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Technical aspects of aortic root sparing surgery: Structural changes occurring during different thawing protocols of cryopreserved human aortic root allografts and the reproducibility of external aortic root annuloplasty using Coroneo ring.

Technické aspekty záchovných operací aortálního kořene: Strukturní změny vzniklé při různých protokolech rozmrazování na lidských kryoprezervovaných allograftech aortálního kořene a reprodukovatelnost externí aortální anuloplastiky za použití prstence Coroneo

PhD Dissertation Theses

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

Obhajoba disertač

Abstrakt

Záchovné operace aortálního kořene použité v léčbě pacientů s aneurysmem ascendentní aorty již nepředstavuji experimentální a nedostatečně probádanou proceduru. Úspěšné záchovné operace aortálního kořene nebo opravné operace aortální chlopně jsou uskutečňovány s cílem nejenom korigovat distrofický aortální kořen, ale jde při nich o obnovu funkčnosti jednotlivých komponentů kořene aorty, co se týká jejich rozměrů a propojenosti. Zvyšující se využití plastik chlopní ukazuje na to, že záchovné operace aortální chlopně a aortálního kořene získávají stále větší význam. Tato Dizertační práce je věnována studiu některých specifický faktorů důležitých pro záchovné operace aortálního kořene, především o stadium strukturních změn vznikajících u kryoprezervovaných alograftů lidských aortálních kořenů a o stadium reprodukovatelnosti procedury implantace (a reimplantace) extraaortálních prstenců Coroneo.

Dizertační práce byla uskutečněna s vědomím, že chirurgická stránka záchovných operací aortálního kořene je na optimální úrovni. V současnosti je pozornost soustředěna na optimalizaci různých technických aspektů, a nikoliv na principialní změny chirurgického zákroku. Tato situace byla východiskem pro experimentální část dizertační práce, která si kladla za cíl zjistit morfologické změny v arteriání stěně, které vznikají při různých postupů rozmrazování zmrazených lidských alograftu aortálních kořenů, vyhodnotit morfologické změny na lidských chlopňových alograftech, které vznikly při různých způsobech rozmrazování, a provést sumární porovnání výsledků aplikace různých protokolů rozmrazování. Dalším cílem této práce bylo prověření reprodukovatelnostu výsledků implantace prstence Coroneo.

Všechny části této práce byly provedeny při dodržení relevantních etických podmínek a po schválení patřičnou etickou komisí před provedením experimentů.

Abstract

Aortic valve-sparing procedures treating patients with aortic root aneurysm and patients with ascending aortic aneurysm and aortic insufficiency are no longer experimental and unproven procedures. A successful aortic valve-sparing or repair operation aims not only to correct the failing part of the aortic root but also to restore the intro- and the inter-component relationship of the aortic root elements to optimal dimensions and relations. Today, conservative aortic valve surgery seems to be developing into aortic valve repair surgery. This Dissertation Theses are devoted to the study of some specific technical aspects of aortic root sparing surgery, namely to the study of structural changes occurring in cryopreserved human aortic root allografts and the reproducibility of Coroneo ring implantation procedure.

The experimental part of these Dissertation Theses was undertaken with the fact in mind that the surgical improvement of the type of surgery described was achieved. The current effort is aimed at optimization of various technical aspects and not on the principal changes of the procedures used. Consequently, this experimental doctoral work was undertaken with the objectives aiming at the assessment of morphological changes of the arterial wall that arise during different thawing protocols of a cryopreserved human aortic root allograft (CHARA) arterial wall, the assessment of morphological changes of CHARA leaflets that arise during different thawing protocols, and at comparing the obtained results with the results arising from the comparison of different thawing protocols of CHARA leaflets. Additionally, reproducibility of Coroneo ring implantation on the aortic annular base under standardized conditions was evaluated.

All parts of this dissertation were performed with keeping up all the relevant and related ethical considerations. Approvals of the Ethical Committees were secured before the work.

Content

1. Introduction

1.1. Aortic valve sparing operations

Aortic valve-sparing operations were refined in order to preserve the native aortic valve during surgery for the aortic root aneurysm and surgery for the ascending aortic aneurysm with associated aortic insufficiency. The aortic root is an ensemble consisting of distinct entities: the aortic valve leaflets, the leaflet attachments, the sinuses of Valsalva, the interleaflet trigones, the sinotubular junction and aorto-ventricular junction [1-3]. Every single constituent of the aortic root has an optimal macroscopic, microscopic structure and anatomical architecture which contributes to the function of the aortic root: intermittent, unidirectional channelling of large volumes of fluid while maintaining laminar flow, minimal resistance, the least possible tissue stress and damage during varying hemodynamic conditions and demands [4-5]. This synchronized dynamic behaviour of all aortic root components has shown to be of a great importance for a specific flow characteristic, left ventricle function and coronary perfusion [6-8]. When any of the aortic root components fail, it is the recognition of the complexity of the structure that has led to the development and advancements in sparing surgical procedures that respect the fundamental anatomical existence of the individual parts of the aortic root [9-11].

1.2. Current situation

Lansac et al. proposed a standardized approach for aortic valve repair addressing both the aorta and the valve, associating physiological reconstruction of the aortic root according to the remodelling technique with the re-suspension of cusp effective height and an expansible subvalvular ring annuloplasty using expansible aortic ring in order to achieve a complete and calibrated annuloplasty in diastole, while maintaining expansibility of the aortic root (Extra-Aortic ™ ,CORONEO, Inc., Montreal, QC, Canada) [12,13]. This solved a problem in the treatment of aortic root aneurysm and the lack of a geometric annuloplasty ring to facilitate reconstruction of the aortic root that restores physiological annular size and geometry during aortic valve repair. Cusp coaptation height was increased, reducing the stress on the cusps, thus protecting the repair [11]. A multi-centric study analysed preliminary results of this new physiological approach to aortic valve repair with subvalvular aortic ring annuloplasty. In this multi-centric study, unselected patients with aortic root aneurysms were enrolled consecutively, regardless of their aortic insufficiency grade, the presence of bicuspid valve or complex valvular lesion. The addition of a subvalvular aortic ring was systematically performed in all cases to reduce the diameter of the native aortic annular base in diastole. The choice of the aortic ring and the tube graft was standardized, based on the criterion of intraoperative measurement of a native aortic annular size with the Hegar dilators. The diameter of the prosthetic aortic ring was undersized by one size to restore a normal STJ/annular base ratio of 1.2 [15]. A calibrated expansible aortic ring annuloplasty (Extra-Aortic™, CORONEO, Inc., Montreal, QC, Canada) in different sizes was developed in order to facilitate technical standardization. The result of this multi-centric analysis showed that the aortic function remained stable in most patients. Among the 126 survivors without reoperation, 115 patients had aortic insufficiency of grade < 2 (91.3%) at the end of the follow-up. Freedom of aortic regurgitation of grade II or more was 87.7% at 3 years evaluation (95%, Cl: 80.3-95.1%) [16].

1.3. Non-surgical technical development

As anatomy of the aorta is already highly detailed and elucidated and because surgical aspects of aortic valve-sparing operations are already mastered, the significant improvement is happening in non-surgical technical areas. Two of these are the use of fibrin sealants and cryopreservation. There, new information that is being obtained, may contribute to improved outcomes of aortic valve-sparing operations.

1.3.1. Fibrin sealants in cardiac surgery

Technical advancement in cardiac surgery is accompanied not only by the change of used surgical techniques but also by the advancement of other technical aspects contributing towards the success of cardiac interventions. One of such areas is a routine use of fibrin sealants.

Presently, sealants are used in in cardiac surgery for several reasons: (a) they should help to control haemostasis through the control of bleeding in the area of surgical intervention (as auxiliary sutures, not as suture replacement); (b) they should seal openings made by standard sutures; (c) they should be useful in sealing off hollow organs of the body. Ideally, they also should (d) improve wound healing and (e) they may be useful in the delivery of medication to tissues exposed during the surgery. Obviously, the use of sealant in surgery should be simple, safe and well tolerated by patients. The process of disintegration of the sealant should not cause an inflammation, immunological or any other type of unwanted or pathological process. And the cost of the use of sealants in surgery should not be prohibitive. The use of sealants and related products (hemostats, glues, adhesives).

1.3.2. Cryopreservation

Cryopreserved human aortic root allografts (CHARA) have been used extensively in cardiac surgery for their advantages over bio-prosthetic and mechanical valves, such as excellent hemodynamic function, very low thrombotic event rates, and mainly their resistance toward infections [17,18].

The first allograft transplants in cardiac surgery were freshly harvested aortic valves. The first fresh aortic valve allograft transplant was performed by Murray in 1956 [19]. Regardless of the fact that the operation had an imperfect hemodynamic outcome, the allograft performance was outstanding with perfect leaflet function. Other early experimental and clinical trials such as Heimbecker et al., Kerwin et al. and Lam et al. supported the superior properties of fresh aortic valve allografts [20-22]. Nevertheless, the era of allograft transplantation in cardiac surgery began after the first successful aortic valve transplantation performed by Ross in early 1962 based on Brewin experimental work [23,24].

The first allograft transplants in cardiac surgery were freshly harvested aortic valves that underwent minimal treatment with no ABO Blood group matching. Remarkably, these allograft transplants showed outstanding durability and performance, giving the basic foundation for this new type of surgical procedures. Due to do the lack of donors, cardiac centres started to treat allografts with antibiotics in order to prevent disease transmission and cryopreserve them in order to prolong their life span. These techniques of allograft processing and cryopreservation led to significant decrease of allografts durability and their clinical performance between the 1960s and early 1970s leading almost to the abandonment of this type of procedures [25]. This was primarily due to irreversible damage to the cells viability and the loss of the structural integrity caused by thawing, resulting in the loss of allografts toughness and elastic properties [26-29]. Technical advances in tissue handling had led to the reintroduction of allograft transplants back into use in cardiac surgery [18]. To date, there are no recommended guidelines for cryopreservation and subsequent thawing of cryopreserved allografts that would eliminate damage to the cellular structures in order to obtain allografts of the highest possible quality and durability.

1.3.3. The lecture learned

Aortic valve-sparing operations treating patients with aortic root aneurysm with or without aortic insufficiency and patients with ascending aortic aneurysm and aortic insufficiency are no longer experimental and unproven procedures. A successful aortic valve-sparing or repair operation aims not only to correct the failing part of the aortic root but also to restore the intro- and the inter-component relationship of the aortic root elements to optimal dimensions and relations.

2. Objectives of the dissertation

As the general situation in the area of our research indicates, as for now, improvement in the type of surgery described relays more on optimization of various technical aspects than on principal changes of the procedures used. Consequently, this experimental doctoral work was undertaken with the following objectives in mind:

1) To assess morphological changes of the arterial wall that arise during different thawing protocols of a cryopreserved human aortic root allograft (CHARA) arterial wall.

2) To assess morphological changes of CHARA leaflets that arise during different thawing protocols.
3) To compare the obtained results with the results arising from the comparison of different thawing protocols of CHARA leaflets.

The hypothesis for the first 3 objectives is that as the complexity of the structures evaluated is significant, the changes in arterial wall structures and in leaflets may be (or may be not) the same regarding the character, complexity and integrity of the tissue. Additionally, the time of thawing (and, consequently, the rate of thawing) may be significant for the outcome of the quality of the tissue that is being prepared for a transplantation.

4) Evaluate reproducibility of Coroneo ring implantation on the aortic annular base under standardized conditions.

This part of the work is meant to clarify whether aortic annular Coroneo ring implantation is reproducible in our conditions and whether standard outcome of the Coroneo ring implantation can be achieved.

3. Methodology

3.1. Ethical statement

All the allografts were harvested in the operation theatre in patients that were organ donors and were pronounced “clinically dead” with compliance to the Czech Republic’s transplants laws.

The study was reviewed and approved by the Ethical Committee of General University Hospital, Prague Czech Republic.

3.2. Allograft processing cryopreservation protocol

All human ARA underwent an initial decontamination according to the standard protocol of the tissue bank. Afterwards, all allografts are stored in an antibiotic cocktail comprising of Cefuroxime 0.2 mg/ml + Piperacilline 0.2 mg/ml + Netilmicin 0.1 mg/ml + Fluconazole 0.1 mg/ml in tissue culture nutrient medium E 199 for 24 hours at + 37 °C. Subsequently, all ARA were moved into the cryoprotectant solution in sterile laminar flow cabinet and packed by double layer technique (sealed in Gambro Hemofreeze bags, NPBI BV, Gambro, The Netherlands). Cryoprotectant used was 10% dimethylsulfoxide in the nutritional source for cell culture E 199. After the packaging was completed, all ARA were cooled at a controlled rate of -1 °C/min from + 10 °C to -60 °C, then rapidly cooled and stored in a cryocontainers in liquid nitrogen at -196 °C.

3.3. Thawing protocols

Experimental work was based on investigating 12 cryopreserved CHARA specimens. CHARA tissue samples were randomly divided into two groups, each group consisting of six samples. All allografts were thawed in their original packaging (packed by double layer technique and immersed in 10% dimethylsulfidoxide). Two thawing protocols were tested:

- Protocol 1: six CHARA specimens were thawed at a room temperature at 23°C. Thawing times were as follows: minimum 2hr 49 min, maximum 4hr 5 min, with the median of 3hr 19 min.

- Protocol 2: six CHARAs specimens were placed directly into a water bath at +37°C. Thawing times were as follows: minimum 26 minutes, maximum 41minutes, with the median of 32 min.

The time variability in both thawing protocols was given by the different allografts sizes (Tables 1 & 2), as well as different amounts of cryoprotectant used for each allograft during the cryopreservation process. After all the CHARA specimens were thawed, parts of an aortic root arterial wall were dissected from each aortic root and fixated in a 4% formaldehyde solution before they were sent for morphological analysis., fixated in a 4% formaldehyde solution and sent for electron microscope testing. The same procedure was followed for samples of non-coronary AV leaflets of each specimen.

3.4. Microscopic slide preparation

After the thawing protocols were completed, a sample of aortic root arterial wall was carefully dissected from each specimen (the arterial wall of non-coronary sinus was harvested from each allograft). After the samples were collected, they were fixed in Baker's solution. Each sample was divided into 5 -10 mm sub-samples. Samples were mounted on convex polystyrene casts with hedgehog-like spines. All samples were washed in distilled water for 5 min and dehydrated in a graded ethanol series (70, 85, 95, and 100%) for 5 min at each level. The tissue samples were then immersed in 100% hexamethyldisilazane (CAS No. 999-97-3; Fluka Chemie AG, Buchs, Switzerland) (HMDS) for 10 minutes and air dried in an exhaust hood at room temperature.

Processed samples were mounted on stainless steel stubs, coated with gold, and stored in a desiccator until they were studied and photographed by Electron Microscope on scanning mode operating at 25 kV – BS 301. Samples were evaluated a scored [30].

3.5. Reproducibility of external aortic root annuloplasty

Human aortic root allografts were used for determining the reproducibility of external aortic root annuloplasty with the use of Coroneo external aortic annuloplasty ring (Extra-Aortic ™, CORONEO, Inc., Montreal, QC, Canada). 18 human aortic root allografts were dissected in a standard manner described by Lansac et al. [12]. This work contains detailed description of the procedure performed.

After the dissection was completed, aortic valve junction (AVJ) was measured with the use of Hegar dilatators. Subsequently, Coroneo ring of the right size was implanted in a standard manner described by Lansac et al. [12]. The size of the Coroneo ring was chosen based on standardized sizing of the aortic annulus. Table 4 [12] on the following page shows the sizing of the aortic annulus.

After the implantation was completed, a new AVJ was measured with the use of Hegar dilatators. For each aortic root allograft, the whole procedure of measuring the AVJ, Coroneo ring implantation with subsequently reduced AVJ measuring was repeated twice. The goal of these experiments was to evaluate the reproducibility of this technique and to determine the effect of variable muscular septum thickness on the reduction of AVJ.

3.5.1. Operation technique

The study of the reproducibility of the implantation of the aortic Coroneo ring was performed according to the reference [12] that contains the complete operation technique description. The details of the technique are shown in parts 3.6.1.1. – 3.6.1.3 of the dissertation.

4. Results

4.1. Assessment of morphological changes of arterial wall arising from the use of different thawing protocols of CHARA arterial wall specimens

Histological analysis of the aortic root arterial wall was as follows:

- Thawing protocol 1(thawing at a room temperature +23°C): All 6 (100%) samples showed loss of the endothelium exposing the basal lamina, damage to the subendothelial layers with randomly dispersed circular defects and micro-fractures. Arterial wall was not contracted (Fig 1). Furthermore, 4 (66%) samples showed no damage to the basal lamina (Score 5), 1(17%) sample showed minimal damage to the basal lamina (Score 5), and 1 (17%) sample showed severe damage to the basal lamina (Score 6).

- Thawing protocol 2 (water bath at +37°C): All 6 (100%) samples showed loss of endothelium from the luminal surface, longitudinal corrugations in the direction of blood flow caused by smooth muscle cells contractions in the tunica media with frequent fractures in the subendothelial layer (Fig 2, Fig 3). Furthermore, 5 (83%) samples showed severe basal lamina damage (Score 6), and 1(17%) sample showed no basal lamina with a severe damage to the internal elastic lamina (Score 6).

Fig. 2. Aortic root arterial wall (thawing protocol 1): Loss of the endothelium exposing the basal lamina, damage to the subendothelial layers with randomly dispersed circular defects and micro-fractures (magnification 560x).

Fig. 3. Aortic root arterial wall (thawing protocol 2): Loss of the endothelium from the luminal surface, longitudinal corrugations in the direction of blood flow caused by smooth muscle cells contractions in the tunica media (magnification 520x).

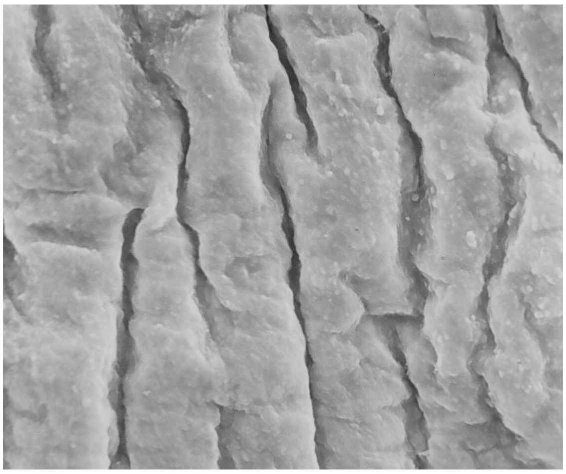
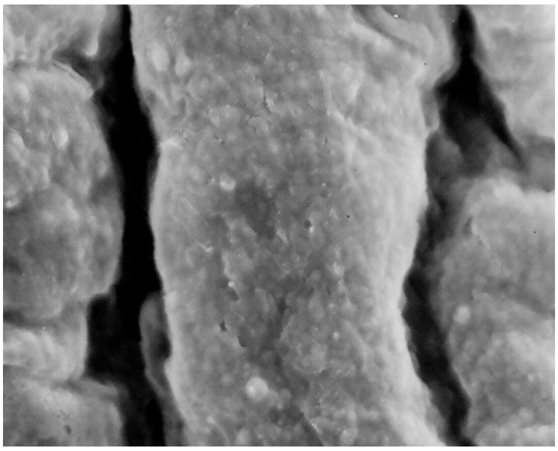


Fig. 4. Aortic root arterial wall (thawing protocol 2): Loss of the endothelium from the luminal surface, longitudinal corrugations in the direction of blood flow caused by smooth muscle cells contractions in the tunica media with frequent fractures in the subendothelial layer (magnification 1700x).



4.2. Assessment of morphological changes of aortic valve leaflets arising from the use of different thawing protocols of CHARA specimens

Histological analysis of the aortic valve leaflets was as follows:

- Thawing protocol 1 (thawing at a room temperature +23°C): 6 (100%) non-coronary AV leaflets showed loos of endothelial cells covering the basal membrane with no damage to the basal lamina (score 5) (Figure 4).
- Thawing protocol 2 (water bath at +37°C): 5 (83%) non-coronary AV leaflets showed loos of endothelial cells covering the basal membrane with no damage to the basal lamina (score 5), 1 (17%) non-coronary AV leaflets showed significant damage to the basal membrane (score 6). (Figure 5).

Fig. 5. Non-coronary aortic valve leaflet (thawing protocol 1): Loss of endothelial cells covering the basal membrane with no damage to the basal lamina (magnification 520x).

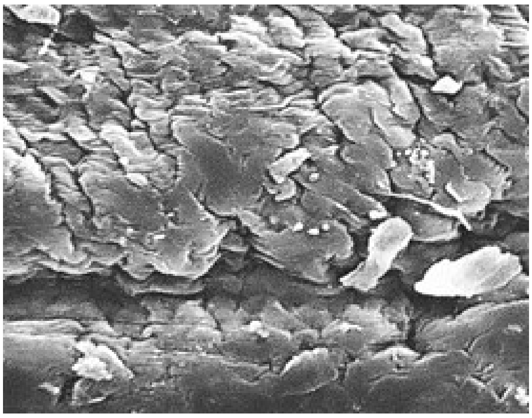
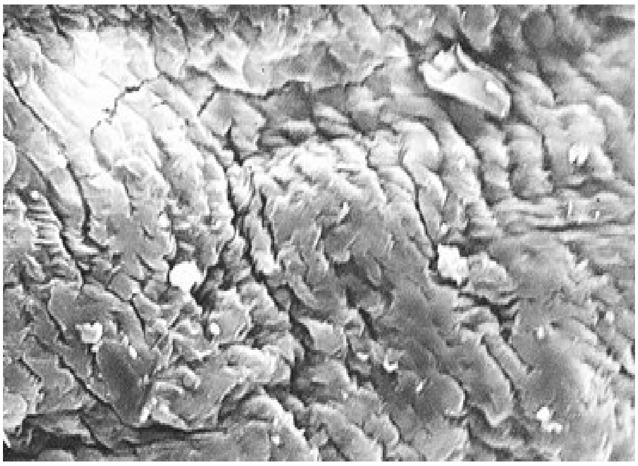


Fig. 5. Non-coronary aortic valve leaflet (thawing protocol 2): Loss of endothelial cells covering the basal membrane and significant damage to the basal membrane (magnification 520x).



4.3. Comparison of the obtained results with different thawing protocols

Based on our results regarding the Objective 1, we have demonstrated that all the samples of CHARAs thawed at the room temperature showed smaller overall structural damage to the arterial and no smooth muscle cell contraction in tunica media when compared to the samples thawed in a water bath. Thawing at a room temperature seems to be gentler and does not lead to so severe damage of the CHARAs arterial wall. Consequently, based on our histological findings, we can conclude that the samples thawed at a room temperature should be in theory of higher quality compared to samples thaw at water bath, thus they should be more suitable for transplantation. This is despite the fact that the thawing of CHARA allograft specimens take longer when performed at the room temperature (median of 3hr 19 min) compared to the thawing in a water bath at +37°C – median time of thawing of a specimen was only 32 minutes.

Our finding shows the importance of technical aspects even for well-established surgical procedures.

In the study regarding our objective 2, the performed experimental work following the structural changes occurring during different thawing protocols on cryopreserved AV leaflets showed that different rates of thawing show identical structural changes. Therefore, the rate of thawing does not play a significant role in minimizing structural changes that occur during thawing of cryopreserved AV leaflet.

Consequently, it was demonstrated that different types of aortic root tissue (arterial wall versus aortic leaflets react differently when submitted to different thawing protocols, aortic leaflets being less sensitive to the thawing process alteration.

4.4. Reproducibility of external aortic root annuloplasty

As techniques for aortic surgery continue to evolve, the reproducibility of any routinely used surgical procedure becomes more significant. Only techniques that are deemed safe and reproducible, and that are associated with reasonable outcomes and are also superior to similar techniques are employed in surgical practice [31].

A dilated aortic annulus that stays untreated represents a highly significant factor contributing to the failure of aortic valve-sparing operations. Aortic annuloplasty reduces the annulus and leads to an increase of the coaptation height. Consequently, this surgical procedure is being used in significant numbers of patients. The importance of this procedure and the benefits of its use were the reason for us to evaluate the reproducibility of the implantation procedure of Coroneo ring at the aortal annular base.

Eighteen samples of aortic annular bases that were made available for this study by the Transplantation Department & Tissue Bank of the Faculty Hospital Motol in Prague were used for this study. This represents a highly significant number and similar studies with a such high number of aortic roots were not published yet (to my best knowledge).

The procedure of implanting Coroneo ring was performed twice on each aortic annular base and the results were evaluated graphically and by statistical methods.

The characterization of all the used aortic annular bases, including an ID, is at the end of this section as is also the comparison of the first and second Coroneo ring implantations at the aortic annular base outcomes.

Fig. 11. Comparison of the change (%) in the size of the aortic annular base after the first (▨) and second (█) Coroneo ring implantation.

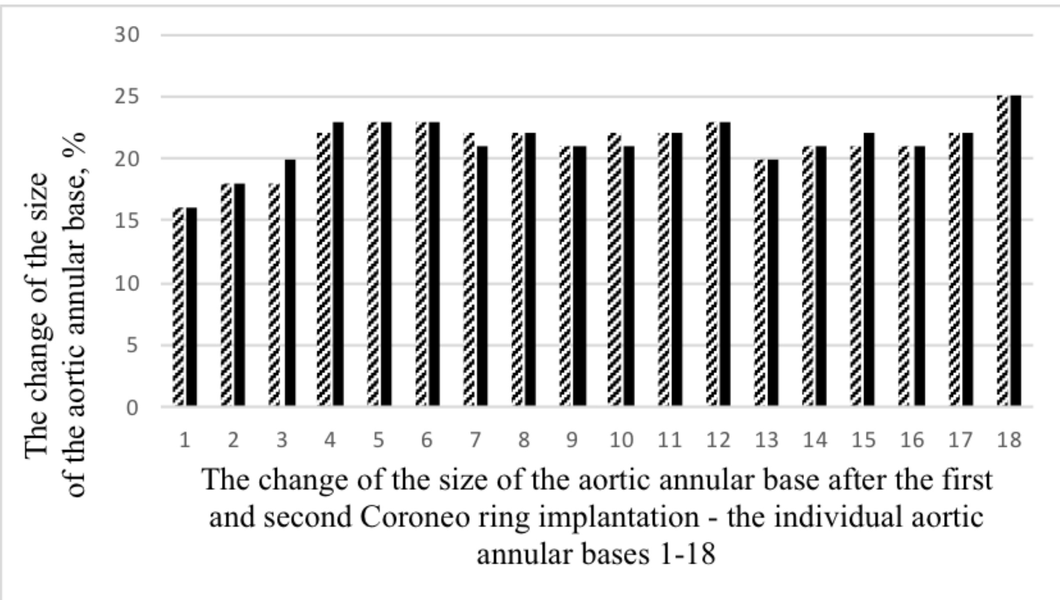


Table 8. The summary of the Coroneo ring implantation results on the size of the aortic annular bases.

No.	ID of the aortic annular base	Aortic annular base, mm	Coroneo ring, mm	Aortic annular base after the implantation of Coroneo ring, mm	
				The first implantation	The second implantation
1	TCAO2009000041A	20	21	16	16
2	TCAO2003000011A	24	23	18	18
3	TCAO2009000020A	22	23	18	20
4	TCAO2009000021A	26	25	22	23
5	03/K057/41	27	25	23	23
6	04/K057/41	26	25	23	23
7	04/K007/41	26	25	22	21
8	03/K042/41	26	25	22	22
9	03/K049/4	26	25	21	21
10	03/K042/42	26	25	22	21
11	TCAO2005000063A	27	25	22	22
12	032/K083	27	25	23	23
13	04/K068/41	25	25	20	20
14	04/K066/41	25	25	21	21
15	05/K027/41	26	25	21	22
16	05/K30/42	25	25	21	21
17	TCAO2004000052A	28	27	22	22
18	03/K044/41	32	29	25	25

Table 9. Comparison of outcomes of the first and second Coroneo ring implantation at the aortic annular base (A) and summary difference between the two implantations performed (B).

A

Parameter	Coroneo ring implantation	
	The first	The second
Number of experiments	18	18
Decrease of the size of the aortic annular base:	3 - 7 mm	3 - 7 mm
	12 - 25%	12 - 25%
Average	4.56 mm/18%	4.61 mm/17%
Median	17%	18.5%

B

Average decrease of the size difference	1%
Median difference of the decrease of the size	1.5%
Average change of the size of the annulus	
between two implantations*	0.60%

*There was no difference between the two implantations outcome in 13 out of 18 experiments.

5. Discussion

5.1. Morphological changes of the arterial wall and aortic root allograft leaflets rising from the use of different thawing protocols

Over the past four decades, cryopreservation of arterial allografts had shown inconsistent results in long-term graft durability. In order to understand the changes that occur during cryopreservation and thawing of allografts, microscopic and immuno-histological techniques are used in order to determine structural and functional changes. One of the most important experimental works performed by M'Bengue-Gaye et al. on a rabbits' carotid arteries clearly showed the effects of cryopreservation on allografts [32]. It had been clearly demonstrated that allograft processing including cryopreservation and thawing are crucial in determining cryopreserved "muscular arteries" allografts durability and clinical performance [33,34].

Immunological reactions caused by cryopreserved arterial allografts are very complex and not fully understood. Arterial allografts are immunogenic as they induce the anti-HLA antibody response in the recipient, thus causing graft rejection. The contribution of anti- HLA class I antibody to the structural allografts degradation is not clear [35].

Although cryopreserved aortic root allograft transplants are associated with outstanding hemodynamic, low thromboembolic events and low risk of endocarditis, the biggest concern is their long-term durability and subsequent risk of reoperation based on the allograft structural degradation related to the degradation of valvular leaflets, thus leading to aortic valve insufficiency [36-39].

Our experimental results show identical structural changes in both examined thawing protocols. Consequently, the faster rate of thawing does not necessarily mean that the aortic valve leaflet will be more structurally damaged or structurally compromised. Therefore, they would not/do not require more frequent observation after implantation.

Another aspect that is thought to contribute to the cryopreserved AV allografts failure is gender mismatch. However, the evidence behind this theory is imprecise as gender matching is not done routinely before these transplants. Böll et al. demonstrated that gender-mismatched vs. gender-matched allografts showed no significant difference in regard to death, need for reoperation and allograft function [40].

Cryopreserved allografts represent a gold standard as a treatment in selected indications such as bacterial endocarditis, Ross procedure. However, there is a grooving evidence that decellularized engineered allografts may be superior to cryopreserved allografts [41]. Decellularized aortic valve allografts have shown outstanding mid-term results after their implantation in terms of their stable structural integrity, low rate of calcification and hemodynamic properties [42,43]. Despite the promising short and mid-term results, long-term results are still not known.

Even thou the efforts in minimizing the damage inflicted by cryopreservation on the AV allografts, there are still many factors that need thorough experimental and clinical examination in order ensure allografts of highest possible quality and durability.

5.2. Reproducibility of Coroneo ring implantation into the aortic root

The aortic annuloplasty ring, Coroneo ring in our study (Fig. 12) [44], is essential for the widespread adoption of aortic valvuloplasty. The introduction of Coroneo ring into everyday surgical practice led to effective standardization through proper selection of patients, assessment of valve performance and prosthetic ring choice. Coroneo ring availability led to improved attention to aortic valve-sparing Techniques for dystrophic disease of aortic root and aortic valve.

Coroneo ring (Extra-Aortic annuloplasty ring) supports dystrophic tissue of the aorta through its attachment on the outside diameter of the aorta to support the external dilated diameter of the aorta. The elasticity of the ring makes an expansion of the tissues during the diastole and systole possible by 10%. Coroneo ring is available in 6 sizes according to the normal diastolic diameters of the aortic annular base. The sizes available are 23, 25, 27, 29, 31 and 33 mm. The company claims that the rings maintain their elasticity for long time after tissue in-grows into the expansible sheaths around the elastic cores of Coroneo ring [44].

Coroneo ring is attached on the outer wall of the aorta as a hoop supporting the tissue against aortic pressure directly. Consequently, the stress concentrating in the aortic wall is reduced. As the attachment sutures do not have to withstand significant forces, they are applied only in numbers preventing migration of the ring.

The performed study was planned as some related technical aspect of cardiac surgeries are still being investigated, even when it is generally recognized that an untreated dilated aortic annulus represents a major risk for failure of aortic valve-sparing operations or repair and that aortic annuloplasty reduces the annulus and increases the coaptation height. The stabilization of all of the components of the aortic root improves the durability of the valve, and the techniques proposed are reproducible and stable in the medium-term. These operations provide satisfactory long-term results for each ascending aorta phenotype with bicuspid or tricuspid valve. The longer follow-up of the patients is ongoing with the AVIATOR registry [45,46].

Our study concentrated on verification of reproducibility of the Coroneo ring implantation procedure and additionally on the effect of re-implantation of Coroneo ring on the aortic annular base size. The obtained data are presented in Tables 5-9 and Figs. 7-12 (of the Doctoral Disertation)..

A ables and a figure in the chapter 4.4. summarize the set of experiments evaluating the effect of Coroneo ring implantation on the size of aortic annular base. The decrease of the size of the aortic annular base was between 3 and 7 mm with the average decrease of 4.56 mm. The size decrease was between 12 and 25% of the aortic annular base before implantation with the average decrease of 18% and median of 17%.

It was shown that the size of aortic annular base decreased in similar manner after the first and second Coroneo ring implantations. The change in the size of the aortic annular base was the same in 13 out of 18 experimental implantations and re-implantations. The second Coroneo ring implantation (second implantation) resulted in an increased aortic annular base diameter in only 3 experiments and in a decreased aortic annular base size in only two experiments. The size difference was only 1 mm in the four mentioned cases and 2 mm in one experiment.

6. Conclusion

Our results on assessing morphological changes of the arterial wall and CHARA leaflets arising during different thawing protocols indicate the following:

1) Thawing protocol that allows tissue samples to thaw at the room temperature provides better outcomes regarding the quality of the thawed tissue compared to thawing in a water bath at higher temperature.
2) The changes observed in the tissue samples of the arterial wall and CHARA leaflets included the loss of the endothelium and an exposition of the basal lamina, damage to the subendothelial layers with randomly dispersed circular defects, micro-fractures, and longitudinal corrugations in the direction of blood flow.
3) Cryopreserved AV leaflets are more stable to the use of different thawing protocols compared to the arterial wall tissue.

Our results on reproducibility of aortic annular base Coroneo ring implantation and re-implantation indicate the following:

4) The reproducibility of this procedure is very high.
5) There is no significant difference between the procedures of implantation and second implantation of Coroneo ring on the aortic annular base.

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Publications of the author:

1. Publication *in extenso* that served as the base for the Disertation:

a) Publications with an IF:

1. **Novotny R.**, Slizova D., Hlubocky J., Krs O., Spatenka J., Burkert J., Fiala R., Mitas P., Mericka P., Spacek M., Lindner J.: Changes arising from different thawing protocols on cryopreserved human allograft's aortic valve leaflets. Accepted for publication 27th March 2017 Adv. Clin. Exp.Med. **(IF 1.179)**
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b) Publication without an IF:

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2. Other publication *in extenso* not related to the Disertation:

a) Publications with an IF:

1. **Novotny R.**, Hlubocky J., Mitas P., Hruby J., Spacek M., Spunda R., Tosovsky J., Lindner J.: Iatrogenic post-catheterization radial arteriovenous fistula in 64-old male patient. J. Imag. Intervent. Radiology. 2015. Vol.2, No. 1:17. **(IF: 0,148)**

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