PARTICULARITIES OF TISSUE REGENERATION OF DECELLULARIZED BOVINE PERICARDIUM XENOGRAFTS USED IN THE RECONSTRUCTION OF THE ANTERIOR ABDOMINAL WALL DEFECTS IN THE EXPERIMENTAL MODEL

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ABSTRACT

The purpose of this study was to evaluate the regeneration potential of decellularized bovine pericardium grafts used as an option to close the abdominal wall defects in the experimental model.

The study group consisted of pigs subjected to reconstructive surgery of congenital abdominal wall defects (group 1 - 3 animals) (fig. 1) and total defect of the abdominal wall created surgically (involving all layers, including the peritoneum) with decellularized bovine pericardium (group 2 - 3 animals). Animals were sacrificed and investigated 30 days (3 animals) and 90 days after surgery (3 animals).

he results of this experimental study have allowed us to conclude the following:

- The acellular bovine pericardium graft is characterized by strength and durability, favorable for the reconstruction of major abdominal wall defects.
- In the regenerative-reparative processes of the acellular bovine pericardium grafts used in the reconstruction of the abdominal wall defects together with fibrogenesis processes, there are also processes of metaplasia with differentiation in chondroblasts which induce the neoformation of cartilage foci in the biological implant, which, then functions as an enchondral ossification factor with the formation of trabecular osteogenesis and ossification foci.
- The results obtained imply the need for further studies to clarify the role of these changes in the development of potential follow-up postoperative complications.

Key words: abdominal wall defects; biological grafts; extracellular matrix; bovine pericardium; biocompatibility

INTRODUCTION

Bovine pericardium is a collagen-rich biological tissue widely used as a natural biomaterial in the modeling of a variety of bioprostheses in cardiovascular surgery [4, 6] in reconstructive surgery of soft tissue defects [9] and complex defects of the abdominal wall [2, 5].

The removal of cellular components from the biological grafts while preserving the extracellular matrix allows for better tissue remodeling due to reduced antigenicity, graft degradation processes, host cell recellularization, formation of a new extracellular matrix and neovascularization [7]. Although the use of decellularized biological implants represents a performance in reconstructive surgery, the long-term interaction of these xenogeneic biomaterials with the local tissues and physiological systems of the patient as well as the regeneration processes induced by them are not elucidated. The possibility of predicting biocompatibility and undesirable consequences of these biological grafts can minimize patient`s risk, which determined the up-to-dateness and novelty of the topic being addressed [7, 10].

The purpose of this study was to evaluate the regeneration potential of decellularized bovine pericardium grafts used as an option to close the abdominal wall defects in the experimental model.

MATERIAL AND METHODS

The study group consisted of pigs subjected to reconstructive surgery of congenital abdominal wall defects (group 1 - 3 animals) (fig. 1) and total defect of the abdominal wall created surgically (involving all layers, including the peritoneum) with decellularized bovine pericardium (group 2 - 3 animals). Animals were sacrificed and investigated 30 days (3 animals) and 90 days after surgery (3 animals). To decellularize the bovine pericardium, sterile SDS solution 0.5% (HiMedia) was used, the decellularization efficiency being histologically confirmed. The decellu-

larized grafts were stored in 50% Glycerol (Alchimia) solution with RPMI (HiMedia) at -80 ° C (fig. 2).

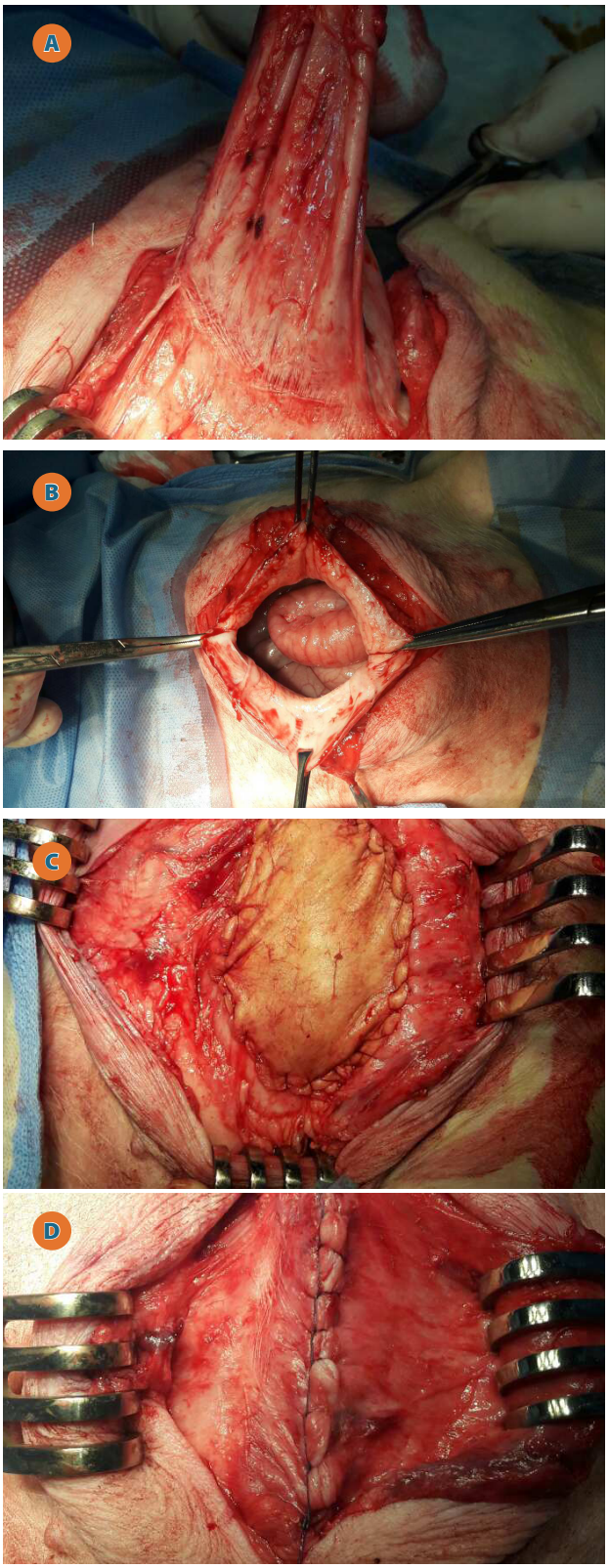

Fig. 1. Stages of experimental surgical reconstruction of the congenital defect of the abdominal wall with decellularized bovine pericardium graft: A - intraoperative aspect of the defect after mobilization; B - excision of membranes; C - closure of the abdominal cavity; D - fixing the implant to the marginal fibrous ring of the defect.

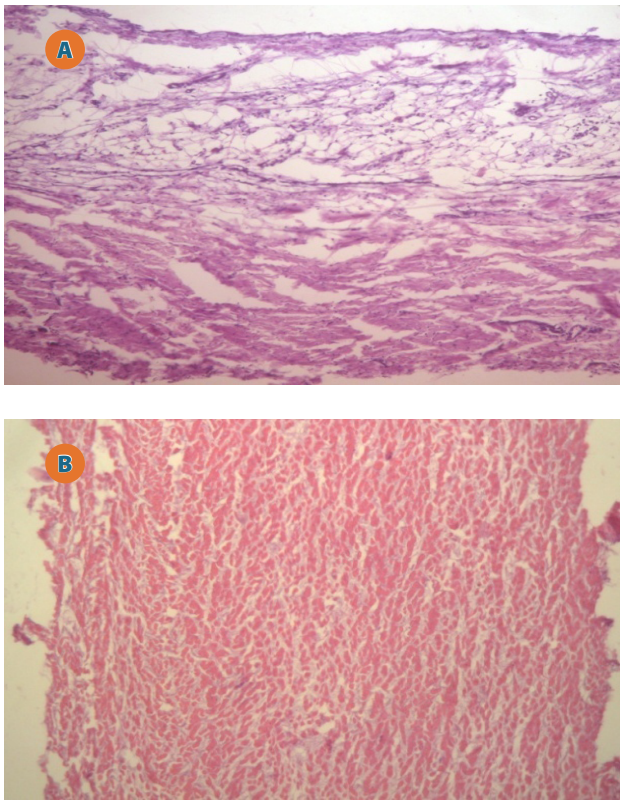

Fig. 2. Histological aspect of the bovine pericardium before decellularization (A), with the preserved differentiated fibrillary connective structure of various density with a marked nuclear-cellular component × 100. VG coloration, and after decellularization (B) with marked bundles of orderly acellular connective tissue among which the elements of the extracellular matrix are revealed. × 25. HE coloration

RESULTS AND DISCUSSION

In study group 1 there were neither postoperative complications, nor incisional hernias of the anterior abdominal wall. In all 3 cases postoperative wounds were primarily scarred (fig. 3A). After the mobilization of the external surface of the bovine pericardium graft area, no lumps were found, the area being fairly compact in the account of the fibrous tissue on which adipose tissue developed (fig. 3B). There were no adhesions at the opening of the abdominal cavity (fig. 3C). At macroscopic examination, the graft presented as a deformed and corrugated flap of pale brown color, more accentuated at the margins, comprised by connective tissue, which is more pronounced from the peritoneal part (fig. 3D).

In study group 2 complications were not recorded either, the postoperative wound healing primarily (fig. 4A, B). At the opening of the abdominal cavity, the left lobe of the liver was adhered to the operative incision region (2 cases), in all 3 cases the omentum was adhered intimately to the internal surface of the implant. No inter-intestinal adhesions were observed in the abdominal cavity (fig. 4C). After the omentum detachment from the

internal surface of the implant, some hemorrhage dots were observed (ig. 4D).

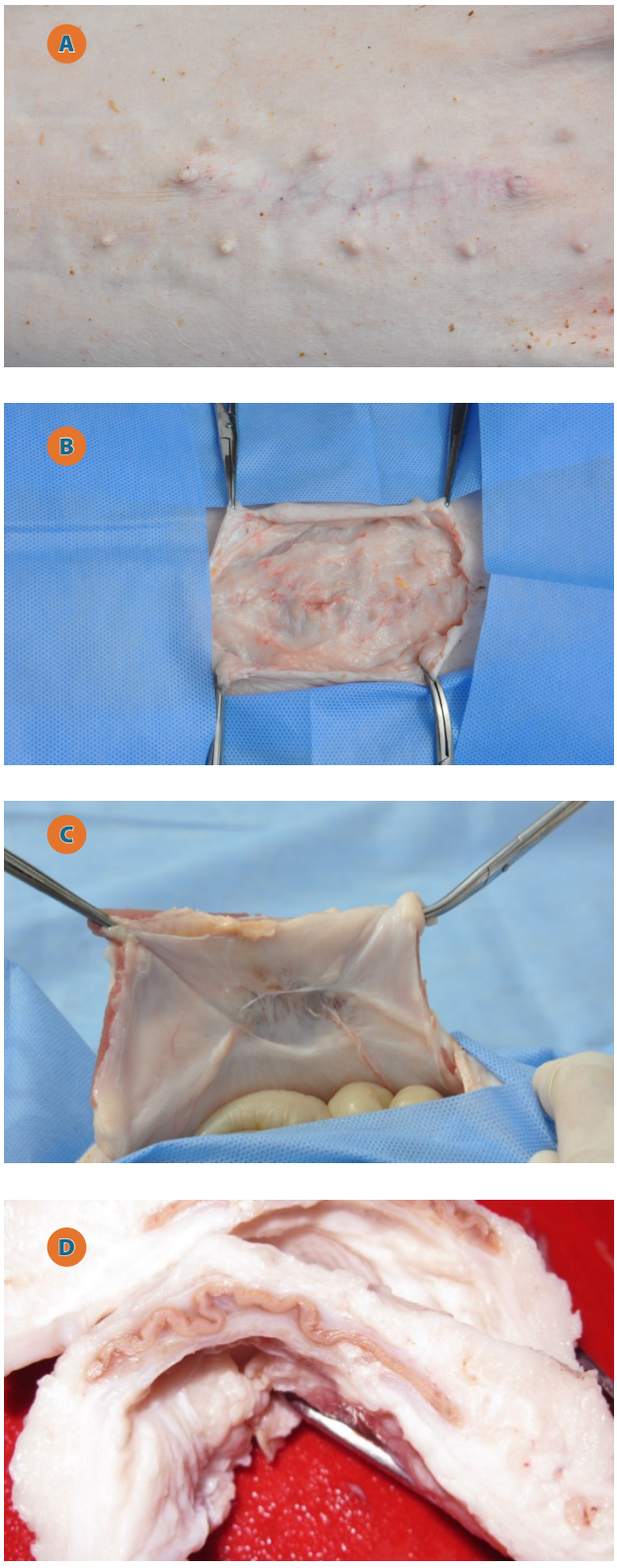

Fig. 3. A - appearance of the postoperative scar; B - macroscopic appearance of the external area of the bovine pericardium implant region; C - macroscopic aspect of the peritoneal surface of the bovine pericardium implant region; D - implant region (macropreparate): 1 - implant graft; 2 - internal peritoneal connective area; 3 - external cellular-adipose connective tissue.

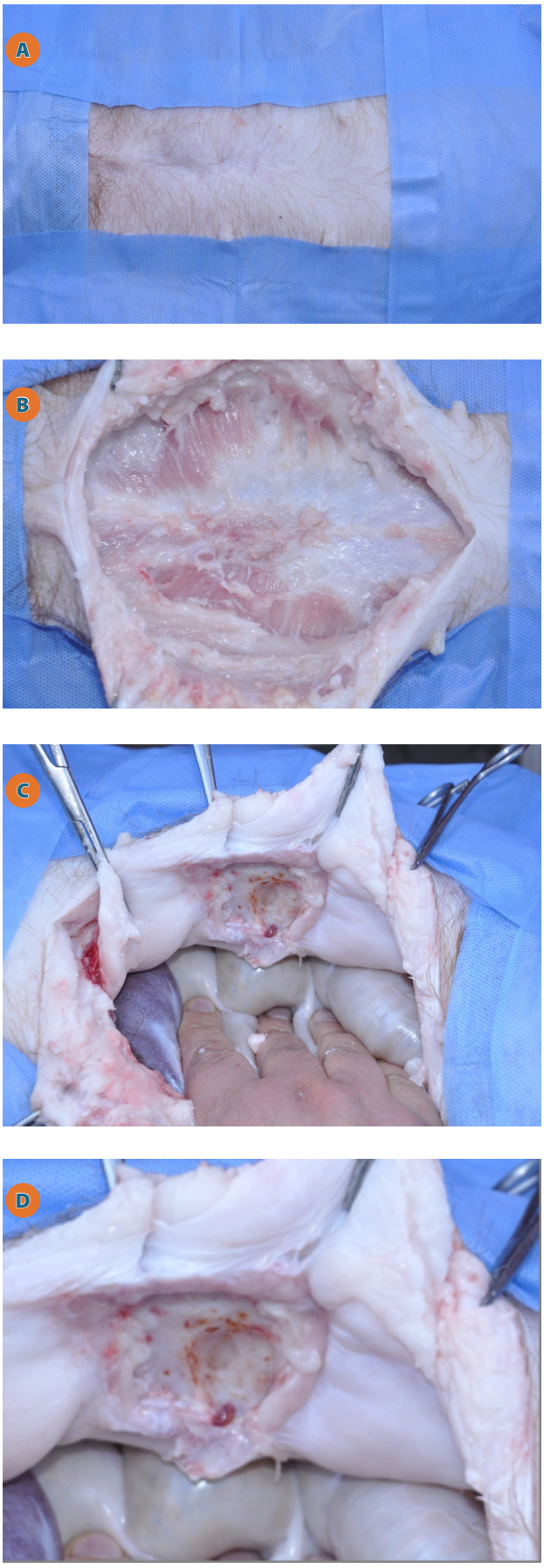

Fig. 4. Macroscopic appearance of the acellular bovine pericardium graft area during animal`s sacrifice (explanations in the text)

After the circular resection of the implant application area, the macroscopic examination revealed that the external surface was dominated by elastic hard fibrous tissue (fig. 5A). The internal surface of the implant represented a tough, trabecular plate, with small hemosiderosis foci. At the periphery there was an incomplete bead of variable thickness (0.2-0.6 cm) and hard (bone) consistency corresponding to the suture line boundaries, 0.5-1.3 cm wide (2 cases), or even 2.3 cm (1 case) (fig. 5 B). In section, the implant area was 1.7 to 2.5 cm thick at the bead level it had sponge-like appearance (fig.5 C).

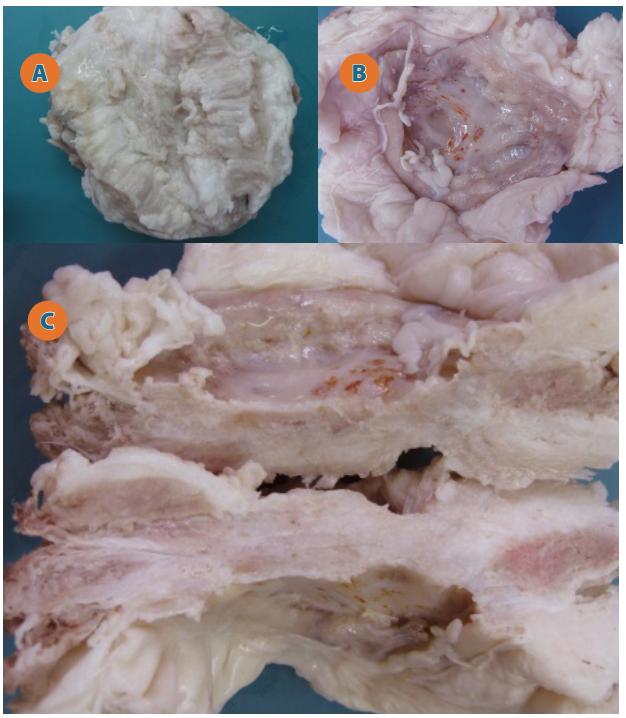

Fig. 5. Macroscopic aspect of the resection piece of the acellular bovine pericardium graft area; A - external surface: 1 - fibrous-connective plate; 2 - muscle tissue; B - the ossified bead-like internal surface; C - implant in perpendicular section: 1- fibrous-connective plate; 2 - osteogenic area in the bead; 3 – osteocartilaginous area in the plate; 4 - muscle tissue.

The results of the histological examination in study group 1 allowed to find that the graft ends were invaded by vascularized fibrous connective tissue. At the boundary between the graft and the proper tissue, the presence of an invasive focal or zonal fibrillar-cellular reaction, with the predominance of fibroblasts of the neoformed connective tissue and penetration in different ratio at the edges of the graft, is observed (fig. 6A). At this level, granulomatous changes, especially of the peritoneal area, of various dimensions, with polynuclear cellular symplasts, some with necrosis in the center, and others with calcinosis, were also revealed. Frequently, there were graft-like tissue fragments in the granuloma area (fig. 6 B, C).

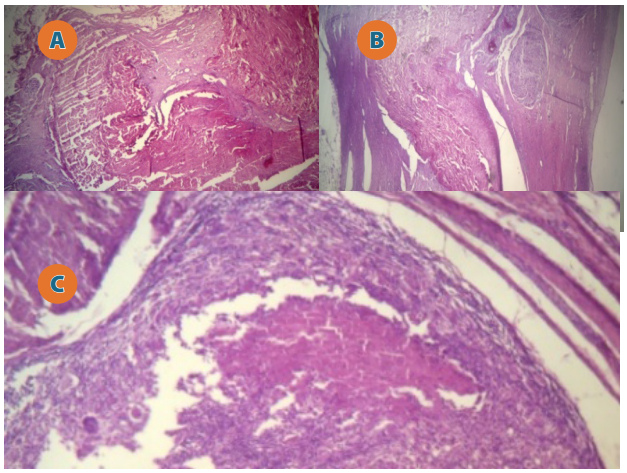

Fig. 6. A - border region: 1 - implant graft; 2 - internal peritoneal connective area; 3 - external connective cellular-adipose area; B - marginal region: 1 - implant graft; 2 - granulomas of polynuclear giant cellular symplasts, with calcinosis; 3 - neoformedfibrillary connective tissue; 4 - proliferative penetration zone of the cellular-fibrillary component of the implant; C - granuloma structure: 1 - necrotizing elements in the granuloma area; 2 - granulomatous tissue of giant cellular symplasts, macrophagic-lymphocytic and fibrillary-connective component.

At the level of active interaction of the neoformed host tissue with the implant graft degeneration area such as hyaline drop-like dystrophy was attested on the graft region, associated with necrolytic degeneration and invasion of the fibroblastic cellular component (fig. 7).

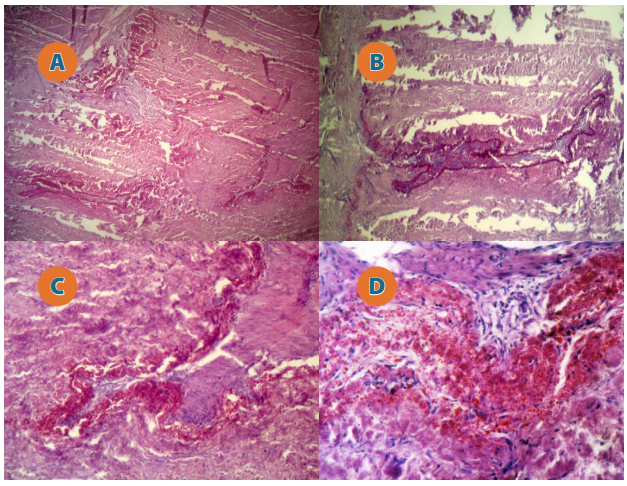

Fig. 7. A - aspects of tissue interaction: 1 - implant graft; 2 - fibrillary cellular connective tissue penetrating into the graft area. B - A sequence at higher magnification: 1 - implant graft; 2 - fibroblastic fibrillary-cellular connective tissue strips penetrating into the graft; 3 - dystrophy; C - interaction area between the graft and the neoformed connective tissue: 1-implant graft; dystrophy zone in the hyaline drop; 2 - invasion of cellular fibrillary connective tissue; D - aspects of degeneration in the hyaline drops of the graft with fibroblasts penetration: 1 - degeneration in graft hyaline drops; 2 - penetration of fibroblasts into the area of degeneration

Concomitantly with the fibroblastic proliferative activity, the presence of lymphocytic and monocytic elements, macrophages, including proliferation of the vascular endotheliocytic component, was attested. The implant fibers underwent intumescent changes (fig. 8 A). At this level, in some areas, a varying intensity of the polymorphic cell component was observed, with the presence in different ratio of polynuclear giant cellular symplasts with phagocytic embedding aspects of the hyaline droplets (fig. 8 B, C, D)

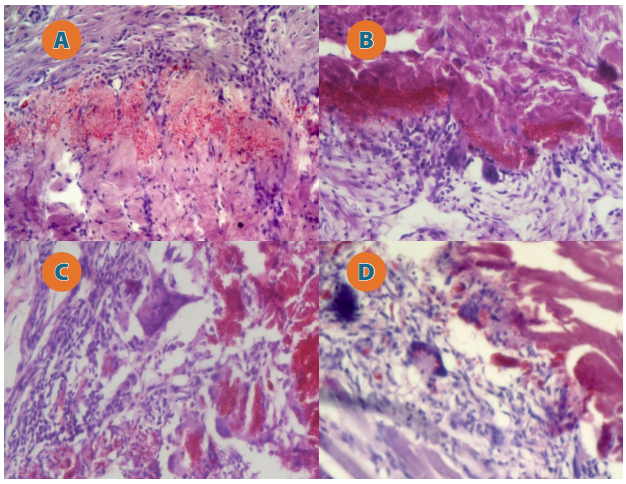

Fig. 8. A - graft-host tissue interaction: 1 - intumescent graft and hyaline degeneration; 2 – fibro-cellular and mixed cellular fibrillary proliferative penetrating processes; B – giant cellular reaction at the tissue intersection line; C - hyaline degeneration foci invaded by polymorphic cellular component with the formation of giant cellular symplasts of macrophagic origin with hyaline incorporation; D - macrophagicsymplasts with polynuclear giants lined with hyaline droplets.

The results of the histological examination, performed in all three cases of the decellularized bovine pericardium graft use in the reconstruction of total abdominal wall defects, including the peritoneal wall (lot 2), revealed that the inflammatory cells practically disappeared 90 days after surgery. At the same time, marked proliferative processes of fibrous tissue with chaotic orientation, with or orientation in connective cords were revealed.

In all three cases, there could be revealed characteristic foci of cartilaginous and bone metaplasia manifested by hypercellularized sections with the presence of fibroblasts and some islets of immature chondric and trabecular tissue, trabecular bone tissue patches in different proportions in the area between the graft and host tissues, sometimes they could also be observed in central areas (fig. 9).

At the bead level and in the underlying area of the bovine pericardium graft, in one case, there were osteogenic mature osteoid plates with random trabecular appearance with diminution of osteoblasts at the trabecular and peri-insular level, where the connective tissue became denser, forming a periosteal strip (fig. 10A). In 2 cases, pathological neoformed tissue was present in some places as an osteo-cartilaginous component (fig. 10B) or sometimes as a trabecular osteogenic plate that could reach in different ratio the central area of the xenogeneic implant (fig. 10C), the intertrabecular fields being lined with acellular adipose tissue. In the central areas of the implant, connective tissue platelets with chaotically oriented cords of collagen bundles in combination with mesenchymal tissue (fig. 10 D, E, F) were frequently observed.

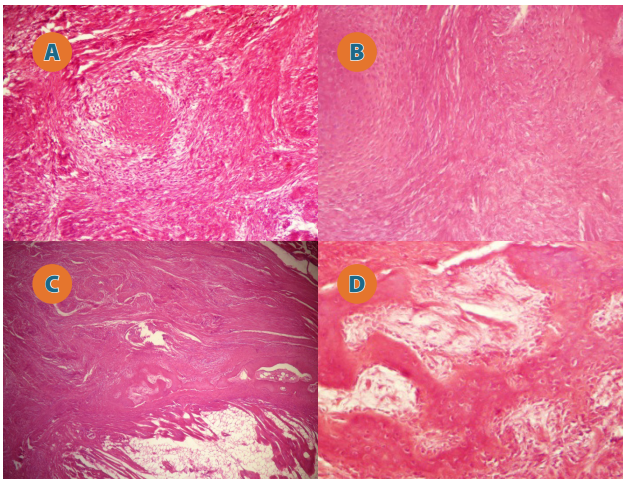

Fig. 9. A - immature mesenchymal cartilage islet in fibroblastic connective tissue mass located 2.5 cm from the border between tissues; B - immature mesenchymal cartilage islet in the connective tissue mass (1) and immature trabecular bone plate (2) in the adjacent area of the bead at the borderline; C - 1.2 cm area from the bead where immature trabecular bone plates are located at the border with the host tissue; D - immature trabecular osteogenic island partially surrounded by cells of osteoblastic (1) and fibroblastic (2) origin.

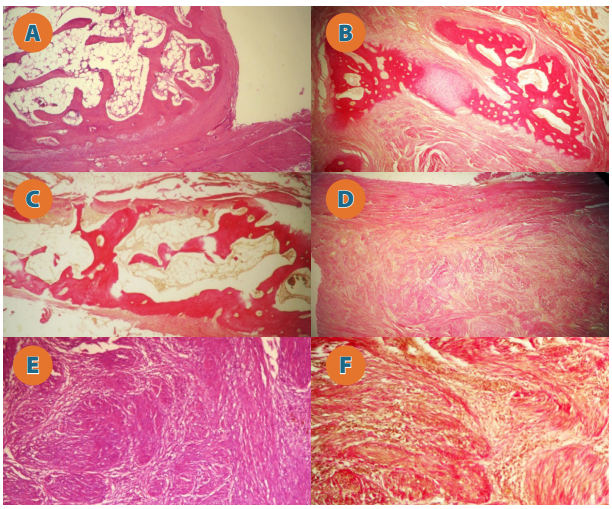

Fig. 10. A - ossified bead area with diminished osteoblasts activity; B - mature osteocartilaginous structures located in the mass of the neoformed connective tissue; C - osteogenic plate in the medial area with periosteum and cellular-adipose tissue; D - neoformed connective plate with chaotically oriented collagen fibers in various ratio; E - neoformed connective tissue cords with active and proliferative aspects of fibroblasts; F - neoformed connective tissue cords with collagen fibers in combination with mesenchymal tissue.

In 2 cases, hemosiderosis foci were observed in the biopsies taken from the central areas towards the internal (peritoneal) surface (fig. 11A). In some sectors, corresponding to the lacunar aspect, a stratification of the connective tissue from the peritoneum was noted, marking a lax aspect with spaced neurovascular bundles, while on the external surface it was much denser with proliferative aspects of fibroblasts (fig. 11B).

In some samples from the central area, lax connective tissue plates were observed in the dense area, at the periphery with lymphocyte infiltrate, sometimes with the neoformed pseudofollicular structures and the presence of hemosiderosis (fig. 11C). In all cases, at a distance of up to 2.2 cm from the borderline, the peritoneal tissue consisted of mature connective tissue, limited in various ratio by fatty tissue. (fig. 11D).

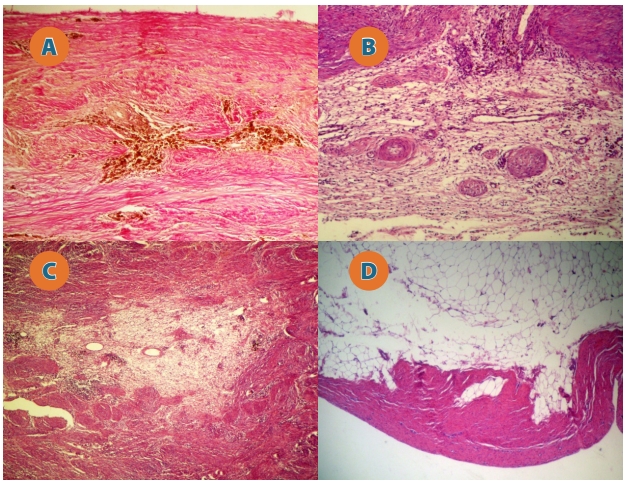

Fig. 11. A - medial area with micro-macrofocal hemosiderosis; B - connective tissue towards the peritoneal and compact area towards the external surface, spaced neurovascular bundles; C – lax mesenchymal connective tissue plates penetrated by vascular network; D - structural aspect of the peritoneum at distance.

In spite of the relevant developments in the use of decellularized bovine pericardium grafts, some complications such as biological matrix deterioration and tissue degeneration due to implant calcification have been identified [3]. Our findings are consistent with the data published by some authors who found the development of osteocartilaginous metaplasia foci in the late postoperative period when using decellularized bovine pericardium xenografts preserved in glutaraldehyde in reconstructive surgery of the major vessels in experimental animal studies [1, 8]. Chondroid and / or bone metaplasia is considered by some authors to be a reversible change involving fibroblasts and myofibroblasts, ischemia being one of the causes [1].

Thus, the results of this experimental study have allowed us to conclude the following:

- The acellular bovine pericardium graft is characterized by strength and durability, favorable for the reconstruction of major abdominal wall defects.
- In the regenerative-reparative processes of the acellular bovine pericardium grafts used in the reconstruction of the abdominal wall defects together with fibrogenesis processes, there are also processes of metaplasia with differentiation in chondroblasts which induce the neoformation of cartilage foci in the biological implant, which, then functions as an enchondral ossification factor with the formation of trabecular osteogenesis and ossification foci.
- The results obtained imply the need for further studies to clarify the role of these changes in the development of potential follow-up postoperative complications.

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