1900, the surgical meshes where first introduced in the form of metal based prosthetics. In the 1950, the synthetic mesh was first described for treatment of abdominal wall hernia (11,27). In 1958, the synthetic meshes such as polypropylene mesh, poly-amide mesh, plastic prosthesis were used for hernia repair (15,28). In 1958 a monofilament polypropylene mesh (marlex, davol Inc, Cranston, RI) was available on the market (25). In 1959, the pellets of polypropylene mesh (marlex mesh) was injected in the abdominal cavity of dogs (7). In 1960, The prosthetic mesh was first time used for ventral hernia

repair in humans (29). Monofilament polypropylene mesh was first utilized in 1962 and treatment of hernias with the use of surgical meshes has been developed since 1963 (19,1). The examination use of polypropylene mesh was reported in ponies in 1969 (30). In 1971, the plastic mesh, Vitafil and fine nylon nets were used for the repair of ventral hernia in 15 buffalo calves and found that all these three synthetic materials were suitable for the repair of hernia (7). In 1986, the polypropylene mesh was described using for method of tension-free inguinal hernia repair (31).

3.2. Definition of Amniotic Membrane

Amniotic membrane is defined as a translucent membrane made out of an inward layer of epithelial cell, planted on a basement layer that along these lines is connected with a thin connective tissue layer by filamentous strands. It is the internal massive part of three layers forming the fetal membrane. An amniotic membrane is gotten from fetal ectoderm by cavitation inside the fetal pack and is bordering over the umbilical thread with the fetal skin (32).

3.2.1. History of Amniotic Membrane

The application of amniotic membrane in the repair of tissue defects have been suggested by some authors, lately. Some researchers have studied the use of amniotic membrane in the reconstitution of tissue lesions, since the first half of the recent century. The tension of injecting amniotic sac in order favoring the open wound granulation and subsequently in eye tissue were noticed in 1910 (33).

In 1995, the use of amniotic membrane was reported and reintroduced in the treatment of ocular lesions and ophthalmology (33). Amniotic membranes were effectively used for wound and reconstructive reason since the early twentieth century. The following uses of human amniotic membrane was reviewed over the twentieth century to consist of some of the programs at some point of that duration. These blanketed reconstructive OB/GYN surgical procedure, dentistry, and neurosurgical and well known surgical applications. A complete evaluation was mentioned of a few 550 instances of skin transplantation on the Johns Hopkins University in 1910. The amniotic membranes were stated on using preserved in pores and skin grafting for burns and ulcers in 1913. The amniotic membrane was first used to restore eye wounds in 1940. Ophthalmologic usage might move directly to be one of the maximum famous packages of the material inside the late twentieth century. In the latter half of the twentieth century, natural amniotic membrane started out for use as a wound masking, starting within the Nineteen Sixties via the quite of the century, with medicine for diabetic neurovascular ulcers, venous stasis ulcers, and numerous kinds of postsurgical and post disturbing wound dehiscence (34,35).

In 1965, the amniotic membrane was mentioned from deliveries may be sterilized and stored for six weeks at 4 °C and used adequately on acute 2nd-degree burns and on skin donor sites. In 2006, scientist advanced techniques for cleansing, making ready and dehydrating human amniotic membranes for surgical use, developing dehydrated sheets of the material that would be reduce into sections and saved. In this shape, the material changed into easy to handle, stable

at room temperature and held a self-existence of up to five years, as showed via a number of standardized exams (34,35).

3.2.2. Advantages of Amniotic Membrane

The amniotic membrane is using in early healing of peritoneal lesions and adhesions control, burns, mouth sores, neo vagina reconstruction, varicose ulcer, ocular lesions and nerve damage (33).

Amniotic membrane has been used for pterygium repair, conjunctival reconstruction, burn medicine, gives a matrix for cell migration and proliferation, is non-immunogenic, promotes increased recovery and enhancement of the wound recovery method reduce inflammation, has antibacterial residences, affords a natural organic barrier and includes some of important growth elements and cytokines. The material gives a natural scaffold for wound recovery and consist of numerous essential increases factors and organic macromolecules essential in wound recovery. Those molecules have been scientifically discovered to confer residences that lesson wound pain, suppress scar formation, suppress infection and offer anti-inflammatory mediators (34,35). Essential amniotic membrane assist supposition that biologically active coatings may be especially beneficial for adhesion prevention and tissue integration in hernia repair (36).

3.2.3. Structures of Amniotic Membrane

Amniotic membrane is structurally composed of the liner of the fetal surroundings in the course of gestation, isolating the growing fetus from the mom in utero. On gross exam the amniotic membrane consists of some of layers that

may be visible and liked with easy dealing with and the naked eye. Amniotic membrane mixed with non-absorbable artificial material and additional matrix (34,37).

3.3. Definition of Mesh

A surgical mesh is defined as a medical device that is applied to give extra support to debilitated or damaged tissue. Surgical mesh is constructed from manufactured material can be (absorbable, non-absorbable or mixture of them) or they are developed from animal tissue (skin or intestine). All meshes that derived from animal tissues are absorbable. Alloplastic mesh is defined as the important one of embedded mesh that is applied in hernia surgery and applied for each clinical state with the true objective that the behavior of the mesh matches the abdominal wall as closely as possible (38).

3.3.1. Types of Mesh

1- Polypropylene mesh (Marlex and Prolene): The most important mesh used for repair of hernias defects, is knitted from monofilament yarn, to a relatively large pore size, in order to allow tissue in growth. polypropylene mesh has three types (monofilament, double filament and multifilament) polypropylene mesh.

The polypropylene mesh is defined as a thermoplastic polymer that is all around tolerated when embedded in vivo, that, is used as standard of examination as part of harmfulness testing of bio materials. It is the most broadly used prosthetic material because of its strong, low cost, has excellent tissue in

corporation, high infection resistance, has resistance to all (acids, alkaloids) and insoluble at room temperature. It is also inert, no carcinogenic and simple to handle. It has a high rigidity and microporous structure permit fiber, resulting in consolidation of the mesh into the abdominal wall to form a strong permanent repair. The polypropylene mesh is one of the most common prosthetic biomaterials used to abdominal wall defects in humans (7,15,18,24,28,29,39-42).

2- Polytetrafluoroethylene (PTFE): Most constantly used in hernia surgery made of an expanded, no absorbable and non-braided biocompatible material.

Especially there are two types of mesh (biological mesh) has revolutionized the treatment of complex abdominal wall hernia and (synthetic mesh) which is made of nylon or gore Tex. (7,14,15,21,43-45).

3- Relon mesh: It is made from non-wet table fiber, can be cut easily and shaped with scissors, and has the desired porosity to facilitate fibroplasia (46).

4- Polyester mesh, multifilament (absorbable and no absorbable) (43).

5- Polyester coated with collagen (7).

6- Polyester monofilament (43,44)

7- Green polyester yarns (14).

8- Nylon mesh (7,46).

9- Carbon mesh (7).

10- Polyethylene terephthalate PET (47).

11- Mersilene mesh (48).

12- Polyglycolic acid (dexon) absorbable (49).

13- Polyglactin (vicryl) absorbable (49).

14- Vypro (Polypropylene and vicryl) (49).

15- Sepramesh: The upper layer is polypropylene, the lower layer seprafilm (Hyaluronic acid / Carboxy-methylcellulose) (50).

3.3.2. Advantages of Mesh

1. Polypropylene meshes are likely the greatly used prosthetic material in mesh repair because they are strong, easy to handle, flexibility characteristic, have excellent tissue incorporation and one of the most inert materials available (29,30,40, 51-54).
2. Providing a support for tissue incorporation, resistance to infection and ability to maintain tensile strength (27,51).
3. Mesh can be put in the sub fascial, extra fascial or intra peritoneal positions (47).
4. The repair of incisional hernia with mesh can be decrease of recurrence rate from 30-50 % to less than 10%. (55).
5. Biomaterials and prostheses mesh represent a main contribution in the repair of abdominal wall disorder (45).

3.3.3. Disadvantages of Mesh

1. Resulting adhesion is one of potential complication (40,41,53,56-59).
2. Intestinal obstruction and incarceration (41,54,56,57,60-62).
3. Perforation or fistula formation (54,57,59,61-63).
4. Foreign body reaction (1).

5. Chronic abdominal pain (24,55).
6. Paresthesia (47).
7. Discomfort or even pain (47).
8. Infections (18,28,41,57).
9. Granulomas (57).
10. Inflammatory response (23).
11. Re operations (22).
12. Including mesh construction which has been responsible for recurrence and pain (64).
13. Skin erosion (51).
14. Abdominal wall stiffness (8).
15. Mesh dislocation and wound fistulas (8).
16. Shrinkage, wrinkling and seroma formation (36,47,53,65,66).
17. Infarction (67).
18. female infertility (23,54,67).
19. Chronic neuralgia (68).
20. Intestinal erosion (68).
21. Persistent incisional drainage and peritonitis (29,39).
22. Susceptibility to bacterial colonization and chronic infection (52).
23. Wound adhesiolysis (65).
24. Migration and rejection of the mesh and mesh-related infections (28,53).

3.4. Definition of Adhesion

Adhesion may be defined as fibrous structures within the abdominal cavity that rise up at injured peritoneal surface, and is outcome of disturbed tissue restore after peritoneal trauma (69). Adhesion compose of fibrous bands that form among tissues and organs, frequently because of damage throughout surgical procedure (70). A situation in which body tissues that are typically separated develop collectively, a fibrous band of scar tissue that binds together typically separate anatomical structures, the union of opposing surfaces of a wound, especially in recovery (71). Abnormal union of physical tissues maximum is not unusual within the abdomen (72).

3.4.1. Abdominal Adhesions

Abdominal adhesions are described as formation of fibrous tissue between small or large intestine loops and peritoneum or with different organs in the abdominal cavity (urinary bladder, gallbladder, liver, uterus, ovaries and fallopian tubes) (73).

3.4.2. Causes of Adhesion

1. The common reasons or the origin of adhesions which are foreign bodies include (prosthetic patches, and starch from gloves) (74).
2. Adhesions are occurring after (small intestinal, large intestinal, ovariectomy and cryptorchidectomy) surgical procedures (74).
3. The previous abdominal surgical treatment is the most common purpose of intraperitoneal adhesions (74).

4. The congenital abnormalities and intra-abdominal inflammatory diseases result in adhesions (74).

3.4.3. Adhesions Lead to

1. Obstruction and strangulation of the bowel. Intestinal obstruction particularly forming within a few hours after operation (74).
2. Pain (74).
3. Ischemia (74).
4. Fibrin deposits onto the damaged tissues and inflammation (74).
5. Intestinal obstruction with abdominal pain (70,73).

3.4.4. The most Important Ways to Prevent Adhesions Formation

- 1- By ways of preventing fibrin deposition; which include;
 - a- The usage of anticoagulant like (heparin, aprotinin, dicumarol, sodium citrate, and noxytiolin) (74).
 - b- Using polyethylene glycol, dextran and povidone iodine (74).
 - c- The usage of prosthetic mesh, free grafts of omentum, tolmetin sodium (74).
 - e- Seprafilm is twice powerful in preventing adhesion formation when compared to just surgical approach alone (70).
- 2- Using sterile surgical tools (74).
- 3- By means of inhibition of fibroblastic proliferation, using drugs along with (cytotoxic drug, ibuprofen, vit E, selenium, sodium hyaluronate, oxyphenbutazone dexamethasone, 5-fluorouracil and carboxymethylcellulose (74).

1- Laparoscopic surgical operation has a reduced risk for developing adhesions (70).

2- Taking precautions during operation to prevent adhesions; such as using starch and latex free gloves, handling tissues and organs gently, not allowing tissues to dry out and shortening surgery time (70).

3- Peritoneal trauma should be reduced. Reduction of damage is possible by way of avoiding hypothermia and desiccation of serosa, limiting manipulation of the peritoneum and by means of reducing the use and fall of foreign substances intra-abdominally (69).

3.4.5. Pathogeneses of Adhesion

Adhesion formation post-surgical operation generally happens when two injured surfaces are closed to each other. Adhesion form as a naturally part of the body's healing procedure after surgical treatment in a comparable way that a scar extends within one tissue across a replicated area including the peritoneal cavity. Intra-abdominal adhesions are most commonly caused by attachment of abdominal organs to the surgical site or to other organs inside the abdominal cavity (70).

Damage to the peritoneum may be due to mechanical injury which includes in surgical procedure, by exposure to foreign substances and by using inflammation diseases (69).

The higher tissue injury, the greater accelerated collagen and fibrin deposition, are making the peritoneal fibrinolysis and growing the adhesive capability of the wound line, progressing to the formation of adhesions (33).

Formation of adhesions is proved to be related with decreased capacity of fibrin in peritoneal cavity. Fibrinolytic capability is reducing by means of the operation time. After surgery there's no tissue on the way to separate synthetic mesh from direction touch with abdominal organs, so bowel and omentum adhesions can occur. Adhesions stand up on the first postoperative day, the rate increases till seventh postoperative day but after that there are not any greater adhesions arises. Mechanical trauma, thermal injury, foreign bodies, chemical injury, bacterial contamination, hypersensitive reactions, irradiation and ischemic injury can lead to damage and next adhesion formation (6).

3.5. Definition of Polyethylene Glycol

Polyethylene glycol is a polyether compound with many programs from commercial production to medicinal drug, Polyethylene glycol is also referred to as polyethylene oxide or polyoxyethylene oxide, depending on its molecular weight, Polyethylene glycol is produced through the interaction of ethylene oxide with water, ethylene glycol or ethylene glycol oligomers (75).

3.5.1. Advantages of Polyethylene Glycol

- 1- Has been suggested to protect against pathogen colonization by way of enhancing colonic barrier function (76).
- 2- Reduce fibrin deposition and adhesion formation (76,77).
- 3- Polyethylene glycol is a safe and non-migrating adhesion barrier, that is used during open surgical and laparoscopic operations due to its easy to use naturally (44).

4- Polyethylene glycol is a suitable non-absorbable fecal indicator for calcium, phosphorus and fatty acids, and has numerous capabilities which commend its use in choice to insoluble chromium sesquioxide and barium sulphate in particular while marking water soluble dietary elements (78).

5- Used commercially and medically in several programs, inclusive of in foods, as surfactants, in cosmetics, in biomedicine, in pharmaceuticals, as solvents, as miserable retailers, in suppository bases, in ointments, as laxative and as pill excipients (75).

6- Used chemically has a low toxicity, flexible, water soluble polymer, it is able to be used to create very excessive osmotic strain, used as polar stationary phase for gas chromatography, in addition to heat transfer fluid in electronic testers (75).

7- Used biologically, Polyethylene glycol is used to pay attention viruses, in blood banking, Polyethylene glycol is used as a potentiate to enhance detection of antigen and antibodies (75).

A number of experimental incisional hernia studies have been undertaken to prevent the complications of the mesh (48,79-85). The purpose of this experimental work is to investigate the effectiveness of polypropylene mesh coated bovine amniotic membrane with 5% Polyethylene glycol 4000 as adhesion barrier in the repair of experimental 2 x 2 cm of abdominal hernias in rats.

4. MATERIAL AND METHODS

The experimental study was approved by Firat University, Chair of The Local Ethics Committee on Animal Experiments, (Date of meeting, 15.06.2016, number of meetings: 2016/12, decision no: 123, Protocol number: 2016/71)

Placenta of bovine was obtained from cattle slaughterhouse. The placenta was washed with sterile saline for clearance of blood clots and tissue residues. Amniotic membrane was separated from chorion by blunt dissection. Later 2.5 X 2.5 cm total 16 amniotic membrane patches were waited for 24 hours in sterile saline at 4 °C that include penicillin 1000 000 I.U. and 1 g streptomycin per one liter. These amniotic membrane patches were used for a week (86).

Thirty-two Wistar albino rats (adult, female, average 250 g) were divided into four groups (every groups include 8 rats). General anesthesia of rats was performed via Ketamine Hydrochloride (Ketalar, Parke-Davis) 80 mg / kg I.M. After general anesthesia the abdominal region is prepared for operation, the rats were identified on the operation table in the supine position and the region will be disinfected and ready for operation with sterile services. After a median incision (4 cm) is made, a 2 cm x 2 cm defect were created in the full thickness of abdominal muscle on the anterior abdominal wall at a distance of 1 cm from the xiphoid process (Figure 1, Figure 2).

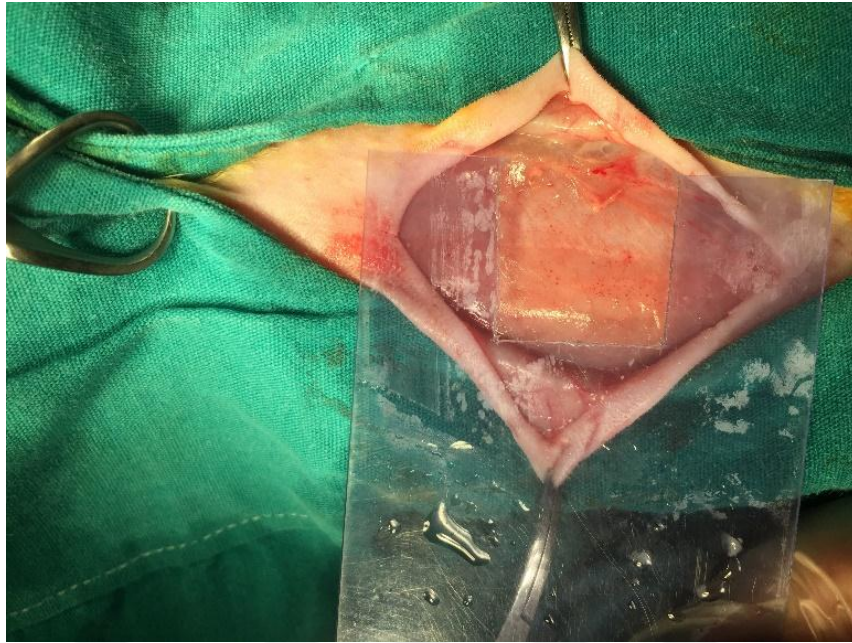


Figure 1: View of the guide used for create a 2 cm x 2 cm defect on the anterior abdominal wall at a distance of 1 cm from the xiphoid process.



Figure 2: Appearance of defect (2 cm x 2 cm).

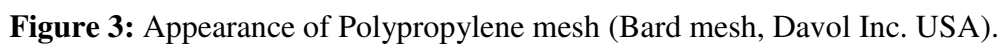
Group I: Polypropylene mesh and 5 ml I.P. 0.9 % NaCl.

Group II: Polypropylene mesh and 5 ml I.P. 5 % Polyethylen
4000 as adhesion barrier.

Group III: Polypropylene mesh, bovine amniotic membrane and
0.9 % NaCl.

Group II: Polypropylene mesh and 5 ml I.P. 5 % Polyethylene glycol as adhesion barrier.

Group IV: Polypropylene mesh, bovine amnion membrane and 5 ml I.P. 5
ethylene glycol 4000 as adhesion barrier.



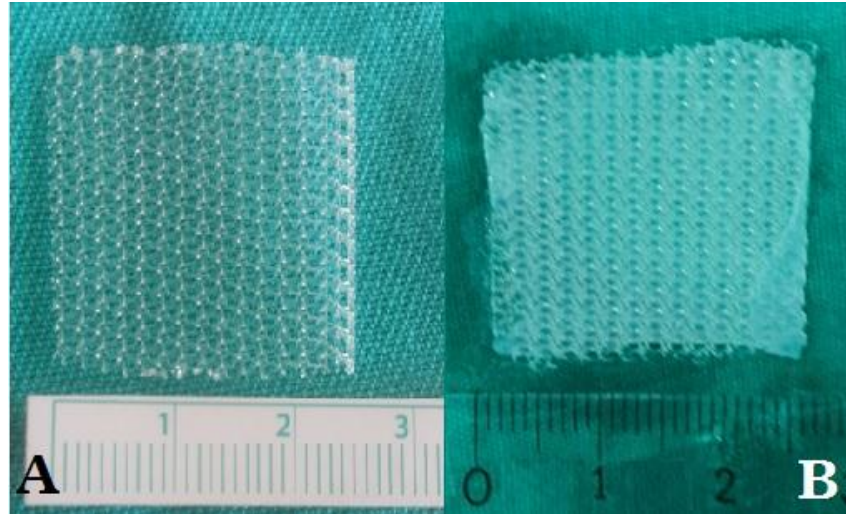


Figure 4: **A:** Polypropylene mesh (2.5 x 2.5 cm) **B:** Polypropylene mesh coated with (2.5 x 2.5 cm) of amniotic membrane.

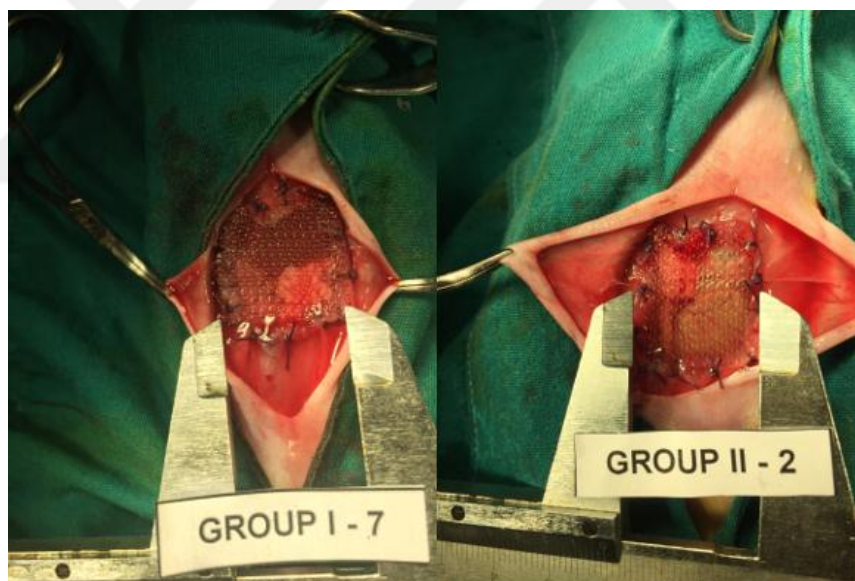


Figure 5: Appearance of implanted polypropylene mesh in Group I and Group II.

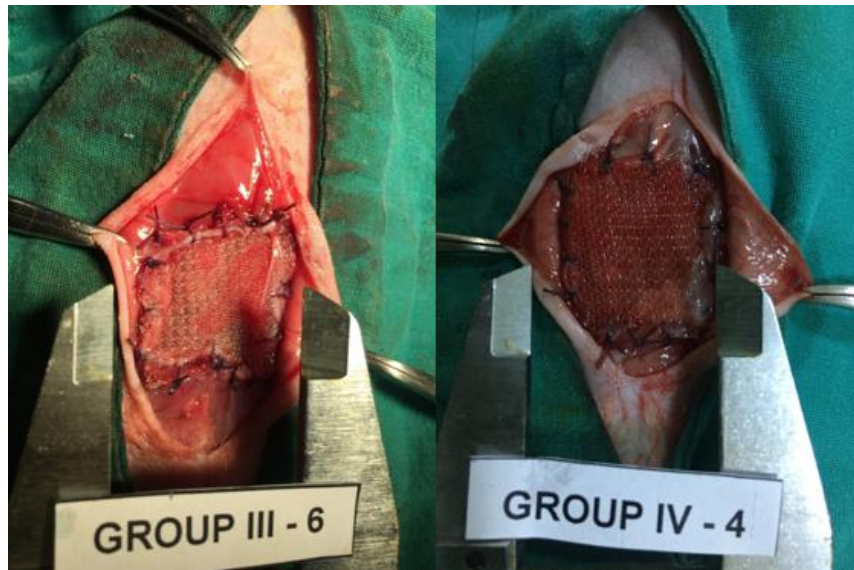


Figure 6: Appearance of implanted polypropylene mesh coated with amniotic membrane in Group III and Group IV.



Figure 7: Appearance of Polyethylene glycol 4000 (Merck,USA).



Figure 8: Protection of the wound line against infections by gauze after the skin closed with simple interrupted sutures.

Penicillin (30,000 U / kg 1x1) and Flunixin Meglumin 2.5 mg / kg 2x1 (Fundamin, Bayer) were administered intramuscularly for 5 days postoperatively in all rats. Water and feed restrictions was not being made.

After 21 days following the operations, a total of 32 rats were euthanized by carbon dioxide inhalation (Figure 9). The abdomen wall was opened in the form of a "U" (Figure 10) and the condition of the grafts was examined macroscopically.



Figure 9: Appearance of carbon dioxide inhalation unit.

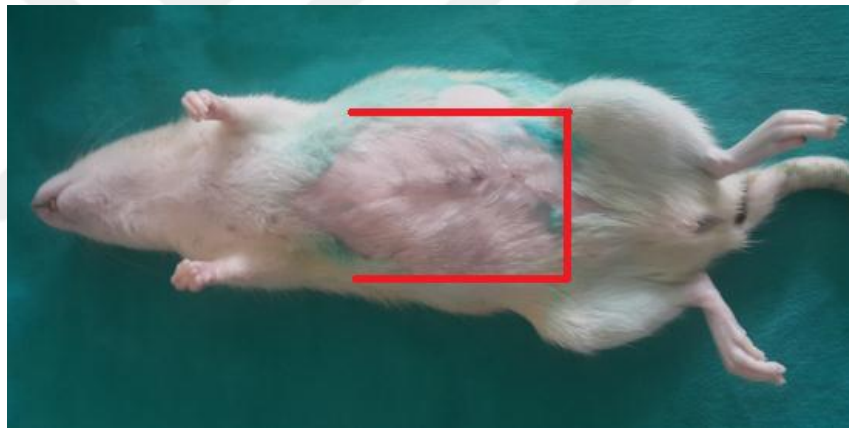


Figure 10: Appearance of “U” shaped incision.

Adhesion formation was evaluated macroscopically and microscopically.

Macroscopic evaluation was performed according to the scoring system (87).

Grade 0: No adhesion.

Grade 1: Blunt dissectible, easily separable filmy adhesions

Grade 2: Freely dissectible mild to moderate adhesions

Grade 3: Difficult dissectible moderate to dense adhesions

Grade 4: Non-dissectible adhesions.

Grafts were excised along with abdominal wall and sent for histopathological evaluation to Firat University Department of Pathology in 10% formalin solution. Five micron thick sections from the tissues embedded into paraffin was obtained. Sections were stained with hematoxylin-eosin (H&E) and were evaluated under light microscope (Olympus BX43, DP72) with respect to fibrosis (**Grade 0:** no fibrosis, **Grade 1:** minimal, loose fibrosis, **Grade 2:** moderate fibrosis, **Grade 3:** florid, massive fibrosis) and inflammation (**Grade 0:** no inflammation, **Grade 1:** large cells, rare, dispersed lymphocytes and plasma cells **Grade 2:** large cells together with increased number of lymphocytes, neutrophils, eosinophils and plasma cells **Grade 3:** multiple mixed inflammatory cells and presence of micro-abscess) (88).

Statistical Analysis: SPSS 22 program was used for statistical analysis. The differences between the groups has been evaluated by Kruskal Wallis analysis of variance and Mann-Whitney U test.

5. RESULTS

5.1. Macroscopic Examination

Group I: Higher adhesions percentage were found in Group 1. There were no abscesses between the polypropylene mesh and visceral organs. Inflammation was found in 2 cases (Figure 16A). Subcutaneous seroma was found in 3 cases. There were adhesions between the intestines and the mesh in two cases (Figure 15A). There were adhesions between the stomach and the mesh in one case (Figure 14B). Other adhesions were formed between the omentum and the mesh. Suture dehiscence was not observed. It was observed mild, moderate or more adhesions, but small bowel obstruction was absence. Wound dehiscence and signs of swelling were clean. Dislocation of propylene mesh was absence (Table 1).

According to the scoring system; It was observed Grade 1 in one case, Grade 2 in 1 case (Figure 14B), Grade 3 in 2 cases (Figure 15A), and Grade 4 in 4 cases (Figure 16A). Grade 0 was not observed in this group (Table 5, Figure 11).

Table 1: Macroscopic evaluation of Group I according to the scoring system.

GROUP I		
CASES	Macroscopic examination	According to the scoring system
1	Difficult dissectible moderate to dense adhesions	Grade 3
2	Non-dissectible adhesions and subcutaneous seroma between skin and polypropylene mesh	Grade 4
3	Freely dissectible mild to moderate adhesions	Grade 2
4	Blunt dissectible, easily separable filmy adhesions	Grade 1
5	Non-dissectible adhesions and inflammation	Grade 4
6	Difficult dissectible moderate to dense adhesions, subcutaneous seroma (between skin and polypropylene mesh)	Grade 3
7	Non-dissectible adhesions and inflammation	Grade 4
8	Non-dissectible adhesions, subcutaneous seroma between skin and polypropylene mesh	Grade 4

Group II: There were no abscesses between the polypropylene mesh and visceral organs. Inflammation was found in one cases (Figure 16B). There were adhesions between the intestines and the mesh in one cases. Other adhesions were formed between the omentum and the mesh. Suture dehiscence was not observed. It was observed mild, moderate or more adhesions, but small bowel obstruction was absence. Wound dehiscence and signs of swelling were clean. Dislocation of propylene mesh was absence (Table 2).

According to the scoring system; It was observed Grade 0 in one case (Figure 12B), Grade 1 in one case, Grade 2 in 3 case, Grade 3 in 2 cases (Figure 15A), and Grade 4 in 1 cases (Figure 16B) (Table 5, Figure 11).

Table 2: Macroscopic evaluation of Group II according to the scoring system.

GROUP II		
CASES	Macroscopic examination	According to the scoring system
1	No adhesion	Grade 0
2	Difficult dissectible moderate to dense adhesions	Grade 3
3	Freely dissectible mild to moderate adhesions	Grade 2
4	Freely dissectible mild to moderate adhesions	Grade 2
5	Freely dissectible mild to moderate adhesions	Grade 2
6	Blunt dissectible, easily separable filmy adhesions	Grade 1
7	Non-dissectible adhesions and inflammation	Grade 4
8	Difficult dissectible moderate to dense adhesions	Grade 3

Group III: There were no abscesses between the polypropylene mesh coated amniotic membrane and visceral organs. Subcutaneous seroma was found in one cases. There were adhesions between the intestines and the mesh in 2 cases. Other adhesions were formed between the omentum and the mesh. Suture dehiscence was not observed. It was observed mild, moderate or more adhesions, but small bowel obstruction was absence. Wound dehiscence and signs of swelling were clean. Dislocation of propylene mesh was absence (Table 3).

According to the scoring system; It was observed Grade 0 in 2 cases, Grade 1 in 2 cases (Figure 13B), Grade 2 in 3 case, Grade 3 in one case (Figure 15B), and Grade 4 in 1 cases (Figure 16B) (Table 5, Figure 11).

Table 3: Macroscopic evaluation of Group III according to the scoring system.

GROUP III		
CASES	Macroscopic examination	According to the scoring system
1	Freely dissectible mild to moderate adhesions	Grade 2
2	Difficult dissectible moderate to dense adhesions, subcutaneous seroma between skin and polypropylene mesh	Grade 3
3	Freely dissectible mild to moderate adhesions,	Grade 2
4	Blunt dissectible, easily separable filmy adhesions	Grade 1
5	No adhesion	Grade 0
6	Blunt dissectible, easily separable filmy adhesions	Grade 1
7	No adhesion	Grade 0
8	Freely dissectible mild to moderate adhesions	Grade 2

Group IV: There were no abscesses between the polypropylene mesh coated amniotic membrane and visceral organs. There were adhesions between the intestines and the mesh in 3 cases. Other adhesions were formed between the omentum and the mesh. Suture dehiscence was not observed. It was observed mild, moderate or more adhesions, but small bowel obstruction was absence.

Wound dehiscence and signs of swelling were clean. Dislocation of propylene mesh was absence (Table 4).

According to the scoring system; It was observed Grade 0 in 4 cases (Figure 12A), Grade 1 in 3 cases (Figure 13A), Grade 2 in one case (Figure 14A). Grade 3 and 4 were not observed in this group (Table 5, Figure 11).

Table 4: Macroscopic evaluation of Group IV according to the scoring system.

GROUP IV		
CASES	Macroscopic examination	According to the scoring system
1	Blunt dissectible, easily separable filmy adhesions	Grade 1
2	No adhesion	Grade 0
3	Freely dissectible mild to moderate adhesions	Grade 2
4	Blunt dissectible, easily separable filmy adhesions	Grade 1
5	No adhesion	Grade 0
6	No adhesion	Grade 0
7	Blunt dissectible, easily separable filmy adhesions	Grade 1
8	No adhesion	Grade 0

Table 5: Comparison of the groups in terms of macroscopic adhesion severity grade.

Groups	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	(n)
Group I	-	1(12.5%)	1(12.5%)	2(25%)	4 (50%)	8
Group II	1(12.5%)	1(12.5%)	3(37.5%)	2(25%)	1(12.5%)	8
Group III	2(25%)	2(25%)	3(37.5%)	1(12.5%)	-	8
Group IV	4(50%)	3(37.5%)	1(12.5%)	-	-	8

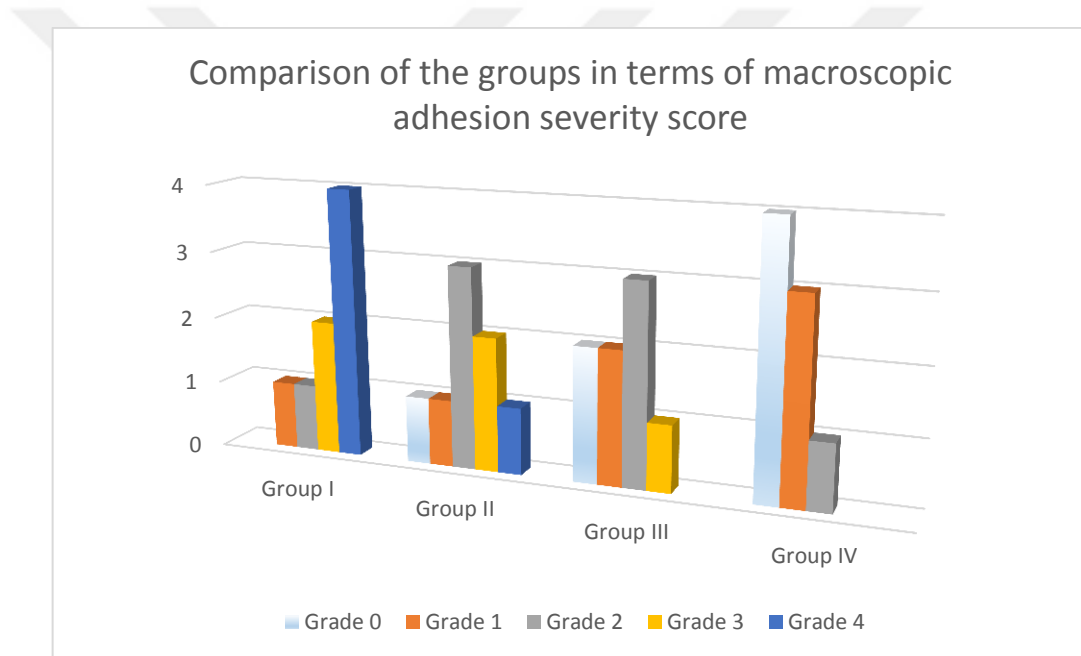


Figure 11: Comparison of the groups in terms of macroscopic adhesion severity grade.



Figure 12: Appearance of Grade 0, No adhesion

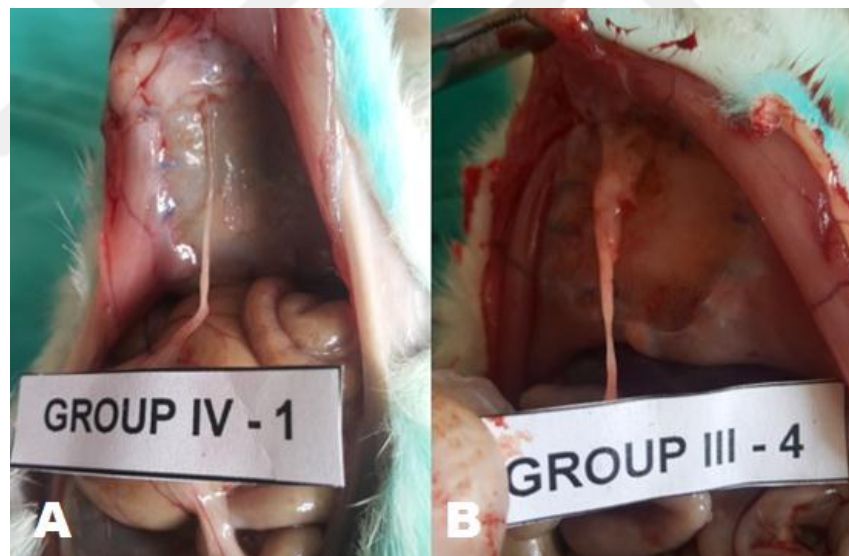


Figure 13: Appearance of easily separable filmy adhesions (Grade 1).

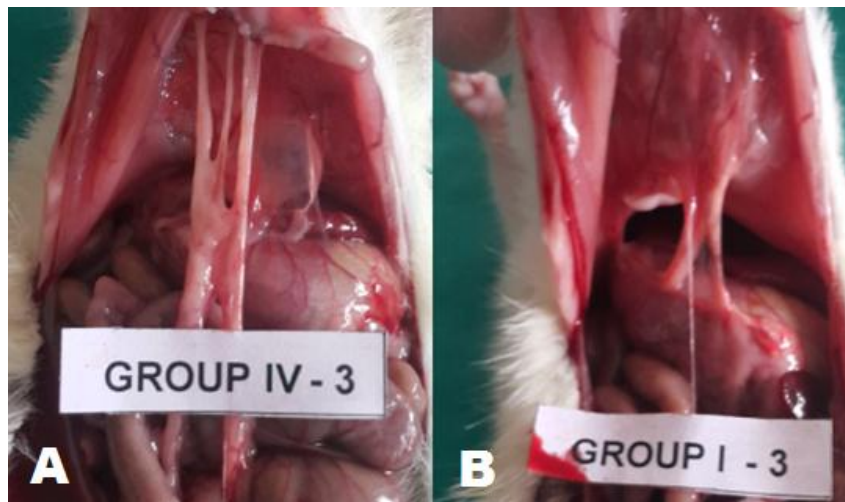


Figure 14: Appearance of moderate adhesions with easy dissection (Grade 2).

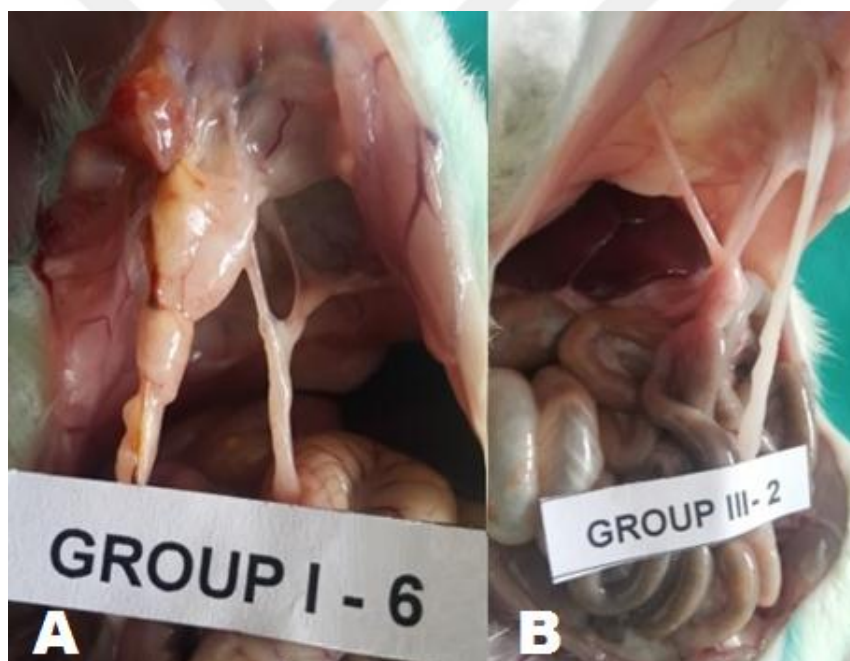


Figure 15: Appearance of dense adhesions with difficult dissection (Grade 3).

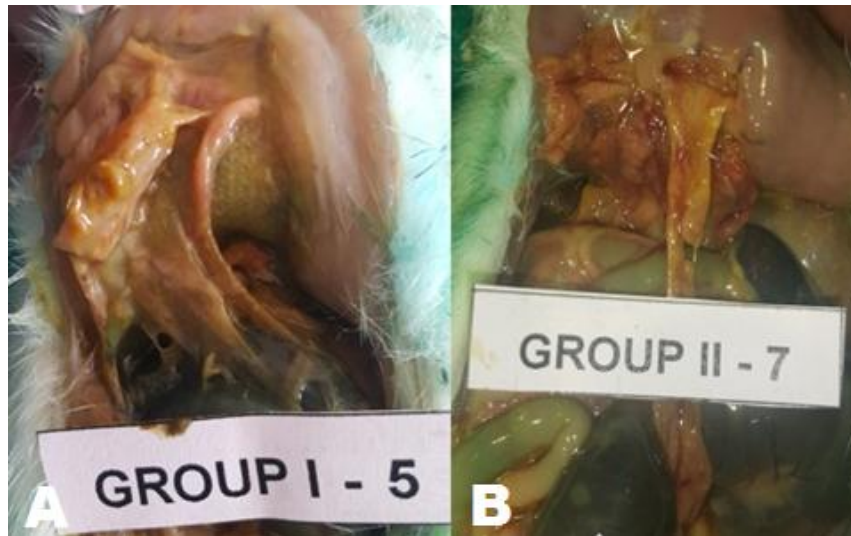


Figure 16: Appearance of non-dissectible adhesions (Grade 4).

5.2. Microscopic Examination

5.2.1. Comparison of The Groups with Regard to Fibrosis

According to fibrosis in Group 1; It was observed Grade 1 in 2 cases, Grade 2 in 2 cases, and Grade 3 in 4 cases. Grade 0 was not observed in Group I.

According to fibrosis in Group II; It was observed Grade 0 in one case, Grade 1 in one case, Grade 2 in 4 cases, and Grade 3 in 2 cases.

According to fibrosis in Group III; It was observed Grade 0 in 2 cases, Grade 1 in 2 cases, Grade 2 in 3 cases, and Grade 3 in 1 cases.

According to fibrosis in Group IV; It was observed Grade 0 in 3 cases, Grade 1 in 3 cases, Grade 2 in 2 cases. Grade 3 was not observed in Group IV (Table 6, Figure 17).

Table 6: Comparison of the groups with regard to fibrosis.

Groups	Grade 0	Grade 1	Grade 2	Grade 3	(n)
Group I	-	2 (25%)	2 (25%)	4 (50%)	8
Group II	1 (12.5%)	1 (12.5%)	4 (50%)	2 (25%)	8
Group III	2 (25%)	2 (25%)	3 (37.5%)	1 (12.5%)	8
Group IV	3 (37.5%)	3 (37.5%)	2 (25%)	-	8

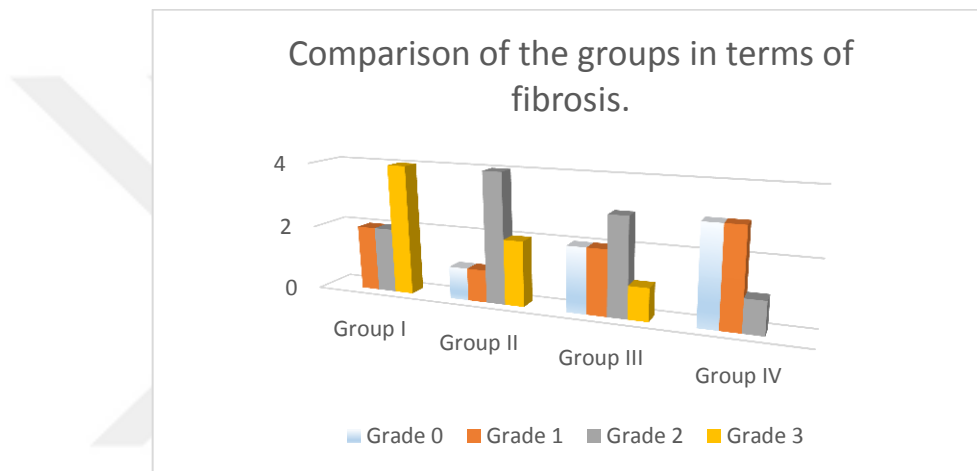


Figure 17: Comparison of the groups with regard to fibrosis.

5.2.2. Comparison of The Groups with Regard to Inflammation

According to inflammation in Group 1; It was observed Grade 1 in 2 cases, Grade 2 in 2 cases, and Grade 3 in 4 cases. Grade 0 was not observed in Group I.

According to inflammation in Group II; It was observed Grade 0 in one case, Grade 1 in one case, Grade 2 in 4 cases, and Grade 3 in 2 cases.

According to inflammation in Group III; It was observed Grade 0 in 2 cases, Grade 1 in 2 cases, Grade 2 in 3 cases, and Grade 3 in 1 cases.

According to inflammation in Group IV; It was observed Grade 0 in 3 cases, Grade 1 in 3 cases, Grade 2 in 2 cases. Grade 3 was not observed in Group IV (Table 7, Figure 18).

Table 7: Comparison of the groups with regard to inflammation.

Groups	Grade 0	Grade 1	Grade 2	Grade 3	(n)
Group I	-	2 (25%)	2 (37.5%)	4 (37.5%)	8
Group II	1 (12.5%)	1 (12.5%)	4 (50%)	2 (25%)	8
Group III	2 (25%)	2 (25%)	3 (37.5%)	1 (12.5%)	8
Group IV	3 (37.5%)	3 (37.5%)	2 (25%)	-	8

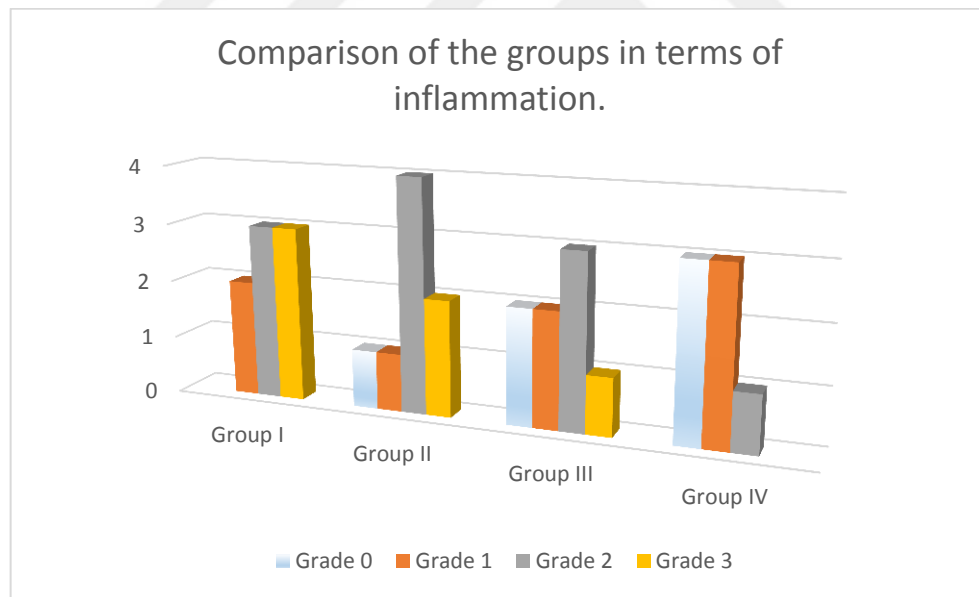


Figure 18: Comparison of the groups with regard to inflammation.

In histopathological examinations by a majority were observed fibrous adhesions and giant cell infiltration due to foreign body reaction in cases of Group I (Figure 19). It was observed common inflammatory cell infiltration and fibrosis

in cases of Group II (Figure 20). It was observed medium inflammatory cell infiltration and fibrosis in cases of Group III (Figure 21). It was observed a large number of small blood vessels, a small number of inflammatory cell infiltration, and fibrosis in cases of Group IV (Figure 22).

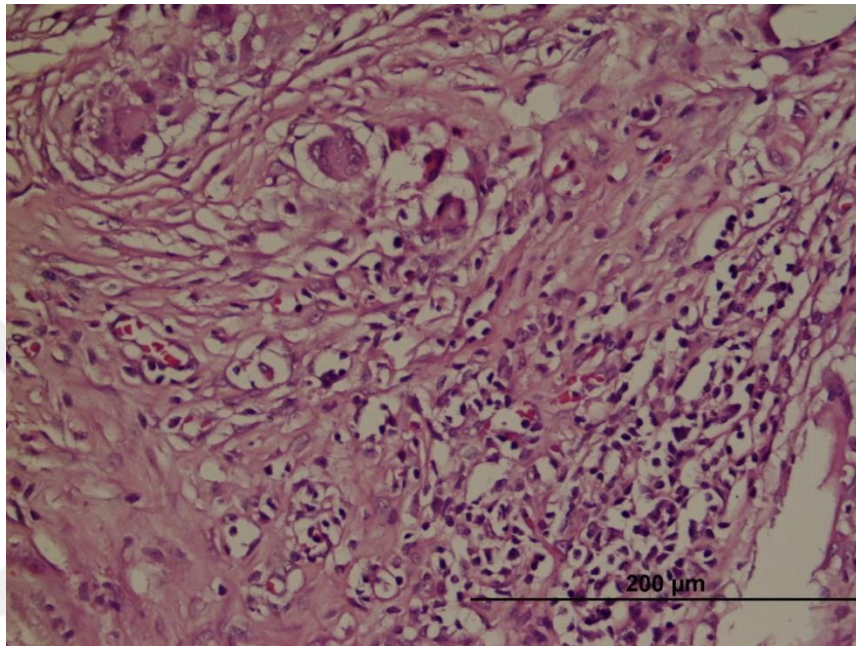


Figure 19: Fibrous adhesions and giant cell infiltration due to foreign body reaction in Group I (H.E).

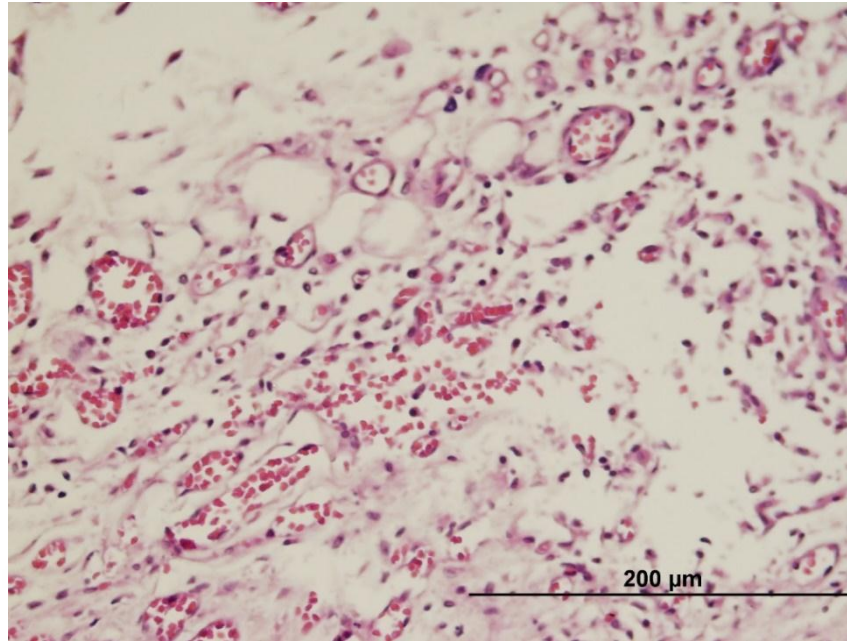


Figure 20: Common inflammatory cell infiltration and fibrosis in Group II (H.E).

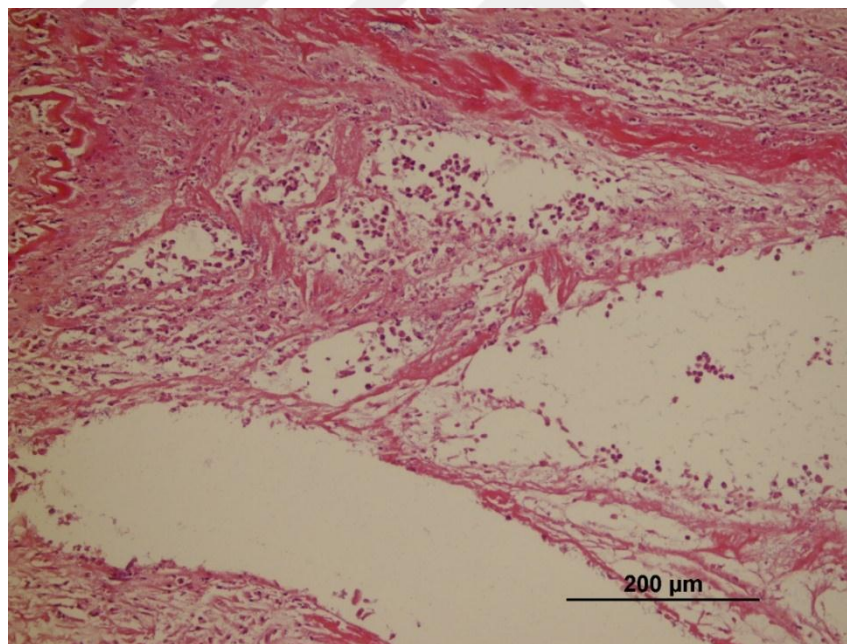


Figure 21: Medium inflammatory cell infiltration and fibrosis in Group III (H.E).



Figure 22: A large number of small blood vessels in Group IV, a small number of inflammatory cell infiltration and fibrosis (H.E).

5.3. Statistical Evaluations

The differences between the groups were evaluated by Kruskal Wallis analysis of variance and Mann-Whitney U test. $P < 0.05$ were considered statistically significant.

Comparison of the groups in terms of macroscopic adhesion severity grade; Group IV (Polypropylene mesh, bovine amnion membrane and 5 % Polyethylene glycol 4000) was significantly different from Group I (Control group) ($p < 0.05$). Group II (Polypropylene mesh and 5 % Polyethylene glycol 4000) was not significantly different from Group III (Polypropylene mesh, bovine amniotic membrane and 0.9 % NaCl) ($p > 0.05$). Group II and Group III were not significantly different from Group I (Control group) ($p > 0.05$).

Comparison of the groups with regard to fibrosis; Group IV (Polypropylene mesh, bovine amnion membrane and 5 % Polyethylene Glycol 4000) was significantly different from Group I (Control group) ($p < 0.05$). Group II (Polypropylene mesh and 5 % Polyethylene glycol 4000) was not significantly different from Group III (Polypropylene mesh, bovine amniotic membrane and 0.9 % NaCl) ($p > 0.05$). Group II and Group III were not significantly different from Group I (Control group) ($p > 0.05$).

Comparison of the groups with regard to inflammation; Group IV (Polypropylene mesh, bovine amnion membrane and 5 % Polyethylene glycol 4000) was significantly different from Group I (Control group) ($p < 0.05$). Group II (Polypropylene mesh and 5 % Polyethylene glycol 4000) was not significantly different from Group III (Polypropylene mesh, bovine amniotic membrane and 0.9 % NaCl) ($p > 0.05$). Group II and Group III were not significantly different from Group I (Control group) ($p > 0.05$).

6. DISCUSSION

Wistar rats are frequently used in experimental studies due to its ability to adapt to an extensive variety of environmental situations. These animals are also isogenic, which means that all are genetically similar individuals (1). Wistar rats were also used in this study because of this feature.

It has been reported that Polyethylene glycol provides good results in prevention of intra-abdominal adhesions (44,89). Polyethylene glycol has been found providing significant reductions in adhesion formation. In the reported study, Polyethylene glycol has been sprayed underneath the mesh during closure of the induced ventral defect with polypropylene mesh. (44). In presented study, 5% Polyethylene glycol 4000 was used in Group II, which used polypropylene mesh only, and Group IV, which used polypropylene mesh covered with amniotic membrane. Especially in Group IV, good results were obtained in terms of prevention of adhesions.

The foreign body reaction to polypropylene mesh is much less pronounced than that to many different mesh materials (56). But the polypropylene mesh is placed directly on the intra-abdominal organs, it can lead to serious complications such as dense adhesions, fistula and seroma. To prevent this negative situation, pre peritoneal (sub lay) placement may be preferred (47,49). Presence of a mesh in a living tissue may supply rise to special stages of infection, thrombosis, calcification, fibrosis and contamination (19). In Group I, the polypropylene mesh directly contacted the internal organs, resulting in dense adhesions and subcutaneous seroma.

Prosthetic meshes are divided into macro and micro pore meshes in keeping with their pore size, the pore size describes the size of fenestration in the mesh. Macro pore meshes ($>75\text{ }\mu\text{m}$) offer better tissue ingrowth/host integration in which as meshes with small pore size ($10\text{-}75\mu\text{m}$) or no pores contain a risk of encapsulation thus resulting in reducing integration into the abdominal wall, Micro pore meshes are historically regarded as causing a minimum adhesion formation, at the same time as macro pore mesh may additionally result in a disordered neo peritonealization and therefore probably cause more adhesions (25). Differences in pore size were suggested as a reason for differences in the inflammatory reaction to surgical meshes. Determined an increased foreign body response with polypropylene meshes with smaller pores (47). The pore size of mesh is vital in the improvement and preservation of abdominal adhesions and tissue ingrowth (62). Klinge et al., (59) assumed an impaired fluid transport through small pores to be responsible for an accentuated tissue reaction. In order that the mesh used in this study had 10 mm pore size.

In general, adhesions rise up from any tissue damaged in the first week after injury, also adhesions generally consist of omental fat and formed mainly at the edges of the mesh and at the fixating sutures (22,33). Prosthetic mesh edge exposure is a main source of adhesions, specifically when the mesh edge is adjacent to the peritoneal cavity (40). It was observed that Grade 1 and Grade 2 adhesions formed at the edges of the mesh and at the fixation sutures in this study.

Suture material additionally performs an important role in infection, and for this cause monofilament materials have been widely recommended because they have fewer tendencies to harbor microorganisms (30). In this study, absorbable,

with antibacterial protection, braided Vicryl was used. Vicryl have tension durable for 2-3 weeks and absorbed in 55-70 days. So that the applied mesh was securely fixed to the abdominal wall.

It was reported that skin healing usually happened with 7-8 days of surgery (46). In this study, similar results were observed.

The continuous suture pattern was used within the inlay technique, in which the breakdown of one stitch results in the dehiscence of the whole suture line (66). It was observed that interrupted sutures used for fixation of the implant in the interlay method provided multiple factors of no tension fixation which helped divide stress evenly over the mesh and reduced mesh folding and bulging (66). In order that the suture pattern used in this study was simple interrupted suture to reduce dehiscence of the whole suture line.

For surgical repair of abdominal hernias usage of prosthesis; appropriate surgical repairing approach, strength of the material, tissue compatibility, case of suturing, protection method of material and cost are important factors for attention to select a material which has less probability of being rejected, less tissue reactions and no damaging results in other organs (65).

Polypropylene mesh is very strong, inert, and immune for contamination. The polypropylene mesh is easy to deal with, the cut edges do not fray, and granulation tissue is able to develop via its spaces. From the literature it seems that herniorrhaphy with polypropylene mesh offers very good effects in horses and cattle (9). Polypropylene has been shown to be appropriate because it is one of the most inert materials available and therefore is useful in the presence of infection and contamination (30). It is still the simple prosthetic material used for

hernia repair (19). The propylene mesh was selected for wide use in medical practice, because it has a highly affordable value. It approximates the standards of an ideal material and its surgical approach for the correction of abdominal hernias is widespread due to its advantages including less tissue response, sterilized and handling (1). In order to that the polypropylene mesh was used in this study. Although it is a suitable material, direct contact of polypropylene mesh with abdominal organs has caused intensive adhesion formation in Group 1.

Absorbable meshes only provide a temporary solution in hernia repair. Therefore, a mesh used for hernia repair should be non-absorbable (69). In order that non-absorbable polypropylene mesh that widely used in hernia surgery was used in this study. It was reported that complications related to double application of mesh because of technical difficulties or accelerated mesh rejection and infection (30). Therefore, one layer of polypropylene mesh was used in this study to reduce infection and rejection of mesh.

It was reported that using polypropylene mesh covered by fibrous tissue showed similar results when compared to using only the mesh in regards to tension and histological analysis. In terms of the degree of adhesions, the mesh surrounded through fibrous tissue has caused less intraperitoneal adhesions, with the advantage of reduced postoperative complications consisting of enteric fistulas and difficulty in accessing the surgical cavity in a new exploration (1). Clinically, when polypropylene mesh is to be in direct touch with intra-abdominal contents, application of the bioresorbable membrane over the viscera may also reduce the severity of adhesion formation and likely diminish subsequent complications (61). To prevent adhesions, a few authors propose that the parietal peritoneum must be

preserved during incisional hernia repair because it forms a barrier. When the parietal peritoneum cannot be saved intact, the surgeon may also attempt to place the greater omentum between the abdominal contents and the prosthetic material (10). Experimental research showed that the occurrence of adhesion formation is 80% - 90%. A large peritoneal disorder with direct contact between the mesh and intra-abdominal organs might result in adhesion formation, mechanical bowel obstruction and fistula (14). In order that the amniotic membrane was used in this study to reduce adhesion formation and postoperative complication consisting of enteric fistula.

Amnion membrane has been used correctly in numerous surgical conditions, either as a surface covering (leg ulcers and wound, lining of the cavity following radical mastiectomy, traumatic ulcer, treatment of burns) in order to encourage epithelization, or to prevent adhesion in the abdominal cavity or edema and adhesions following craniotomy cavity and brain surgical procedure (55). Vital amniotic membrane supplied great adhesion prevention and showed properly biocompatibility, causing only a moderate local inflammation response (36). Therefore, polypropylene mesh was coated with bovine amniotic membrane.

Polyethylene glycol is a dependable and effortlessly applied adhesion barrier, and reduces adhesion formation after open and laparoscopic surgical procedure (44,89). A completely extra peritoneal method to mesh placement or a physical barrier in between is needed to reduce adhesions after mesh repair of the abdominal wall (56). Adhesion barrier prevent adhesion formation without activation tissue infection and bacterial growth. They can be used either in laparoscopic procedures or at laparotomy, without or with suturing (14).

Therefore, in the present study the 5% Polyethylene glycol 4000 was used alone and in combination with the bovine amniotic membrane to prevent polypropylene mesh complications.

Intra-abdominal adhesions are located in up to 93% of patient who have undergone intra-abdominal surgery. Usually, most adhesions are asymptomatic, but will, however, reason problems in about 5% of the patient. These postsurgical, adhesion-associated troubles include small bowel obstruction, female infertility, pelvic pain and abdominal pain. The formation of adhesions additionally causes secondary problems like prolongation and risking future intra-abdominal operation (6). Peritoneal trauma including surgical operation is the main cause of intra-abdominal adhesion. Ischemia and foreign body enhance the improvement of adhesions. In order to reduce adhesions via current techniques, peritoneal trauma need to be reduced, inflammatory reaction and coagulation have to be inhibited and surface that are likely to form adhesions should be cleaned to inhibit fibrosis (15). The most vital factors to reduce adhesions are introduction of minimum surgical trauma, reducing trauma to the peritoneum, minimizing preliminary damage , medical interventions within the fibrin formation/degradation balance, avoiding coagulation of exudate, barriers preventing organs from bridging over to other structures within the abdomen and there by forming adhesions, extending touch of surfaces can be reduced, fibroblast proliferation can be stopped or slowed and absence of powdered gloves (6,74).

In various studies; to prevent direct contact with abdominal organs of polypropylene mesh; the part of the mesh that looks inside the abdomen has been covered with human amniotic membrane, stretch film, and seprafilm (36,55,85).

In another study; polyethylene glycol has been sprayed underneath polypropylene mesh (44). It has been reported that the results obtained from these studies are positive. In this study; It was observed that there was no significant difference between Group III (polypropylene mesh covered with bovine amniotic membrane) and Group II (polypropylene mesh and 5% Polyethylene glycol 4000). Group IV (polypropylene mesh, bovine amniotic membrane, 5% Polyethylene glycol 4000) was significantly different when compared to Group I (Control group). In terms of preventing complications of polypropylene mesh; the combined use of 5% Polyethylene glycol 4000 and bovine amniotic membrane were observed to be better than all other groups according to macroscopic and microscopic evaluations.

As a result; polypropylene mesh is widely used in hernia repair because it is cheap and easy to find. However, cause many complications such as postoperative adhesions inflammation, seroma and abscess. Various drug and adhesion barriers have been used to prevent these complications. But the desired result has not been achieved. In this study, bovine amniotic membrane and 5% Polyethylene glycol 4000 were combine used for prevention of complications and were compared with their one by one uses.

According to the results of this experimental study, the combined use of bovine amniotic membrane and 5% Polyethylene glycol 4000 was helpful to prevent the complications of polypropylene mesh.

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8. CURRICULUM VITAE



I born in Sulaymaniyah / Iraq on 25.11.1979. Between 1985 and 1998 years I completed Primary and Secondary School in Sulaymaniyah, Iraq. Between 1999 and 2003 years I completed BSc (Hons) Veterinary Medicine and Surgery, University of Sulaymani, Sulaymaniyah, Iraq. I worked as Veterinary Clinician from September 2003 and March 2015 at Sulaymaniyah Veterinary Clinic, Ministry of Agriculture and Water Source, Erbil, Iraq. I started to my master program at 2015 year (Firat University, Institute of Health Science, Veterinary Program, Department of Surgery). I know Kurdish, Arabic, English, and Turkish languages.

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