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RESULTS OF A STUDY OF THE EFFECTIVENESS OF DIRECT CORONARY OXYGEN PERSUFFLATION AS A DONOR HEART CONDITIONING METHOD

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Objective: to evaluate the technical feasibility as well as functional, metabolic and structural integrity of donor heart myocardium after 4 hours of direct intracoronary oxygen persufflation in an experiment.**Materials and methods.** Mini-pig siblings aged 3 months with a body weight of 23–36 kg were used as the experimental model. In the control group (n = 8), donor hearts were cold preserved by injecting 2 liters of Bretschneider cardioplegic solution (Custodiol®, Germany, HTK) into the aortic root. In the experimental group (n = 8), modified HTK solution (with 40 mg/L hyaluronidase added) was used to initiate cardioplegia, then moistened carbogen (95% O_2, 5% CO_2) was injected into the ascending aorta, maintaining 40–45 mm Hg aortic root pressure. The hearts were stored in an mHTK solution at 0–4 °C. After 3 hours of donor heart preservation, orthotopic heart transplantation (OHTx) was performed. In the post-transplant period, we studied central hemodynamic parameters, myocardial oxygen consumption, level of myocardial ischemia markers (troponin I, TnI; creatine phosphokinase-MB, CPK-MB; lactate dehydrogenase, LDH), and histological signs of structural cellular injury. **Results.** Sixteen OHTx surgeries were performed during the study. At 120 minutes after restoration of spontaneous cardiac activity, cardiac output was 2.99 [4.85; 3.17] L/min and 2.48 [2.04; 2.92] L/min ($p > 0.05$) in the control and experimental groups, respectively. Changes in LDH, TnI and lactate levels in the blood flowing from the coronary sinus were significantly higher in the early reperfusion period. However, there was no statistically significant difference between the groups ($p > 0.05$). Myocardial oxygen consumption in the control and experimental groups was 8.2 [7.35; 9.35] ml-O_2/min/100 g and 7.7 [6.75; 10.12] ml-O_2/min/100 g, respectively ($p > 0.05$). Morphological examinations also showed no significant myocardial ischemia injury in the persufflation group compared to the control group. **Conclusion.** The experiment showed the technical feasibility and safety of direct intracoronary oxygen persufflation for 4 hours at the ex vivo donor heart conditioning stage. At the same time, experimental data showed no significant advantages of coronary persufflation over the standard protocol of cold preservation of donor heart with Bretschneider cardioplegic solution.

Keywords: oxygen persufflation, heart preservation, end-stage chronic heart failure, expanded donor criteria, cardiac output, heart transplantation, cold heart preservation.

INTRODUCTION

Organ shortage is largely determined by the geographical location of donor bases and transplant centers. The search for new strategies for prolonged conditioning of donor organs continues. As before, cold preservation of donor heart with the Bretschneider cardioplegic solution is the most frequently used method of transplant preservation in Russia and Europe. However, after four hours of preservation with Bretschneider, graft function can already be compromised, especially in elderly donors [1, 2]. This organ storage method is the greatest risk factor for primary allograft dysfunction and death [3]. Increasing cold ischemia time from 3 to 6 hours doubles the 1-year mortality after transplantation compared with a 50% reduction in predicted 1-year mortality if the ischemia period is less than 1 hour [4]. According to Kobashigawa J. et al., ischemia longer than 4 hours significantly increases the risk of primary graft dysfunction, which is associated with 8% 30-day mortality and increased mortality at 5 and 15 years after heart transplantation [5].

The optimal method of donor organ preservation includes three main aspects: hypothermia, composition of the preserving solution, and oxygenation [6]. If the first two conditions are fulfilled and can be corrected in any of the cold preservation methods, tissue enrichment with oxygen is associated with a number of problems. It has previously been shown that changing the formulation of the preservation solution (even with available ma-

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croergs and buffers) to remove metabolic waste had little effect on the functional outcome of transplantation, while the quality of oxygenation had a huge impact. Numerous variations of adjuvant cardioprotective prescriptions, including a wide range of pharmacological, metabolic and physical agents, have so far not resulted in any significant success [7].

Under natural conditions, the oxygen transporter substrate is blood hemoglobin, that is why the most physiological way of oxygen delivery to graft cardiomyocytes is continuous ex vivo perfusion of the graft with donor blood or macroergic substrate. The TransMedics system (Massachusetts, USA) is the first commercially available device for transporting a donor heart in a normothermic perfusion state. The perfusate is a proprietary infusion solution with the addition of insulin, antibiotic, methylprednisolone, sodium bicarbonate, multivitamins and fresh donor blood [8]. However, such methods are expensive and require constant monitoring, thereby complicating the organ transport stage [9–12].

In 1902, Rudolf Magnus made an unexpected observation while perfusing an isolated cat heart [13]. Despite the emptying of the reservoir storing liquid perfusate and pressurized air mixture delivery to the coronary channel, the heart continued to contract rhythmically for 9 minutes. In spite of a number of successes achieved in subsequent researches on cardiac preservation by feeding the oxygen mixture into the coronary channel (the term 'coronary oxygen persufflation' (COP) officially replaced 'gaseous oxygen perfusion' in 1971 [14]) interest in these works was reduced from 1960 till 1990s in favor of studies on liver and kidney perfusion [15]. However, after the 2000s, interest in long-term cardiac preservation by coronary persufflation was revived; the results of several studies proving the physiological possibility and efficiency of long-term (up to 14 hours) cardiac conditioning by persufflation, including after short (up to 16 minutes) periods of thermal ischemia have been published [15–18].

Not only the safety but also the very idea of performing PRA is still subjected to serious criticism by clinicians, despite the results of studies proving high efficiency of COP as a method of long-term graft conditioning. The purpose of this study was to technically adapt the COP technique to the current clinical protocol for OHTx and evaluate the effectiveness of this technique in comparison with the accepted cold heart preservation technique.

MATERIALS AND METHODS

Preparation of experimental animals, anesthesia

Piglets (mini-pigs) aged 3 months were used as the experimental model. Animal care, experiment support, observation and withdrawal of animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 3, 1986). The study protocol was approved by the local bioethics committee, Meshalkin National Medical Research Center, Novosibirsk (protocol No. 1 of October 12, 2020).

On the day of implantation, all animals were premedicated on an empty stomach with a combination of atropine and zoletil-100. The dose was selected individually according to mass-growth parameters. After the onset of sleep, the surgical field and the neck vascular catheterization area were prepared. Then the animals were transported to the operating room and fixed in the supine position for subsequent tracheal intubation, placement of central arterial and venous catheters. The experiment was performed under endotracheal anesthesia with sevoflurane and myorelaxation (pipecuronium bromide). Artificial ventilation (AV) was maintained using anaesthesia workstation FabiusPlus (Dräger, Germany) with positive inhalation pressure (20–30 cm H_2O) and exhalation pressure (5–8 cm H_2O) with 8 mL/kg breathing volume and 12–14 breaths per minute frequency. During the experiment, we monitored invasive (intra-arterial) blood pressure (IBP) by catheterization of the left common carotid artery, central venous pressure (CVP) by catheterization of the right external jugular vein, heart rhythm disturbances (electrocardiography), body temperature, blood gas composition, and activated clotting time (ACT). Suprapubic cystostomy was implemented to monitor diuresis. Blood analysis was performed using automated hematology analyzer XT-4000i (Sysmex, Germany) according to the manufacturer's guidelines. Central hemodynamics parameters were studied by catheterization of the right heart with a Swan–Ganz catheter. The measurements were performed in the donor after anesthesia and beginning of AV, then after implantation of the donor heart into the recipient's body within two hours after the end of cardiopulmonary bypass according to the protocol (Fig. 1).

Vital parameters were recorded using an IntelliVue MP70 patient monitor (Philips, Netherlands). The study protocol included blood sampling from the coronary sinus to measure myocardial ischemia markers – TnI, CPK-MB, LDH, lactate, as well as myocardial biopsy of the left ventricular apex myocardium before and after donor organ ischemia period.

Myocardial oxygen consumption was calculated according to the formula:

$$\text{LV } O_2 \text{ cons.} = \frac{([O_2]_a) - ([O_2]_{cs}) \times \text{CAF}}{\text{LVmass}},$$

$$\text{ml-}O_2\text{/min/100 g},$$

where, $[O_2]_a$ is arterial blood oxygen content, $[O_2]_{cs}$ is coronary sinus oxygen content, CAF is coronary arterial flow, LV mass is left ventricular myocardium mass.

Blood oxygen content was calculated using the formula:

$$O_2 = \frac{\%O_2\ Sat \times [Hb] \times O_2\ capacity\ of\ Hb\ (1.34\ ml - O_2/g)}{100},$$

$$ml\text{-}O_2/dl.$$

Surgical technique of the experiment

Donor: heart explantation and heart preservation technique

Donor piglets with an average body weight of 33 ± 3.2 kg received premedication and anesthesia according to the technique described above. In all cases, access to the heart was performed through a median sternotomy. After heparin injection in a dose of 3 mg/kg of body weight, a 7 Fr cardioplegic cannula was placed in the aortic root. In the control group after vena cava occlusion, the aorta was clamped and cardioplegia was performed by injecting 2 liters of Bretschneider cardioplegic solution (Custodiol®, Germany, HTK) into the aortic root at 75 mm Hg pressure for the first minute and then at 40 mm Hg for the subsequent 9 minutes. The hearts were then stored in the appropriate solution at 0 to 1 °C. In the experimental group, the hearts were subjected to persufflation according to the technique described in Fischer J. [19]. We used modified HTK (mHTK) solution (with 40 mg/L of hyaluronidase added) to initiate cardioplegia, then we placed an aortic valve blocker that was cut from glove rubber in the form of trefoil and fixed with one knot stitch in the center. Moistened carbogen (95% O_2, 5% CO_2) was introduced into the ascending aorta through a transverse incision or through the brachiocephalic trunk, maintaining pressure in the aortic root at 40–45 mmHg. The heart was placed in a plastic bag filled with mHTK solution and surrounded by ice chips. Drainage tubes were placed in the right and left ventricular cavity, the free ends of which were left in the solution to determine free gas escape. After 3 hours of preservation, we proceeded to graft preparation and implantation to the recipient.

Recipient: donor heart implantation

Piglets weighing 25 ± 1.7 kg underwent median sternotomy. After heparin injection at a dose of 3 mg/kg of body weight, appropriate cannulas were inserted into the right common carotid artery and vena cava. After initiation of cardiopulmonary bypass (CPB), the donor heart was explanted leaving a wide cuff of pulmonary veins; the recipient's body was cooled to 28 °C. Orthotopic implantation of the donor heart was performed using bicaval technique by consecutive anastomosis of the

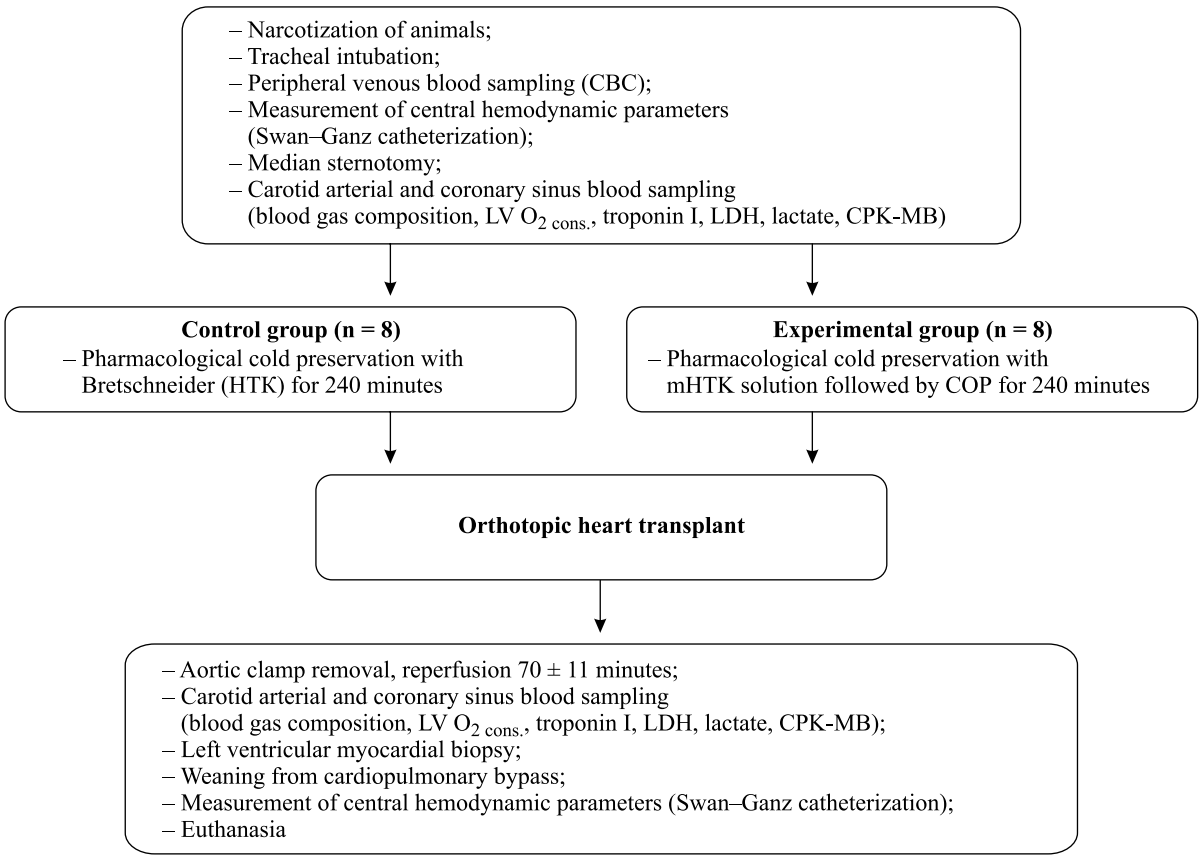

Fig. 1. Study protocol

left atrium, pulmonary trunk, aorta, inferior and superior vena cava. For the purpose of immunosuppression, all recipients received pulse methylprednisolone therapy (Metipred®Orion, Portugal) at a 1,500 mg dose before aortic clamp removal and reperfusion. In the persufflation group, donor heart implantation was performed without cessation of coronary gas supply up to formation of aortic anastomosis. Cardiac reperfusion was started with a 10-minute warming of the heart with oxygenated modified Krebs–Henseleit solution, containing only 50 μmol/L calcium and 15 μmol/L adenosine at 50 mm Hg pressure to remove gas bubbles from the capillary bed. During the first minutes of reperfusion, blood samples were taken from the arterial line of the heart-lung machine and the coronary sinus in order to calculate myocardial oxygen consumption and determine the level of myocardial ischemia markers. Thirty minutes after the clamp was removed from the aorta, myocardial biopsy of the left ventricular apex was performed. The recipient's body was gradually warmed and weaned from CPB. After 2 hours of observation, euthanasia was performed by injecting 100 mL of 4% potassium chloride solution under general combined anesthesia (4–7 mg/kg propofol, 0.006–0.008 mg/kg fentanyl, and 2–4 vol% sevoflurane inhalation).

Myocardial specimens for histological examination were excised from the apical region of the left ventricle, fixed in 10% formalin solution on phosphate buffer (pH 7.4) and embedded in paraffin. 5 μm thick sections were prepared on a Microm HM 550 microtome and stained with hematoxylin and eosin according to the van Gieson method with a combined dyeing of elastic fibers with orsein; a Schiff test was also performed. Histology and morphometric studies were performed using a software-microscope complex that included a light microscope (Carl Zeiss), an AxioCam MRc digital video camera, and a Pentium 4 computer.

Statistical processing was performed using Statistica 10.0 software (StatSoft Inc., USA). The normality of distribution was tested using the Shapiro–Wilk test, followed by estimation of equality of variance using Levene's test. If there was normal distribution in the experimental groups and there was intergroup equality of variance, further processing was performed using parametric statistics – Student's t-test. Nonparametric statistical methods were used for distributions other than normal. Differences between the groups were considered significant at $p < 0.05$.

RESULTS

A total of 16 OHTx surgeries were performed during the study. Donor heart ischemia time in the experimental and control groups was 248 ± 12 and 242 ± 10 minutes ($p > 0.05$), respectively; implantation time did not differ significantly between the groups – on average 47 ± 6 and 39 ± 7 minutes ($p > 0.05$), respectively. In all experiments, reperfusion time was 60 ± 8 minutes, after which infusion of cardiotonic drugs (dopamine 10 μg/kg/min, adrenaline 0.1 μg/kg/min) was started for all recipients and gradually weaned from CPB. Changes in cardiac output (CO) were assessed at three points: point 1 – immediately after weaning from CPB; point 2 – 60 minutes after independent graft functioning; point 3 – 120 minutes after independent graft functioning (Table 1). In both groups, there was a significant decrease in CO after weaning from CPB in comparison with the baseline values. However, the differences between the groups were statistically non-significant ($p > 0.05$).

In the persufflation group, recovery of cardiac pump function required more active antiarrhythmic and cardiotonic support. In all cases in the COP group, there was stable ventricular fibrillation, and restoration of correct rhythm required multiple (up to 10) attempts at defibrillation. In contrast, all animals in the control group showed spontaneous recovery of coordinated heartbeats.

Changes in LDH, TnI and lactate concentrations in blood flowing out of coronary sinus are presented in Table 2. Comparative analysis revealed no statistically significant difference between the groups ($p > 0.05$).

Myocardial oxygen consumption was significantly lower in the persufflation group after reperfusion ($p = 0.011$). However, when compared between groups, oxygen consumption did not differ ($p > 0.05$).

The histological picture of myocardial parenchyma and stroma of control and experimental animals was generally similar. When subjected to H&E stain, muscle fibers of normal size, sarcoplasm of muscle segments were uniformly and moderately accepting of eosin (Fig. 2). Transverse striation was clearly detected in the longitudinally cut fibers, and areas of mild myofibril contracture were noted in some places. The nuclei of muscle fibers were mostly medium-sized, oval bacilliform

Table 1

Changes in cardiac output (L/min)

Group	Baseline	After CPB	In 60'	In 120'
Control (n = 8)	3.36 [3.36; 3.97]	2.35* [2.14; 2.71]	3.03 [2.96; 3.34]	2.99 [4.85; 3.17]
Experimental (n = 8)	3.72 [3.15; 4.28]	2.15*$^{\#}$ [2.01; 2.42]	2.95$^{\#}$ [2.25; 3.12]	2.48$^{\#}$ [2.04; 2.92]

Note: *, $p < 0.05$ versus baseline; $^{\#}$, $p > 0.05$ versus control group.

or elongated, uniformly stained dark blue with clumps of chromatin with distinct nuclei.

In the control group, the epicardial stroma was moderately and irregularly edematous. The arteries and veins were with wide oval lumen; around part of vessels, there was slight perivascular edema, single lymphocytes in capillaries. In the experimental group, unlike the control group, the marginal standing of lymphocytes in capillaries was diffuse; there was slight perinuclear edema in some cardiomyocytes; vessel dilatation with round contour preservation. In both groups, endothelial cells were evenly distributed, flatly arranged and retained their integrity (Fig. 3).

DISCUSSION

Deficit of ischemia time is one of the main factors limiting the geography of donor bases and, accordingly, the possibilities of donor potential. Currently, donor heart ischemia time in clinical practice is limited to 3–5 hours in the case of cold preservation [20]. Unfortunately, the current cold preservation method involves replenishment of all deficits during ischemia except one – oxygen. Donor organ perfusion systems, as well as hyperbaric oxygenation devices, are not widely used in clinical practice due to its cumbersomeness and high cost of its consumable components [20–22]. In contrast to continuous perfusion methods with oxygen-containing preservation solution or blood, COP technique that was discovered more than a century ago does not require complex perfusion equipment. Persufflation is a combination of primary cardiac arrest by cold method followed by continuous antegrade delivery of gaseous oxygen into coronary arteries.

Despite the results of numerous studies demonstrating high efficacy of COP as a method of long-term (14 hours) graft conditioning, so far it has had no impact on the attitude of clinicians towards the idea of intentional filling of the coronary bed with gas mixture [9–11, 23]. The first full-fledged studies on the efficacy and safety of coronary persufflation were performed in 1959; Sabiston D. et al. in a series of experiments on anterograde COP (A-COP) with humidified carbogen gas (95% O_2, 5% CO_2) showed that canine hearts can continue to contract for 5 hours (2.5–8 hours) ex vivo while maintaining normothermia [24]. In the next series of experiments, the authors performed A-COP in situ for 25–30 minutes, after which they were able to restore normal coronary blood flow. At the same time, a majority of animals had complete restoration of the hemodynamic function of the heart. The main conclusions of this study were that: the heart is able to use gaseous oxygen by direct persufflation; successful restoration of myocardial contractility is possible after A-COP and coronary reperfusion with blood.

Later in 1960, Talbert J. et al. introduced the concept of retrograde COP (R-COP) [25]. At that time, retrograde perfusion of oxygenated blood through coronary sinus was actively used to maintain cardiac rhythm and protect the heart against short-term ischemia during open aortic valve interventions [26, 27]. In their studies, the authors injected carbogen through the coronary sinus, which allowed to maintain heart beats for an average of 3.5 hours, and in case of additional cannulation of the anterior heart veins, up to 5.5 hours. Later in 1966, Camishion R. et al. published an article describing the results of R-COP in aortic valve interventions [23]. The term ‘persufflation’ officially replaced ‘gaseous oxygen perfusion’ in 1971 [14] after which the interest in research on persufflation declined significantly.

In the 90’s, persufflation became a subject of research again. In 1998 for instance, Kuhn-Regnier F. et al. published a study on the use of A-COP as a method of heart conditioning before orthotopic allotransplantati-

Table 2

Changes in the levels of biochemical markers in blood flowing out of the coronary sinus

Indicator \ Group	Control (n = 8)		Experimental (n = 8)	
	before OHTx	after OHTx	before OHTx	after OHTx
LDH, U/L	429.85 [355.8; 546.3]	693.60* [491.25; 778.87]	442.05 [329.4; 555.8]	773.25*$^{\#}$ [654.35; 948.67]
TnI, pg/mL	5.15 [2.35; 8.17]	48.45* [26.53; 73.75]	4.85 [2.55; 7.37]	67.10*$^{\#}$ [27.78; 104.8]
Lactate, mmol/L	1.45 [1.12; 2.02]	9.55* [8.53; 10.25]	1.30 [1.05; 2.12]	9.55*$^{\#}$ [8.53; 10.25]
CPK-MB, U/L	204.00 [166.5; 324]	326.15* [225.5; 453.25]	168.00 [118; 324]	376.15*$^{\#}$ [225.5; 535.75]

Note: LDH, lactate dehydrogenase; CPK-MB, creatine phosphokinase; OHTx, orthotopic heart transplantation; *, $p < 0.05$ versus pre-heart transplant level; $^{\#}$, $p > 0.05$ versus control group.

Table 3

Myocardial oxygen consumption (ml-O_2/min/100 g)

Group	Baseline	After reperfusion	P
Control (n = 8)	9.15 [7.17; 11.9]	8.2 [7.35; 9.35]	0.31
Experimental (n = 8)	10.6 [8.18; 15.42]	7.7$^{\#}$ [6.75; 10.12]	0.011

Note: $^{\#}$, $p > 0.05$ versus control group.

on in the experiment [28], a similar study was published by Fischer J. et al. [29]. The average graft ischemia time in these studies was 14.5 hours. The authors described significant advantages in recovery of cardiac output, coronary blood flow, left ventricular pressure and myocardial relaxation after a long period of A-COP compared to an isolated cold preservation group [30].

Given pilot study results, it remains unclear why COP has not received widespread support. Perhaps, the "barrier" of direct and intentional introduction of air mixture into the vascular bed, formed by the general perceptions of clinicians about the danger of embolism, still makes them skeptical about the safety of COP. The technical conduct of persufflation, given the need for continuous gas delivery to the aortic root throughout the cardiac implantation stage, has no significant impact on the course of operation. Moreover, we did not obtain evidence indicating a negative effect of COP on cardiac pump function restoration compared with the control group; the study of myocardial ischemia markers revealed a significant increase in LDH, TnI, CPK-MB, and lactate concentrations in blood flowing from the coronary heart both in the persufflation group and in the control group. Morphological studies also showed no significant ischemic myocardial damage compared with the control group. The integrity of the endothelial lining of vessels and their patency were preserved. Besides, it is necessary to take into account the possible effect of the absence of any crossmatch selection on the results, with the development of acute graft rejection.

CONCLUSION

In the course of the experiment, the technical feasibility and safety of direct intracoronary oxygen persufflation at the stage of donor heart conditioning ex vivo was proved. At the same time, experiments found

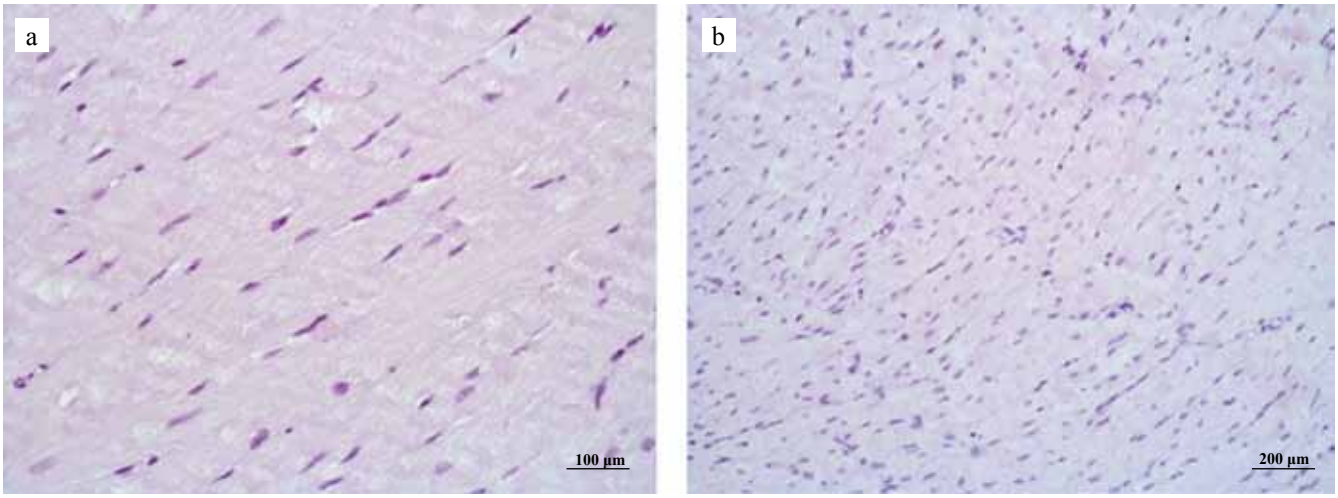

Fig. 2. Left ventricular myocardium with preserved muscle fiber diameters and mild contractures; a, control group. H&E staining. 400× magnification; b, experimental group. H&E staining. 200× magnification

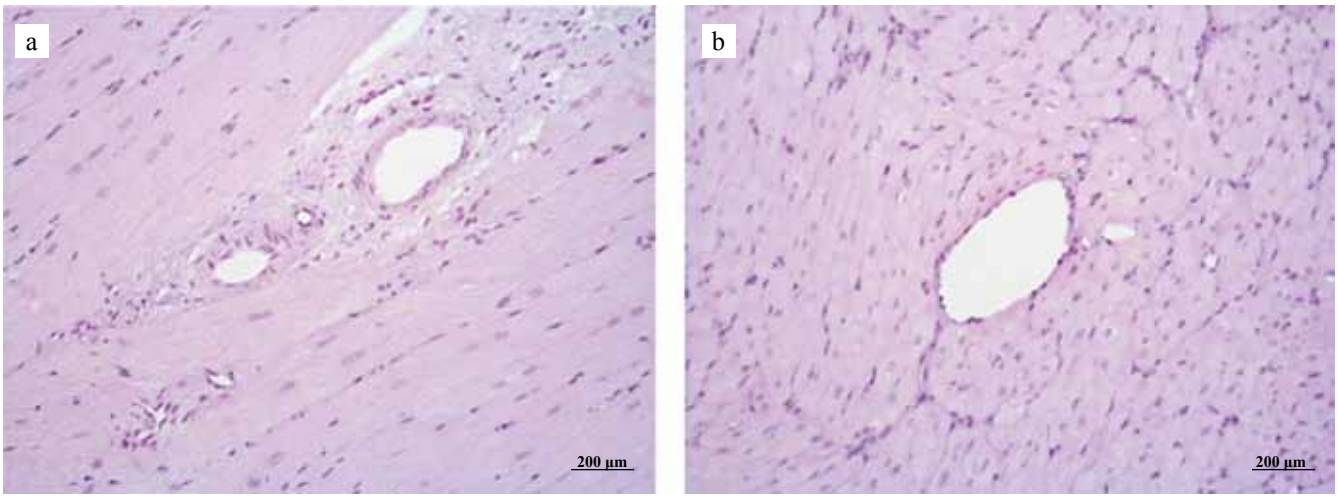

Fig. 3. Left ventricular intramyocardial vessels. Preserved endothelial lining; a, control group; b, experimental group. H&E staining. 200× magnification

no significant advantages of coronary persufflation over the standard protocol of cold preservation of donor heart by Bretschneider cardioplegic solution. The absence of significant differences in functional, biochemical and structural integrity of the graft between the groups may be due to the short organ preservation period and short observation period, which requires more extensive and long-term observations.

The authors declare no conflict of interest.

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