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# HYPOTHERMIC MACHINE PERFUSION OF A DONOR KIDNEY USING AN EXPERIMENTAL DEXTRAN-40-BASED PRESERVATION SOLUTION AND ORTHOTOPIC TRANSPLANTATION (EXPERIMENTAL STUDY)

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**Objective:** to evaluate the efficacy of hypothermic machine perfusion (HMP) of a donor kidney obtained from a non-heartbeating (NHB) donor, using an experimental dextran-40-based preservation solution, in subsequent orthotopic transplantation in a rabbit model. **Materials and methods.** Twenty grey giant rabbits weighing 2,500–3,100 g, divided into donors (n = 10) and recipients (n = 10), were used in the study. After obtaining kidney from an NHB donor, *ex vivo* HMP of the left donor kidney using a dextran-40-based preservation solution was performed and peripheral vascular resistance (PVR) parameters were measured. This was followed by bilateral nephrectomy and orthotopic transplantation. The follow-up period was 12 days. Creatinine levels, urea levels, and glomerular filtration rate (GFR) were measured during follow-up. **Results.** During *ex vivo* HMP of donor kidneys from NHBs, PVR dropped progressively from 1.90 ± 0.27 mmHg/mL/min to 0.72 ± 0.09 mmHg/mL/min at $p < 0.001$. In the early post-transplant period (during the first 2 days after implantation), creatinine and urea levels were moderately elevated compared to normal. Creatinine and urea levels were 91.07 ± 11.49 μmol/L at $p < 0.011$ and 9.09 ± 1.06 mmol/L at $p < 0.009$ on day 2, respectively, but by day 12, they reverted to physiologic values, which were 77.17 ± 10.19 μmol/L at $p < 0.019$ and 4.88 ± 0.54 mmol/L at $p < 0.022$, respectively. These findings were correlated with GFR values, which ranged from 26.29 to 26.74 mL/min/1.72 $m^2$ in mean values over the course of a 12-day follow-up period. **Conclusion.** *Ex vivo* HMP using dextran-40-based preservation solution has a positive effect on the kidney at 30 minutes of warm ischemia following asystole and achieves satisfactory graft function over 12 days of follow-up.

*Keywords: transplantology, orthotopic kidney transplantation, ex vivo hypothermic kidney perfusion, dextran 40.*

## INTRODUCTION

Today, kidneys are the most commonly transplanted organ [1]. Kidney transplantation (KTx) is the treatment of choice for end-stage chronic kidney disease (CKD). However, before receiving a new organ, such patients must wait for a donor kidney while on renal replacement therapy (RRT). The number of people on RRT varies considerably from country to country. In Iceland, Norway and Australia, for example, there are about 100 people on RRT per million population. This figure is more than 300 per 100,000 in the USA and Taiwan. In Russia, about 50 people per million population go through this procedure per year [2]. The only definitive way to help such patients is by KTx [3].

Despite significant advances in transplantation of solid organs, especially the kidney, and better surgical techniques, issues surrounding the rehabilitation of donor organs from non-heartbeating (NHB) donors remain unresolved [3]. At present, the possibility of working with kidney transplants from suboptimal donors is a relevant direction of modern transplantology. Experimental study of new solutions and perfusion-preservation techniques is of considerable importance [4].

World literature presents many animal models for experimental studies. Each of the described models has advantages and disadvantages [5]. A rabbit animal model was chosen for the experimental study to evaluate the efficacy of hypothermic machine perfusion (HMP) of a left kidney transplant obtained from an NHB donor, using an experimental dextran-40-based preservation solution, with subsequent orthotopic transplantation.

**The aim of this study** is to evaluate the efficacy of HMP of a donor kidney, obtained from an NHB donor, using an experimental dextran-40-based preservation solution at subsequent orthotopic transplantation in a rabbit model.

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## MATERIALS AND METHODS

The study was conducted on male gray giant rabbits weighing 2,500–3,100 g (N = 20). All experimental animals were divided into two equal groups, donors (N = 10) and recipients (N = 10). The study was performed in accordance with the rules of laboratory practice in the Russian Federation: order No. 755 of the USSR Ministry of Health, dated August 12, 1977; order No. 267 of the Russian Ministry of Health, dated June 19, 2003; Law "On the Protection of Animals from Cruelty", dated December 1, 1999. Permission to conduct this study was obtained from the ethics committee at Tver State Medical University (protocol dated May 11, 2018).

The study was conducted according to the following protocol: effective circulatory arrest was simulated in the donor within 30 minutes after preliminary heparin injection, cold cardioplegia and removal of the left donor kidney, *ex vivo* HMP of donor kidney, orthotopic transplantation of left donor kidney to the recipient after preliminary bilateral nephrectomy. During the *ex vivo* HMP procedure using an experimental dextran-40-based preservation solution, peripheral vascular resistance (PVR) indicators were determined. In order to assess graft function, the animals were followed up in the postoperative period for 12 days. Immunosuppressive therapy with methylprednisolone was administered daily during the entire follow-up period and blood samples were taken to study biochemical parameters – markers of renal function. After the end of the follow-up period, the histologic material of the graft was taken, and the animal was withdrawn from the experiment.

### Donor nephrectomy

For preoperative preparation, the donor animal was injected subcutaneously with Telazol 100 (Zoetis, Spain) 50 mg. Intravenous catheter Vasofix Certo 22 G (BBraun, Germany) was placed in the marginal ear vein. Atropine 0.2 mg and dexamethasone 2 mg intravenously were used for premedication. The animal was shaved and positioned on its back. Telazol 100 0.5 mL and Xyla (Interchemie, Netherlands) 0.5 mL (10 mg) were injected intravenously. A laparotomy was performed and the abdominal aorta and inferior vena cava with renal arteries and veins branching from them were visualized. The posterior leaflet of the parietal peritoneum was opened near the renal hilum, after which the left renal artery (RA) and left renal vein (RV) were isolated and mobilized. In addition to the renal vessels, the left ureter was also isolated and mobilized over a sufficient length (about 7–9 cm from the kidney). Next, heparin 5000 units was injected intravenously, the exposure time was 3 minutes. Next, a vascular clamp was placed on the main vessels above the renal arteries and 10 mL of 4% potassium chloride was injected intravenously to stop cardiac activity. After 30 minutes of exposure after asystole, a 20 G intravenous catheter was inserted through the aorta into the left RA, the left RV was crossed, and cold cardioplegia of the left kidney was initiated with 60 mL Custodiol 4 °C solution.

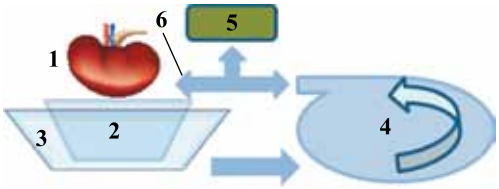


Fig. 1. Schematic diagram of the extracorporeal hypothermic perfusion device for *ex vivo* donor kidney preservation. 1, donor kidney; 2, organ reservoir with perfusate; 3, ice reservoir; 4, peristaltic pump; 5, invasive pressure measurement unit; 6, temperature sensor

### *Ex vivo* HMP

The extracorporeal circuit was pre-assembled for *ex vivo* HMP of donor kidney. The perfusion circuit consisted of a stepper roller pump, an invasive pressure measurement unit, a temperature sensor, two containers (one for positioning the kidney transplant and the other for ice), perfusion lines and a perfusion cannula. A schematic representation of the extracorporeal circuit is shown in Fig. 1.

The perfusion circuit was filled with 100 mL of dextran-40-based solution 4 °C, and heparin 1000 IU was added. After cold cardioplegia preservation, a 20 G intravenous catheter was inserted into the RA of the left donor kidney, deaeration was performed and the perfusion line was connected to the catheter. The *ex vivo* HMP of the donor kidney was performed with pressure regulation. The target pressure was 30 mmHg, with acceptable ranges of 28 to 36 mmHg. The initial volumetric perfusion rate was 5 ± 1.1 mL/min with a gradual increase to 50 ± 15 mL/min over 30 minutes. PVR was an estimated index calculated by the formula: R = P/V, where R is resistance, P is pressure in mmHg, and V is volumetric perfusion rate in mL/min; this indicator reflects the compliance of the renal graft vascular bed, which was a predictor of renal function recovery after implantation. The procedure lasted for 240 minutes. At the end of hypothermic perfusion, we performed another cold cardioplegia using Custodiol solution.

### Orthotopic transplantation of left donor kidney

After a preliminary 4-hour fast, the recipient animal was prepared for surgery. Induction of anesthesia was performed similarly to the donor stage. Vital functions of recipient animals were assessed by pulse oximetry ($SpO_2$ >90%, HR = 180–230/min), and respiratory movements were visually counted (HR >45/min). At laparotomy, bowel loops were removed to the right side and wrapped in sterile napkins pre-moistened with warmed

saline. After isolation and mobilization of recipient's renal vessels and isolation of ureters, heparin 100 units was injected intravenously, and bilateral nephrectomy was carried out. Blood flow through the RA and RV was stopped by applying bulldog clamps. The stumps of the vascular pedicle of the right kidney were sutured and tied with Prolene 5.0 suture material, the ureter was ligated caudally to the bladder with Prolene 5.0 suture material. The recipient's left RA and RV stumps were left sufficient for anastomosis, their length was 3–4 cm for RA and RV. First, end-to-end anastomosis was performed between the renal vein of the donor kidney and the recipient's left RV stump. The posterior wall was sutured with Prolene 6.0 suture material using continuous wraparound sutures, and the anterior wall was sutured with interrupted sutures. After venous anastomosis and checking the quality of venous suture hemostasis, an end-to-end arterial anastomosis was made between the RA of the donor kidney and the recipient's left RA stump using Prolene 7.0 suture material. The arteries were anastomosed using interrupted sutures. Before applying the last suture, deaeration was performed, the artery was treated with Lidocaine 2% to prevent vasospasm, methylprednisolone 20 mg was administered intravenously, and blood flow was started (Fig. 2).

Urine coming out of the ureteral stump after blood flow via the renal arteries was resumed was a sign that kidney function had recovered. Sixty minutes after the procedure started, Telazol 100 0.5 mL and Xyla 0.5 mL were injected intravenously to maintain anesthesia. Ureteral anastomosis was the last stage in the kidney transplant procedure. End-to-end ureteral anastomosis between the recipient's ureteral stump and the ureter coming from the donor kidney was performed using a stent – a 20 G IV catheter – and left inside the lumen. Four interrupted sutures were applied with Prolene 7.0 suture material evenly around the entire circumference of the ureter, after which the kidney was fixed to the renal bed with separate interrupted sutures between the fatty capsule of the kidney and the surrounding tissues. After kidney transplantation, the intestinal loops were placed back into the abdominal cavity and the wound was sutured layer-by-layer.

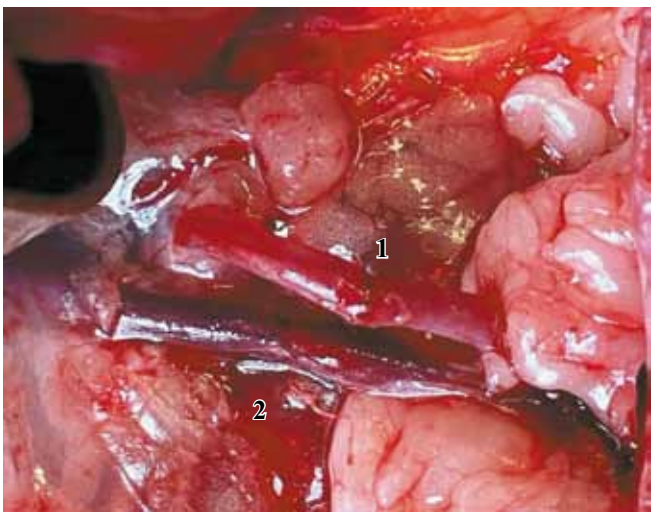


Fig. 2. Vascular anastomoses performed during donor kidney implantation. 1, renal artery anastomosis; 2, renal vein anastomosis

Material for histologic examination was collected on day 12 by routine excisional kidney biopsy, fixed in 10% neutral buffered formalin (Biovitrum, Russia), dehydrated in 8 changes of isopropanol, starting with 50% aqueous isopropanol, poured into Histomix paraffin medium (Biovitrum, Russia) using the ESD-2800 filling system (MedTekhnikaPoint, Russia). Thin paraffin sections, 4–6 μm thick, obtained on a semi-automatic rotary microtome ERM 3100 (Hestion, Australia) were stained with hematoxylin and eosin. Microscopic study of the obtained experimental material was performed using an Olympus CX21 microscope at low (100×), high (400×) magnifications and oil immersion (1000×). Microphotographs were obtained with a digital camera MC-10 (LOMO, Russia), the obtained images were processed in MCview software.

Statistical analysis was performed using the StatTech v. 3.1.10 program (developer StatTech LLC, Russia). Quantitative indicators were evaluated for conformity to normal distribution using the Shapiro–Wilk test (number of subjects less than 50). Quantitative indices having normal distribution were described using arithmetic mean (M) and standard deviations (SD), 95% confidence interval (95% CI) limits. One-way analysis of variance with repeated measures was used to compare three or more related groups on a normally distributed variable. Statistical significance of changes in the indicator over time was assessed using Pillai's Trace. Posterior analysis was performed using paired Student's t test with Holm correction. When comparing three or more dependent populations whose distribution differed from normal, the nonparametric Friedman test was used with post hoc comparisons using the Conover–Iman criterion with Holm correction. Results were considered statistically significant at $p < 0.05$.

## RESULTS

### Dynamics of changes in peripheral vascular resistance during hypothermic perfusion

During *ex vivo* HMP of the donor kidney, parameters such as RA pressure and volumetric flow rate, were recorded. Based on these parameters, an objective index – PVR, measured by an invasive method directly in the RA for 240 minutes – was calculated, which is depicted in Fig. 3.

In the PVR study, high values were recorded at the beginning of the procedure and after 30 minutes, which was reflected by values of 1.90 ± 0.27 mm Hg/mL/min, and

were a consequence of asystole preceding withdrawal for 30 minutes. However, by the end of the procedure, PVR values decreased and corresponded to physiologic values (0.72 ± 0.09 mmHg/mL/min) for experimental animals.

## Dynamics of changes in biochemical parameters after transplantation

The main recorded biochemical indicators in the post-transplant period were creatinine (Fig. 4) and urea (Fig. 5) levels, as well as the calculated index – glomerular filtration rate (GFR), calculated according to the Schwartz formula: GFR = 36.5 X (length of the rabbit from head to tail in cm/serum creatinine in μmol/L), which is graphically depicted in Fig. 6.

Creatinine, as the main marker of renal function, reflected the excretory function of the graft in recipient animals. On the second day after transplantation, creatinine levels increased to 91.07 ± 11.49 μmol/L in all the animals. However, by the end of the follow-up period, the level did not exceed the limit values of physiologic norm – 77,17 ± 10,19 μmol/L in total. The nonlinearity of the graph of creatinine level fluctuations reflects the recipients' physiologic activity in the posttransplant period.

Changes in urea levels also reflected the state of excretory function of the graft. On the second follow-

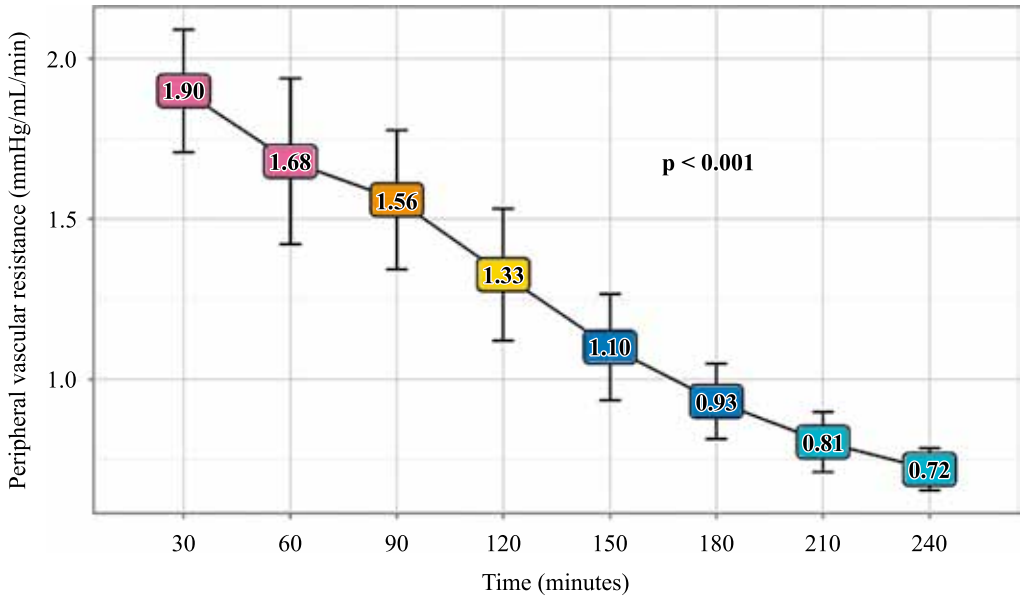


Fig. 3. Changes in peripheral vascular resistance during *ex vivo* hypothermic perfusion of donor kidney. The graph is represented by mean values, vertical lines denote standard deviations, p is statistical significance

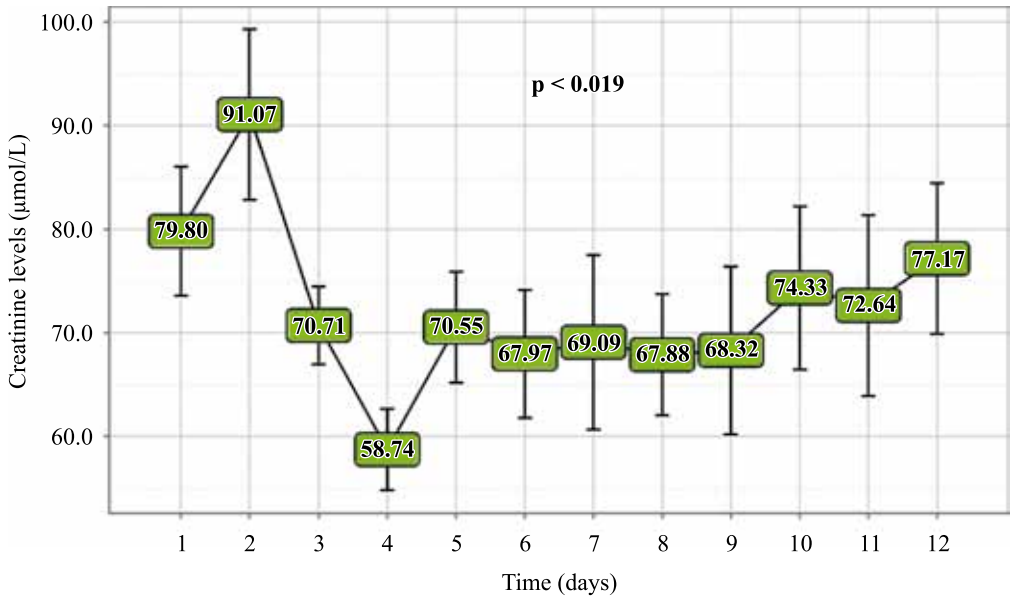


Fig. 4. Changes in creatinine levels after donor kidney transplantation in recipient animals. The graph is represented by mean values, vertical lines indicate standard deviations, p is statistical significance

up day, there was a peak in average urea levels up to 9.09 ± 1.06 mmol/L, which correlated with creatinine levels. However, the urea concentration curve was descending throughout the entire follow-up period, and on day 12, the indicators reached a physiologic norm, 4.88 ± 0.54 mmol/L.

GFR was an objective indicator of the functional status of the graft, adjusted for the small body surface area of the experimental animals. In the early post-transplant period, GFR varied from 26.29 to 29.18 mL/min/1.72 $m^2$ in mean values, and by the end of the follow-up period, the mean GFR value was 26.74 ml/min/1.72 $m^2$. Changes in GFR over 12 days did not go beyond the physiological norms of experimental animals.

Evaluation of biochemical parameters clearly demonstrate the preservation of excretory function of the transplant for 12 days, and the physiologic values of GFR suggest that the functional status of the donor kidney is satisfactory.

## Post-transplant histological study

According to the histological study (Fig. 7) of 10 biopsy specimens obtained from recipient rabbits, morphological changes are represented by the presence of signs of dystrophic changes in the form of intracellular

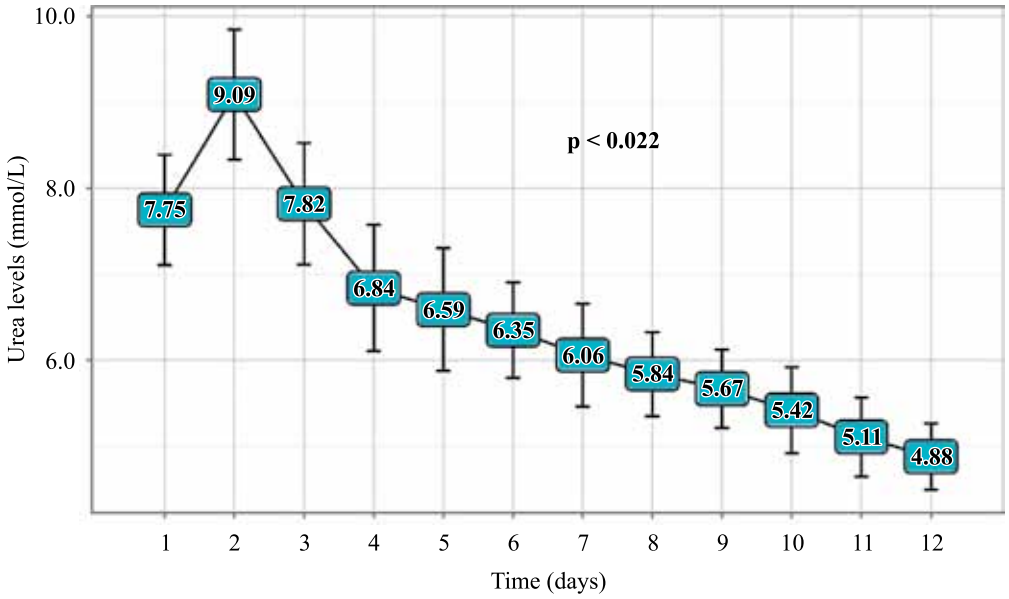


Fig. 5. Changes in urea levels after donor kidney transplantation in recipient animals. The graph is represented by mean values, vertical lines indicate standard deviations, p is statistical significance

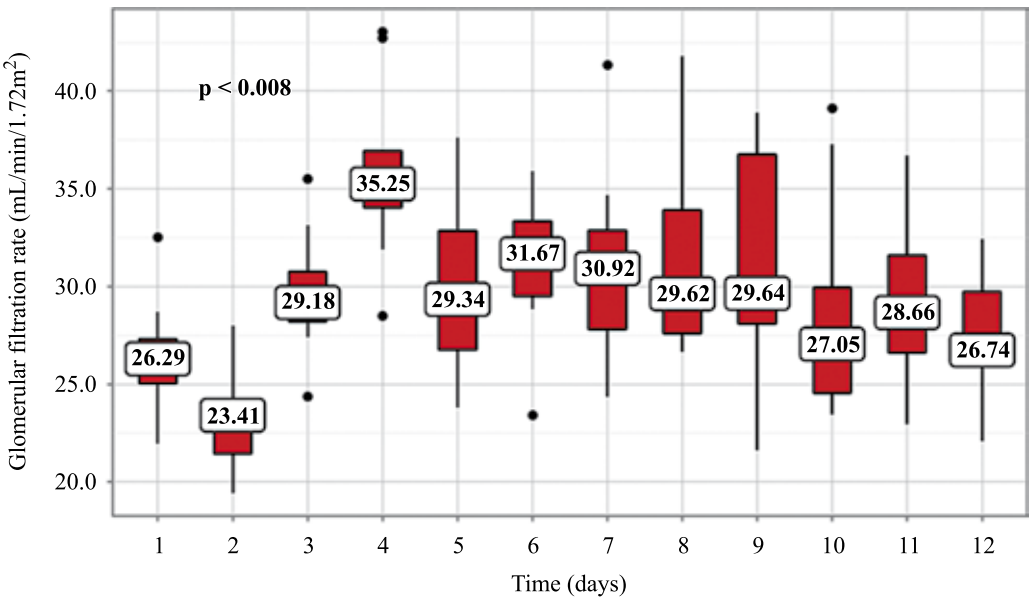


Fig. 6. GFR changes after donor kidney transplantation in recipient animals. The graph is represented by mean values, vertical lines denote standard deviations, p is statistical significance

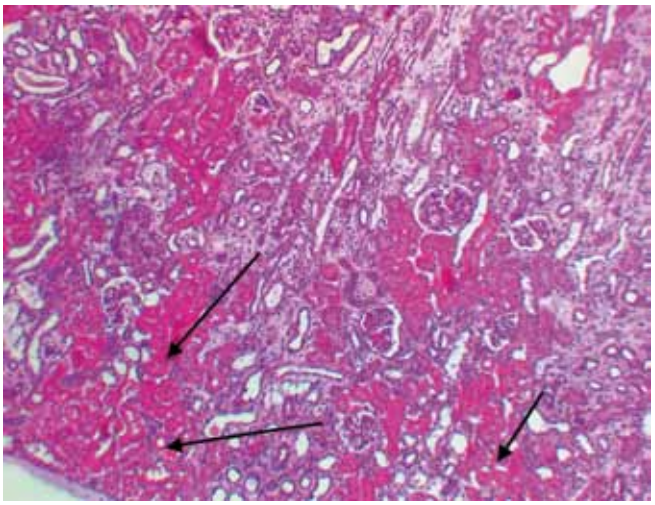

Fig. 7. Histological kidney tissue slide at 12 days after transplantation. Arrows indicate foci of renal tubule epithelium necrosis. H&E stain, magnification 100×

inclusions and diffuse foci of epithelial necrosis (marked by black arrows in the figure) of both proximal and distal tubules. Polymorphic cellular infiltration is also present, occupying from 10% to 25% of the parenchyma. The presence of signs of tubular atrophy in no more than 25% of the cortex indicates the onset of transplant glomerulopathy [7].

## DISCUSSION

One possible path for modern transplantology is to enable the use of donor organs in cases of circulatory arrest [8]. *Ex vivo* perfusion of donor organs has allowed the donor pool to grow by over 25% to date, and this figure continues to rise annually. Thus, an experimental study of hypothermic perfusion, the influence of *ex vivo* perfusion technique, as well as subsequent clinical implementation will ensure a future increase in the number of transplants [9].

Investigation of new perfusion solutions is extremely important. Currently, the choice of perfusion agent for *ex vivo* hypothermic or normothermic machine perfusion remains an open debatable issue [10]. Despite the existing and implemented clinical protocols, an ideal perfusion agent has not been identified [11]. This study describes the use of a dextran-40-based preservation solution. Due to the high colloidal and rheological properties of dextran-40, as well as its moderate molecular size, this particular component has an advantage for use in *ex vivo* hypothermic perfusion of donor kidney. For example, Lindell et al. studied the effect of the commonly used potassium-rich hydroxyethyl starch (HES) – containing perfusion solutions for hypothermic perfusion of donor kidneys, Belzer MPS and UW Viaspan. Despite successful procedures, the results described moderate interstitial graft edema and moderate post-transplant changes [12]. In turn, because dextran-40 has the special ability to bind water molecules, it can prevent interstitial edema of donor kidney tissue during prolonged perfusion. Moreover, unlike albumin solutions and HES-containing agents, dextran molecules prevent the destruction of the renal structural unit without rupturing or occluding the nephron [13].

During *ex vivo* HMP of the donor kidney, high PVR values were observed, which was a consequence of a preliminary 30-minute asystole in the donor. Throughout the perfusion procedure, PVR decreased in all cases, and returned to normal at the end of perfusion, proving the efficacy of *ex vivo* HMP for rehabilitation of expanded criteria donor kidneys. After transplantation of such kidneys, physiologic indicators of biochemical markers of renal function were observed, not exceeding borderline values of the maximum permissible limits [14]. Graft function was preserved in all cases, which is evidence of the effectiveness of *ex vivo* HMP and correlates with the data obtained during perfusion.

## CONCLUSION

In addition to demonstrating the preservation of the functional characteristics of the graft after transplantation in the rabbit experiment, the experimental study showed the admissibility of using a dextran-40-based preservation solution as a perfusion agent for *ex vivo* hypothermic machine perfusion of donor kidney. The findings of this work clearly indicate the need for a deeper investigation into the pathophysiological aspects of the effect of dextran-40-induced hypothermic perfusion on kidney graft, both in the early and the long-term postreperfusion periods.

*The authors declare no conflict of interest.*

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