The Heart Surgery Forum #1999-16834
2 (1):60–69, 1999

Online address: www.hsforum.com/vol2/issue1/1999-16834.html

Bovine Pericardium Used as a Cardiovascular Patch

(#1999-16834)

Dr. Pires

Adilson Casemiro Pires, Wladimir Faustino Saporito, Luís Eduardo Villaça Leão, Vicente Forte, Sigmar Horst Cardoso, Osiris Ramaciotti

Paulista Medical School, São Paulo Federal University and ABC Medical School, São Paulo, SP, Brazil

ABSTRACT

Background: The use of glutaraldehyde preserved bovine pericardium in valvular prostheses is well known. Although widely used clinically as patch material, bovine pericardium has not been extensively studied in this setting.

Methods: With this objective, 21 dogs received a standard bovine pericardial patch to partially replace the aortic, left atrial, or pericardial walls. The dogs were randomly divided into three groups according to implant duration. Group 1 consisted of 6 dogs evaluated surgically after 33 to 43 postoperative days, Group 2 with 7 dogs reoperated after 120 to 165 days, and Group 3 with 8 dogs reoperated after 225 to 305 days.

Results: Microscopic and macroscopic evaluation demonstrated: 1) the wrinkled surface of the bovine pericardium adhered to neighboring structures whereas the smooth surface did not adhere to the epicardium; 2) the bovine pericardial patch did not show structural changes in any of the implant sites; 3) the final left atrial patch was significantly smaller than the aortic and pericardial patches for Group 2 and Group 3 dogs; 4) the atrial patch area decreased significantly, whereas the aortic and pericardial areas did not change over time; 5) the pericardial implant was significantly thinner than the aortic and left atrial patches for Group 3 dogs; 6) a layer of fibrous connective tissue was formed on the smooth surface of the left atrial and aortic patches. The internal apposition fibrasis was significantly thicker in the left atrium than in the aorta in Groups 1 and 2; 7) the internal fibrasis layer of the atrial and aortic patches was calcified in Groups 2 and 3; and 8) the internal apposition tissue of the atrial and aortic patches showed neoformation of elastic fibers which clearly increased with implant duration.

Conclusions: The fate of implanted glutaraldehyde-preserved bovine pericardial patches in cardiovascular applications depends on three factors: 1) the contact surface, 2) the tension it is subjected to, and 3) contact with blood flow.

Address correspondence and reprint requests to: Adilson Casemiro Pires, Av. George Corbisier, 845 – Cj. 204, Jabaquara, São Paulo – SP, Brazil, CEP: 04043-000

INTRODUCTION

Glutaraldehyde preserved bovine pericardium is one of the most useful biological patches in cardiovascular surgery. It has been used for the correction of many cardiac abnormalities such as the replacement of atrial, ventricular, arterial and venous structures and in the repair the pericardial sac [Cliff 1973, Gervin 1975, Ionescu 1977, Braile 1981, Gallo 1982, Braile 1983, Revuelta 1983, Lukács 1984, Yakirevichs 1984, Abdulai 1985, Crawford 1986, Mills 1986, Arnoni 1987, Cochran 1987, Moraes 1987, Bongiovani 1988, Casagrande 1988, Évora 1988, Moraes 1988, Souza 1988, Barbero-Marcial 1989, Dallan 1989, Eng 1989, Gabbay 1989, Loures 1989, Moraes 1989, Palma 1989, Pieracciani 1989, Wanderley 1989, Braile 1990, Ribeiro 1990, Braile 1991, Salles 1991, Leão 1992]. Although widely used, there are few reports of clinical failures resulting from its use. Malfunctions have been reported when used in the atrium, in Mustard operations [Couchran 1987], the Senning operation [Bullaboy 1989], and as a pericardial substitute [Skinner 1984, Gallo 1985, Bortolotti 1986, Gabbay 1990]. This result is opposed to the results obtained when used as valvular prosthesis, especially calcification. This difference and the experimental studies by Gabbay et al. [Gabbay 1990] and Bortolotti et al. [Bortolotti 1986] have lead us to further investigate the cardiovascular applications of this material. Therefore, the objective of our study was to experimentally investigate the biologic fate of glutaraldehyde-preserved bovine pericardial patches implanted into the left atrium, aorta, and pericardial sac.

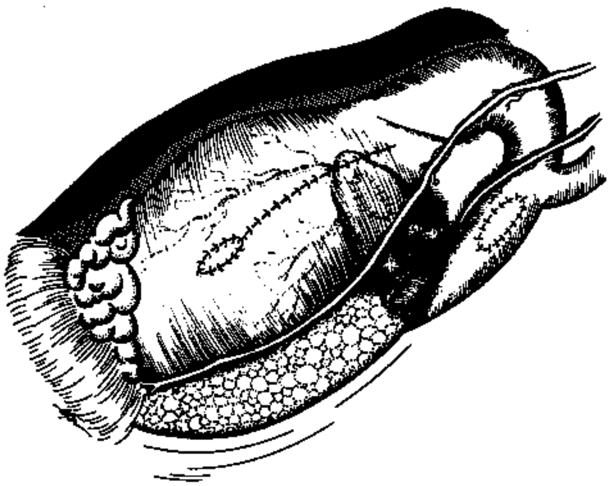

Figure 1. Illustration showing the operation with the atrial, aortic, and pericardial patches.

MATERIALS AND METHODS

Twenty-one adult dogs with undefined breeds and weight ranging from 11 kg to 35 kg were utilized for this study. Standard, commercially available glutaraldehyde-preserved bovine pericardial patches were cut according to a specific shape and were implanted in these experimental animals. Identical-sized elliptical patches were constructed with a long axis dimension of 1.5 cm and a short axis dimension of 1.0 cm. These elliptical patches were implanted to partially substitute for surgically created defects in the left atrial wall, thoracic aorta, and native pericardial membrane.

All surgeries were carried out through a small left thoracotomy with the animals under general anesthesia with orotracheal intubation and controlled mechanical ventilation. The recipients' native pericardium was opened parallel to the phrenic nerve and the left atrial wall was tangentially clamped with a Derra vascular clamp. A 1.5-cm long atriotomy was performed. The opening was then closed using the patch material sutured to the edges of the defect with continuous polypropylene 5-0 stitches. The smooth surface of the pericardial patch was always aligned facing the interior of the left atrium. The same procedure was used in the descending aorta. Then the majority of the animals' native pericardial sac was closed with separate cotton 2-0 stitches. An opening of 1.5 cm was left in the pericardiotomy near the diaphragm which was closed with the bovine patch using continuous polypropylene 5-0 stitches keeping the smooth surface facing the interior of the pericardium (Figure 1).

All dogs were transferred to a kennel where they received humane postoperative care and feeding. They were reoperated at different time intervals. After recording a detailed description of the visual findings at reoperation, the animals were sacrificed using an intravenous injection of potassium at 19.1%. Pieces of their respective patches were removed and stored in formaldehyde solution.

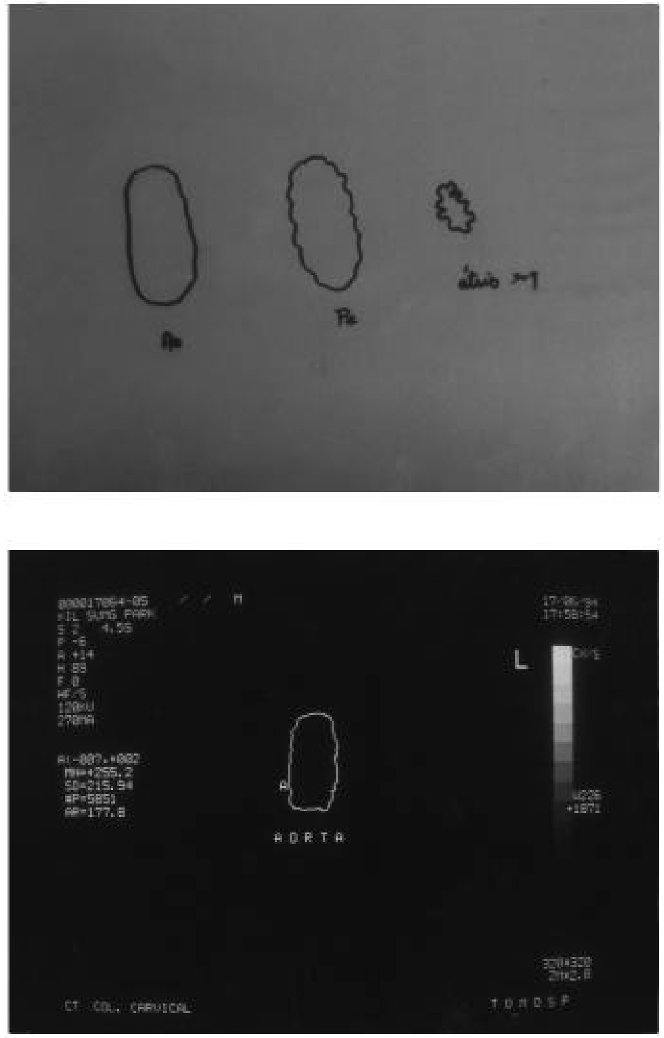

Figure 2. A) Overhead projector image marking patch borders; B) Tomography image showing the measurement of the aortic patch area.

In the macroscopic study, adherence of the patch to the surrounding structures was subjectively evaluated. Depending on the difficulty of obtaining a cleavage plane between the structures, we recorded a 0, +, ++, and +++ for no adherence, minimal, moderate or intensive adherence, respectively. In addition to adherence, the size of each patch was taken into consideration. For this evaluation, the patch area was maintained with a photocopy of each piece. The patch borders, seen from the interior of the left atrium, the aorta and pericardium were established. They were transferred to an overhead projector image and the areas were calculated by computerized tomography (Figure 2).

For the microscopic study, pieces of retrieved patch material were blocked in paraffin, subjected to 16 intercalated cuts of 5 to 8 microns, and then stained with Hematoxylin-eosin, Masson trichome or by Verhoeff's method.

To evaluate patch performance, the dogs were divided into 3 groups according to implant duration as follows: Group 1 included 6 dogs reoperated and sacrificed

Table 1. Identification and quantification of adhesions in smooth and wrinkled surfaces of atrial, aortic and pericardial patches.

				Wrinkled Adherence			Smooth Adherence
DOG	GENDER	WEIGHT	POD[1]	Ao[2]	LA	Pe[4]	Pe[4]
1	F[5]	25.4	160	+	++	+	0
2	F	23.0	153	+	++	+	0
3	M[6]	17.5	040	+	++	+	0
4	M	35.0	175	+++	++	+	++
5	M	13.8	305	+	+++	+	0
6	M	14.3	298	++	++	+++	0
7	M	13.5	121	+	+	++	+
8	M	10.3	298	++	++	+	0
9	M	16.7	289	++	++	++	0
10	M	20.0	136	+	++	+++	0
11	M	17.2	225	+	++	++	0
12	M	11.4	120	+	++	+	0
13	M	17.0	284	+	++	+	0
14	F	13.9	282	+++	+++	++	0
15	M	21.7	302	+	++	+++	0
16	M	22.0	147	++	+++	+++	0
17	F	14.3	043	+++	+	+++	+++
18	M	12.5	041	+	+	++	+
19	M	11.7	033	+	++	+	0
20	M	21.0	033	+	+	+	+
21	F	18.6	033	++	++	+	0

[1]Postoperative Day; [2] Aorta; [3] Left Atrium; [4] Pericardium; [5] Female; [6] Male.

between postoperative days 33 and 43. Group 2 included 7 dogs reoperated and sacrificed between 120 and 165 postoperative days. Group 3 included 8 dogs reoperated between postoperative days 225 and 305.

Analyses for nonparametric tests were used, taking into consideration the parameters or the variability of the measurements carried out. The following tests were used:

1) Friedman's variance analysis, to compare the parameter values in the left atrium and pericardium. Groups 1, 2 and 3 were separately analyzed. When a significant difference was observed, the multiple comparison test was done.

2) Kruskal-Wallis variance analysis was used to compare the three groups according to the parameters established. The left atrium, aorta and pericardium were separately analyzed. When a significant difference was observed, the multiple comparison test was done.

3) Wilcoxon's test for two non-independent samples was used when the thickness of the internal fibrotic layer of the left atrium and the aorta were compared.

For all tests, the rejection level for the null hypothesis was 0.05 (alpha $\leq$ 0.05), and significant values were marked with an asterisk.

RESULTS

Early Results

All dogs tolerated the surgery. There were no intraoperative deaths. The presence of atrial fibrillation was a common occurrence during atrial manipulation which usually returned to normal sinus rhythm spontaneously after left atrial declamping.

The bovine pericardium proved to handle easily and permitted polypropylene sutures to be passed without resistance. In addition to the identification of the incision borders its malleability allowed good coaptation to the structures to which it was sutured, giving an excellent hemostatic result. Blood loss was minimized by the impermeability of the material.

One dog (4.7%) was reoperated due to total dehiscence of the operative wound. Three (14.2%) developed operative wound infection.

Reoperation and sacrifice

Macroscopic appearance: Adherence to the wrinkled surface of the patch was observed in all cases in each group. The patches implanted in the aorta and pericardium adhered to the lung, whereas the atrial patches adhered to the parietal pericardium. A different result was observed on the smooth surface of the bovine pericardial patch implanted in the native pericardium. It was free of adherence in most cases (Figure 3 and Table 1).

The pericardial and aortic patches had clear borders, with relatively preserved thickness, whereas the atrial patch was thickened with unclear borders and had a rough aspect (Figure 4). The measured surface areas of the atrial patches were smaller than for the aortic and pericardial patches in all groups. However, this difference was not statistically significant for atrial and pericardial patches in Group 1 animals (Table 2). Kruskal-Wallis analysis showed the atrial patches decreased in size over time whereas the aortic and

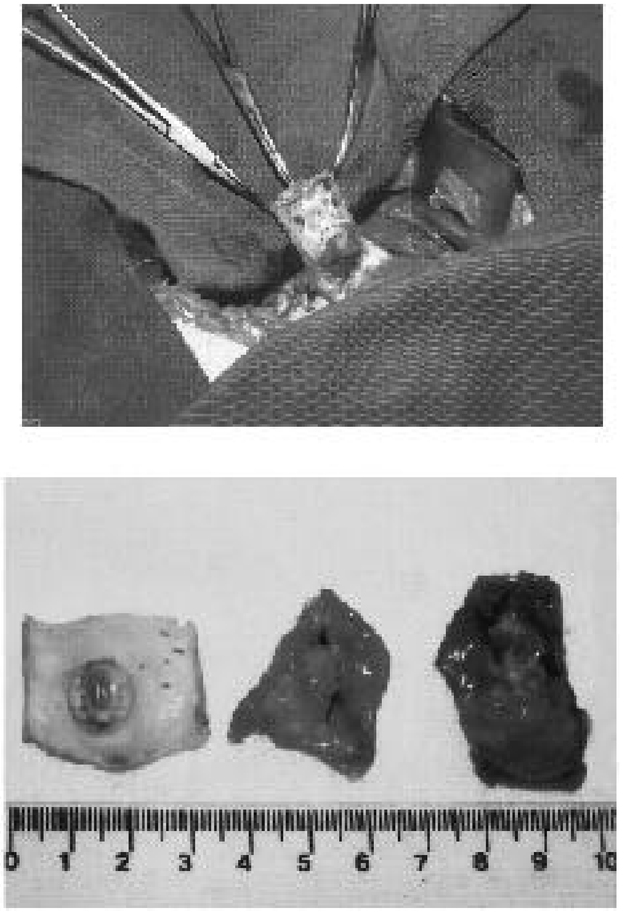

Figure 3. A) Atrial patch; B) Aortic, pericardial, and atrial patches.

pericardial patches were unchanged over time. Two dogs (numbers 7 and 10) showed large thrombi adherent to the atrial patch surfaces.

Microscopic appearance: Histologic testing did not show alterations in the structure of the bovine pericardial patch in any of the 63 pieces studies. The explanted patches were made up of dense fibroelastic collagen connective tissue with parallel and compact fibers. No degenerative, inflammatory or metaplastic reactions were observed in the patches themselves. The patch was straight or mildly bent when implanted in the pericardium or aorta, whereas it was undulated or folded in the left atrium (Figure 4).

The patch was connected to the surrounding structures by a thin covering of collagen connective tissue which also involved the sutures. The patch, the aorta, and pericardium were coapted by apposition or mild superposition of the borders (Figure 5).

In patches implanted in the native pericardium, the regeneration of mesothelial cells in the coaptation zone and the presence of pulmonary tissue or adipose tissue adhered to the wrinkled face were observed in addition to the integration tissue.

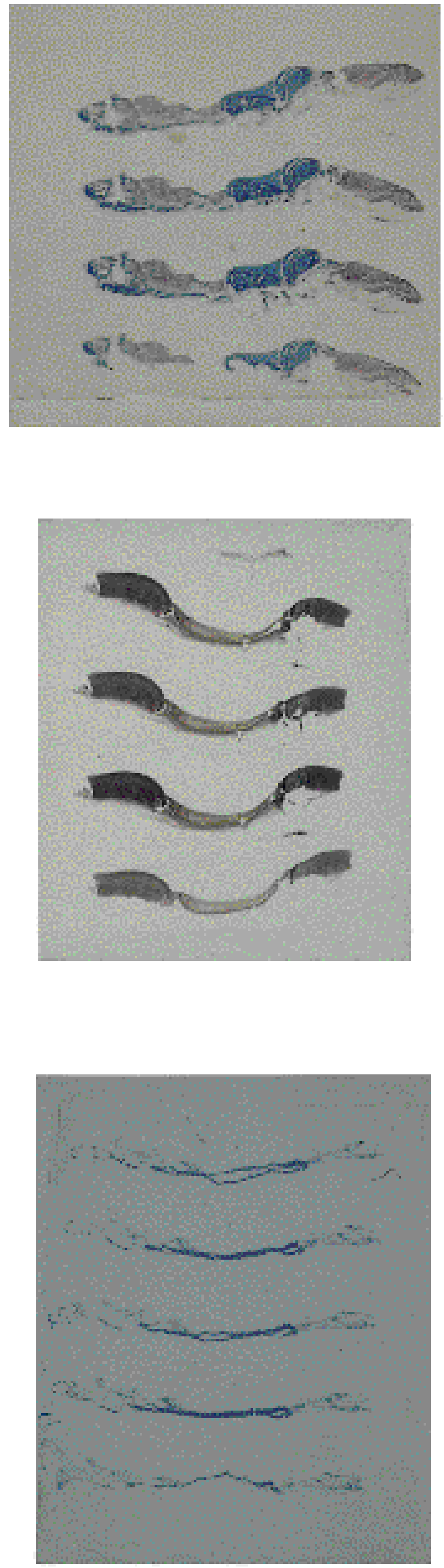

Figure 4. A) Atrium. Structural continuity between the patch (center) and the atrial wall; magnified 2x Masson trichome; B) Aorta. Structural continuity between the patch (center) and the aorta; magnified 2x Verhoef;C) Pericardium. Bovine pericardial patch with free smooth surface; magnified 2x Masson trichome.

Table 2. Groups 1, 2 and 3 dogs, according to area values, in mm^2, of the aortic, atrial and pericardial patches.

Group 1			Group 2			Group 3		
Ao[7]	LA	Pe[9]	Ao[7]	LA	Pe[9]	Ao[7]	LA	Pe[9]
63	12	48	47	21	76	53	03	40
49	21	49	62	23	51	62	10	48
43	25	71	70	50	50	55	14	56
64	46	57	45	16	64	71	29	43
64	36	59	58	24	64	44	20	42
63	37	62	60	30	83	42	08	80
			54	29	66	58	20	60
						75	05	52
M=57.7	29.5	57.7	56.6	27.6	64.9	57.5	13.6	52.6

FRIEDMAN' s Variance Analysis
(Aorta x Atrium x Pericardium)
critical x 2r = 5.99

Group 1	Group 2	Group 3
cal x 2r = 10.17 multiple comparison test Ao > atrium	Cal x 2r = 9.85 multiple comparison test Ao and pericardium > atrium	cal x 2r = 12.58 multiple comparison test Ao and pericardium > atrium

KRUSKAL–WALLIS' s Variance analysis
(GROUP 1 x GROUP 2 x GROUP 3)
critical H = 5.99

Aorta	Atrium	Pericardium
critical H = 0.39	critical H = 8.22 multiple	critical H = 4.62

Multiple comparison Test
Group 1 = Group 2 = Group 3

M=Mean; [7]Aorta; [8]Left atrium; [9]Pericardium.

The bovine pericardial patches implanted in the left atrium and in the aorta showed a different result than the patch implanted in the native pericardium. The smooth surface in contact with the circulatory flow showed a deposition of fibrous connective tissue. This tissue, called internal apposition fibrosis (Figure 6) showed a different result between the left atrium and the aorta. It was thicker in the left atrium than in the aorta. This difference in thickness was statistically significant for Groups 1 and 2 (Table 3). Calcification was observed in the internal apposition fibrosis and was worse in the left atrium. Neoformation of elastic tissue was observed in the aorta and was not observed in the left atrium. Both the calcification and the elastic neoformation showed a clear relationship with implantation duration (Figure 7 and Table 4).

DISCUSSION

Bovine pericardium was introduced into cardiovascular surgery by Ionescu et al. in 1972 and introduced into Brazilian surgical practice by Braile et al. in 1983 [Ionescu 1972, Braile 1983]. Although its performance in the setting of fabricated bioprosthetic valves is well known [Ionescu 1977], it has not been extensively studied as a vascular patch. The widespread use of pericardial patches is contrasted by the small number of experimental studies verifying its effectiveness. Therefore, we postulated that the study of this biologic material subjected to different implant conditions (in the atrium, aorta, and pericardium) would be useful.

The operative results, with no deaths, is partially due to the characteristics of the patch which is easy to handle and malleable providing satisfactory surgical hemostasis and healing. There was no late mortality or clinical complications related to the patch, corroborating the use of the patch in cardiac repairs.

In our study, a clear difference was observed between the smooth and wrinkled surfaces of the patches. All specimens showed the wrinkled surface of the patches would adhere to surrounding structures, whereas the smooth surface implanted in the native pericardium was free of adhesions in most of the cases. The consistent results allowed the conclusion it may be useful clinically as a pericardial substitute. These results were compatible with those obtained by Gallo et al. in 1978, Meus et al. in 1983 and Bortolotti et al. in 1986 [Gallo, 1978, Meus 1983, Bortolotti 1986]. Our excellent experimental results contrast with other publications reporting intensive epicardial reaction, fibrosis, and adhesion formation when bovine pericardial

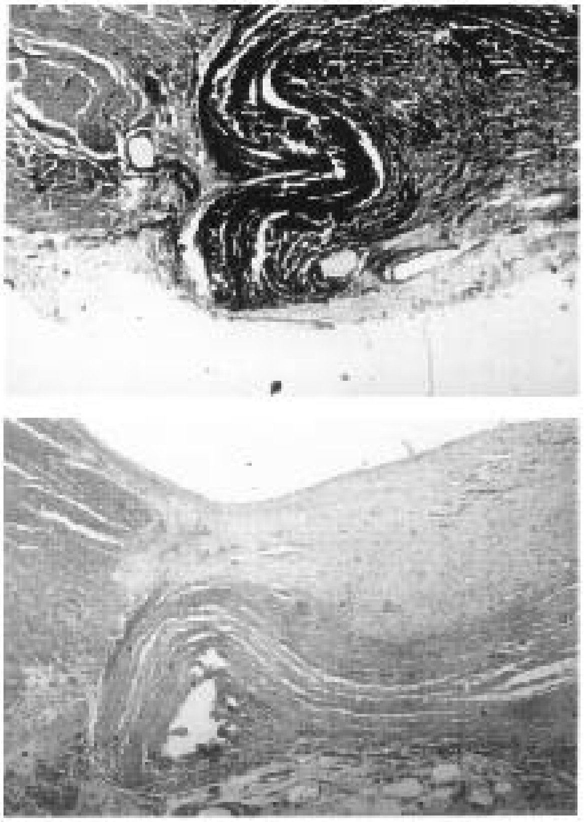

Figure 5. Atrium. A) Coaptation of the borders by invagination of the pericardial patch. Integration tissue represented by collagen connective tissue, suture thread surrounded by a uniform circle of collagen connective tissue; magnified 40X, Verhoeff; B) The patch, below and right is coapted to the atrium with border invagination. The integration tissue is represented by a thin collagen connective tissue. Internal apposition fibrosis connected to the atrial wall;magnified 40X. Hematoxylin-eosin.

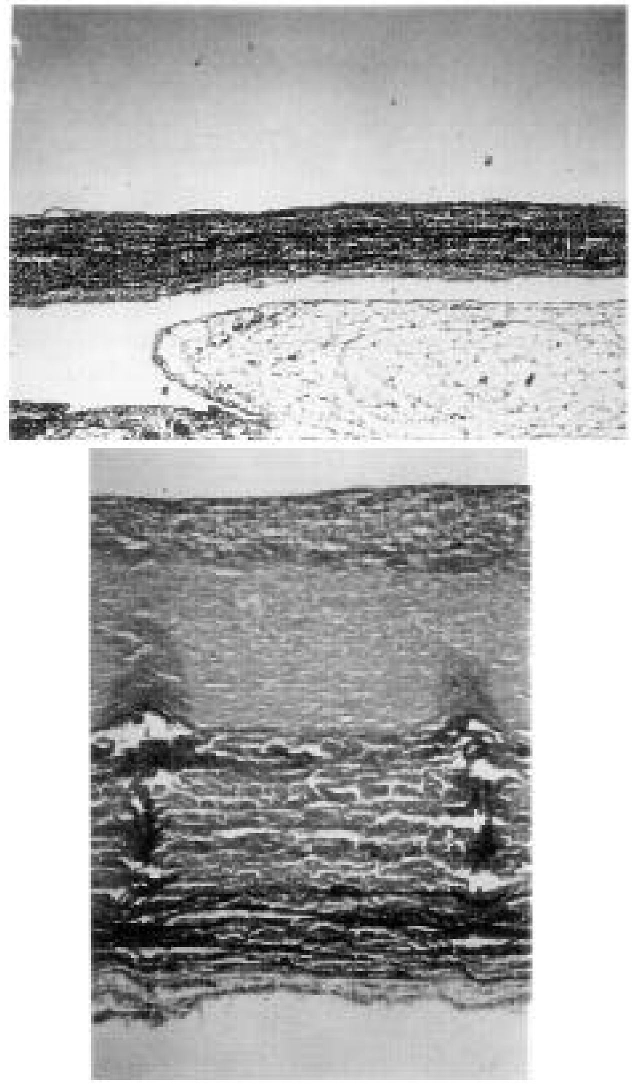

Figure 6. Pericardium. A) Bovine pericardial patch with preserved structure and free smooth surface. Elastic fibers may be observed in black, adhered adipose tissue on the wrinkled surface; magnified 35X. Verhoeff; B) Smooth surface of the bovine pericardial patch covered by internal apposition tissue. Structure of the normal patch, and above the newly formed elastic tissue; magnified 100X. Verhoeff.

patches were used as pericardial substitutes [Skinner 1984, Gallo 1985, Oppie 1986, Eng 1989].

These same discrepancies led Gabbay et al. to study the genesis of pericardial adhesions [Gabbay 1989]. They observed important adhesion formation when the animal was subjected to extracorporeal circulation. Based on studies by Gervin et al. in 1973 reporting decreased pericardial fibrinolytic activity when undergoing trauma, they suggested the same mechanism is responsible for the adherence genesis, that is a decrease in pericardial fibrinolytic activity resulting from extracorporeal circulation [Gervin 1973]. This change in the fibrinolytic activity was clinically observed by Nkere et al. in 1973 [Nkere 1973]. They observed decreases in tissue plasminogen activator concomitant to pericardial mesothelial inflammatory changes. According to Cliff et al. and Robison et al., two important factors should be considered in the genesis of pericardial adhesions: 1) decreased pericardial fibrinolytic activity, and 2) the presence of blood in the pericardium [Cliff 1973, Robinson 1983]. In our study, the pericardium was extensively opened, repaired and often blood was extravasated from the suture of the atrial patch and yet few adhesions were observed.

It should be stressed that adherences were found between the wrinkled surface of the patch implanted into the left atrium and the dog's pericardium, suggesting a different response of the two patch surfaces and indicating the presence of another patch-related factor, in addition to those shown in pericardial adhesion formation. The favorable results in our experiments, particularly minimal shrinkage, fibrosis, and calcification, suggest bovine pericardium may be clinically useful as a pericardial substitute. On the other hand, the role of extracorporeal circulation in the genesis of pericardial adhesions needs to be established.

Table 3. Group 1, 2 and 3 dogs according to thickness, in mm^2, of aortic, atrial and pericardial patches.

Group 1			Group 2			Group 3		
Ao[7]	Ae[11]	Pe[12]	Ao	Ae	Pe	Ao	Ae	Pe
1.0	1.3	0.6	1.0	1.5	0.6	1.0	0.8	0.6
0.9	1.3	0.6	0.8	2.1	0.6	0.8	1.5	0.6
1.0	2.1	0.6	0.8	1.8	0.6	1.3	1.8	0.6
0.8	1.0	0.6	0.9	4.0	0.6	1.0	1.8	0.6
0.9	1.8	0.6	1.0	1.5	0.6	1.0	1.5	0.6
0.9	1.5	0.6	1.5	1.3	0.6	1.0	1.0	0.6
			0.8	2.1	0.6	1.2	3.2	0.6
						1.4	1.3	0.6
M=0.92	1.5	0.6	0.97	2.04	0.6	1.09	1.61	0.6

Variance Analysis by FRIEDMAN (Aorta x Atrium x Pericardium) critical x 2r = 5.99		
Group 1 cal x 2r = 12 multiple comparison test Atrium > pericardium	Group 2 cal x 2r = 12.29 multiple comparison test Atrium > pericardium	Group 3 cal x 2r = 12.97 multiple comparison test Atrium and Ao > pericardium
Variance analysis by KRUSKAL –WALLIS (GROUP 1 x GROUP 2 x GROUP 3) critical H = 5.99		
Aorta critical H = 0.39	Atrium critical H = 8.22 multiple	Pericardium critical H = 4.62
Multiple comparison test Group 1 = Group 2 = Group 3		

M=Mean; [10]Aorta; [11]Left atrium; [12]Pericardium.

The patches also showed significant macroscopic differences. In the aorta and pericardium the borders were clear and their original form was unaltered. However, in the left atrium the patch was thicker and the borders were not well established. The atrial patch area was smaller than the pericardial and aortic surface areas, although there was no statistical significance between atrial and pericardial patches for Group 1 dogs. In addition to the size difference, Kurskal-Wallis analysis showed the atrial patch size decreased over time (Table 2).

The factors that could influence this difference are: 1) the different pressures subjected to the patch, and 2) the coaptation of the patch to the structures. The patch implanted in the left atrium was subjected to low tension and, as a result, it was undulated or folded (Figure 7) whereas the patches implanted into the aorta or pericardium, due to higher pressure, were straight or curved. The patch area was a result of the different patch formats.

There were no microscopic changes in the patch structure at any of the implant positions. The changes reported by Ferans et al. in 1980, Levy et al. in 1983, and Shoen et al. in 1985 for porcine bioprosthesis and those reported by Rossi et al. in 1986, Ferrans et al. in 1987, and Dahm et al. in 1990 for bovine pericardium were not observed in our canine study [Ferrans 1980, Levy 1983, Shoen 1985, Rossi 1986, Ferrans 1987, Dahm 1990]. These authors reported collagen fiber degeneration resulting from

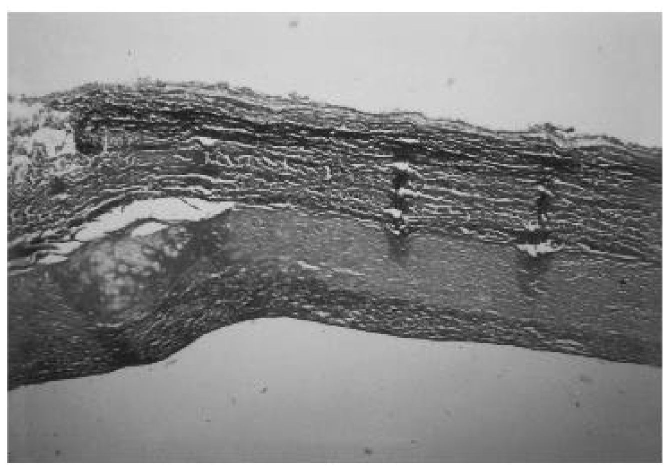

Figure 7. Aorta. Minimal internal apposition fibrosis limited to bone-cartilage metaplasia, to the right, and a continuous newly formed tissue capsule, above. The bovine pericardial patch, below, shows a preserved structure; magnified 35x. Verhoeff.

Table 4. Evaluation of aortic, arterial and pericardial patches: internal apposition fibrosis, bone-cartilage metaplasia and elastic neoformation for group 1, 2 and 3 dogs.

			Fibrosis			Metaplasia			Elastic		
G[13]	DOG	POD[14]	Ao[15]	LA[16]	Pe[17]	Ao	LA	Pe	Ao	LA	Pe
	19	33	+	++	0	0	0	0	+	0	0
	20	33	+	++	0	0	0	0	+	0	0
1	21	33	+	+++	0	0	0	0	+	0	0
	03	40	+	+	0	0	0	0	+	0	0
	18	41	+	++	0	0	0	0	+	0	0
	17	43	+	++	0	0	0	0	+	0	0
	12	120	+	++	0	0	+	0	++	0	0
	07	121	+	+++	0	0	0	0	++	0	0
	10	136	+	++	0	0	0	0	++	0	0
2	16	147	+	+++	+	+	+++	0	++	0	0
	02	153	+	++	0	0	0	0	++	0	0
	01	160	++	++	0	+	+	0	+++	0	0
	04	175	+	+++	0	0	+	0	+++	0	0
	11	225	+	++	0	0	+	0	+++	0	0
	14	282	0	++	0	++	+	0	+++	0	0
	13	284	++	++	0	+	+	0	+++	0	0
3	09	289	+	++	0	+	+	0	+++	0	0
	06	298	+	++	0	+	+	0	+++	0	0
	08	298	+	+	0	0	0	0	+++	0	0
	15	202	+	+++	0	++	++	0	+++	0	0
	05	305	++	++	0	++	++	0	+++	0	0

[13]Group; [14]Postoperative Day; [15]Aorta; [16]Left Artrium; [17]Pericardium

inflammatory infiltration with lymphatic-histiocytic invasion and calcification. In our study, the preservation of patch structure may be related to the fact that only the smooth surface is in contact with blood flow. In contrast, in valvular prostheses and subcutaneously implanted patches, as was the case in the studies mentioned above, both surfaces were in contact with blood flow. In this patch surface there was internal apposition tissue deposition, which apparently isolated the patch from the action of blood elements for a longer time. All of the patches implanted in the left atrium and aorta showed this layer by postoperative day 33.

It is relevant that Rossi et al. in a study of subcutaneously implanted pericardium in rats observed calcification in the patch structure [Rossi 1986]. They reported patch calcification 24 hours after the implant which became progressively greater over time. The presence of internal apposition tissue was not reported. In this study fibrous tissue was not observed in the patches implanted in the native pericardium without direct contact with blood flow. Thus, similar to the findings of Gabbay et al. and Bortolotti et al., these data show that bovine pericardium seems to be influenced by the implant site and by direct blood contact [Gabbay 1984, Bortolotti 1986]. In our study there was calcification in the internal apposition fibrosis layer. The patch induced a surface healing, which does not change the patch structure itself. This scar tissue and the patch make up the surgical patch. The latter may suffer from biological tissue degeneration, as it is the case of the calcification observed in internal apposition tissue.

The pericardium became connected to the structures similar to the integration of the suture thread (polypropylene), which is a synthetic material. This finding suggests the conclusion that bovine pericardium treated and implanted in these conditions acts as an inert material. Internal apposition fibrosis was observed in all of the cases of patches implanted in the left atrium and the aorta. This fibrosis was thicker in the atrium than in the ventricle. There was a statistically significant difference for Groups 1 and 2, and the fibrosis did not increase over time.

Fibrous tissue with the same characteristics was reported in both surfaces of valvular prostheses and with neointima of vascular conduits [Ishihara 1981, Ferrans 1987]. No fibrosis was observed in patches implanted in the pericardium. This suggests that this tissue may be formed by the fibrous organization of blood elements deposited on the smooth surface of the patch. This layer is subject to the same changes reported by Arbustini et al. in 1983 who observed cartilage metaplasia and ossification in the fibrous tissue covering biologic tissue prosthesis [Arbustini 1983]. This metaplasia indicates the transformation of fibroblasts into osteoblasts. This calcification, presented in Table 4(), shows a clear association with increasing duration of implant. It should be reminded there was no calcification in the patch structure itself.

Another marked change in the internal apposition tissue was the finding of elastic neoformation around the aortic patches. Just as with calcification, it showed a clear association with time. It seems to indicate a morphological-functional adaptation of the internal apposition fibrosis related to the elastic properties of the aorta since it was not observed in any of the atrial patches. This neoformation may be produced by the elasticity found in bovine pericardial patches.

The favorable results obtained in this study in terms of minimal structural change, shrinkage, fibrosis, and calcification suggests glutaraldehyde preserved bovine pericardium may be clinically useful as a substitute for aortic and pericardial tissue. However, it is somewhat less satisfactory when used to replace defects in the left atrium due to shrinkage and occasional thrombus formation.

REFERENCES

1. Abdulali SA, Silverton NP, Yakirevich VS, Ionescu MI. Right ventricular outflow tract reconstruction with a bovine pericardial monocusp patch. J Thorac Cardiovasc Surg 89:764–71, 1985.
2. Arbustini E, Jones M, Ferrans VJ. Formations of cartilage in bioprosthetic cardiac valve implanted in sheep:a morphologic study. Am J Cardiol 55:632–43, 1983.
3. Arnoni AS, Falcão HCB, Oliveira JB, et al. Tratamento cirúrgico do abscesso aórtico com descontinuidade ventrículo esquerdo-aorta em endocardite infecciosa. Rev Bras Cir Cardiovasc 1:53–60, 1987.
4. Barbero-Marcial M, Riso AA, Verginelli G, Pileggi F, Jatene AD. Técnica para correção de “truncus arteriosus” tipos I e II sem condutos extracardíacos. Rev Bras Cir Cardiovasc 4:1–8, 1989.
5. Bongiovani HL, Ribeiro PJF, Évora PRB, Brasil CF, Reis CL, Sgarbieri RN. Plastia valvar aórtica por ampliação de válvula com pericárdio bovino: nota prévia. Rev Bras Cir Cardiovasc 3:130–3, 1988.
6. Bortolotti U, Zussa C, Factor S, Frater RWM. Glutaraldehyde treated auto, homo and xenograft pericardium in atrial, mitral, aortic and pericardial applications. Life Support Systems 4:148–50, 1986.
7. Braile DM. Prótese valvular de pericárdio bovino: desenvolvimento e aplicaçáo clínica em posiçáo mitral [Tese. Doutorado] Sáo Paulo: Escola Paulista de Medicina da Universidade Federal de Sáo Paulo, 110.
8. Braile DM, Bilaqui A, Ardito RV, et al. Alargamento da raiz da aorta com ìpatchî de pericárdio bovino preservado em glutaraldeído. Arq Bras Cardiol 41:289–96, 1983.
9. Braile DM, Bilaqui A, Ardito RV, et al. Prótese de pericárdio bovino: 44 meses em 164 pacientes. In: Anais do 9^{0} Congresso Nacional de Cirurgia Cardíaca. São Paulo, SP, Sociedade Brasileira de Cirurgia Cardiovascular, 1981.
10. Braile DM, Mustafá RM, Santos JLV, et al. Correçáo da geometria do ventrículo esquerdo com prótese semi-rígida de pericárdio bovino. Rev Bras Cir Cardiovasc 6:109–15, 1991.
11. Bullaboy C. Bovine pericardium:another cautionary note. Ann Thorac Surg 48:743, 1989. (Correspondence)
12. Casagrande ISJ, Puig LB, Salles CA, Ricardo OP. Tubo de pericárdio bovino corrugado recoberto com Dacron: novo modelo de prótese vascular. Rev Col Bras Cir 15:153, 1988. (Abstract)
13. Cliff WJ, Grobety J, Ryan GB. Postoperative pericardial adhesions:the role of mild serosal injury and pilled blood. J Thorac Cardiovasc Surg 65:744–50, 1973.
14. Cochran RP, McGough EC. Bovine pericardial:a source of pulmonary venous obstruction in the Mustard operation. Ann Thorac Surg 44:552–3, 1987.
15. Crawford FA, Sade RM, Spinale F. Bovine pericardium for correction of congenital heart defects. Ann Thorac Surg 41:602–5, 1986.
16. Dahn M, Lyman WD, Schewell AB, Factor SM, Frater RWM. Immugenicity of glutaraldehyde-tanned bovine pericardium. J Thorac Cardiovasc Surg 99:1082–93, 1990.
17. Dallan LA, Oliveira SA, Ramires JA, Sabino Neto A, Verginelli G, Jatene AD. Tratamento cirúrgico da comunicaçáo interventricular pós infarto agudo do miocãrdio: conduta atual. Rev Bras Cir Cardiovasc 4:64–74, 1989.
18. Eng J, Ravichandran PS, Abbott CR, Kay PH, Murday AJ, Shreiti I. Reoperation after pericardial closure with bovine pericardium. Ann Thorac Surg 48:813–5, 1989.
19. Évora PRB, Ribeiro PJF, Brasil JCF, et al. Experiencia com dois tipos de técnicas para tratamento cirúrgico da insuficiéncia mitral: I. Prótese com preservaçáo de elementos do sistema valvar. II. Plastia valvar com reconstruçáo e avanço da cúspide posterior. Rev Bras Cir Cardiovasc 3:36–49, 1988.
20. Ferrans VJ, Boyce SW, Billingham ME, Jones M, Isihara T, Roberts WC. Calcific deposits in porcine prostheses:structure and pathogenesis. Am J Cardiol 46:721–34, 1980.
21. Ferrans VJ, Tomita Y, Hilbert SL, Jones M, Roberts WC. Pathology of bioprosthetic cardiac valves. Human Pathol 18:586–95, 1987.
22. Gabbay S. The need for intensive study of pericardial substitution after open heart surgery. Transac ASAIO 36:789–91, 1990.
23. Gabbay S, Bortolotti U, Factor S, Shore DF, Frater RWM. Calcification of implanted xenograft pericardium:influence of sitiun and function. J Thorac Cardiovasc Surg 87:782–7, 1984.
24. Gabbay S, Guindy AM, Andrews JF, Amato JJ, Seaver P, Khan MY. New outlook on pericardial substitution after open heart operations. Ann Thorac Surg 48:803–12. 36, 1989.
25. Gallo JI, Artinano E, Duran CMG. Clinical experience with glutaraldehyde-preserved heterologous pericardium for the closure of the pericardium after open heart surgery. J Thorac Cardiovasc Surg 30:306–9, 1982.
26. Gallo JI, Artinano E, Duran CG. Late clinical result with the use of heterologous pericardium for closure of the pericardial cavity. J Thorac Cardiovasc 89:709–12, 1985.
27. Gallo JI, Pomar JL, Artinano E, Val F, Duran CMG. Heterologous pericardium for the closure of pericardial defects. Ann Thorac Surg 26:149–54, 1978.
28. Gervin AS, Jacobs G, Hufnagel HV, Mason KG. Surgical trauma and pericardial fibrinolytic activity. Ann Surg 41:225–6, 1975.
29. Ionescu MI, Smith DR, Sutherland TW, Wooler GH. Heart valve replacement with aortic heterografts. Ann Thorac Surg 13:1–14, 1972.

30. Ionescu MI, Tandon AP. Heart valve replacement with Ionescu-Shiley pericardial xenograft. J Thorac Cardiovasc Surg 73:31–42, 1977.
31. Ionescu MI, Tandon AP, Macartney FJ. Long-term sequential hemodynamic evaluation of right ventricular outflow tract reconstruction using a valve mechanism. Ann Thorac Surg 27:426–34, 1979.
32. Ishihara T, Ferrans VJ, Jones M, Boyce SW, Roberts WC. Structure of parital pericardium and of unimplanted Ionescu-Shiley pericardial valvular bioprostheses. J Thorac Cardiovasc Surg 81:747–57, 1981.
33. Leão LEV, Succi JE, Gerola LR, Lima PR, Braile DM, Gimenes AC. Sistematizaçáo das reoperações sobre a valva mitral. Rev Soc Cardiol Estado de Sáo Paulo 2:72, 1972.
34. Levy RJ, Wolfrum J, Schoen FJ, Hawley M, Lund SA. Inhibition of calcification of bioprosthetic heart valve by local controlled-release diphosphonate. Science 228:190–1, 1985.
35. Loures DR, Ferreira MJA, Almeida RS, et al. Aneurisma adquirido de aorta ascendente em criança. Rev Bras Cir Cardiovasc 4:94–9, 1989.
36. Lukács L, Zaborszky B, Sórkozy K, Órvay A. Reconstruction of the right ventricular outflow tract with bovine pericardial monocusp patch. Tex Heart Inst J 11:234–7, 1984.
37. Meus PJ, Wernly JA, Campbell CD, et al. Long-term evaluation of pericardial substitutes. J Thorac Cardiovasc Surg 85:54–8, 1983.
38. Mills AS, Salem W. Complications associated with the use of heterologous bovine pericardial closure. J Thorac Cardiovasc Surg 92:446-54, 1986.
39. Moraes CR, Rodrigues JV. Conduto valvulado de pericárdio bovino: relato de caso. Arq Bras Cardiol 43:173–7, 1987.
40. Moraes CR, Rodrigues JV, Gomes CA, et al. Coarctaçáo da aorta: resultados de cirurgia e análise crítica de diversas técnicas. Rev. Bras Cir Cardiovasc 4:168–72, 1989.
41. Moraes CR, Rodrigues JV, Gomes CA, et al. Operaçáo de Rastelli utilizando-se conduto valvulado de pericárdio bovino: experiencia inicial. Rev Bras Cir Cardiovasc 3:84–92, 1988.
42. Nkere UU, Whawell SA, Thompson EM, Thompson JN, Taylor JN. Changes in pericardial morphology and fibrinolytic activity during cardiopulmonary bypass. J Thorac Cardiovasc Surg 106:339–45, 1993.
43. Opie JC, Larrieu AJ, Cornelli IS. Pericardial substitutes: delayed reexploration and findings. Ann Thorac Surg 43:383–5, 1987.
44. Palma H, Juliano JA, Cal RGR, et al. Tratamento dos aneurismas da aorta descendente por endoprótese (tromba de elefante). Rev Bras Cir Cardiovasc 4:190–4, 1989.
45. Pieracciani G, Carvalho JL, Jesus Filho AR, et al. Aneurismas não rotos dos seios de Valsalva com insuficiência aórtica e mitral: relato de caso. Rev Cir Cardiovasc 2:25–9, 1989.
46. Revuelta JM, Ubago JL, Duran CMG. Composit pericardial monocusp patch for the reconstruction of rigth ventricular outflow tract: application in 7 patients. Thorac Cardiovasc Surg 31:156–9, 1983.
47. Ribeiro PJ, Bongiovani HL, Évora PRB. Plástia aórtica por ampliaçáo de válvula(s) com pericárdio bovino. Rev Bras Cir Cardiovasc 5:99–105, 1990.
48. Robison RJ, Brown JW, Deschner WP, Highes B, King H. Prevention of pericardial adhesions with Dextran 70. Ann Thorac Surg 37:488–90, 1984.
49. Rossi MA, Braile DM, Teixeira MDR, Carillo C. Calcific degeneration of pericardial valvular xenografts implanted subcutaneously in rats. Int J Cardiol 12:331–9, 1986.
50. Salles CA, Puig LB, Casagrande IS, et al. Crimped bovine pericardial conduit for arterial reconstruction. Eur J Cardiothorac Surg 5:273–9, 1991.
51. Schoen FJ, Levy RJ, Nelson AC, Bernhard WF, Nashef A, Hawley M. Onset and progression of experimental bioprosthetic heart valve calcification. Lab Invest 52:523–32, 1985.
52. Skinner JR, Kim H, Toon RS, Kongtahworn C, Phillips SJ, Zeff RH. Inflammatóry epicardial reaction to processed bovine pericardium: case report. J Thorac Cardiovasc Surg 88:789–91, 1984.
53. Souza LCB, Chaccur P, Dinkhuysen JJ, et al. Tratamento cirúrgico da lesão do óstio da coronária esquerda. Rev Bras Cir Cardiovasc 3:182–8, 1988.
54. Wanderley Neto J, Torres LDF, Dourado GO, et al. Tratamento cirúrgico da ectasia anulo aórtica com tubo valvado. Rev Cir Cardiovasc 2:37–41, 1989.
55. Yakirevich VS, Abdulali SA, Abbott CR, Ionescu MI. Reconstruction of pericardial sac with glutaraldehyde-preserved bovine pericardium. Tex Heart Inst J 3:238–42, 1984.