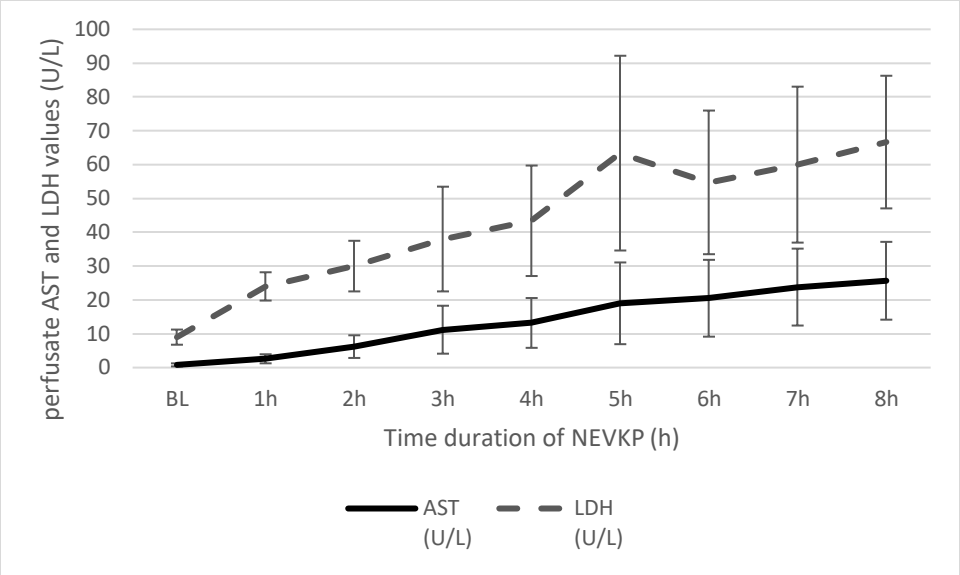

Figure S1. perfusate AST and LDH values during 8h of NEVKP. The values are presented as mean values +/-standard deviation

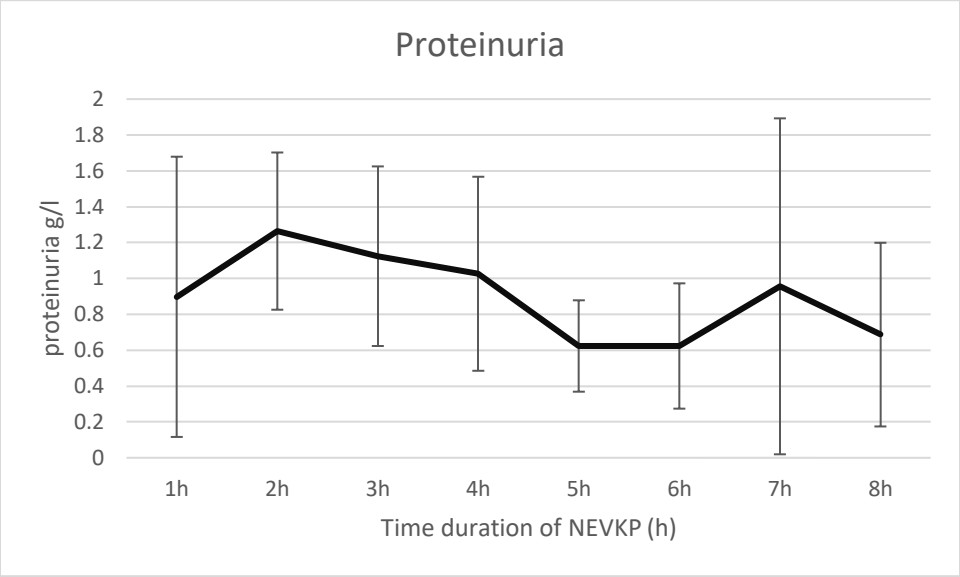

Figure S2. Proteinuria during the perfusion. The values are presented as mean values +/-standard deviation

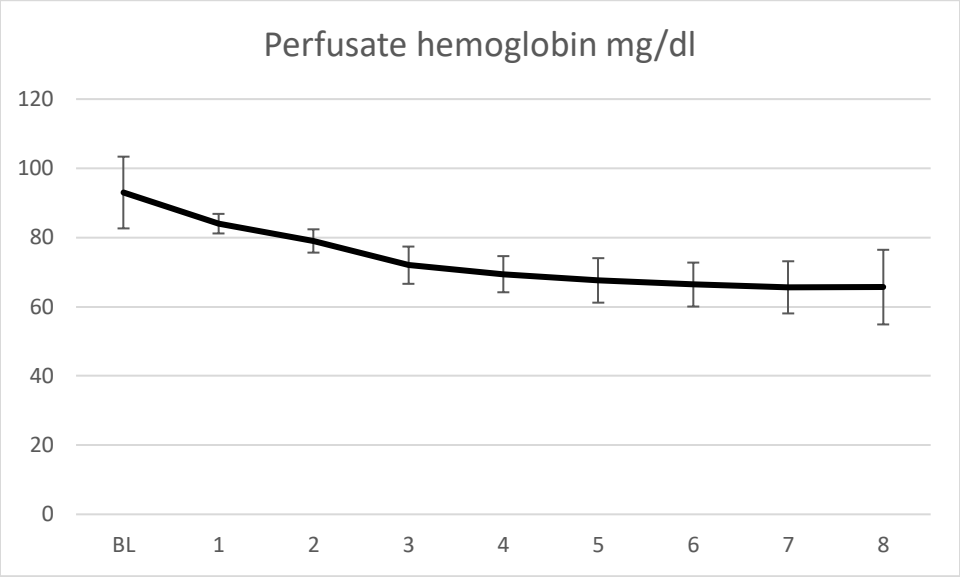

Figure S3. The change in perfusate hemoglobin levels during 8h of NEVKP. The values are presented as mean values +/-standard deviation

SUPPLEMENTARY DIGITAL CONTENT

Materials and methods

Animals

3 months old male Yorkshire pigs (~30kg) were utilized and acclimated 1 week prior to experimentation. All animals were randomly assigned to one of the three groups. Average animal weights were similar in each group (NEVKP group: 30.7±1.3kg, no preservation group 30.8±1.5kg, SCS group 29.4±2.1kg; p=0.49). Water and food was provided ad libium. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care of Laboratory Animals" published by the National Institutes of Health.

Whole Blood, Serum, and Urine Measurements

Blood gas analysis (RAPID Point 500 Systems, Siemens AG, Berlin, Germany) were performed during the perioperative period (baseline, prior to retrieval, prior to autotransplantation, hourly after reperfusion in the first 3hr, and daily during the postoperative follow up. Serum samples were also analyzed daily for creatinine, blood urea nitrogen and AST (Piccolo Xpress, Union City, Canada) prior to the transplant and during the postoperative follow up. Further serum and urine analyses were performed in the Core Laboratory on the Abbott Architect Chemistry Analyzer using the manufacturer's reagents (Abbott Laboratories, Abbott Park, IL, USA). Twenty-four-hour urine collection was performed using a metabolic cage to investigate the creatinine clearance before transplantation (day -1), on postoperative day 3 (pod 3). Additional serum samples were frozen down to -80°C until ELISA analysis. Neutrophil gelatinase-associated lipocalpin (NGAL), one of the most specific early renal injury biomarkers that has

recently emerged in clinical laboratory practice to predict acute kidney injury (AKI) (19), was measured using a porcine (NGAL) ELISA kit (Bioporto, Hellerup, Denmark). Serum samples were taken baseline (BL), first (1POD) and third postoperative morning (3POD).

Histology

Under anesthesia, baseline needle biopsy was performed from the contralateral kidney during the retrieval, and from the graft kidney 30 minutes after the reperfusion during the transplant procedure. On the 3rd postoperative day the pigs were euthanized, under deep anesthesia a wedge biopsy of renal tissue was performed. Tissue was placed in 10% neutral buffered formalin and transferred to 70% alcohol after 48h. Following paraffin-embedding, sectioning, and staining, 3-μm periodic acid-Schiff (PAS) stained sections were used to score tubular injury, edema, fibrosis and interstitial inflammation on a scale of 0 to 3 blinded to the experimental group. Tubular injury including brush border loss, tubular dilatation, epithelial vacuolation, thinning and sloughing, and luminal debris were scored in 10 high power fields (HPF) and averaged to assess overall tubular injury. Interstitial inflammation was scored in 10 low power fields and averaged. Glomerular shrinkage was assessed. TUNEL staining was performed according to standard protocol. A semi-quantitative TUNEL score was used, assessed in 25 high-power fields and averaged. According to the manufacturer's instructions, polyclonal rabbit anti-human Ki67 antibodies (Cat# ab15580, 1/5000, ABCAM) were used for immunohistochemistry staining for needle-biopsy samples taken 30 minutes after reperfusion. The positive cells were counted and averaged in 10 high-power fields under light-microscope.

Statistical Analysis

SPSS software version 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Values are presented as means ± standard deviation. For multiple comparisons, one-way analysis of variance (ANOVA) was used with Tukey a post hoc test to identify significant differences between the selected groups. Repeated measures ANOVA was used to compare continuous repeated variables over time such as postoperative serum creatinine and BUN with post hoc Bonferroni correction. For comparison of non-parametric variables, such as histology and TUNEL scores, Kruskal-Wallis test was used. To test normally distributed continuous parameters over time within the same group, such as perfusion parameters, a paired T-test was used. Significance was defined as $p<0.05$.

BGA analysis, osmolarity and oncotic pressure values	***Baseline values from the perfusate (n=5)***	***Physiologic values in venous samples from Yorkshire pigs (n=20)***
pH	7.32±0.02	7.46±0.06
pCO2	36.1±5.5 mmHg	43.5±7.3 mmHg
pO2	633±21 mmHg	47.5±7.3 mmHg
HCO3-	20.2±2.8 mmol/l	30.3±2.4 mmol/l
Hb	105±14 g/l	104±10 g/l
O2 sat	99.9%	-
Na+	142±0.8 mmol/l	137±3.9 mmol/l
K+	3.5±0.1 mmol/l	3.9±0.5 mmol/l
Ca2-	1.36±0.15 mmol/l	1.25±0.1 mmol/l
Cl-	108±2 mmol/l	101±2 mmol/l
Glu	4±0.4 mmol/l	4.7±2.5 mmol/l
Lac	10.4±0.8 mmol/l	0.94±0.2 mmol/l
Osmolarity	286±4 mosmol/l	282±2 mosmol/l
Oncotic pressure	11±0.9 mmHg	14±0.8 mmHg

Table S1. comparison of perfusate composition, osmolality and oncotic pressure parameters with venous blood samples. The perfusate composition was designed and optimized based on series of measurements for electrolyte composition and osmotic and oncotic features of venous samples taken from heathy pigs. Oncotic pressure was adjusted by using double reverse osmosis water

(DRO). STEEN solution is a buffered extracellular solution optimally designed to ex vivo perfusion systems.