Particularities of the regenerative processes in the reconstruction of diaphragmatic defects with decelularized grafts of porcine pericardium in experimental model

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Abstract

Particularitățile proceselor regenerative în reconstrucția defectelor diafragmatice cu alogrefe decelularizate de pericard porcin în model experimental

Actualmente se depun eforturi considerabile în elaborarea unor materiale biologice decelularizate ca alternativă de corecție chirurgicală a defectelor diafragmatice.
Scopul acestui studiu a fost evaluarea eficienței, siguranței și particularităților proceselor de regenerare și remodelare tisulară ale alogrefelor decelularizate de pericard porcin crioprezervate, utilizate în reconstrucția defectelor diafragmatice, create chirurgical în model experimental la porci.
Lotul de studiu a inclus 6 purcei cu greutatea de 10 kg, supuși laparotomiei subcostale stângi cu reconstrucția defectului diafragmatic, creat chirurgical, cu grefe decelularizate de pericard porcin (lotul 1 - 3 animale) și peritoneu porcin (lotul 2 - 3 animale).
Exameul radilogic efectuat în loul 1 la 15 zile postoperator a stabilit o configurație normală a neohemidiafragmului creat, la a 60 zi postoperator fiind documentată eventrația nesemnificativă a neohemidiafragmului. În lotul 2 la a 15 zi postoperator a fost observată o eventrație neînsemnată a neohemidiafragmului, ulterior, ambele animale acestui lot decedînd subit la a 54 și a 60 zi postoperator din cauza dehiscenței grefei.
În lotul I au fost observate zone cu o fibrilogeneză atipică (malformativă) care se evidențiau printr-un aspect multichistic sau lacunar-cavitar, cu suprafețe interne tapetate cu epiteliu mezotelial peritoneal unistratificat aplatizat fără aspecte de activitate mitotică. Formațiunea cavitar-chistică putea fi și o pseudotumoră chistică cu septuri mai subțiri, cu o carcasă redusă din fibre de colagen. La animalele lotului 2 în ambele cazuri s-a constatat un hemidiafragm cu defect persistent determinat de reabsorbția parțială a materialului biologic, necătînd că putea fi observat și zone de țesut neoformat.
Așadar, grefele decelularizate de peritoneu porcin utilizate în reconstrucția chirurgicală a diafragmului sunt caracterizate de o biorezistență redusă, care poate contribui la reabsorbția parțială a acestui material biologic cu dezvoltarea unor complicații grave. Grefele decelularizate de pericard au o biorezistență acceptabilă comparativ cu cele de peritoneu porcin, aceste date sugend necesitatea unor studii suplimentare pe termen lung, care ar avea ca scop obținerea unor rezultate mai durabile.

Cuvinte cheie: diaphragmatic defects, biological grafts, porcine pericardium, regenerative processes

Abstract

Considerable efforts are currently underway in the development of decellularized biologic materials as an alternative to surgical correction of diaphragmatic defects.

The aim of this study was to evaluate the efficiency, safety and particularities of tissue regeneration and remodeling processes of allograft decellularized cryopreserved porcine pericardium, used in the reconstruction of diaphragmatic defects, surgically created in experimental model in pigs.

The study group included 6 pigs weighing 10 kg, subjected to left subcostal laparotomy with surgical reconstruction of diaphragmatic defect, with decellularized grafts of porcine pericardium (1-3 animals) and porcine peritoneum (group 2- 3 animals).

The radilogic examination performed in group 1 at 15 days postoperatively a normal configuration of the neohemidiaphragm created, at 60 postoperative day being documented the insignificant eventration of neohemidiaphragm. In group 2 to 15 postoperatively, a slight incidence of neohemidiaphragm was observed, after which both animals of this group died suddenly at 54 and 60 days postoperatively due to graft dehiscence.

In 2 cases areas with atypical (malformative) fibrillogenesis were observed, which were evidenced by a multicystic or lacunar-cavity aspect, with internal surfaces covered with unistratified peritoneal mesothelial epithelium flattened without aspects of mitotic activity. The mesothelial cavity-cystic aspect has also been shown on border, in some cavities being present fibro-epithelial conjunctival micro-polyps. The cavity-cyst formation could also be a cystic pseudotumor with thinner septa, with a reduced collagen fiber housing. In the animals of lot 2, in both cases, there was a hemidiaphragm with persistent defect determined by the partial reabsorption of the biological material, although neoformed tissue areas could be observed.

Therefore, decellularized porcine peritoneum grafts used in surgical reconstruction of the diaphragm are characterized by a low bioresistence which can contribute to the partial reabsorption of this biological material with the development of serious complications. Decellularized grafts of porcine pericardium have an acceptable bioresistence compared to porcine peritoneum, and this data suggests the need for further long-term studies that would seek to achieve more sustainable outcomes.

Keywords: gastroschisis, omphalocele, newborn, associated congenital malformations, prognosis

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Introduction

Congenital diaphragmatic defects remain an important cause of morbidity and mortality in newborns, the size, anatomical details or their type directly influencing the prognosis [1, 36]. The surgical treatment of these defects significantly evaluated from emergency surgery to delayed interventions, with the elective plasty of the diaphragmatic defect, performed after stabilizing the general condition of the child and of the cardio-respiratory functions [27, 29]. Along with new modalities of diagnosis and treatment in neonatal intensive care [13, 35] including with the support of ECMO [15], were described several surgical techniques for repair and reconstruction of diaphragmatic defects [18, 19, 43], including minimally invasive [20, 38, 40, 51]. The indisputable advantages of contemporary methods of surgical treatment of diaphragmatic hernias have not reduced the early recurrence rates, which constitute 3-50% [10, 36]. To improve the situation, biological grafts with integration properties in the host tissues are used more frequently in the primary reconstruction of the diaphragmatic defects, and subsequently they are replaced [3, 4].Several biological grafts have been created, and some acellular tissues are approved in clinical practice without identifying the ideal material, including: human dermal acellular matrix [2, 9], porcine intestinal submucosa [31], collagenous material derived from porcine dermis [28], bovine pericardium [25]. These biological materials have been shown to be effective in repairing human tissue [5], some of them being used as an option in the reconstruction of diaphragmatic defects [17, 23, 47].

The aim of this study was to evaluate the efficiency, safety and particularities of tissue regeneration and remodeling processes of allograft decellularized cryopreserved porcine pericardium, used in the reconstruction of diaphragmatic defects, surgically created in experimental model in pigs.

Material and Methods

The study group included 6 Landrace pigs of 3 weeks of age and weighing 8.9-9.3 kg, undergoing general anesthesia and sacrificed over 60 (group I - 3 animals) and 90 respectively. days (group II - 3 animals), according to the requirements in force, the study being approved by Bioethics Committee.

The animals underwent left subcostal laparotomy (3 animals) and left postero-lateral thoracotomy (3 animals), with the surgical modeling of a circular defect of 4 cm in diameter in the left hemidiaphragm, after which it was closed with decellularized grafts of cryopreserved porcine pericardium (fig. 1). In the control group, the diaphragmatic defect was closed with decellularized porcine peritoneum (2 animals).

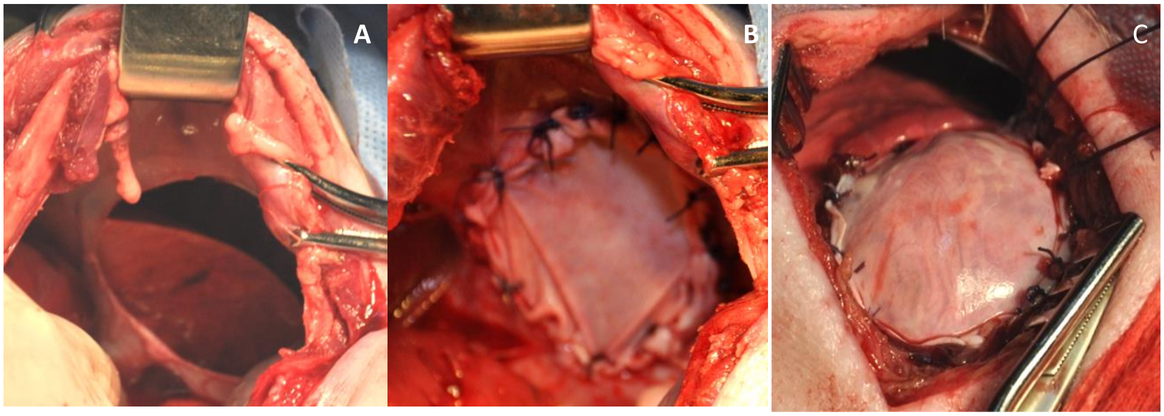

Fig. 1. The intraoperative aspect of the surgical modeling with decellularized biological graft of a diaphragmatic defect in animal experiment: A - the intraoperative aspect of the diaphragmatic defect created surgically by abdominal approach; B - the intraoperative aspect of closing the diaphragmatic defect with pericardial graft by abdominal approach; C - the intraoperative aspect of closing the diaphragmatic defect with pericardial graft through thoracic approach

Porcine pericardium and peritoneum were obtained immediately after slaughter of the animal at the slaughterhouse, and subsequently transported to the laboratory in 0.9% sodium chloride isotonic solution.
The processing of biological material included several stages:

- Decontamination: in antibiotic coctail (gentamicin, lincomycin, amphotericin B) for 6 hours;
- Decellularization: use of sterile 0.5% Sodium Duodecyl Sulfate solution, the solution being changed every 24 hours for 72 hours;
- Washing of the decellularization membrane: under sterile conditions with isotonic solution of 0.9% sodium chloride, in abundance;
- Repeated decontamination for 24 hours in the antibiotic cocktail; control of the efficiency of histologically confirmed decellularization.

As a control of the morphological particularities of hemidiaphragm tissue maturation in the pigs involved in the study, in 6 cases the contralateral hemidiaphragm from the same pigs and the same slaughter period was used. In order to assess the efficiency of the reconstruction of the diaphragmatic defect, the radiological examination was performed after 2 weeks, 2 months and 3 months from the time of the intervention.

For the histological investigations tissue samples (3 pieces with dimensions of 1.0 x 1.0 x 0.5 cm) were taken from the central, intermediate and peripheral areas sutured to the host tissues and at a distance of 1.5-2 cm. Once they have been fixed to the ground. Formol of 10% for 6-12 hours, were treated according to the standard protocol for histopathological investigations, using the network of histoprocessing and automated staining "Diapath". For the histological tests, at the SLEE MAINZ-CUT 6062 microtome microtome (Germany), 3-4 sections with a thickness of ≈ 3-4 μ, colored using the conventional hematoxylin-eosin (H&E) method were obtained.

Results and discussion

At the radiological examination, performed 15 days postoperatively, in the control group, where in the reconstruction of the diaphragmatic defect was used decellularized porcine peritoneum, an ascending position of the left hemidiaphragm was determined.

When using porcine pericardial grafts, the configuration of the hemidiaphragm undergoing surgery was normal and was maintained in this condition for 30 days after surgery (fig. 2).

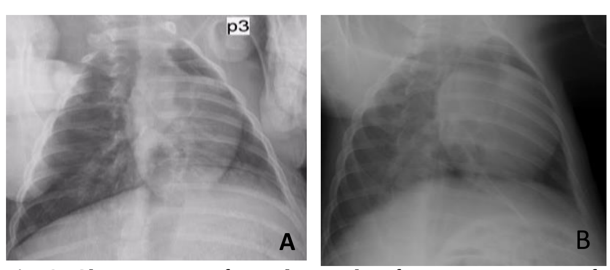

Fig. 2. Chest x-ray performed 2 weeks after reconstruction of the diaphragmatic defect with allograft of: a - peritoneum, b - pericardium.

Both animals in the control group died at the 54th and 60th day, respectively, after the intervention due to the recurrence of the diaphragmatic defect, complicated with strangulation, in both cases being observed, at necropsy, the subtotal resorption of the peritoneum graft (fig. 3).

Fig. 3. The appearance of the hemidiaphragm undergoing reconstruction with decellularized graft of porcine peritoneum that has been resorbed subtotal, generating a persistent defect: 1 - fibro-muscular area; 2 - the limits of the plastic defect; 3 - unformed tissue reminiscent of graft

All animals in groups I and II remained alive, but showed signs of eventualization of the reconstructed hemidiaphragm (fig. 4).

Macroscopic exploration of the hemidiaphragm undergoing reconstruction showed that the development of the event, confirmed radiologically, is determined by the significant thinning of the graft following the regeneration and remodeling processes (fig. 5).

The histological examinations consisted of the evaluation of the process of neoformation of the connective tissue (fibrillogenesis), the presence of the inflammatory process and the reminiscences of the allografts according to the mentioned areas, as well as the evaluation of the activity of the fibroformed connective tissues.

In both lots, during the mentioned periods of sacrificing and examination, the presence of unformed connective tissue with insignificant deviations between lots of study was attested. The results obtained according to the evaluation indices are presented in tab.1.

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Fig. 4. Chest radiography of the laboratory animals from group II performed 60 days (A) and 90 days postoperatively (B). Viewing the event of the left hemidiaphragm

Table 1. Dynamism of normal and malformative fibrillogenesis

Indices	Depending on lots	
	Lot I (n=3)	Lot II (n=3)
Fibrillogenesis		
Mature	2	3
Intermediate (mature and imature)	2	-
Atypically (malformative)	1	1
Inflamation		
Polymorphonuclear (PMN)	2	-
Mononuclear (MN)	3	1
Granulomatous (GM)	2	1
Allograft residues		
In the contact area (graft-recipient)	1	-
In the new-formatted plate	3	-

In the morphological explorations, performed at the level of the hemidiaphragm after the use of pericardial grafted grafts, the presence of regenerative processes and neoformation of the connective tissue in the form of elastic bands, endowed with the vascular network, was found, the suture material persisting only at the border line. A more expressed and ordered differentiation of the aponevrotic conjunctival fascicular layers was observed in the animals in group II.

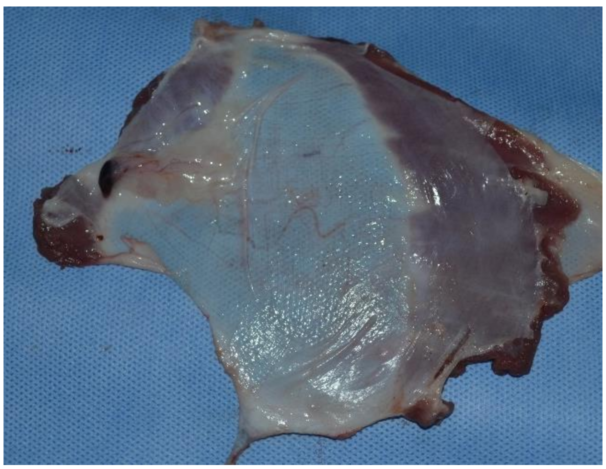

Fig. 5. Macroscopic appearance of the hemidiaphragm undergoing reconstruction with decellularized porcine pericardium.

The presence of the inflammatory process, discreetly marked by the polymorphonuclear inflammation adjacent to the allograft reminiscences, in association with mononuclear was observed, especially of the lymphocyte components which, in the border area with the graft, had the appearance of nodular or pseudofolicular structures. The reminiscences of the graft were circumscribed and of granulomatous inflammation. In the samples taken from the intermediate zone, with a predilection towards the center, in the uncomplicated cases, in both batches there were attested maturations of the newformed tissue in the form of strips with dense and lax collagen fibers, covered with mesothelial peritoneal lining. In areas with adhesive processes, the newformed tissue was much looser, in the form of adhesive bundles. In some areas towards the center, the newformed tissue showed only a conjunctival band with a reduced maximum density of collagen fibers, covered in lot I of focal granules, which occupied up to 95% of its thickness.

In some areas, there was a segmental disorder of the new-formed tissue with the presence of allograft segments, in the form of small islets and plaques, of the polymorphocellular active inflammatory processes and of the lymphocyte component in the nodules, from the host tissues, to the border between the graft and the host. In the presence of the inflammatory process there was a partial dehiscence of the border between the reminiscent graft and the host tissue. The presence of adhesion processes with the pleura and the capsule of the liver induced the disjunction from the beginning of the new-formed tissue plates lined with mesothelium. In 2 cases areas with atypical (malformative) fibrillogenesis were observed, which were evidenced by a multicystic or lacunar-cavity aspect, with internal surfaces covered with unistratified peritoneal mesothelial epithelium flattened without aspects of mitotic activity. The mesothelial cavity-cystic aspect has also been shown on border, in some cavities being present fibro-epithelial conjunctival micro-polyps. The cavity-cyst formation could also be a cystic pseudotumor with thinner septa, with a reduced collagen fiber housing.

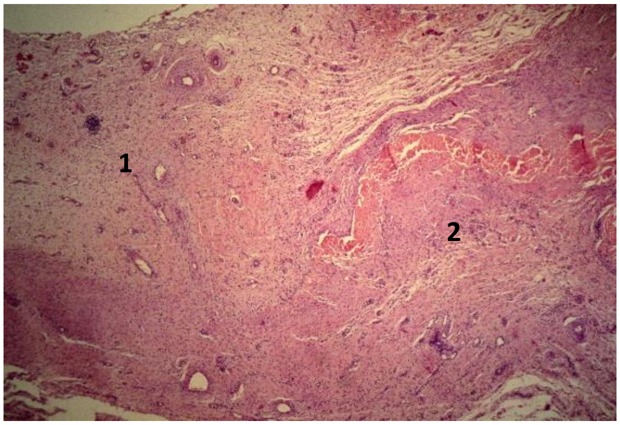

Fig. 6. Strips of newformed tissue of different density and fibroblastic cellular activity: 1 - of lax type; 2 - of dense type. Color. H-E

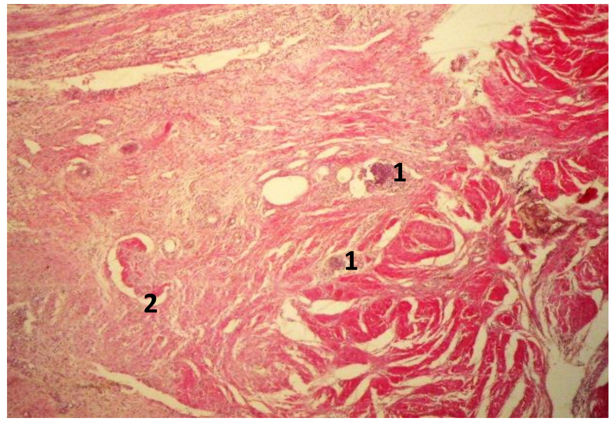

Fig. 7. The graft-host border area with lymphoid follicles (1) and graft reminiscences (2). Color. VG

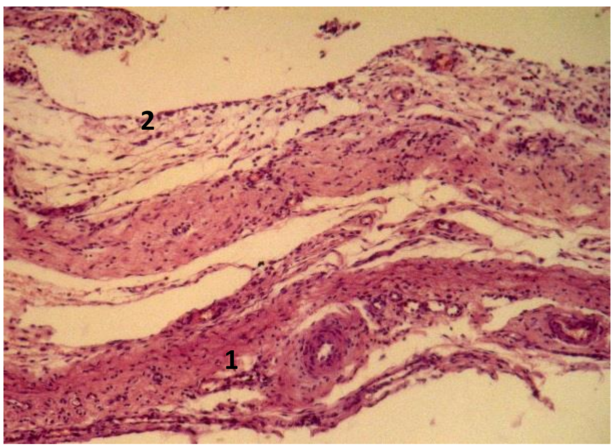

Fig. 8. Strips of fibrillar conjunctival newformed tissue (1) and loose tissue lined with peritoneal mesothelioma (2). Color.

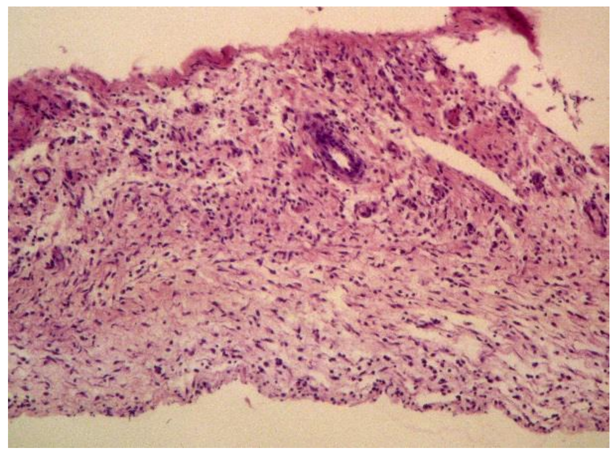

Fig. 9. New-formed tissue in the central area. Color. H-E

Fig. 10. New-formed tissue with focal granulomatous infiltrates. Color. H-E

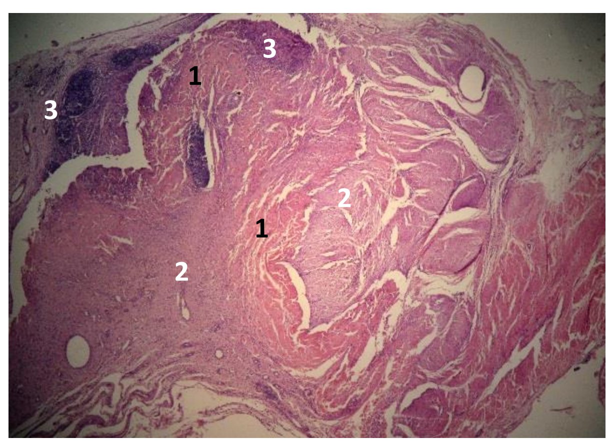

Fig. 11. Allograft residues (1) embedded in new-formed tissue (2) with the persistence of an active polymorphocellular inflammatory process (3) with a pseudofollicular lymphocyte component. Color. H-E

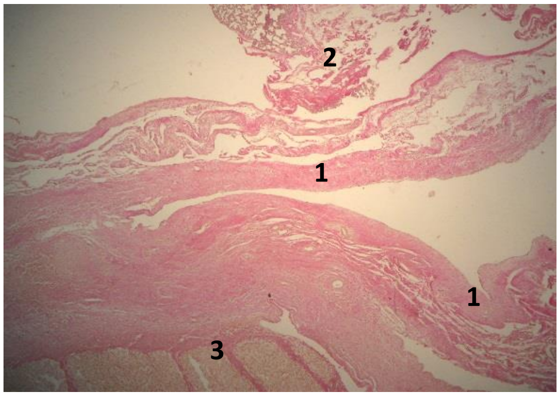

Fig. 12. Dehiscence in 2 connective plates of newformed tissue (1) by retractile-scar aspects with pleura (2) and liver (3). Color. H-E

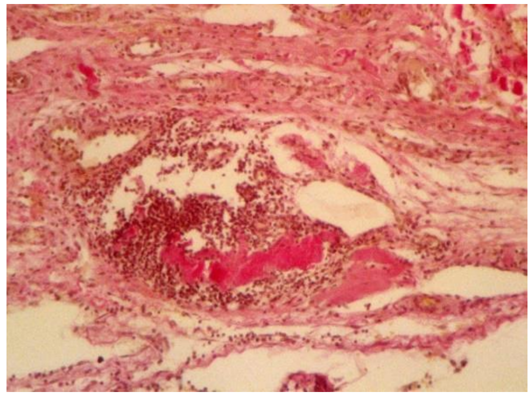

Fig. 13. Reminiscences of allograft with active polymorphocellular inflammatory reaction in the area of newformed tissue. Color. VG

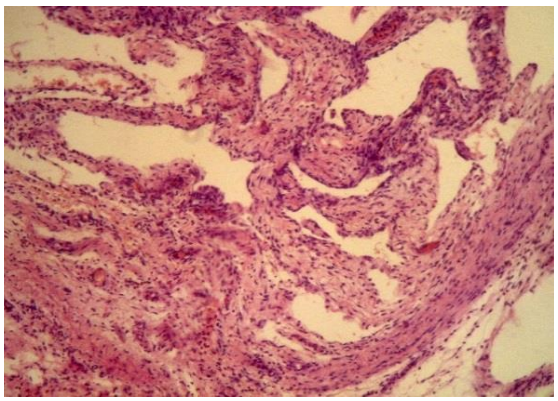

Fig. 14. Atypical-malformative appearance of fibrillogenesis in mesothelium-lined cavity-cystic structures. Color. H-E

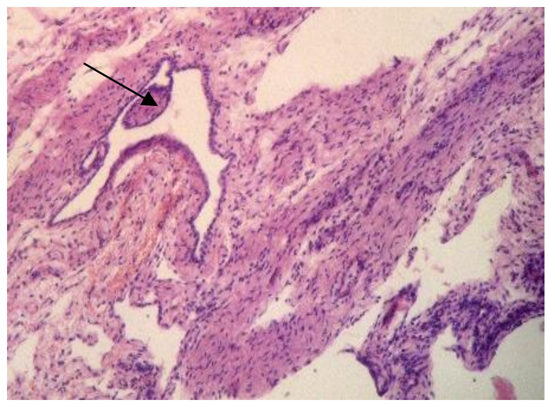

Fig. 15. Cystic structures covered with flaking or viable mesothelium with the presence of micropolyps. Color. H-E

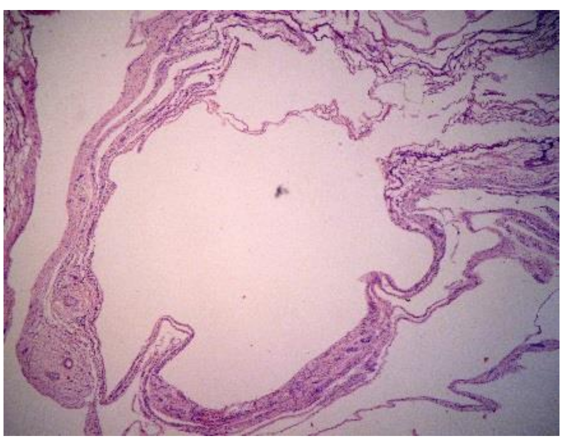

Fig. 16. Multicyctic cavity process with thin conjunctival septum. Color. H-E

Lymphocyte mononuclear cellular elements were present throughout the septa.

The extracellular matrix, derived from decellularized tissue, is increasingly used in regenerative medicine [8]. Decellularized pericardium, by endogenous cell repopulation, has been shown to be an optimal biological material for tissue regenerative processes [30].

The potential benefits of using bovine or porcine pericardial tissue are determined by the accessibility of this biological material, the structural and functional similarity with human tissue, the acceptable immunogenicity and biocompatibility provided by the decellularization process [7, 12]. Recent studies have suggested that porcine pericardium, due to its collagen bundle structure and regenerative properties, may be considered a suitable alternative to bovine pericardium in the treatment of cardiovascular disease [22, 46]. There are experimental studies to test the safety and efficacy of porcine pericardium in duraplasty [41].

In reconstructive operations of diaphragmatic defects, xenopericard grafts are rarely used, with unique cases of bovine pericardium being used in adult or elderly patients [39, 42, 50].

Conclusion

1. Cryopreserved decellularized grafts of porcine pericardium, used in the experimental reconstruction of diaphragmatic defects, have acceptable tissue biocompatibility and greater durability, compared to decellularized bovine peritoneum grafts, but a low bioresistence, after certain periods of time, with mechanical degradation properties and graft formation.
2. The result of this study revealed some morphological aspects of the regenerative-adaptive processes that take place using the porcine pericardial graft and the specific resorption capacities of this biological material, with the non-formation of a plate in which fibrilogenesis processes predominate, at different stages. of maturation.
3. When using the porcine pericardium in the reconstruction of the diaphragmatic defects, a very differentiated fibrilogenesis has been found, in some areas with small deviations, including the presence of a dematurity attested by the variety of collagen fibers and of the cellular fibroblastic component, the latter showing different fibriloblastic activity.
4. The atypical-malformative peculiarities of fibrilogenesis, attested in the mentioned cases, are, in our opinion, a consequence of the fibrilogenesis disorder that could have evolved, following mechanical actions, through dehiscences, gradual ruptures, later with epithelialization through the peritoneal mesothelioma invasion. epithelialization process, accumulation of serous fluids with evolution in hygroma.
5. The obtained results suggest the need for further long-term studies, which would aim to improve the methods of processing this biological material and to obtain more sustainable results.

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