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NORMOTHERMIC *EX VIVO* LUNG PERFUSION USING A DEVELOPED SOLUTION FOLLOWED BY ORTHOTOPIC LEFT LUNG TRANSPLANTATION (EXPERIMENTAL STUDY)

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The continued unavailability of adequate organs for transplantation to meet the existing demand has resulted in a major challenge in transplantology. This is especially felt in lung transplantation (LTx). LTx is the only effective method of treatment for patients with end-stage lung diseases. Normothermic *ex vivo* lung perfusion (EVLP) has been proposed to increase the number of donor organs suitable for transplant – EVLP has proven itself in a number of clinical trials. The ability to restore suboptimal donor lungs, previously considered unsuitable for transplantation, can improve organ functionality, and thus increase the number of lung transplants. However, widespread implementation of *ex vivo* perfusion is associated with high financial costs for consumables and perfusate. **Objective:** to test the developed solution on an *ex vivo* lung perfusion model, followed by orthotopic LT under experimental conditions. **Materials and methods.** The experiment included lung explantation stages, static hypothermic storage, EVLP and orthotopic left LTx. Perfusion was performed in a closed perfusion system. We used our own made human albumin-based perfusion solution as perfusate. Perfusion lasted for 2 hours, and evaluation was carried out every 30 minutes. In all cases, static hypothermic storage after perfusion lasted for 4 hours. The orthotopic single-lung transplantation procedure was performed using assisted circulation, supplemented by membrane oxygenation. Postoperative follow-up was 2 hours, after which the experimental animal was euthanized. **Results.** Respiratory index before lung explantation was 310 ± 40 mmHg. The PaO_2/FiO_2 ratio had positive growth dynamics throughout the entire EVLP procedure. Oxygenation index was 437 ± 25 mm Hg after 120 minutes of perfusion. Throughout the entire EVLP procedure, there was a steady decrease in pulmonary vascular resistance (PVR). Initial PVR was 300 ± 100 dyn×s/cm^5; throughout the EVLP, PVR tended to fall, reaching 38,5 ± 12 dyn×s/cm^5 at the end of perfusion. **Conclusion.** A safe and effective EVLP using our perfusate is possible. The developed orthotopic left lung transplantation protocol under circulatory support conditions, supplemented by membrane oxygenation, showed it is efficient and reliable.

Keywords: lung transplantation, donation, perfusate, perfusion.

INTRODUCTION

Shortage of donor organs remains a major challenge in transplantology. The situation is especially critical in areas such as LTx. LTx is the only effective method of treatment for patients with end-stage lung diseases. In order to increase the number of donor organs that are suitable for transplant, EVLP is proposed. EVLP has shown to be effective in a number of clinical trials [1, 2].

The ability to restore suboptimal donor lungs, previously considered unsuitable for transplantation, allows for improving organ functionality. However, the spread of *ex vivo* perfusion technology in clinical practice is associated with high financial costs for consumables and perfusate [3]. Swedish company XVIVO leads the global market in terms of production of perfusate for donor lungs. The company accounts for about 70% of the market. Due to the small production capacity of this monopolistic company, countries that are just introducing *ex vivo* perfusion technology are experiencing a certain deficit [4].

Ex vivo perfusion is beginning to develop in Russia. Its active introduction in the country is being restrained by the high cost of the original perfusate (Steen Solution

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TM). In previous articles, we have presented a successful experience in using our Russian-made human albumin-based solution as perfusate. The only disadvantage of this solution is that it must be prepared before each EVLP procedure.

The purpose of the presented pilot study is to test our developed solution under experimental conditions on an *ex vivo* lung perfusion model followed by orthotopic LTx.

MATERIALS AND METHODS

Isolated lungs obtained from Romanov sheep weighing 25–30 kg were used in the experimental study. The experimental work program was approved by the Committee on Biological Safety and Bioethics, Shumakov National Medical Research Center of Transplantology and Artificial Organs. The work was performed in compliance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [5].

The experiment included lung explantation stages, static hypothermic storage, EVLP and orthotopic left LTx.

Donor and recipient were narcotized with zolazepam solution at 10 mg/kg, followed by a combination of isoflurane 2.5% to 3% vol. inhalation anesthetics. During explantation, central pressure monitoring and artificial ventilation were performed in the volume control mode at the rate of 8–10 ml/kg; peak inspiratory pressure did not exceed 25 cm a.c., positive end-expiratory pressure did not exceed 5 cm H_2O, respiratory rate was 25 bpm, and depth of anesthesia was adjusted using an isoflurane vaporizer. The explantation technique was described in detail in our previous article [6]. Celsior solution was used as a preserving agent.

Perfusion was performed in a closed circuit using Ex Stream perfusion system (Transbiotech, Russia); a balloon with deoxygenating mixture (N_2, 86%; CO_2, 8%; O_2, 6%) was connected to the oxygenator. A centrifugal CPB pump with a hydrophilic head was installed in the trunk system between the cardiotomy reservoir and oxygenator. The trunk after the oxygenator was connected to an 18 Fr cannula installed in the pulmonary artery. Outflow was performed actively through a funnel-shaped cannula sutured to the left atrial area. Pressure measurement in the trunk system was performed by installing three invasive sensors: the first was installed after the oxygenator to measure pressure in the proximal part of the perfusion system, the second was installed directly in the pulmonary artery cannula to measure perfusion pressure in the pulmonary artery. The third sensor measures pressure in a cannula inserted into the left atrium to monitor left atrial pressure. The lung graft at the moment of perfusion was placed in a chamber designed to allow *ex vivo* perfusion under sterile conditions [7]. We used our own Russian-made human albumin-based perfusion solution as perfusate. The perfusate was 1.5 liters in volume in all groups. Red cell mass was prepared by centrifugation of whole leukocyte-free blood for 15 minutes at 3,500 rpm. Meropenem 1000 mg, methylprednisolone 1000 mg, and short-acting insulin 10 units were added to the perfusate. The target hematocrit level was 15%. A general view of the perfusion system is shown in Fig. 1.

The perfusion time was 2 hours, evaluation was done every 30 minutes. PVR, dynamic compliance, respiratory index, glucose utilization rate and lactate gain were assessed. At the end of perfusion, the lungs were re-preserved with 2,000 mL of experimental solution antegrade. Static hypothermic storage after perfusion lasted for 4 hours in all observations. Postoperative follow-up was 2 hours, after which the experimental animal was euthanized.

The orthotopic left LTx procedure consisted of 3 stages:

Stage 1 (Anesthesia of experimental animal): Was performed in the same manner as in the donor procedure. All transplant surgeries were performed under cardiopulmonary bypass. Ex Stream device (Biosoft-M, Russia) with centrifugal pump Rotaflow (MAQUET, Germany) and oxygenator Affinity Fusion (Medtronic, USA) with a set of lines, were used as auxiliary circulation. Catheterization of the external jugular vein and common carotid artery for invasive monitoring was performed on the right side; arterial and venesection was performed on the left side, followed by cannulation after systemic heparinization. A 20 Fr venous cannula was inserted to the 20–25 cm mark, which corresponded to its position in the vena cava sinus. A 12 Fr arterial cannula was inserted into the common carotid artery to a 5–6 cm depth. After cannula positioning, artificial circulation was started at half of the calculated volume of 1,500 mL/min.

Fig. 1. General view of the closed perfusion system

Stage 2 (Left-sided thoracotomy, pneumonectomy): The skin incision was made at the level of the 5th intercostal space, wound edges were separated with a retractor. For better visualization of anatomical structures of the root, ventilation was stopped, and artificial circulation was switched on at the full estimated volume. When isolating the root, the pulmonary artery was first isolated and taken into a tourniquet, then pulmonary veins were isolated and taken into a holder. After all vascular structures were isolated, the pulmonary artery was sutured with a stapler, the pulmonary veins were ligated manually. The left main bronchus was sutured and crossed last with an 0.5 cm indent from the tracheal bifurcation. At the end of pneumonectomy, meticulous hemostasis was performed.

Stage 3 (Left lung transplantation): The lung graft was removed from a sterile bag containing a preservative and placed on a manipulation table, the left lung was separated. The pulmonary artery was severed from bifurcation, the left main bronchus was crossed with an indentation of one semicircle from the bifurcation. After the graft was placed in the wound, the bronchial anastomosis was placed first, using continuous locking PDS 4/0 sutures (Ethicon, USA). Next, atrial anastomosis was performed using Prolen 5/0 (Ethicon, USA) continuous winding suture on an atraumatic needle, and the pulmonary artery anastomosis was performed last, also using Prolen 7/0 (Ethicon, USA) continuous locking suture on an atraumatic needle.

Upon completion of the anastomoses, we resumed artificial ventilation, opened the clamp on the pulmonary artery and under visual control performed graft deaeration through the untied atrial anastomosis. Then the knot was tightened and tied at the atrial anastomosis, hemostasis control was performed. After hemodynamics stabilization, artificial circulation was stopped, and protamine was injected in the calculated dose. The observation period was 4 hours, the thoracotomy wound was not sutured. Blood samples were taken selectively from the left pulmonary veins every hour. Obtained data were plotted (pCO_2, pO_2 and respiratory index were assessed).

All experiments ended with taking graft biopsy specimens followed by morphological study. Microscopic analysis was performed using a light microscope, photos were taken using a digital camera. The obtained sections were evaluated for vascular thrombosis, hemorrhages, interstitial and alveolar edema development as well as cellular infiltration.

The experiment was terminated by euthanizing the animal with an injection of lethal dose of potassium chloride to trigger a cardiac arrest.

RESULTS

Respiratory index before donor lung explantation was 310 ± 40 mmHg. Throughout the entire *ex vivo* perfusion procedure, PaO_2/FiO_2 increase maintained positive dynamics. After 120 minutes of perfusion, the oxygenation index was 437 ± 25 mm Hg, which is a good indicator of restoration of respiratory lung function (Fig. 2).

From the beginning of perfusion, lactate level in the perfusate was 1.2 mmol/L, and a gradual increase in lactate level was noted throughout the *ex vivo* perfusion procedure. At the end of the EVLP procedure, lactate levels in the solution were 7.4 mmol/L, indicating adequate metabolism in the perfused lungs (Fig. 3).

Throughout the entire *ex vivo* perfusion procedure, PVR decreased steadily. Initial PVR was 300 ± 100 Dyn×s/cm^5, throughout the *ex vivo* perfusion, PVR tended to fall. At the end of perfusion, the PVR index was 38.5 ± 12 Dyn×s/cm^5, dynamics of PVR changes are shown in Fig. 4.

The PaO_2/FiO_2 ratio was 345 ± 25 mm Hg 60 minutes after orthotopic left LTx; the respiratory index was 360 ± 25 mm Hg after 120 minutes. Dynamics of the respiratory index are shown in Fig. 5).

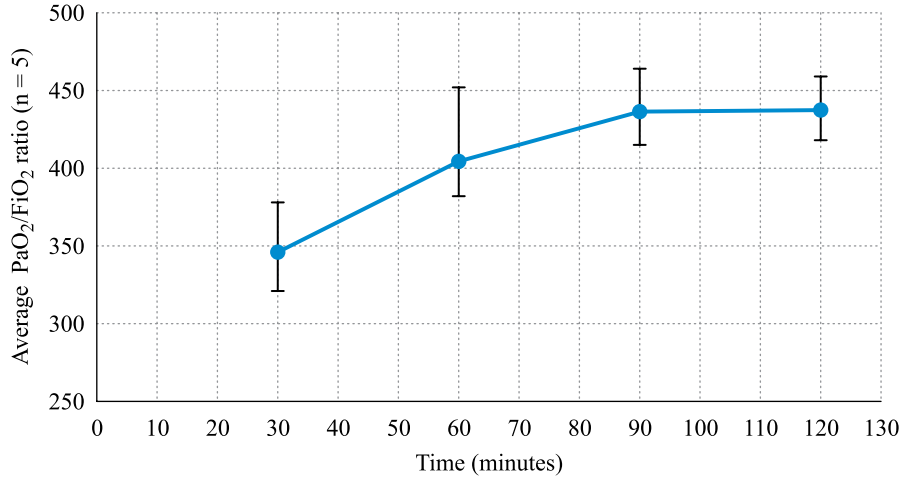

Fig. 2. Dynamics of the PaO_2/FiO_2 ratio at the *ex vivo* perfusion stage

Changes in lung compliance indices (dynamic compliance) during normothermic *ex vivo* machine perfusion procedure are shown in the graph (Fig. 6). The positive increase in values from the moment perfusion was initiated to the final measurement indirectly indicated a decrease in the amount of extravascular water in donor

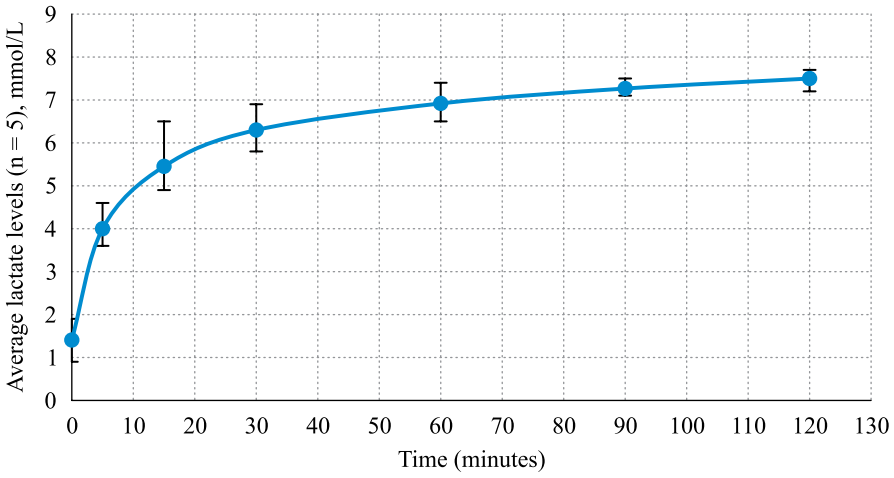

Fig. 3. Lactate levels during *ex vivo* perfusion

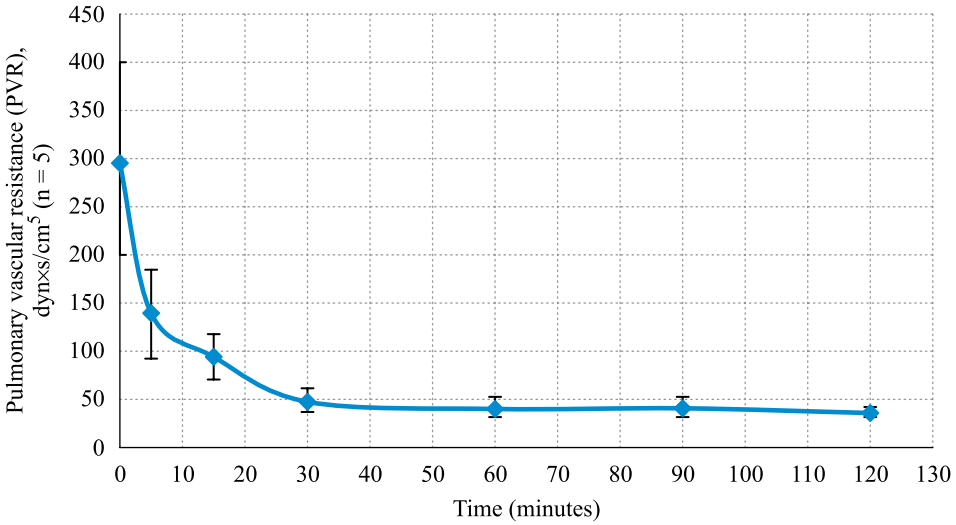

Fig. 4. Pulmonary vascular resistance dynamics

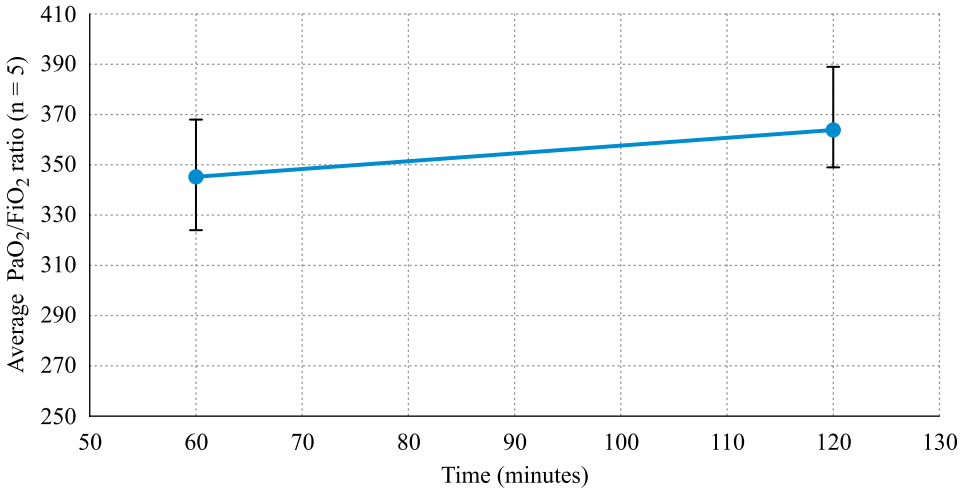

Fig. 5. Oxygenation index dynamics after transplantation

lungs, being a criterion for EVLP efficiency, and reflected positive dynamics of the functional status of the graft.

Throughout the entire *ex vivo* perfusion procedure, airway resistance decreased steadily. At the beginning of perfusion, resistance was 1.4 ± 0.5 cm H_2O/L/sec. At the end of perfusion, resistance was 0.7 ± 0.4 cm H_2O/L/sec. Dynamics of airway resistance during *ex vivo* perfusion are shown in Fig. 7.

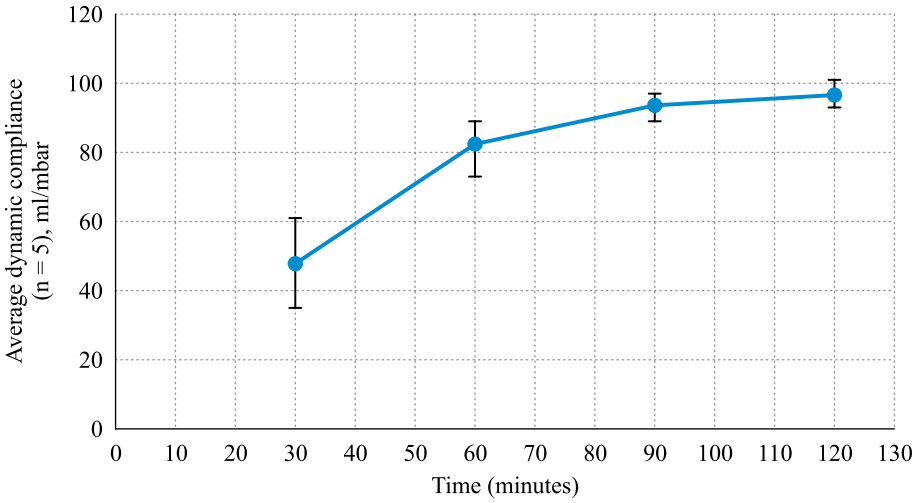

Fig. 6. Dynamic compliance dynamics

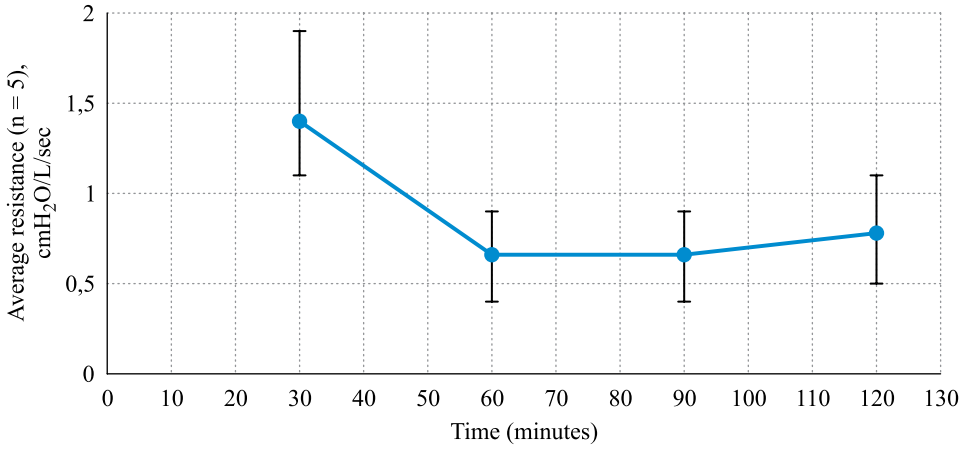

Fig. 7. Airway resistance dynamics during *ex vivo* perfusion

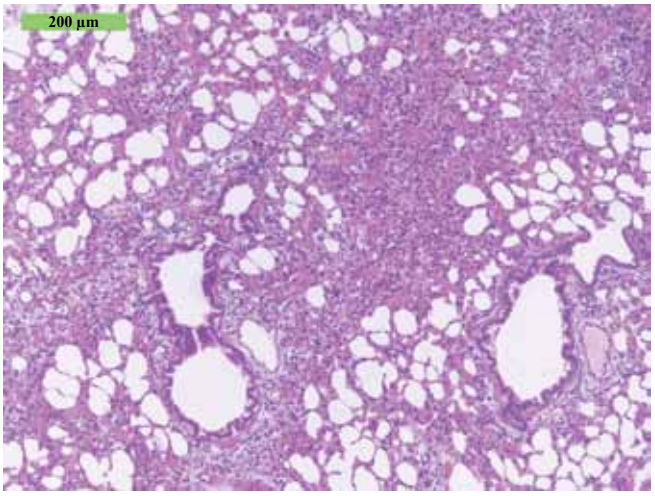

Fig. 8. Histological pattern, area of atelectasis taken before perfusion

Morphological data

Before *ex vivo* perfusion, histological material was taken from the atelectasis zones mainly in the posterior basal regions. Sections showed a classical picture of atelectatic pulmonary parenchyma in the form of recessed alveolar air spaces (Fig. 8).

Morphological examination of lung fragments from zones of expanded massive atelectasis, obtained after 120 minutes of perfusion, showed that the architectonics of the pulmonary parenchyma were preserved in all observations. Well swollen alveoli were noted in most sections. Microatelectatic zones were distributed heterogeneously and were found only in separate segments. Alveolar air spaces, as well as peribronchovascular interstitium were slightly thickened (Fig. 9).

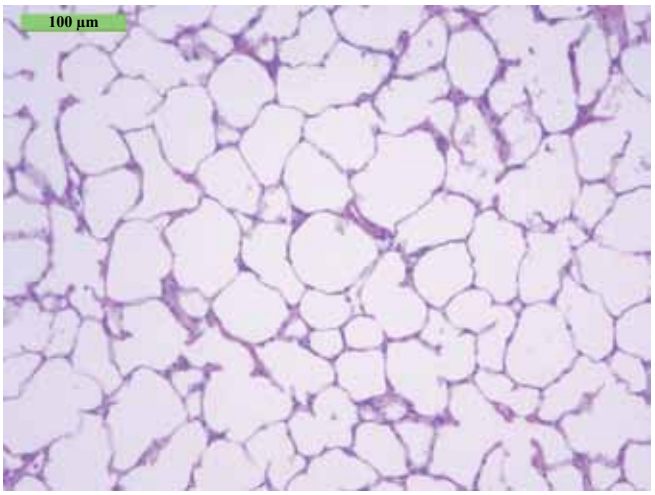

Fig. 9. Histological pattern, areas of expanded atelectasis, after *ex vivo* perfusion

DISCUSSION

The high demand for normothermic EVLP procedure creates prerequisites for coming up with alternative most technologically profitable solutions. More and more often in world literature, there are works featuring modifications of the original solution, and attempts are also being made to create a solution on the basis of official drugs commonly available in the clinic [10].

The developed original native solution for normothermic EVLP that is based on commonly available registered drugs is certainly the optimal solution for overcoming the existing challenges. In previous works, experimental testing of perfusion solutions prepared on the basis of drugs commonly available in the clinic achieved positive results. However, after *ex vivo* perfusion, LTx was not performed under experimental conditions [11, 12]. In this study, the main task was to create a model on a large animal, which is as close to clinical conditions as possible. The EVLP procedure was performed according to the previously described protocol using the original solution. After *ex vivo* perfusion, lung graft was preserved before the upcoming transplantation. The research work resulted in the development of a single-lung transplantation protocol on an experimental ram model.

Several animal models have been proposed in the world literature. However, in the present study, preference was given to rams due to the most surgically acceptable anatomy of the main vessels in the neck, which is necessary for adequate anesthetic support [13, 14]. In addition to setting up central access and invasive arterial pressure monitoring, central vessels can be cannulated on the contralateral side to implant a mechanical circulatory support system supplemented with membrane oxygenation, which was also implemented in this work. During transplantation, a circulatory assist device was implanted in order to prevent hydrostatic pulmonary edema and maintain adequate hemodynamics. Performing orthotopic LTx under assisted circulation significantly increases the duration of the experimental work, but at the same time avoids many complications and brings the experiment as close as possible to real clinical practice [15–17]. The experimental study achieved satisfactory oxygenation index values during *ex vivo* perfusion and two hours after orthotopic LTx. Dynamic compliance indicators during the early post-transplant period indirectly reflected the degree of ischemia-reperfusion injury in the donor lung; and values >50 mL/bar along with other indices suggest a low risk of early primary graft dysfunction. Limit airway resistance values during normothermic machine perfusion procedure and in the early post-transplant period remained within the limits of physiological norm; they were significantly lower than the generally accepted constants during artificial ventilation of the lungs. Low airway resistance is an indirect sign of satisfactory functional status of donor lungs, and also testify to the absence of parenchymal edema, accompanying primary graft dysfunction.

The dynamics of a decrease in PVR during perfusion indicates its adequacy and effectiveness. Experimental orthotopic single-lung transplantation under circulatory support with the use of peripheral cannulation of the central vessels showed easy implementation and high efficiency. The absence of pulmonary parenchymal edema and absence of pathological changes during perfusion and after orthotopic transplantation, according to histological study results, indicate that the technique is effective and safe [18, 19].

CONCLUSION

It is possible to conduct a safe and effective normothermic EVLP procedure using our own-made perfusion solution. The developed orthotopic left LTx protocol under assisted circulation, supplemented by membrane oxygenation, is efficient and reliable.

The authors declare no conflict of interest.

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