HISTOLOGICAL EVALUATION OF THE EFFICIENCY OF HUMAN AMNIOTIC MEMBRANE USED IN EXPERIMENTAL RECONSTRUCTION OF THE ANTERIOR ABDOMINAL WALL DEFECTS

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Abstract

The purpose of the study was to conduct a morphopathological evaluation of the efficiency of human amniotic membrane used in experimental reconstruction of abdominal wall defects. The study group included 20 Californian rabbits, of both sexes, with the body weight ranging from 2300 to 2500 g. The abdominal wall defect was performed surgically under aseptic conditions resecting a fragment, 10cm x 5cm in size, involving the muscular-aponeurotic layer and parietal peritoneum. Animals subjected to the intervention of reconstruction of the anterior abdominal wall defect were divided into 4 groups consisting of 5 animals, depending on the method of implant processing and application. Unabsorbable polyester mesh of *Erceokaque* type was used in the abdominal wall reconstruction in the control group. The mesh was fixed to the abdominal wall layers but it was not covered by skin. In group 1 there was used the amniotic membrane treated with 0.1% glutaraldehyde protected externally with *Stypro* preparation. In group 2 there was used the amniotic membrane treated with 0.5% formaldehyde. In lot 3 the cryopreserved amniotic membrane and biological implants were protected by suturing the skin and subcutaneous layer. The study results allowed to conclude that the use of the amniotic membrane as implant, treated with glutaraldehyde and formalin, does not provide a long-term stability, the cryopreserved amniotic membrane having some advantages, namely, implant elasticity and stability of fixation sutures, as well as a marked reparative-regenerative activity. The amniotic membrane may be considered a useful temporary substitute for the peritoneum and a promising non-adherent adjuvant in reconstructive interventions of the abdominal wall defects with a viscero-abdominal disproportion. The obtained results justify the need to continue clinical trials to evaluate the efficacy and safety of the application of this biological material.

Key words: abdominal wall defects, amniotic membrane, graft, peritoneal adhesions.

Introduction

The correction of abdominal wall defects in children is a real challenge for paediatric surgeons, several reconstructive methods being proposed, including the use of protein synthetic and biological materials (3, 4). their characteristic varying by strength, biodegradability, resistance to infection and formation of adhesions (10).

The synthetic grafts, first introduced by Usher F.C. (1958) (23), are quite widely spread in surgery due to their acceptable biocompatibility and mechanical stability, but as nonresorbable material, they are quite frequently associated with various adverse postoperative effects, including persistent pain, hematomas, wound erosions, enterocutaneous fistulas, development of a significant intra-abdominal adhesive process, susceptibility to infection. The synthetic material does not increase in size as the child (6, 9, 13) is growing. In this context, the use of biological grafts, derived from animal and human sources, in the reconstruction of the anterior abdominal wall defects, including congenital defects, have certain advantages over synthetic prosthetic materials (2, 17).

Adhesive disease is a serious complication after corrective operations of congenital defects of the anterior abdominal wall (24). Several studies have found that amniotic membrane has several beneficial features. Some authors suggest using the amniotic membrane to cover the abdominal cavity organs in cases when there is no peritoneum (1), it having marked anti-adherent properties (12, 25). At the same time, some authors put into question the anti-adhesive effect of the amniotic membrane after interventions of closing the abdominal wall defect (15).

Purpose

The purpose of the study was to carry out a morphopathological evaluation of the efficiency of the human amniotic membrane used in experimental reconstruction of abdominal wall defects.

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Material and methods

The study group included 20 Californian rabbits, of both sexes, with the body weight of 2300 to 2500 g. The abdominal wall defect was performed surgically in aseptic conditions, under general anesthesia, resecting a fragment of 10cm x 5cm in size, involving the muscular-aponeurotic layer and parietal peritoneum (14, 18).

Animals subjected to the intervention of reconstruction of the anterior abdominal wall defect were divided into 4 groups consisting of 5 animals, depending on the method of implant processing and application. In the control group, the nonresorbable polyester mesh of *Erceokaque* type, (Fig. 1A) was used in the abdominal wall reconstruction, the mesh being fixed to the abdominal wall layers without being covered by skin. In group 1, there was used the amniotic membrane treated with 0.1% glutaraldehyde, protected on the outside with *Stypro* preparation (Fig. 1 B). In lot 2 there was used the amniotic membrane treated with 0.5% formaldehyde (Fig. 1C) and, in lot 3 – the cryopreserved amniotic membrane was used (fig. 1D), biological implants being protected by suturing the skin and the subcutaneous layer.

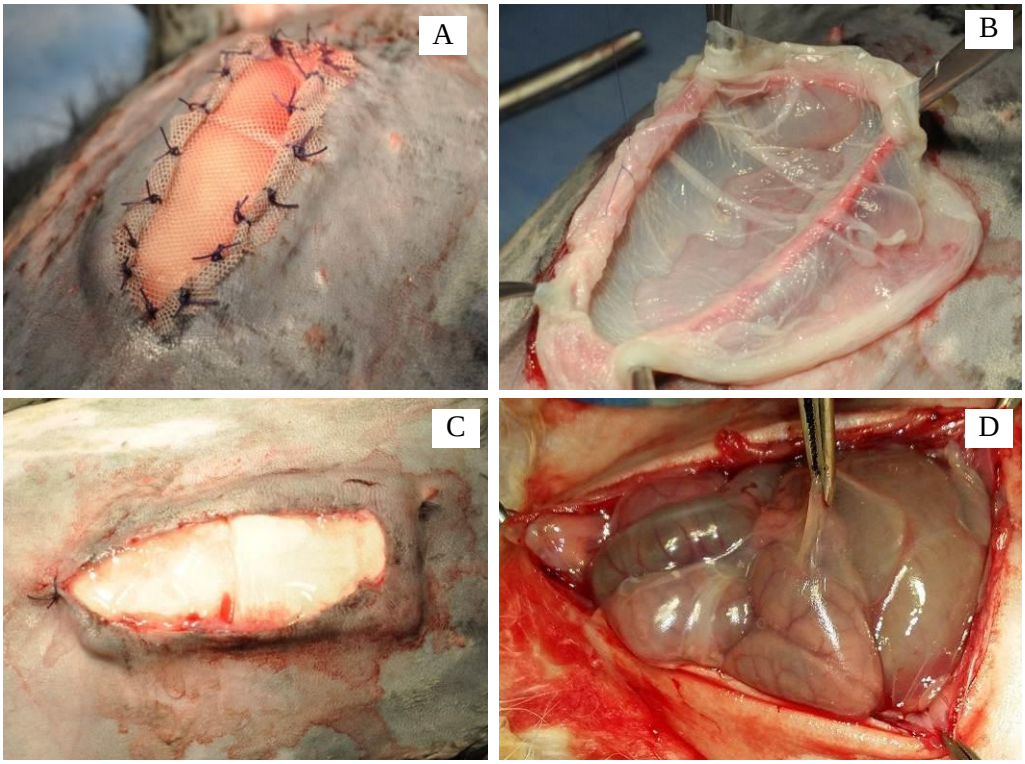

Fig. 1. Macroscopic appearance of the reconstruction of the abdominal wall defect with nonresorbable polyester mesh, *Erceokaque* type (A), amniotic membrane treated with 0.1% glutaraldehyde (B), amniotic membrane protected with Stypro (C), cryopreserved amniotic membrane (D).

In our research the grafts were treated at *Tissue Engineering and Cell Culture Laboratory*, State University of Medicine and Pharmacy "Nicolae Testemitanu". From graft harvesting to graft transplantation a series of systematized SOPs stages (standard operating procedures) are followed, structured according to the source used for the graft preparation.

The harvesting of the human amniotic membrane was performed immediately after birth by Caesarean section, to avoid contamination of the umbilical-placental complex through the birth pathways, from adult donors aged 25-35 years, after obtaining consent for harvesting, in compliance with the legislation in force (Law no. 42 of 06.03.2008 on transplantation of organs, tissues and cells).

After separation, the amniotic membrane was placed in decontamination medium comprising: RPMI (HiMedia), lincomycin, vancomycin (World Medicine), gentamicin (KRKA), hepes (HiMedia) 1 ml, is incubated at $+4^0$C for 12 hours, after which it is cut into strips of required size and placed in a double container in 50% of glycerol solution (Alchimia) with RPMI (HiMedia) and kept in the freezer at -80^0C.

Another method of the amniotic membrane preparation was to preserve the grafts in weak glutaraldehyde solution (0.1%) and formalin (0.5%). The graft is preserved in 0.5% neutralized formaldehyde solution, 7.3-7.4 pH, prepared in isotonic sodium chloride solution at $+4^0$C. The solution is daily changed for 10 days, then once a week up to 30 days. The first 30 days are the period of quarantine, as well as

sterilization and decrease of immunogenic properties. They were used after the serological and bacteriological validation of the grafts. The preparation of the amniotic membrane in glutaraldehyde and formalin allows to stabilize the implant, enhancing the biomechanical and enzymatic resistance (Spollensak G. et al., 2004).

The serological control is performed for the following tests: -Ac HIV 1+2, Ac HCV, HBV: (Ag HBs),(Ac anti HBs), syphilis.

Before use, during harvesting and after preparation some bacteriological tests are performed to establish the sterility, using 2 ml of transport liquid and 3-4 small portions of tissue, 5 x 5 mm in size, in various graft sectors in Thioglycolate Medium (HiMedia) and Sabouraud Dextrose Broth (HiMedia). The incubation is performed for 7-10 days to get final results.

The histological pieces were obtained by sectioning (four of each sample), using the semi-automatic microtome SLEE MAINZ-CUT 6062, 2.5 - 3µ thick. At the staining stage, the following methods were used: *hematoxylin-eosin* (H&E) and *Van Gieson* (VG).

Results and discussions

The animals were inspected daily to find the development of any complications. Animal sacrifice was made on the 7th day after surgery.

In spite of the postoperative wound infection in the control group, found in 4 animals, the dehiscence of sutures retaining the synthetic mesh was found in a case of animal`s death. Infection and wound dehiscence with eventration of the intestinal loops were found in 2 animals in group 1, and an animal died. Partial wound dehiscence with no signs of infection and development of incisional hernia were revealed in 3 animals in group 1, and in 2 animals in lot 2. In lot 3 partial dehiscence of the postoperative wound was recorded in a case (Fig. 2). The postoperative wound dehiscence was mostly caused by the implant rupture at the level of fixation sutures or less in central regions.

During the macroscopic evaluation of the peritoneal cavity, the development of an adherence process of various intensity was found in all cases of the control group, most often the synthetic mesh adhering to the omentum and intestinal loops, being observed the presence of some hard removable adhesions (3 cases), in four cases relatively easily removable inter-intestinal adhesions being detected with no signs of intestinal obstruction. In case of dead animals there were found purulent interintestinal collections and signs of diffuse peritonitis. There was revealed the development of an intestinal fistula in the control group. In a case in group 1 there was found the omentum adhering to the line of contact between the implant and the defect edge. In groups 2 and 3, even in cases of partial dehiscence of the fixation implant sutures no significant changes were observed along with the development of an adhesive process (Fig. 3).

The histological investigations of the samples taken in the study groups proved common and particular changes of the morphogenesis of reparative-regenerative processes at the tissue level and a correlation between the animal tissues and biological implant. The latter manifests asynchronously by the neoformation of granulations of various degree of maturity, expressed by fibroblastic elements, and of angiogenesis with persisting inflammatory response at the implant border, characterized by a various ratio of the cellular extrinsic and intrinsic component, with the predominance of polymorphonuclear cells (PMN).

The examination of tissue samples harvested from the contact line with the synthetic mesh (control group) did not reveal the presence of detersive reactions or formation of polynuclear giant symplasts, the latter being present in sutures. In these cases, an active regeneration was marked at the contact line with the synthetic mesh, which manifested by granulations rich in fibroblastic and capillary cells.

The changes in the intestinal visceral peritoneum in control animals showed reactive or proliferative processes.

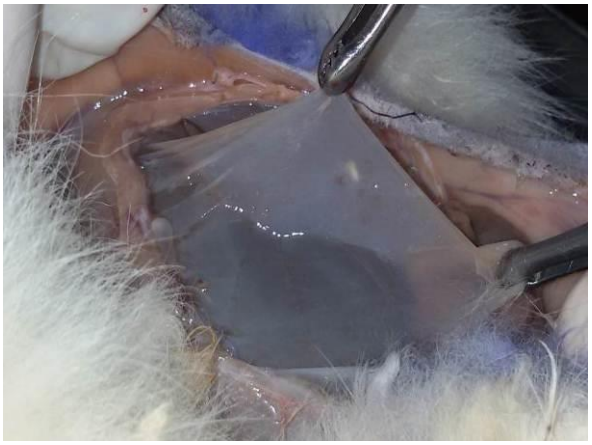

Fig. 2. Macroscopic appearance of the cryopreserved amniotic membrane fixed to the musculofascial layer with the preservation of the integrity of fixation sutures on the 7th day after surgery.

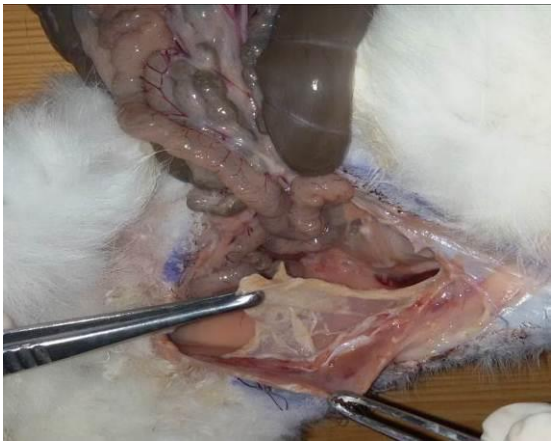

Fig. 3. Amniotic membrane treated with formalin on the 7th day. There is partial membrane rupture at the level of fixation sutures. The organs of the peritoneal cavity with no adhesions.

The animals in group 1, in large areas in the suture line between the musculo-peritoneal plane of the abdominal wall and amniotic membrane there was revealed the neoformation of granulation tissue in plateaus with various degree of maturity, with reduced PMN presence, the latter being more marked at the boundary with the connective layer of the amniotic membrane (fig. 4 A). It alternated with areas characterized by the proliferative mesenchymal-cellular anchoring features penetrating the connective matrix of the amniotic membrane, reaching the limit of the compact layer, sometimes its fasciculation being observed (Figure 4 B).

In those areas, at the level of the connective matrix of the amniotic membrane there was also found a hypercellularization on the account of fibroblasts, with the presence of some endothelial buds which emphasizes fibrillogenesis and angiogenesis activity (Fig. 4 C). A feature, found in the correlations between the granulation tissues and amniotic membrane, is active generalization of granulations on its surface, thus encompassing the amniotic membrane in various ratio. There were observed small areas of penetration and disjunction of the amniotic epithelium, most often bypassing the epithelium and forming small fissures (fig. 4 D).

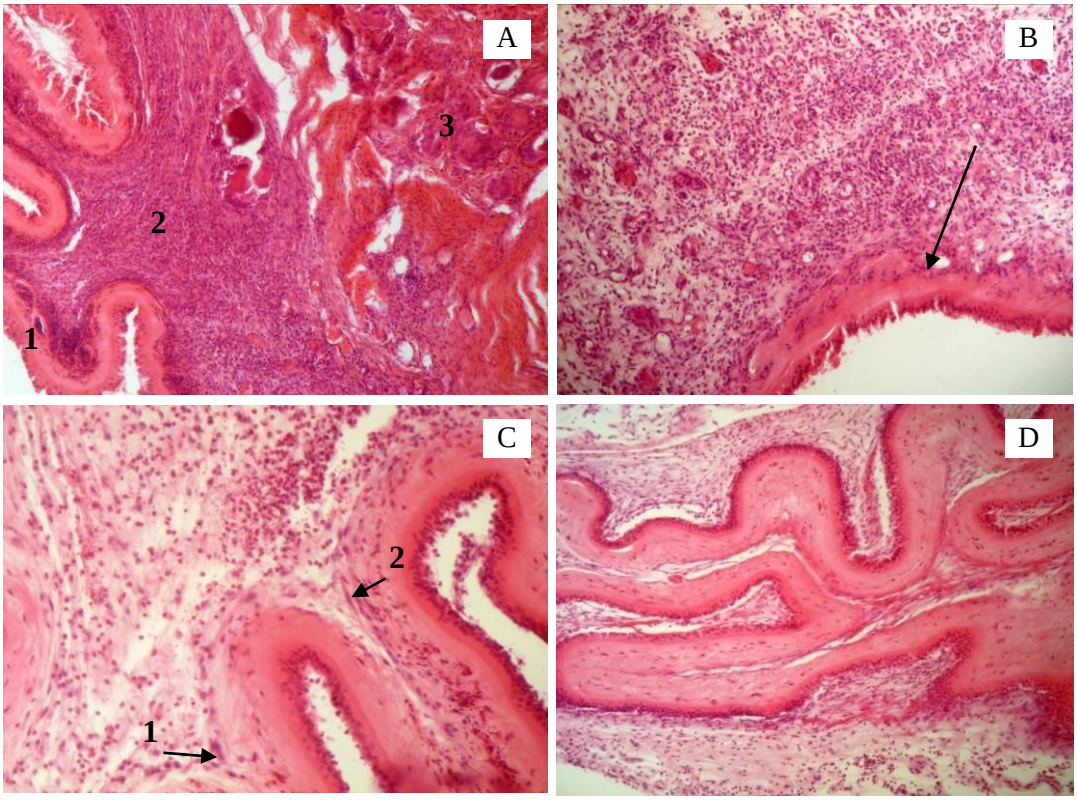

Fig. 4. A) Immature granulations in the plateau at the contact line: 1 - amnion; 2 - immature hypercellularized granulation tissue; 3 - abdominal muscular zone. x 75; B) Aspects of proliferative mesenchymal-cellular anchoring of the granulation with the amnion. x 125; C) Cellular-tissue elements in the cellular-mesenchymal anchoring area 1-endothelial buds; 2 fibroblasts. x 125; D) Aspect of granulations in strips embedding the amnion: 1-amnion; 2-granulations with reduced number of PMN. x100. *Color. H-E.*

At the border with the amnion, the granulation tissue had variable thickness, on some areas it being excessive and deformed. In close proximity there was often found the persistence of active inflammatory processes, characterized by PMN elements forming a fold (layer) with an abundant eosinophilic content that penetrated the area of the compact connective layer, with degeneration and disjunction phenomena with no detachemnt Fig. 5 A, 5 B). Thus, on some areas the loose connective zone of the amnion was invaded by immature granulations (Figure 5 B). At a higher magnification, a conventional cascade stratification of the cellular mesenchymal components of the immature granulation can be observed at the contact line, in which the angio-fibroblastic layer was followed by proliferative processes of fibroblasts and endothelial buds with the invasion of the loose connective (matrix) layer of the amnion, followed by the cellular PMN component which migrated in the dense connective layer of the amnion.

Examination and testing of the connective tissue revealed small precipitations and threadlike deposits of collagen at the level of granulations (fig. 5 C). There were also revealed reparative-regenerative processes of the

muscular and interstitial connective tissues, the regeneration of the connective tissue being predominant. Simultaneously with the changes described, the presence of microbial colonies was observed in some samples with the PMN predominance (fig. 5 D), which in fact accounts for polymorphism.

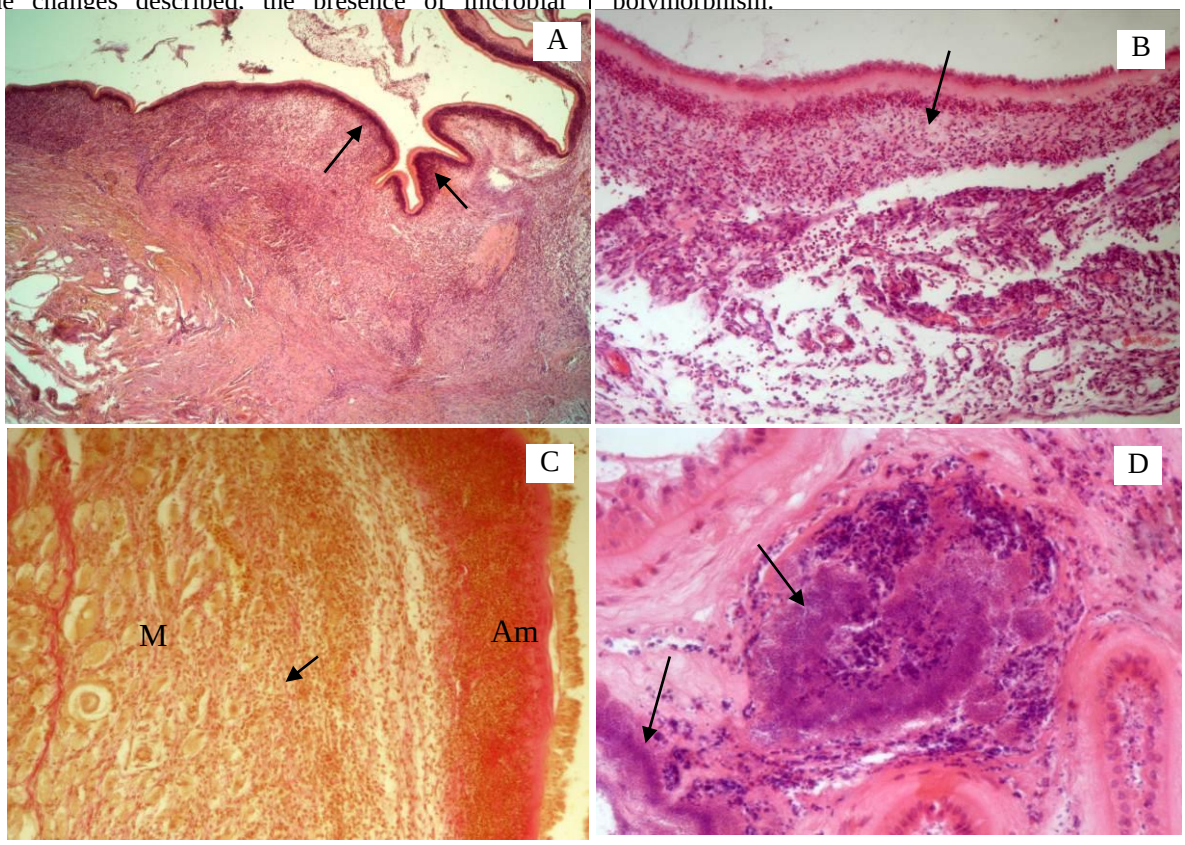

Fig. 5. A) Excessive granulations associated with the inflammatory process actively expressed by the PMN at the border with the amnion. x 25; B) Imature eosinophilic granulations in the loose connective layer (matrix) of the amnion. x 100; C) Precipitates and collagen filiform deposits in the area of granulations between the muscle area (M) and amnion (Am) x 100; D) Bacterial microcolonization with PMN and lymphocytic reaction at the level of the connective matrix of the amnion x 200. *Color. H-E*

In the area of the newly formed granulation tissue there were observed polynuclear cellular symplasts frequently adjacent to the host muscle area, characterized by deformed hypertrophic myocyte elements, dystrophy and polynuclear myogenic giant cells, reflecting the regenerative processes of the muscle area (Fig. 6 A). Giant cellular symplasts were also found adjacent to sutures, associated with the foreign-body type reaction (Fig. 6 B). In areas with the partial dehiscence of sutures fixing the amnion, the peritoneum showed an exudative inflammatory polymorphocellular serous-macrophagic reaction associated with active proliferative fibroblastic processes (Fig. 6 C). The inflammatory peritoneal process in the presence of microbial colonies revealed the presence of visceral focal proliferative- fibroblastic peritonitis (Fig. 6 D).

At the contact line of the amniotic membrane with the peritoneal stroma, the regenerative granulation processes manifested an activity analogous to that described in tha host muscle area, in 2 cases the proliferative-fibroblastic mesenchymal ones being predominant, and the PMN component being much lower. The correlations with the connective (matrix) layer of the amniotic membrane revealed a neoformation of the intimately anchored granulation tissue, also concrescent to it up to the dense connective membrane (fig. 7 A, B). In areas opposed to the epithelial layer of the implant there were revealed amniotic microcalcifications along with microfissures of its detachment; polynuclear giant symplasts being revealed at the level of granulations. In those areas, the amniotic element was intact or with some modifications of intumescence, circumscribed within the granulation of cellular reaction in polynuclear symplasts (Fig. 7 B). In these cases, when testing the connective tissue in the granulation area, the presence of threadlike collagen deposits was revealed on the 7th day, some resembling fine connective fibers.

Another phenomenon observed in the correlations of the newly formed granulation tissue with the amniotic membrane, particularly in the presence of the degenerescence of the amniotic layer, was the presence of pronounced cells at the level of the compact connective layer of the implant (Fig. 7 C), which became more pronounced, with an appearance of free or chain migration. On some areas fibroblast penetration from the granulation

region was observed, some fissures being present at this level, through which a trend of the cellular component penetration could be seen. Simultaneously, in areas with active regenerative processes, in the area of granulations, there were found only their residues as dense connective bundles comprised by spiral fibroblasts (Fig. 7 D).

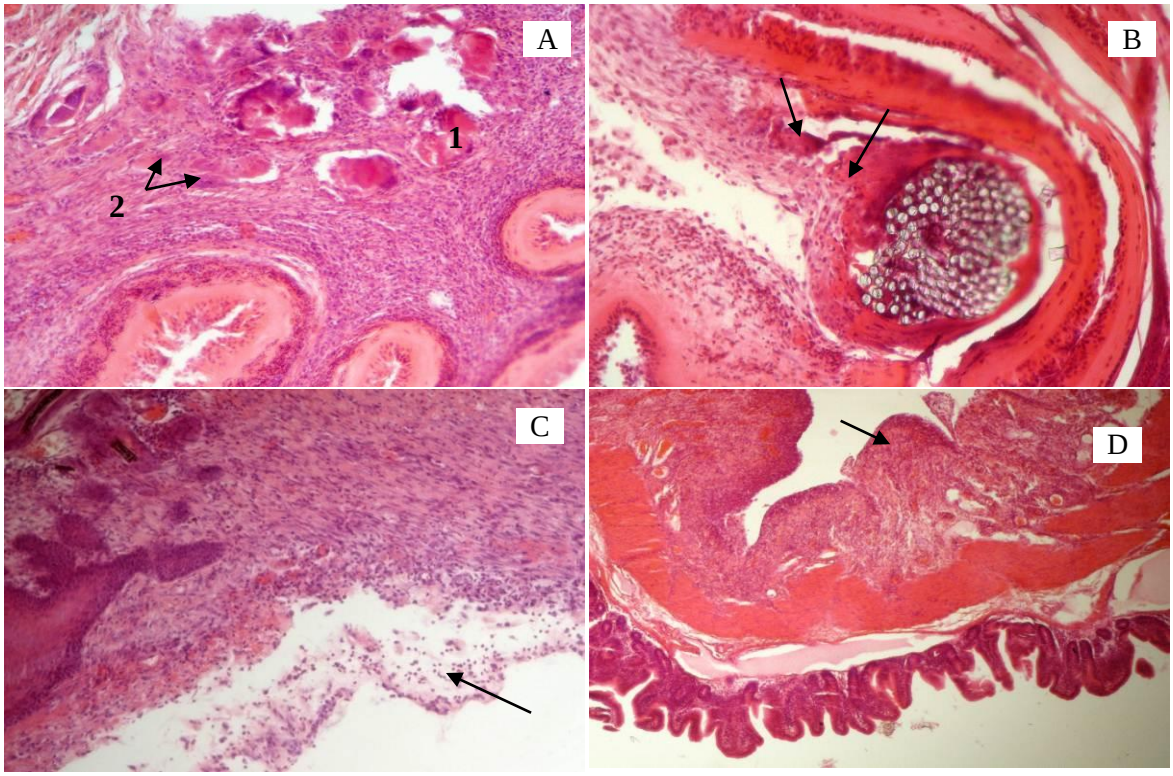

Fig. 6. A) Dystrophic and regenerative processes of the host muscular area 1 - hypertrophic myocyte cells with dystrophy; 2 - polynuclear giant cells of muscular origin in formation x 100; B) Foreign-body reaction with polynuclear symplasts around the suture wires. x 125; C) Area of the peritoneum contact line. Proliferative fibroblastic and exudative serous processes. x 75; D) Proliferative visceral fibroblastic peritonitis *Color. H-E.*

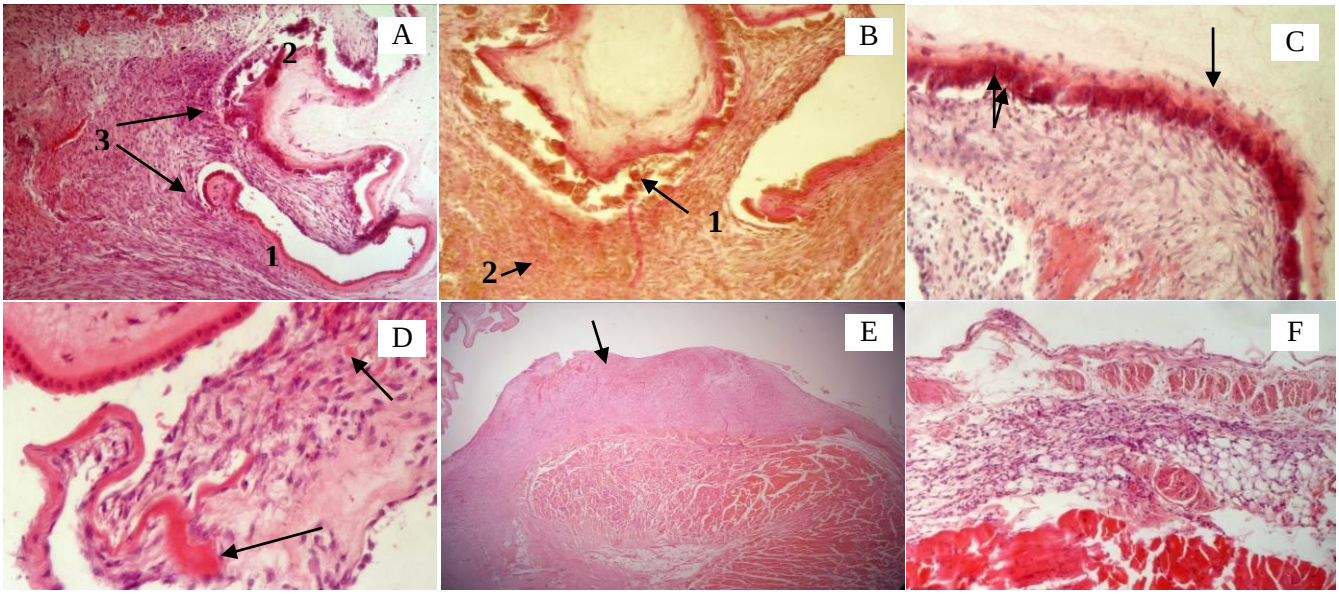

Fig. 7. A) Granulations in the peritoneal-mesenchymal zone-: 1 - intimate consolidation with matrix layer of the amnion; 2 – focal tension relieving of the amnion in the amniotic area; 3 - cellular polynuclear symplasts x 75; 4 - Amniotic membrane partially embedded in the granulation tissue B) Picture preceding amplification: 1- reaction in polynuclear giant cellular symplasts; 2 - threadlike deposits of collagen and fine connective fibers. x 120; C) Transepithelial migration of fibroblasts at the border with the amnion x 100; D) Bundles of the dense connective amnion in the granulation area embedded by spiral fibroblasts x 120; E) Processes of excessive and proliferative-fibroblastic peritoneal granulations nearby the contact line x 75; F) Distant view of the reactive peritoneal edema and cellular-adipose tissue, x 75. *Color. H-E.*

In samples taken in cases of the dehiscence of fixation sutures of the amniotic membrane, at the level of the adjacent peritoneum, including cases with the amniotic membrane detachment there were detected monstrous massive granulations with active proliferative fibroblastic processes (Fig. 7 E). The remote parietal peritoneum had no inflammatory changes, only a moderate reactive edema being present (Fig. 7 F).

In group 2, in the correlation with the contact surface of the amnion, there were revealed reparative-regenerative processes and correlation of tissues with the implant analogous to group 1. In the area of granulation plates, in various ratio, there were present the PMN elements with marked eosinophilia, mainly in areas overlapping with the amniotic epithelium. In some areas the granulations interposed between the very muscle area and the amnion were excessive. In group 2, compared to group 1, the amniotic component of the amnion manifested degenerescence, and sometimes it was absent (Fig. 8 A). In various areas nearby the very muscle area could be seen polynuclear muscular giant symplasts differentiated in myoblastic bud cords. When overlapping with the epithelial-cellular surface, as in previous cases, there were revealed signs of detachment of the amnion, in some areas there were focal calcifications of the epithelium; areas of partial detachment of the amnion and embedding of granulations being common (Fig. 8 B).

Eosinophilic cellular component manifested mosaic and disorderly character in the contact areas, which was confirmed by the absence of polymorphonuclear elements and eosinophils, including cases with overlapping epithelial cellular layer of the amniotic membrane, the proliferative fibroblastic cellular tissue manifesting phenomena of invading the epithelium area (fig. 8 C). These phenomena were marked with the decrease of the inflammatory process and the prevalence of the proliferative fibroblastic process. Compared with group 1, in group 2 there were more frequently observed polynuclear muscular cellular elements, sometimes in plateaus (Fig. 8 D). Frequently, the eosinophilic component was found perivascularly, including the boundary between the muscular tissue and granulations (fig. 8 E, F), being more frequent in overlapping areas with the epithelial-cellular surface. We should note that in study group 2 the polynuclear symplasts were frequently present in the area of sutures.

From the adjacent peritoneum there were found proliferative fibroblastic processes with the presence of polymorphonuclear cells and eosinophilic component. At the level of mesenterium, the presence of edema and polymorphonuclear cells are revealed regionally.

In cases with no dehiscence of sutures fixing the amniotic membrane, from the visceral peritoneum of the small and large intestines no inflammatory changes or vascular cellular reactions were observed in this study group.

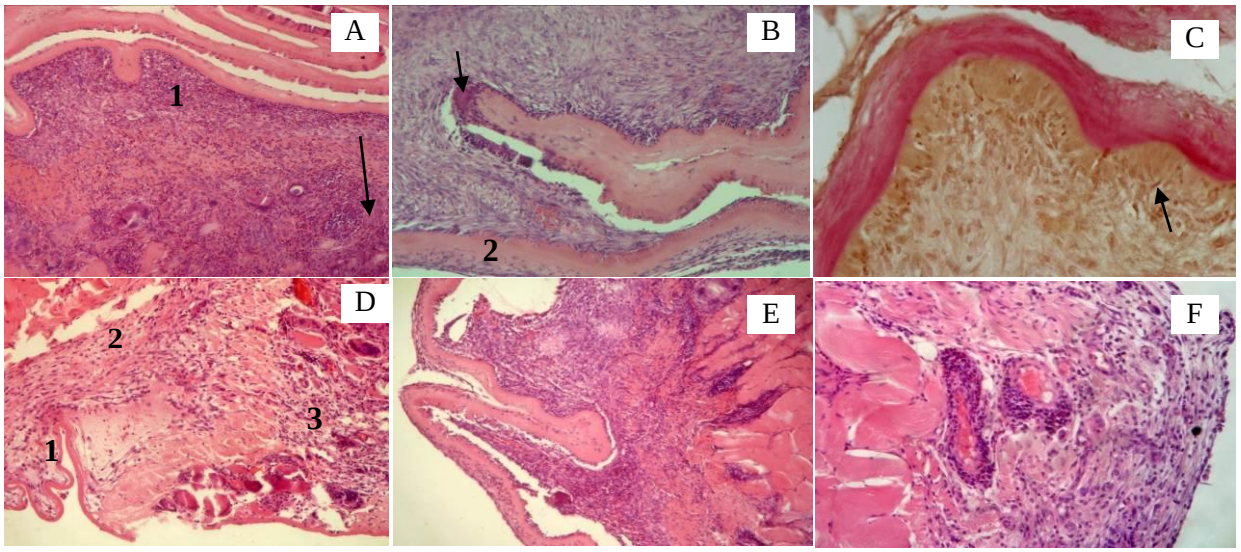

Fig. 8. A) Plate of immature granulations interposed between the muscle area and the amnion: 1- hipercellularization with PMN - eosinophils; 2- polynuclear muscular symplasts arranged (→) in small cords.x 25; B) Aspects of animal tissue correlation with the implant: 1- amnion with epithelial calcification and partial detachment; 2 - amnion lacking amniotic component embedded in granulation x 75 Color. H-E; C) Contact area - granulation plateau with phenomenon of PMN penetration and fibroblast implantation in amniotic component. x 200, Color. VG; D) Processes of regeneration of the connective-muscular tissue: 1 - amnion; 2 -granulations; 3- muscle tissue regeneration and angiogenesis. x 100; E) detachment phenomenon of the amnion when overlapping with the amniotic surface, immature granulations with PMN associated with perifocal vasculitis at the level of muscle layer, x 75; F) Polymorphocellular perivasculitis with predominance of eosinophilic component on the border with the musculo-fascial layer x 100. *Color. H-E*

The histological investigations in lot 3 have not confirmed any significant deviations of the reparative-regenerative processes of the tissue components and correlations between the tissues and amnion implant. There were frequently found morphological processes analogous to those found in lots 1 and 2, namely, a more marked activity of the regenerative processes and cellular-eosinophilic component. As in previous groups, the foreign-body type reaction with the presence of polynuclear giant cellular symplasts are frequently observed in the area of sutures, sometimes the degeneration of muscle fibers included in the area of monstrous massive granulations. Compared with the previous lots, in group 3 the PMN elements were found not only in the matrix or connective dense layer of the amnion, but dispersed throughout the area, often invading the epithelial cellular layer, which reveals the migration capacity of the cellular component in the implant area.

In group 3, between the granulation tissue with proliferative fibroblastic activity and epithelial-cellular surface there was found a more pronounced intimate relationship with more active angiogenesis peculiarities, such as areas of granulations penetration in the connective dense layer of the amnion with more pronounced adhesion and anchoring.

From the connective matrix of the amniotic membrane, in the contact areas there were found proliferative angio-fibroblastic processes supplemented by polymorphonuclear elements, penetrating in depth, partially involving the dense connective membrane, the latter having dystrophic changes. In this group the proliferative fibroblastic and angiogenetic processes with the capillary neoformation were comparatively more obvious and active. Also, in this lot the amniotic epithelium had degenerative alterations with no calcifications or foreign-body type reactions, the granulation tissues being frequently less huge or monstrous.

In most cases (4 cases) in group 3, the intestinal loops showed no pathological changes, except for a case, where some insignificant inflammatory changes were observed in the mesenteric region.

On the 7th day, in the nearby ares of the musculo-fascial layer involved in suture and in areas adjacent to the contact area with no necrotic, exudative or detersive changes, there was recorded the development of myocytes in myosymplasts, then in polynuclear myoblasts, followed by the appearance of cellular cords improperly oriented towards the granulation tissue of the contact line. It can be considered a remultiplication of the muscle fiber myocytes being significant in the plasty and regeneration processes of the muscle tissues.

Thus, the study results allowed to reveal some features of the morphogenesis of reparative-regenerative processes of the animal muscular-fibrillar tissues and correlations with the human amniotic membrane used as implant. The histogenesis of regenerative processes was found to be comparable to the normal histogenesis, characterized by the connective tissue formation manifested on the 7th day by the neoformation of the proliferative-cellular immature and mature fibrovascular granulations. In the same period, the histogenesis of regenerative processes in correlation with the implant had a mosaic appearance, being regionally retarded, characterized by an active persistence of exudative inflammatory processes, cellular degeneration and the presence of infiltrating neutrophils, more pronounced in groups 1 and 2 compared to group 3. In our opinion, this phenomenon can be influenced both by the processing method of the implant and the physiological individual peculiarities of animals. As seen in this study, the regeneration by substitution with the connective tissue evolves synchronously with the regeneration of fibrillar-muscular elements.

The PMN persistence in various areas is a reaction of the inflammatory process directed towards the implant degenerescence due to their proteolytic peculiarities, expressed in lot 3 by their more accessible migration to the implant structure. The tendency to form generalized granulations on the implant surface, as well as its anchoring and embedding was another feature found between tissues and implant.

The formation of more excessive granulations with an ugly aspect, found in some areas, is not a pathological reparative-regenerative organization but rather the result of small detachments caused by physiological animal behavior and mechanical factors. Thus, they mark the reparative-regenerative activity of *per secundam* healing, which is more pronounced in cases of dehiscence. The regenerative pathological processes as foreign-body type reactions with polynuclear giant symplasts were frequently found around sutures, as well as perifocally in the very degenerative muscle tissues as a result of the prevalent activity of both the neoformation of granulations, and the lack of exudative-detersive residues removal, they remaining embedded in the granulations. In the same context the foreign-body type reactions to amniotic epithelial elements were seen in the first lot. Adherent processes found at the peritoneal level in implant cases are conventionally pathological being determined not by implant itself, but by intraperitoneal eliminations of exudate or necrotic-detersive masses in the regeneration area.

The amniotic membrane is known in transplantology more than 100 years, when it was proposed by Davis J.M. (1910) as skin transplant (5). During the 40s of the last century the anti-adherent property of the amniotic membrane was discovered, contributing to significant advances in many surgical fields (11, 21). Subsequently, this fetal membrane was used in ocular lesions, burns and

chronic skin ulcers, in the treatment of Stevens-Johnson syndrome, as well as in reconstructive surgery of tendons, nerves, bladder and vagina, in order to prevent adhesions (7,20,22). The use of amniotic membrane in the reconstruction of congenital abdominal wall defects in newborn was proposed in 1971, which led to a decreased mortality in cases of gastroschisis (8). In 1975, J.H. Seashore and coauthors proposed swine skin grafts and human amniotic membrane as biological dressings in the treatment of gastroschisis and omphalocele, these biological grafts being considered useful in the adjuvant management of these cases (19).

The increased interest in the amniotic membrane is caused by reduced antigenicity, antimicrobial, anti-inflammatory and anti-adherent properties, this biological material representing a substrate for the growth of tissues etc. Given these properties, several authors tried to use the amniotic membrane in combination with other synthetic materials in the treatment of abdominal wall defects, these studies being on the experimental stage (1, 16).

Conclusions

1. The use of the amniotic membrane as implant, treated with glutaraldehyde and formalin, in spite of its durability, biomechanical strength and acceptable biocompatibility, does not provide long term stability, its application resulting in the dehiscence of the post-operative wound particularly within the fixation sutures. In this context, the cryopreserved amniotic membrane has some advantages, namely, implant elasticity and stability of fixation sutures, as well as marked reparative-regenerative activity.

2. The detachment phenomenon of amniotic membrane is determined by epithelial-cellular overlapping, this indicating the incorrectness of the implant application.

3. The amniotic membrane may be considered a useful temporary substitute for the peritoneum and a promising anti-adherent adjuvant in reconstructive interventions of the abdominal wall defects with viscero-abdominal disproportion, the results justifying the need to continue clinical trials in order to assess the efficacy and safety of this biological material application.

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