DOI: 10.15825/1995-1191-2021-3-82-89

EVALUATION OF THE EFFICACY OF A NOVEL PERFUSION SOLUTION FOR NORMOTHERMIC *EX VIVO* LUNG PERFUSION COMPARED WITH STEEN SOLUTION™ (ANIMAL EXPERIMENTAL STUDY)

S.V. Gautier[1, 2], O.M. Tsirulnikova[1, 2], I.V. Pashkov[1], N.V. Grudinin[1], D.O. Oleshkevich[1], D.M. Bondarenko[1], N.P. Mozheiko[1], A.A. Karpov[3], N.S. Bunenkov[4]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Sechenov University, Moscow, Russian Federation

[3] Almazov National Medical Research Centre, St. Petersburg, Russian Federation

[4] Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation

Respiratory diseases, together with infectious complications and hereditary lung diseases, rank third in international mortality statistics. Today, lung transplantation is a recognized method of treating end-stage lung diseases. However, the number of transplant surgeries performed is not much. This is down to the high requirements on the condition of a potential lung donor and directly on the quality of the donor lung. This has significantly limited the number of optimal donors. Rehabilitation of donor lungs to optimal gas exchange indicators can be achieved and objectively assessed in the course of *ex vivo* lung perfusion (EVLP). The EVLP procedure is widespread in leading transplantation centers in Europe and North America. It allows to significantly expand the pool of donor lungs, thereby serving a greater number of patients in need of lung transplantation. The possibility of EVLP procedure using publicly available perfusion equipment was demonstrated. The optimized protocol fully demonstrated its reliability and efficiency. The developed perfusion solution had no statistically significant differences in comparison with the Steen SolutionTM, which in the future will serve as an alternative for EVLP procedure.

Keywords: lung transplantation, donation, ex vivo lung perfusion.

INTRODUCTION

Respiratory diseases, together with infectious complications and hereditary lung diseases, rank third in international mortality statistics. Progression of respiratory diseases often confronts physicians and patients with the need for lung transplantation. Chronic obstructive pulmonary disease (COPD), cystic fibrosis, interstitial pulmonary fibrosis, primary pulmonary hypertension, and mucoviscidosis are the most frequent indications for lung transplantation, and the effectiveness of transplantation in malignant tumors is reported [1]. According to reports from the World Health Organization, about 3,973 lung transplantations are performed annually [1]. In the Russian Federation, 164 lung transplantations were performed from 2009 to 2019. The small number of lung transplants is due to the complexity of the surgical intervention and, to a greater extent, to the high requirements imposed on the condition of the potential lung donor and directly on the quality of the donor organ. This ultimately leads to significant limitations in the number of optimal donors. In order to provide the majority of patients in need of lung transplantation, the possibility of using lungs from suboptimal donors is being considered [4]. The main requirement for lungs from suboptimal donors is having the functional capacity to provide sufficient oxygenation of the recipient's blood [11]. Rehabilitation of donor lungs to the level of optimal values of gas exchange parameters can be achieved and objectively assessed during ex vivo lung perfusion (EVLP) [10, 12]. EVLP is widely used in leading transplant centers of Europe and North America, it allows to significantly expand the pool of donor lungs and thus provide more patients in need of lung transplantation [2, 4]. The first prospective, non-randomized clinical trial of EVLP efficacy was the HELP study, published in 2011. A team led by Cypel (Toronto, Canada) performed 4-hour extracorporeal perfusion of 23 donor lungs. The donor lungs in the study group were classified as high-risk with PaO_2/FiO_2 values <300 mmHg. In 20 cases, EVLP achieved satisfactory gas exchange and oxygenation, which allowed to transplant the rehabilitated lungs. When compared with the control group of recipients who underwent optimal donor lung transplantation during the same period, there were no differences in 30-day mortality, duration of postope-

Corresponding author: Nikita Grudinin. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (903) 805-63-58. E-mail: Zbignev.religa@mail.ru

rative ventilation, bronchial complications, or length of stay in the intensive care unit [3].

Thus, today EVLP can be of great interest as an effective way to increase the number of lung transplants through rehabilitation of suboptimal donor organs.

Taking into account the necessity of wide implementation and active use of this technique within the framework of national clinical transplantology, it seems advisable to study and master it in experimental conditions on big animals.

Objective of the study: to evaluate the possibility of using ex vivo donor lung perfusion in experiment using routine components of extracorporeal perfusion, artificial ventilation, and invasive monitoring on a biological lung model of a large experimental animal (ram); to evaluate the effectiveness of our own perfusion solution in an EVLP experiment.

MATERIALS AND METHODS

Male Romanov rams weighing 50 kg were used in the experiments. The experiment program was approved by the biosafety and bioethics committee. The experiments were performed in compliance with the rules of the European Convention for the Treatment of Laboratory Animals and the 2010/63/EU Directive.

The study design involved two series of experiments: Group 1 (control) consisted of lungs of rams perfused with Steen Solution TM (n = 5), Group 2 (experiment) included lungs of rams perfused with our own solution (n = 5). Functional capacity of the lungs was assessed according to the following parameters: oxygenation index, dynamic compliance, pulmonary vascular resistance, histological changes.

The experiment included stages of donor anesthesia, lung explantation, static hypothermic storage for 4 hours, EVLP initiation. The rams were kept in standard pens with adlibitum water supply under a 12-hour day-night cycle.

Donor anesthesia stage: on the day of the experiment, the animal was sedated in the pen 60 minutes before surgery with Zoletil 100™ solution at a 15 mg/kg dose. During sedation, the animal was taken to the operating room and the surgical field and vascular access points were shaved. The animal was positioned on the operating table in the supine position and ECG monitoring was connected in standard leads. Aseptic catheterization of the external jugular vein with a 7 Fr dual-lumen central catheter and the common carotid artery with a 5 Fr catheter was performed for invasive arterial pressure (AP) monitoring. Hemodynamics were monitored via a Philips™ monitoring system. After setting vascular access and providing invasive AP monitoring, central venous pressure (CVP) monitoring, intravenous premedication was performed: lornoxicam 8 mg, metoclopromide 10 mg, chloropyramine 20 mg, infusion of 4.2% sodium bicarbonate solution 200 ml at 200 ml/hr, infusion of Sterofundin isotonic solution at 100 ml/hr. Anaesthetic induction: atropine 1 mg intravenously, methylprednisolone 500 mg, Zoletil 100™ 10 mg/kg, tracheal intubation with an 8.0 intubation tube. Mechanical ventilation was performed using Draeger Fabius plus anesthetic breathing apparatus in the volume control mode at 8–10 ml/kg, peak inspiratory pressure did not exceed 25 cm H_2O, positive end expiratory pressure did not exceed 5 cm H_2O, breathing rate was 20 d/min, anesthetic depth was controlled using isoflurane vaporizer, optimum anesthesticization for explantation surgery was achieved at 3 vol% vaporizer setting. To maintain hemodynamics, a 160 ng/kg norepinephrine solution was infused via a syringe dispenser.

Donor lung procurement. Surgical access through median sternotomy. The pericardium was opened longitudinally, and the aorta and pulmonary artery were divided bluntly. After administering sodium heparin at a 300 units/kg dose, purse-string sutures were applied on the aorta and the pulmonary artery. The aorta was cannulated with a 7 Fr catheter to collect donor blood. The pulmonary artery was cannulated with a 20 Fr cannula. The first stage was preparation of the animal's autologous blood into a hemocontainer with citrate preservative. Erythrocyte mass was obtained by centrifugation of whole blood purified from leukocytes. Upon completion of blood banking, prostaglandin E1 solution ("Vasoprostane", Bayer Schering Pharma, Germany) was injected into the pulmonary artery in a 20 μg dose. Before preservation was started, mechanical ventilation parameters were changed with a 4–5 ml/kg tidal volume. In order to decompress pulmonary circulation, the left atrial appendage was widely dissected, then 1 liter of cold 0.9% NaCl 4 °C with addition of 25,000 units of sodium heparin was injected anterogradely through the pulmonary artery. Preservation was performed with 2 liters of Celsiore™ 4 °C solution. After the perfusion of the preservation solution was completed, the lungs were explanted. For ease of removal, the donor heart was removed while maintaining the maximum length of the pulmonary artery to connect it to the EVLP circuit. Intubation tube #8 was inserted into the trachea at the middle third level, followed by fixation with a synthetic tape. Above the tracheal intubation site, extracorporeal suture was applied. After tracheal intubation, several recruitment maneuvers were performed with subsequent clamping of the intubation tube with a main clamp in order to keep the lungs in a flattened state. Upon completion of explantation, the lungs were placed in a sterile plastic bag filled with 500 ml Celsior preservative solution (manufactured by Ganzyme, France). The bag was hermetically sealed and placed in a thermocontainer for static cold storage for 4 hours.

EVLP perfusion circuit assembly included $^1/_4$ size trunk lines connected to a cardiotome reservoir and a Terumo Corp. membrane oxygenator CapioxRX15™.

A hydrocirculatory heat exchange element, a deoxygenating mixture containing 86% N_2, 8% CO_2, 6% O_2, and an oxygen-air mixture were connected to the oxygenator. A Stockert Sorin 5™ circulatory machine roller pump was installed in the piping system between the cardiotomy reservoir and the oxygenator. The tubing after the oxygenator was connected to a cannula installed in the pulmonary artery. Pressure in the trunk system was measured by installing two invasive sensors: the first was installed after the oxygenator to measure pressure in the proximal part of the perfusion circuit, the second was directly in the pulmonary artery cannula to measure perfusion pressure in the pulmonary artery. Upon completion of the prescribed cold preservation period, the graft was removed from the sterile bag and placed in a prone position in a sterile container equipped with a recess with a drainage hole communicating with the cardiotomy reservoir. Perfusion of the graft was performed through the pulmonary artery; perfusate was drained passively through the pulmonary veins. Perfusate was collected by gravity into the cardiotomy reservoir.

The EVLP circuit diagram is shown in Fig. 1.

In the control group, Steen Solution™ with the addition of red blood cell mass was used as perfusion solution, in the study group – our own solution with the addition of albumin and red blood cell mass in specified proportions. The volume of perfusate in both groups was 2 liters. Erythrocytic mass was harvested by centrifugation of whole leukoreduced auto blood at 3500 rpm for 15 minutes. The following were added to the perfusate: cefepime 1000 mg, methylprednisolone 1000 mg, insulin 4 units, and glucose 40% 5 ml. Target hemoglobin level was maintained at 30 g/L, hematocrit index was 10%–15% in both groups.

The maximum perfusion duration did not exceed 180 minutes.

EVLP initiation: The initial temperature was 15 °C, target pulmonary artery pressure was 4–10 mmHg. Perfusion rate was adjusted based on pulmonary artery pressure, at the beginning of perfusion it was 150–200 ml/min. Gas-air mixture flow where FiO_2 <0.5 was set, corresponding to target minimum pO_2 values >100 mmHg. A deoxygenating mixture was required to achieve gas homeostasis, where pCO_2 40 to 50 mmHg, the flow rate corresponded to a 1:1 perfusion rate. The ionic and gas composition of the perfusion solution was monitored using an ABL 800™ gas analyzer. When all parameters were stabilized, the perfusion rate was gradually increased to 1200 ml/min for 20 minutes, and the perfusate was warmed to 32 °C. After the temperature reached 340 °C, mechanical ventilation (MV) was started. The MV parameters were protective in nature and consisted of tidal volume of 6 to 8 ml/kg, positive-end expiratory pressure (PEEP) of 5 cm H_2O, respiratory rate (RR) – 16/min. The main goal of protective MV was to optimize the volume and pressure in the airways, while avoiding damage to atelectasized sections of the alveoli. Recruitment maneuver was performed not more frequently than once every 10 minutes to prevent ventilator-associated graft damage of the graft. Respiratory oxygen fraction did not exceed 50% (FiO_2 <0.5). Perfusate gas composition was monitored at a preset frequency. After reaching the target temperature of 37 °C for 20 minutes, the volumetric perfusion rate was increased to 100% of cardiac

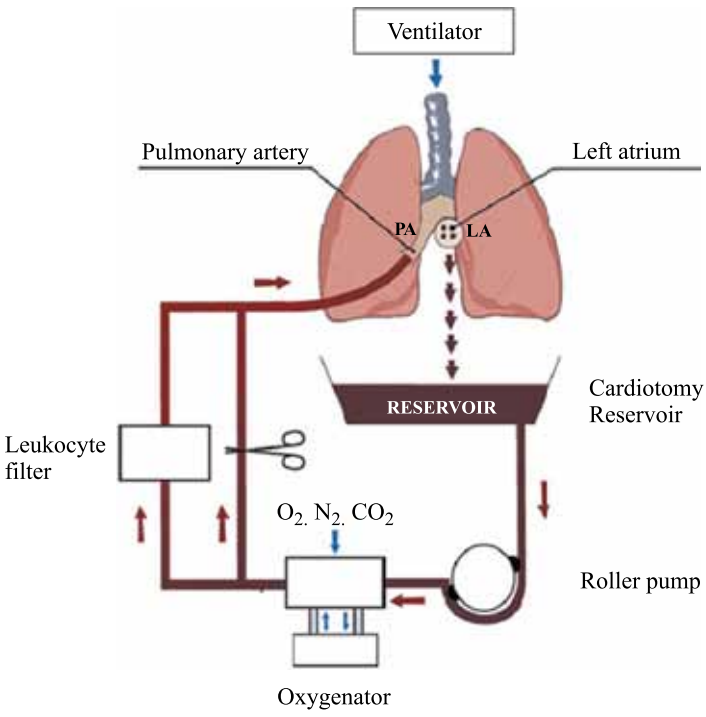

Fig. 1. Diagram of EVLP open circuit

output – 1700–1900 ml/min. Perfusion lasted no more than 180 minutes.

Graft assessment after EVLP procedure

After the graft was warmed up to 37 °C and parameters of gas and ionic perfusate composition stabilized, oxygen fraction on inspiration decreased to 21% (FiO_2 = 0.21), clinical, imaging and laboratory assessment of gas composition was performed. The surgeon palpated and visually assessed the homogeneity of the parenchyma and the absence of lung infiltrative changes. X-ray examination was performed to assess residual atelectasis and pulmonary parenchymal edema. Bronchoscopy was performed to sanitize the tracheobronchial tree and identify indirect signs of pulmonary edema. Gas and ionic composition of perfusate was assessed every 15 minutes. At the end of the EVLP procedure, biopsy specimens were taken for morphological study.

RESULTS

In both groups, the mean PaO_2/FiO_2 value at the time of explantation was 220 ± 25 mmHg.

PaO_2 values were similar in both groups before donor lung explantation (control: 220 ± 20.25 mmHg vs. experiment: 230 ± 10.20 mmHg, P = 0.606). Similarly, there were no statistically significant differences in PaO_2 values after lung perfusion 350 ± 20.53 mmHg, P = 0.348). Both groups showed significant improvement in lung function after EVLP procedure (Fig. 2).

Both groups demonstrated significant improvement in dynamic compliance in perfusion compared with baseline (P < 0.0001). The dynamics of the studied index in the comparison groups were comparable. However, after three hours, the final compliance in the experimental group tended to be higher than in the control group (Experimental 38.8, Control 29.3, P = 0.22) (Fig. 3).

According to perfusion results, both groups demonstrated decrease in pulmonary vascular resistance (PVR). There was no statistically significant difference between the experimental and control groups (P = 0.39) (Fig. 4).

Morphological examination data

In both groups, histological examination showed no significant differences and structural damage to the pulmonary parenchyma (Fig. 5–6).

In both groups, the lung parenchyma had histological signs of functioning tissue without pathological changes. Well swollen alveoli were noted in most sections. Microatelectatic areas were distributed non-uniformly in both groups and were found only in some sections.

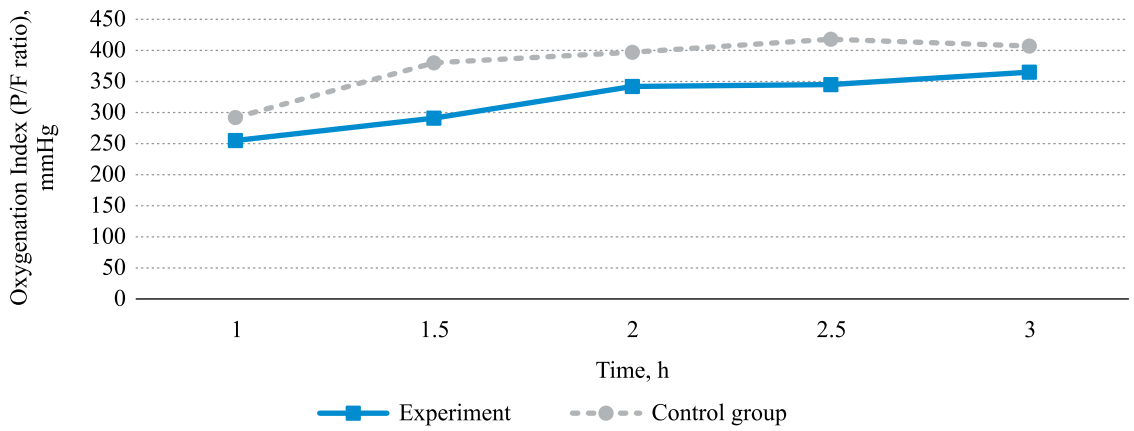

Fig. 2. Oxygenation Index

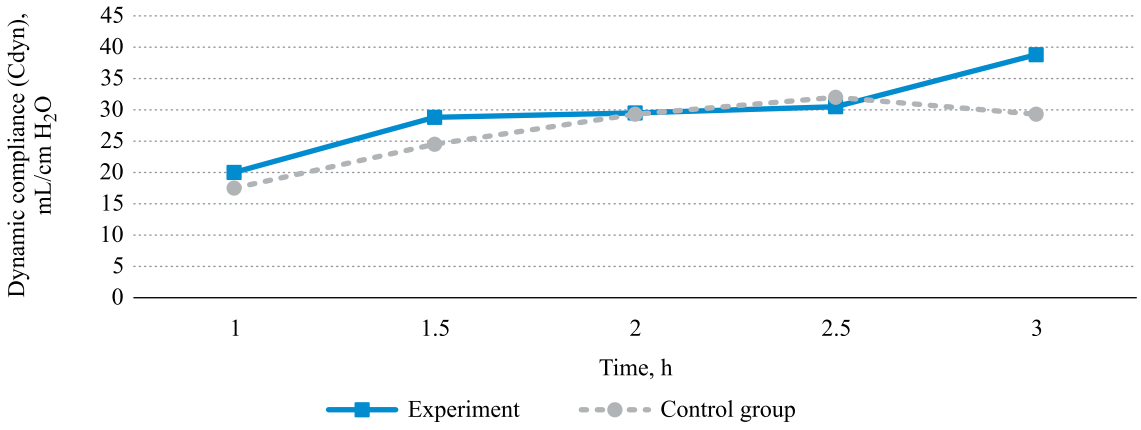

Fig. 3. Dynamic compliance

DISCUSSION

Over the years, lung transplantation has established itself as a radical and effective treatment for patients with end-stage respiratory diseases [13, 14]. Despite the development of technology and improvements in immunosuppressive therapy protocols, long-term survival rates in lung transplant recipients remain the lowest among all solid organ recipients [12, 15]. The reason is high susceptibility of transplanted lungs to the influence of both external and internal negative factors [9]. The same circumstance is also relevant for lungs of a potential donor at the conditioning stage. Strict ventilation modes, contamination by hospital flora, consequences of ineffective hemodynamics, aggressive cardiorespiratory resuscitation and other circumstances lead to donor lung damage. As a result, about 70% of potential lung transplants are rejected due to their unsuitability, and only 20–30% are considered suitable for transplantation [4, 8, 16]. The emergence of a procedure for normothermic perfusion of donor lungs ex vivo has opened up new horizons in the development of lung transplantation worldwide [17, 18, 23]. In 2006, Stig Steen et al. (Lund University Hospital, Sweden) reported the first results of successful single lung transplantation after EVLP procedure. In 2009, Cypel et al. (Toronto, Canada) presented their own protocol, which later became the most physiological and successful, allowing long-term perfusion over 12 hours [5, 15, 22]. The EVLP procedure allowed to increase the pool of donor lungs several times, thereby increasing the number of transplants [4, 17].

Several patented perfusion systems have been developed, including XVIVO perfusion system (XVIVO Perfusion AB, Gothenburg, Sweden), Vivoline LS1 system (Vivoline Medical, Lund, Sweden) and organ care system (OCS lung) (Transmedics, Andover, Mass, USA) [6, 17, 18]. XVIVO perfusion system uses Steen cell-free solution (Vitrolife, Gothenburg, Sweden), and Vivoline system uses a mixture of Steen solution and erythrocyte mass. OCS Lung uses a combination of OCS Lung Solution and erythrocyte suspension [7, 14, 18]. Steen Solution is a solution containing dextran and albumin that mimics extracellular electrolyte concentrations [8, 14, 15]. OCS Lung Solution is a similar buffer solution containing dextran instead of albumin [5, 8, 20, 21].

Our study was based on the perfusion protocol developed by Steen et al. in Sweden for lung assessment

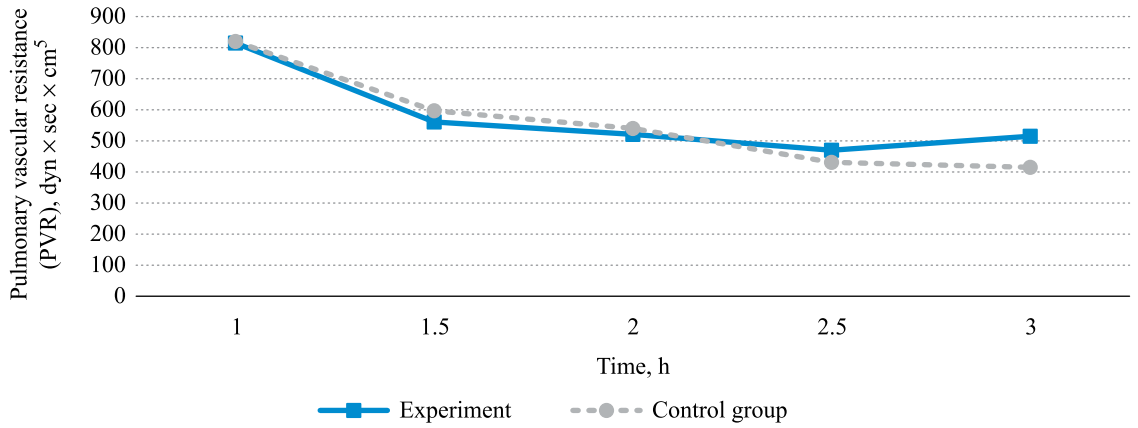

Fig. 4. Dynamics of changes in pulmonary vascular resistance

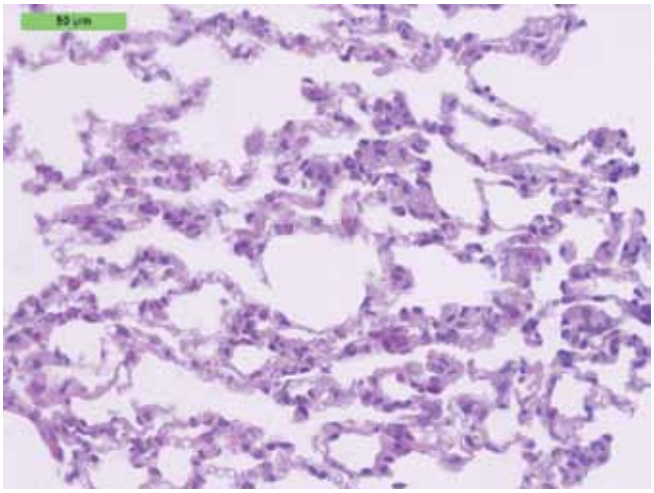

Fig. 5. Experiment

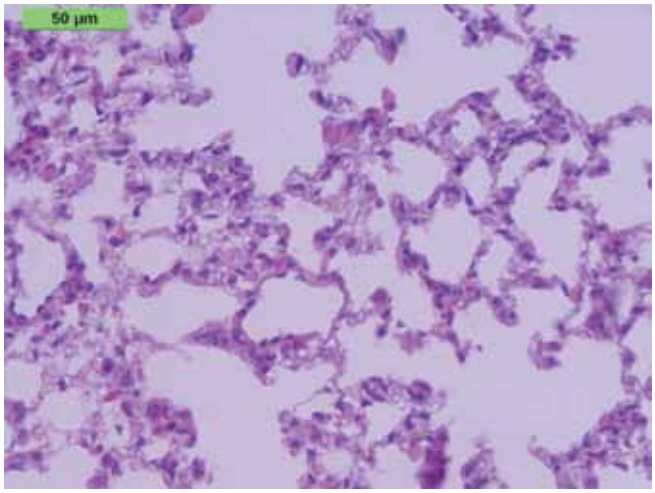

Fig. 6. Control

and rehabilitation. The study managed to demonstrate feasibility of the procedure and reproducibility of EVLP results in an experiment on ram model using routine technical components widely used in specialized medical institutions. The experimental conditions and the adapted perfusion protocol were as close to clinical practice as possible. Evaluation of the efficacy of the proprietary perfusion solution was performed within the standard ex vivo perfusion protocol undertaken in both groups. The perfusion solution, Steen Solution™, considered the gold standard in EVLP procedures worldwide, was used as a control. The results obtained in both groups show no statistically significant differences. The lungs of rams in the comparison groups were similar in functional and histological characteristics, and therefore the differences found after perfusion completion can be attributed to the lung preservation quality. For histological examination, a score based on semi-quantitative analysis of the changes observed in conventional light microscopy was used. The comparison groups showed the same degree of tissue injury after cold ischemia and at the end of perfusion. This may indicate that the developed solution is effective when compared with that of Steen Solution™.

CONCLUSION

Results of our pilot study demonstrate that performing the EVLP procedure using commonly available perfusion equipment is feasible. The optimized protocol fully demonstrated its reliability and efficiency. The developed perfusion solution had no statistically significant difference with Steen Solution™ solution. This will allow it to be used as an alternative for EVLP procedure in the future.

The authors declare no conflict of interest.

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The article was submitted to the journal on 30.06.2021