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Long-term Transplant Function After Thrombolytic Treatment Ex Vivo of Donated Kidneys Retrieved 4 to 5 Hours After Circulatory Death

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Background. Using a novel thrombolytic technique, we present long-term transplant function, measured by creatinine and iohexol clearance, after utilizing kidneys from porcine donors with uncontrolled donation after circulatory deaths, with 4.5–5 h of warm ischemia. **Methods.** Pigs in the study group were subjected to simulated circulatory death. After 2 h, ice slush was inserted into the abdomen and 4.5 h after death, the kidneys were retrieved. Lys-plasminogen, antithrombin-III, and alteplase were injected through the renal arteries on the back table. Subsequent ex vivo perfusion was continued for 3 h at 15°C, followed by 3 h with red blood cells at 32°C, and then transplanted into pigs as an autologous graft as only renal support. Living-donor recipient pigs that did not receive ex vivo perfusion, and unilateral nephrectomized pigs served as the controls. **Results.** Pigs in the study group (n = 13), surviving 10 d or more were included, of which 7 survived for 3 mo. Four animals in the living-donor group (n = 6) and all 5 nephrectomized animals survived for 3 mo. Creatinine levels in the plasma and urine, neutrophil gelatinase-associated lipocalin levels, Kidney Injury Marker-1 expression, and iohexol clearance at 3 mo did not differ significantly between the study and living-donor groups. Histology and transmission electron microscopy after 3 mo showed negligible fibrosis and no other damage. **Conclusions.** The present method salvages kidneys from extended unontrolled donation after circulatory death using thrombolytic treatment while preserving histology and enabling transplantation after ex vivo reconditioning, with clinically acceptable late function after 3 mo, as measured by creatinine and iohexol clearance.

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INTRODUCTION

According to the Global Observatory on Donation and Transplantation, less than 10% of the global need for donor organs is met.[1] The primary source of donor organs (77.3%) used in transplantation originates from donation after brain death, although an increasing number of organs

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are donated after circulatory death (DCD).[1] Different types of donors are classified according to the Maastricht scale.[2] Other means of solving organ shortages include living donors, which contribute to a substantial number of transplantations in many countries. Regenerative medicine[3,4] and xenotransplantation[5] are alternative approaches to increase the number of available organs; thus far, they have not contributed to a larger donor pool, although recent clinical attempts are encouraging.[6,7] Until recently, less than 5% of deceased donors were controlled DCD. The numbers are improving, but the total number of deceased donors has not increased, yielding a donation of around 20 donors per million people (PMP). The UK has 30%–40% controlled DCD but still has a similar number of donors as PMP.[8] Although uncontrolled DCD (uDCD), that is, patients dying from circulatory arrest outside the hospital, accounts for only 2.4% of the total number of organs from DCD donors in Europe,[8] it is by far the most promising source of potential organs. By limiting the donors to 65 y of age or younger, not dying of intoxication or suicide, and excluding all intercurrent diseases that could result in kidney disease we arrived at 30 potential donors, in a city of 5–600 000 inhabitants. This translates to 50 donors PMP, or 100 kidney PMP. These numbers translate to 50% of the number of kidney transplantations performed yearly in Gothenburg (from deceased donors). The hurdles that must be overcome to make this option more readily available are a simplified process for organ retrieval and a reliable method for reducing the negative effects of warm ischemia time (WIT), resulting in an increased frequency of delayed graft function in kidneys transplanted from uDCD donors.

In a recent study,[9] we reported a novel method to salvage kidneys from extended uDCD. The protocol allowed the retrieval of uDCD kidneys in an ethically and clinically acceptable manner, without the need for extracorporeal circulation or rapid procurement by using a new thrombolytic treatment procedure utilizing Lys-plasminogen and tissue plasmin activator (tPA) (alteplase) ex vivo to clear the capillaries of the kidney from fibrin. We hypothesized that the most important factor causing ischemia-reperfusion injuries (I/R-Is) and delayed graft function in uDCD, and possibly in donation after brain death kidneys, is the formation of fibrin thrombi in the capillaries, as demonstrated by immunohistochemistry (IHC) and transmission electron microscopy (TEM). A recent study by DiRito et al[10] who examined the effects of plasminogen and tPA on marginal kidneys in an ex vivo normothermic machine perfusion model, found that clearing fibrin from capillaries in tubules resulted in decreased vascular resistance. Plasminogen occurs naturally as Glu-plasminogen in human plasma and is converted naturally to the more effective Lys-plasminogen after first binding to fibrin.[11] There is a risk of rethrombosis due to activated endothelium and platelets after dissolving the clot despite the presence of naturally occurring antithrombin-III (AT-III). We used a direct thrombin inhibitor (argatroban)[12] and a platelet inhibitor (abciximab)[13] to prevent clot formation owing to the activated endothelium and platelets after the clots had been dissolved.

To differentiate between allogeneic responses and ischemia-reperfusion reactions, we designed an autotransplantation model for uDCD as described in previous publications.[9,14] A short period of 30 min as an "allogeneic" graft in the donor did not result in rejection (unpublished data), indicating that we primarily studied I/R-I. In addition, by applying a "sham" procedure to one group, we challenged a pig with the same surgical trauma as the study group by switching one kidney from the left side to the right side in a living-donor autotransplantation procedure, resulting in the only remaining difference being ischemic trauma in the study group.[14]

The present study aimed to report the recovery of renal function, measured as creatinine and iohexol clearance, after 3 mo of observation following transplantation of uDCD kidneys treated ex vivo with Lys-plasminogen, AT-III, and tPA, according to the protocol described in detail in our recent article.[9]

MATERIAL AND METHODS

Animals

Swedish domestic pigs (n = 38), weighing 30–50 kg, were used in this study. The study design is summarized in Figure 1 and Table 1. An autotransplantation model for uDCD has been developed to avoid allogeneic reactions.[9,14]

Ethical Permit

The Regional Animal Experiment Ethics Committee approved this study (Dnr 5.8.18-13977/2018; Dnr 5.8.18-09474/2019; 5.8.18-09182/2020).

Anesthesia and Experimental Equipment

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

Blood Grouping

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

Red Blood Cell Washing

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

Ex Vivo Perfusion Device

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

DCD Protocol

This protocol has been recently described in detail.[9] Briefly, donor pigs were anesthetized and maintained under normoventilation. The blood gases, blood pressure, weight, and length of the pigs were recorded. The ventilator was turned off and the time of asystole, which occurred within 15 min in all pigs, was recorded as the time of death. Pigs were left at room temperature for 2 h before a small midline incision was made and ice slush (2–3 liters) was poured into the abdomen. Temperature probes were placed in the liver and right flank muscles close to the right kidney. Each pig was left for 2 additional hours before both kidneys were removed en bloc with the caval vein and the aorta.

Perfusion Procedure

The perfusion procedure has been recently described in detail.[9] See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473. Thirteen pigs that received

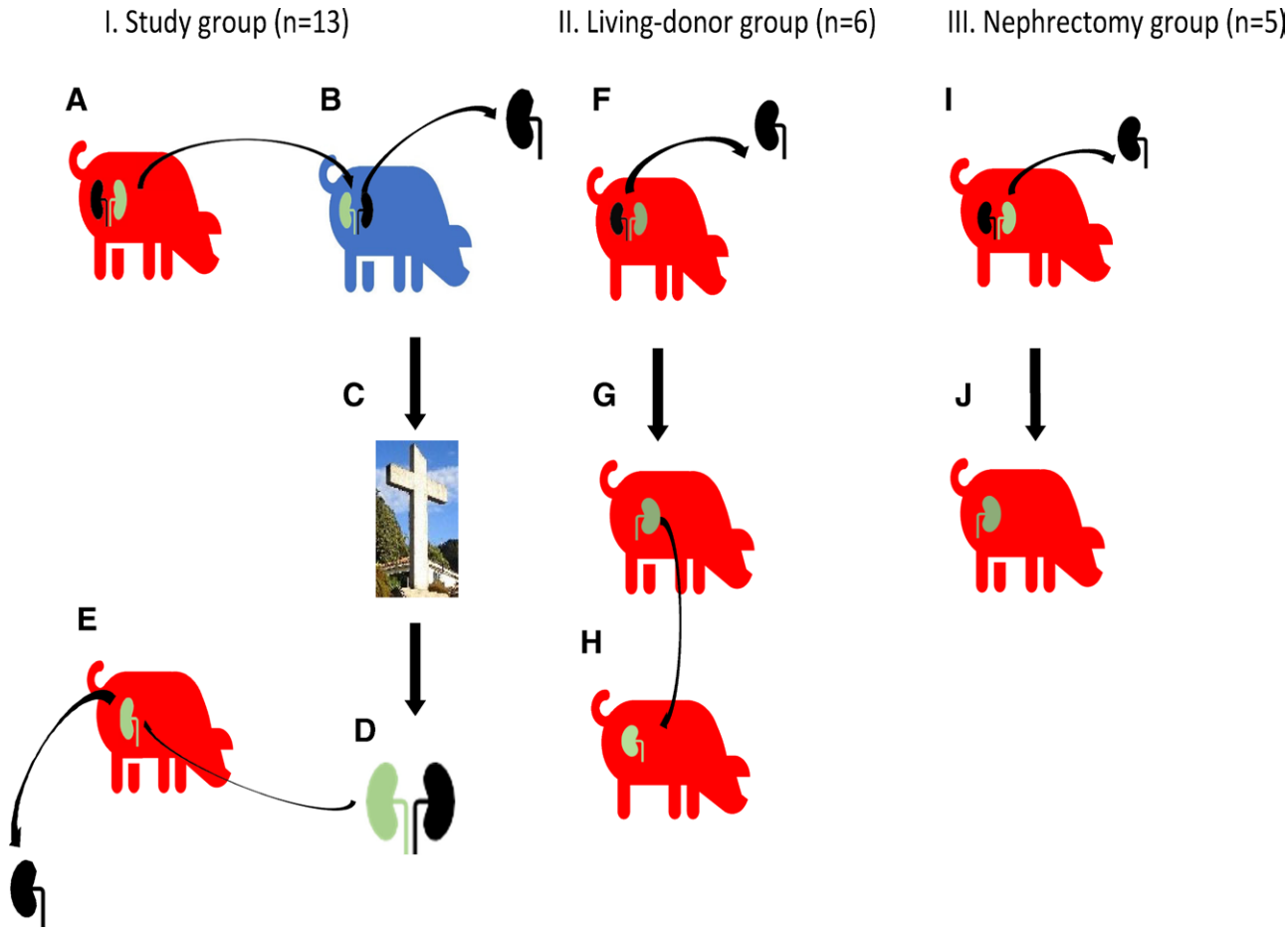

FIGURE 1. The study group I (A–E) is seen to the left: (A) The left kidney, in green, was transplanted from the recipient (red pig) to the donor pig (blue pig) after (B) the right kidney of the donor had been removed and discarded. C, The donor pig was then converted to a uDCD pig. D, After 4.5 h, both the remaining donor kidney and the transplanted recipient kidney (in green) were removed en bloc with vena cava and aorta, followed by treatment according to the protocol. E, After completed reconditioning, the original recipient kidney, in green, was transplanted back to the recipient after the remaining right kidney of the recipient had been removed. The transplanted kidney is now an autotransplant, in a uDCD model. The recipient is followed for 3 mo.The live-donor group II (F–H) is seen in the middle (F) The right kidney is removed from a healthy pig. The pig is kept under anesthesia during the same time period as the study group. G, After 15–16 h, the remaining left kidney is removed and flushed with StoreProtect. H, After the flush, the kidney is transplanted back to the right side of the pig, without any reconditioning protocol, and then observed for 3 mo.The nephrectomy group III (I,J) is seen to the right: (I) The right kidney is removed and the pig is observed for 3 month with no surgery (J) until the end of the experiment. uDCD, uncontrolled donation after circulatory death.

TABLE 1.

Groups included

Group	Recipients (n)	Donors (n)	Treatment	Total (n)
I	13	13	uDCD, perfused with base solution and given thrombolysis before being transplanted	26
II	6	N/A	One kidney was removed and after 16 h anesthesia the remaining kidney was transplanted to the contralateral side thrombolysis	6
III	5	N/A	One kidney was unilaterally removed	5
Sum	24	13		37

I, One kidney was transferred from the recipient (n = 13) to a pig and subsequently converted into an uDCD donor (n = 13). After reconditioning, the same kidney is transplanted back into the recipient.[9,14]

II, One kidney was removed, and the pig was kept under anesthesia at the same time as in Group I. The remaining kidney was removed and switched to the contralateral side (sham procedure) to achieve similar surgical and anesthetic trauma as in Group I.

III, One kidney was unilaterally removed from healthy pigs.

Groups I-III: Used for histology and TEM studies.

TEM, transmission electron microscopy; uDCD, uncontrolled donation after circulatory death.

the study protocol underwent autologous transplantation as previously described[9,14] and were observed for up to 3 mo.

Two groups served as controls for the transplanted study group kidneys:

II. Living-donor Group (n = 6)

Owing to complex and extensive surgery, a "sham" procedure was designed.[14] Six pigs were anesthetized and the right kidney was removed. The abdomen was closed and the pigs were anesthetized for 15–16 h to simulate the study group, after which the remaining left kidney was removed and flushed with StoreProtect before being transplanted on the right side. After 90 min of observation, the abdomen was closed and the pig was returned to its housing for a 3-mo follow-up. Thus, these otherwise untreated controls can be compared to a living donor or a kidney autotransplantation.

III. Nephrectomy Group (n = 5)

Pigs were nephrectomized on the right side, returned to their housing, and observed for 3 mo.

The kidneys of groups II and III were not subjected to perfusion treatment.

Blood Gas and Creatinine Analysis

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

IOHEXOL Clearance

Iohexol concentrations in the serum were analyzed using UPLC-MS/MS at the Department of Laboratory of Clinical Chemistry, Sahlgrenska University Hospital, using a modified version of the method described by Annesley.[13] See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

Arterial Flow

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

Histology

Representative histological materials from the 3 groups were analyzed in this study (Table 1). See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

Enzyme linked ImmunoSorbent Assay

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

IHC

IHC was performed to determine the presence/absence of Kidney Injury Marker-1 (KIM-1). See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

TEM

See Supplemental Materials and Methods, SDC, http://links.lww.com/TP/C473.

WinSTAT Statistics

WinSTAT Statistic for Excel (Robert Fitch software) was used for data analysis. The Mann-Whitney U-test was used for comparisons between groups, and the Wilcoxon signed-rank test was used for comparisons within groups. Values are presented as means or medians with ranges. Origin Pro was used for the graphical presentation of the scientific data.

RESULTS

Ex Vivo Flow and Resistance During Perfusion

Please see the Figure S1-S2, SDC, http://links.lww.com/TP/C473.

Survival and Renal Function After Transplantation

Study Group

Thirteen pigs were transplanted and survived for 10 d or longer, and 7 of these pigs reached 90 d. See the Results S2, SDC, http://links.lww.com/TP/C473 for details. The complications in the entire study group are summarized in Table 2 and further discussed in the SDC, http://links.lww.com/TP/C473 section.

Creatinine: Renal function, reflected by the creatinine level, measured after nephrectomy, 10 d after transplantation, and at the 1-mo and 3-mo time points in the study group was not significantly different from the values in the living-donor and nephrectomy groups (Figure 2A–D). Urine creatinine levels were high as expected and were not significantly different among the 3 groups (see Figure S3, Results S2, SDC, http://links.lww.com/TP/C473), indicating healthy kidneys. All pigs that survived for 3 mo were in excellent condition, and the macroscopic appearance of the study kidneys reaching the 3-mo endpoint appeared normal at exploration (Figure 3A). The cut surface of the explanted kidneys appeared macroscopically normal (Figure 3B) as well, compared with the living-donor kidneys (Figure 3C–D) and kidneys in the nephrectomy group (Figure 3E–F). The duration of anesthesia without any kidney during transplantation was approximately 30 min.

IOHEXOL clearance: Clearance at 3 mo compared to the living-donor group and healthy pigs with 2 kidneys are shown in Figure 4. There was no significant difference in clearance, as measured using the Mann-Whitney U-test.

Living-donor Group

Six animals were transplanted in the living-donor group, and 2 animals had technical complications. One animal had arterial thrombosis after reperfusion and was therefore excluded from the study. Another animal was excluded after experiencing bleeding from the venous anastomosis due to a fracture of the Prolene suture when manipulating the abdominal organs closing the wound. The remaining 4

TABLE 2.

Complications in study groups pigs

Complication	Number	Comments
Anesthesia complication	1	Day 10
Infections	5	Day 14, 35, 38, 72, 75
Total	6	

One animal died of anesthesia-related complications during the blood sampling on d 10. 5 pigs in the early series died of infectious complications, with normal kidney FUNCTION.

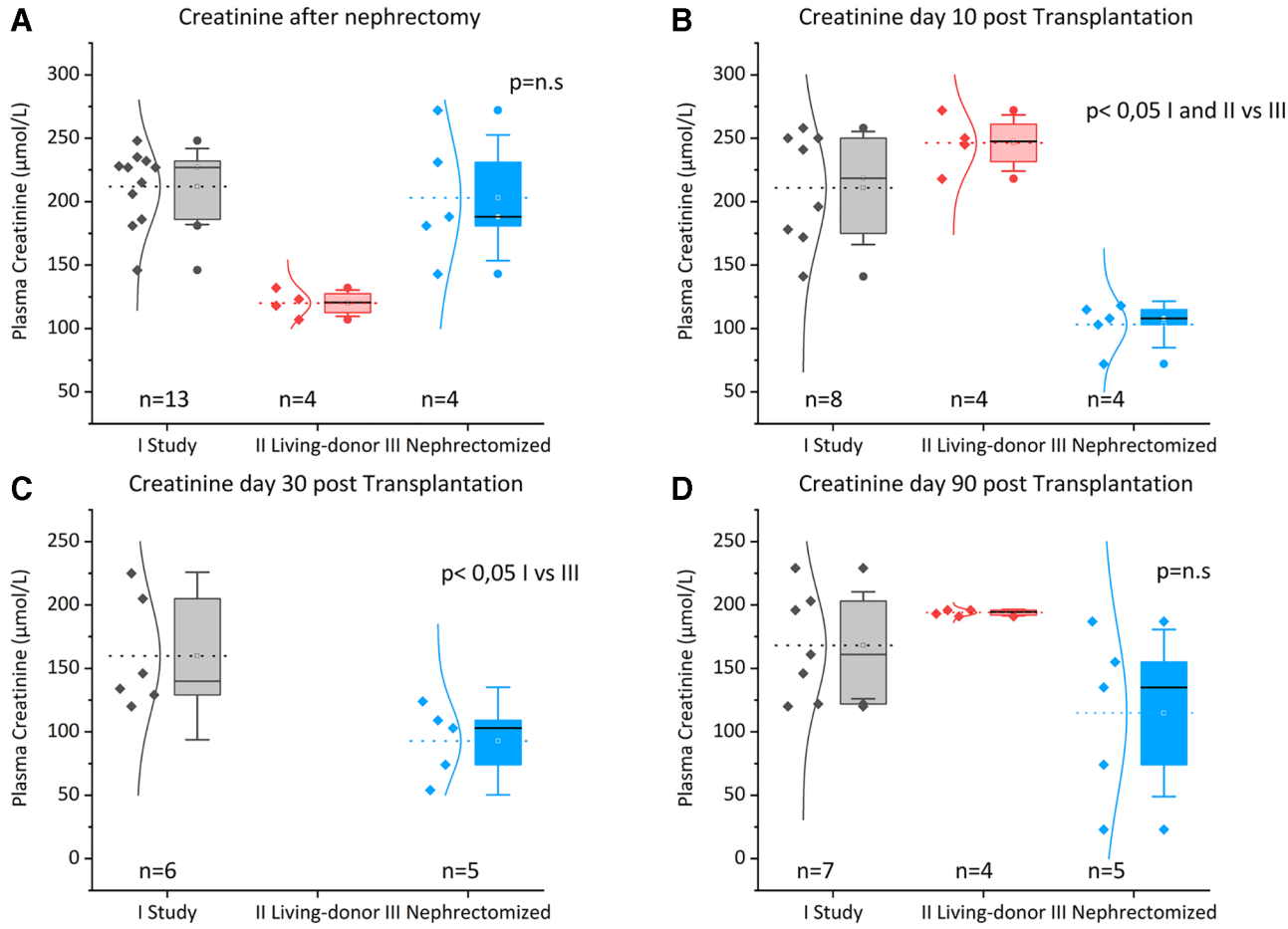

FIGURE 2. Box chart of creatinine in anesthetized pigs receiving study (black) and live-donor (red) group kidneys. Unilaterally nephrectomized controls, in blue, served as a second control group. The box shows 25%–75% percentile range of datapoints. Diamonds, in black, red, or blue, show individual datapoints, round symbols show outliers. Whiskers show 1 SD, mean values are marked as a dotted line, median value as a solid line. A, normal distribution line is plotted beside the data points. In (A) creatinine was measured in anesthetized pigs, after nephrectomy, of the study group (n = 13) kidneys in black, in the live-donor group (n = 4) kidneys in red and in the nephrectomy group (n = 4) kidneys in blue. In (B) creatinine was measured at 10 d after transplantation of the study group (n = 13) kidneys in black, in the live-donor group (n = 4) kidneys in red and in the nephrectomy group (n = 5) kidneys in blue. In (C) creatinine was measured at the 1-mo timepoint in the study group (n = 6) kidneys, in black, and nephrectomy group (n = 5) kidneys in blue, no samples were drawn from the sham group at the 1-mo timepoint. In (D) creatinine was measured at the 3-mo timepoint after transplantation of the study group (n=7) kidneys in black, in the live-donor group (n = 4) kidneys in red and in the nephrectomy group (n = 5) kidneys in blue. No statistical difference in creatinine levels was observed between the groups at the 3-mo timepoint.

animals were included in the study and reached the 3-mo endpoint.

Creatinine: At 10 and 30 d after transplantation, creatinine was significantly higher in the study and the living-donor group compared with the unilaterally nephrectomized pigs. No significant difference in creatinine levels compared with the study group was observed in plasma (Figure 2) or urine (see Results S2, SDC, http://links.lww.com/TP/C473) after 3 mo. Animals in the living-donor group did not have kidneys for approximately 30 min during transplantation.

IOHEXOL clearance: No significant difference in clearance compared with the study group was observed (Figure 4).

Nephrectomy Group

Five animals that were nephrectomized on the right side were used as controls. Creatinine returned to prenephrectomy levels during the observation period (Figure 2); however, the increase in creatinine level after nephrectomy was smaller than that in the other groups and returned to normal within 10 d. Animals in this group had at least 1 functional kidney at all the time points. All animals survived for 3 mo, with a microscopic appearance similar to that of the kidneys in the study and living-donor groups.

Arterial Flow

Arterial flows of the study, living-donor, and nephrectomized control groups at the 3-mo time point did not show any significant differences between the groups.

Neutrophil Gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) levels in urine after induction of anesthesia, 90 min after reperfusion and after 3 mo, did not show any significant differences between the 3 groups (Figure 5).

KIM-1

In biopsies taken 90 min after reperfusion, only 1 of the 4 transplanted kidneys showed positive staining for KIM-1 in both the study and living-donor groups (Figure 6). After 3 mo, 2 out of 4 transplanted kidneys in the study group, 3 out of 4 in the living-donor group, and 2 out of 5 in the

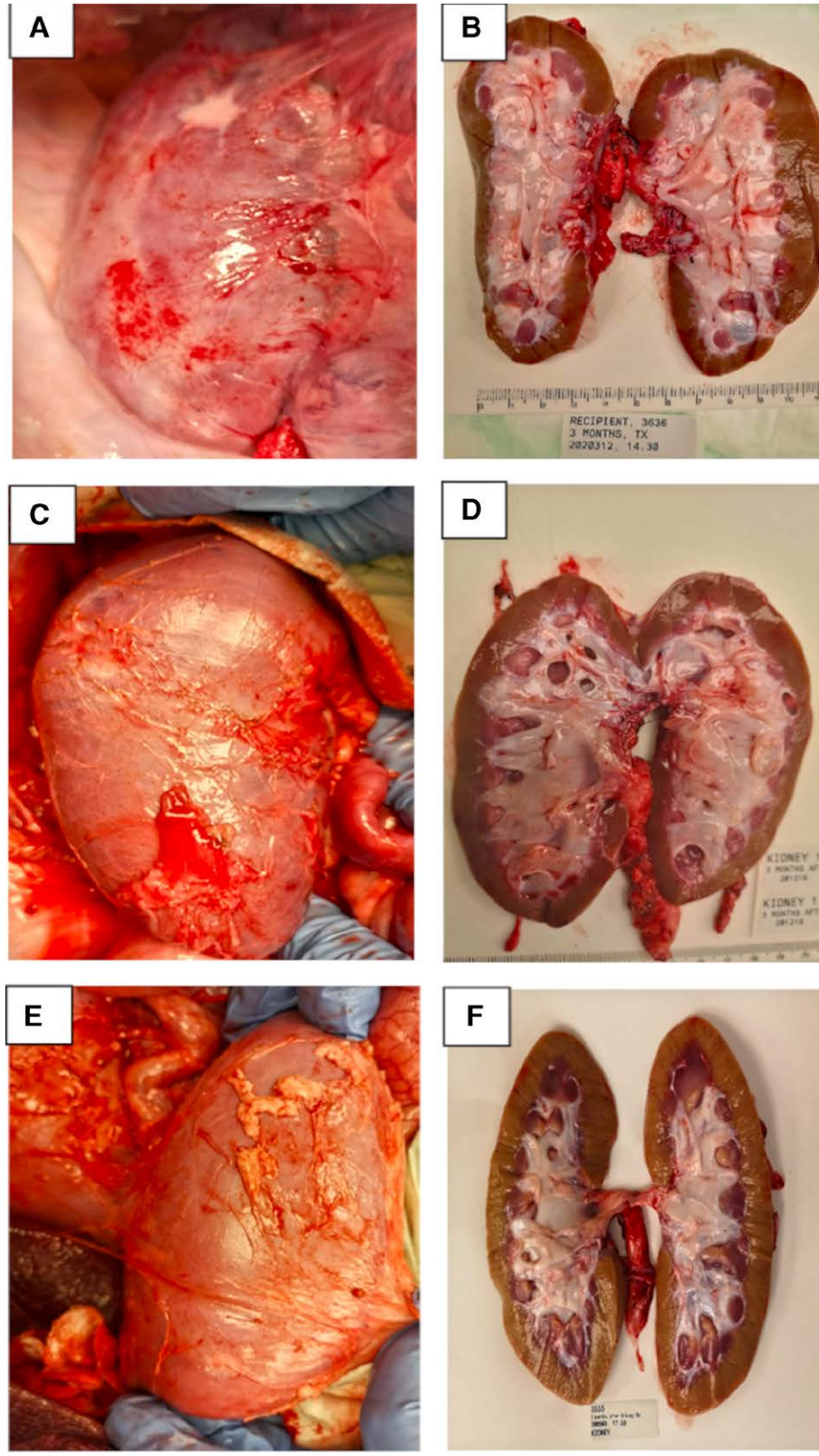

FIGURE 3. Normal looking kidney 3 mo after transplantation, in the study group (A), cut surface of the same kidney to the right (B). Normal looking kidney 3 mo after transplantation, in the live-donor group (C), cut surface of the same kidney to the right (D). Nephrectomy group kidney at exploration (E), and cut surface of the same kidney at the 3-mo time point to the right (F).

nephrectomized control group (Figure 7) stained positively for KIM-1. The amount of positive staining was the highest in the living-donor group, followed by the study group, and lowest in the nephrectomized control group. Some normal kidneys also showed positive staining (Figure 7) for KIM-1.

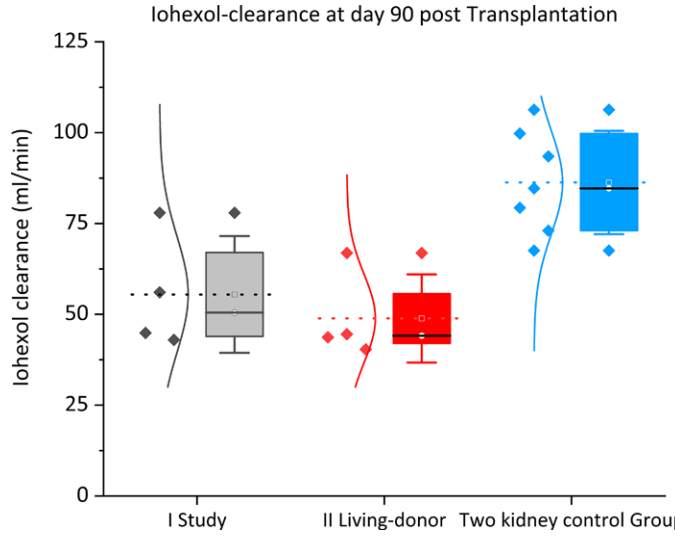

FIGURE 4. Box chart of iohexol clearance in at 3-mo after transplantation. The box shows 25%–75% percentile range of datapoints. Diamonds, in black, red or blue, show individual data points, round symbols show outliers. Whiskers show 1 SD, mean values are marked as a dotted line, median value as a solid line. A normal distribution line is plotted beside the data points. The black box shows data from study group animals surviving 3 mo (n = 4). The red box shows data from live-donor group pigs surviving 3 mo (n = 4). The blue box shows data from pigs with 2 normal kidneys (n = 7). There was no significant difference in clearance between the study and the sham groups.

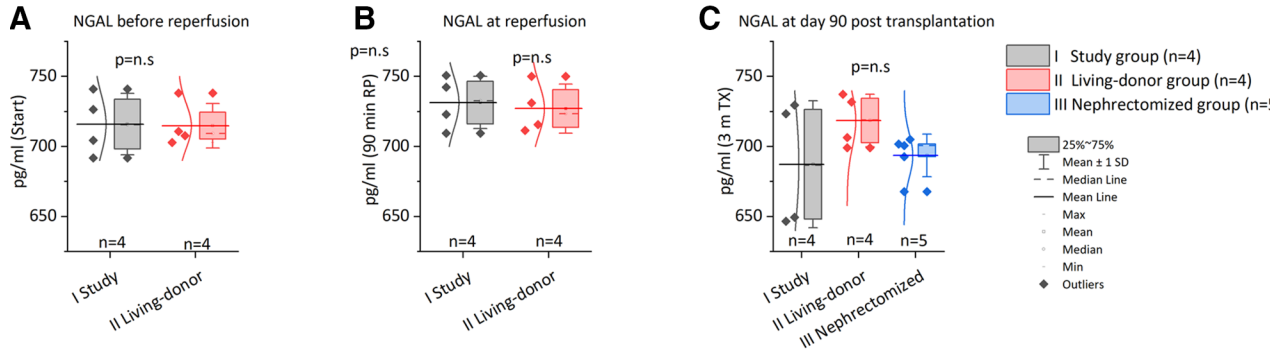

FIGURE 5. NGAL levels at start, 90 min after reperfusion of the transplanted kidney and 3 mo after transplantation. There was no difference between the study group I (n = 4) and the live-donor group (n = 4). At 3 mo comparison included the Nephrectomized Group (n = 5) as well, showing no statistical difference in NGAL expression. NGAL, neutrophil gelatinase-associated lipocalin.

Histological Changes in 3-mo Biopsies

Study Group

In all samples, slight signs of focal tubular injury were observed as a simplification of the proximal tubular epithelium. No inflammatory infiltrates were observed and there were no signs of tissue edema. A minimal degree of fibrosis, amounting to 2%–3% of the cortical tissue, was observed. The histological appearances of the glomeruli, vessels, and interstitium were unremarkable (Figure 8A,B).

Living-donor Group

Kidney histology in 3 of the 4 pigs was unremarkable (Figure 8C,D). The fourth kidney displayed a wedge-shaped area measuring 3×4 mm, with acute tubular injury and secondary inflammatory cell infiltrates. Signs of chronic tubular injury such as tubular atrophy and fibrosis were also observed in this area. Neutrophil infiltration was occasionally observed. Microscopic areas of tubular atrophy, interstitial fibrosis, and secondary inflammatory infiltrate were diffusely scattered throughout the tissue. However, the total degree of focally distributed cortical interstitial fibrosis and tubular atrophy was less than 10% (results not shown).

Nephrectomized Group

No significant signs of kidney injury were observed (Figure 8E and F).

TEM Images

TEM images of biopsies taken at 3 mo from the 3 groups are shown in Figure 9. The tissues of the study group showed an overall preserved architecture, but focal signs of tubular injury were observed in the form of epithelial vacuolization. In some tubules, epithelial swelling led to occlusion of the lumen and reduction of the apical brush border (Figure 9A,B). In the living-donor group, some apical blebbing was focally observed in the otherwise normal tubular compartments. The apical brush border is also reduced (Figure 9C). The ultrastructure of the kidney tissue showed a relatively normal morphology in control animals. Peritubular capillaries were patent, and tubular cells showed preserved apical membranes and normal mitochondrial contents (Figure 9D).

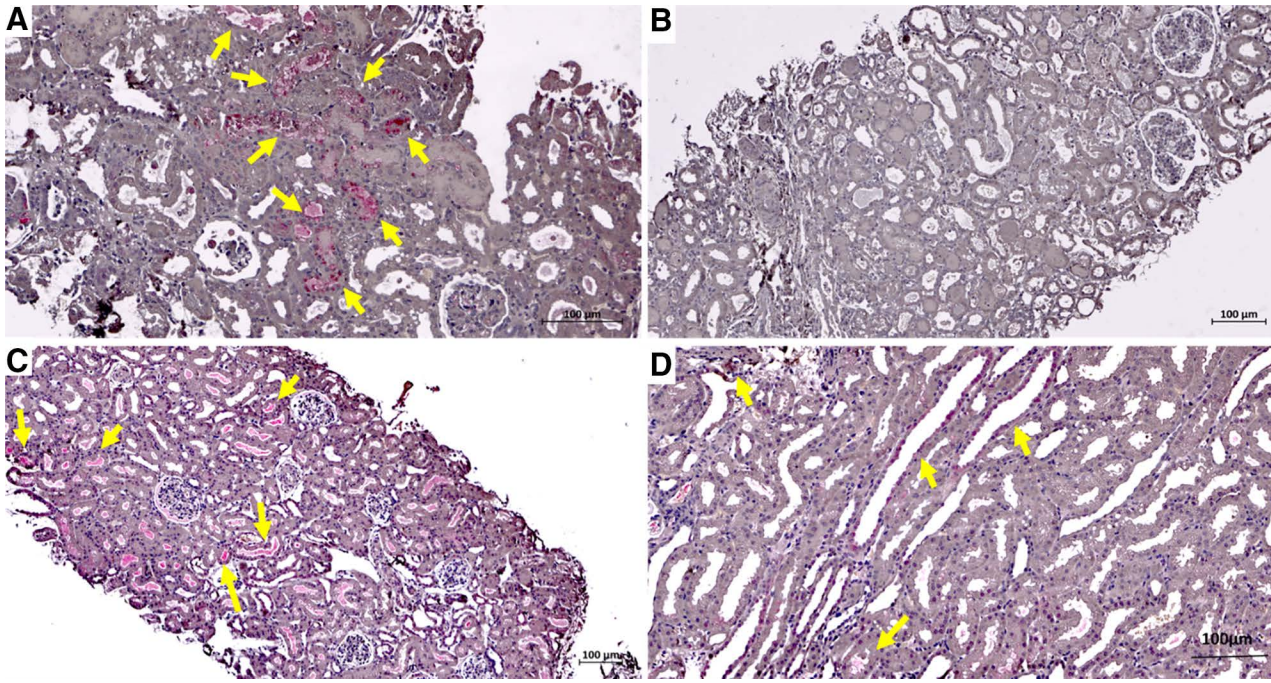

FIGURE 6. IHC staining of KIM-1 in biopsies taken after 90 min of reperfusion in the study group I (A and B) and in the live-donor operated group II (C and D). (A) and (C) represent positively stained biopsies for KIM-1 in groups I and II respectively. Whereas (B) and (D) represent negatively stained biopsies for KIM-1 in the same groups. IHC, immunohistochemistry; KIM-1, Kidney Injury Marker-1

DISCUSSION

In this article, we report 3-mo results from a novel and clinically relevant method of extended uDCD, using thrombolytic treatment with Lys-plasminogen and tPA (alteplase), and ex vivo perfusion in pigs. This model allows for >4 h of ischemia after circulatory arrest, with surprisingly few histological changes after reconditioning and preserved function after 10 d, as recently reported.[9] The present data show that long-term renal function, as reflected by creatinine and iohexol clearance measurements, was preserved 3 mo after transplantation. These results are comparable to the creatinine and iohexol clearance observed in living-donor pigs. No significant signs of fibrosis or long-term damage were observed in the kidney.

A key consideration in the present extended uDCD model design is the effective management of a typical daily clinical scenario. Using thrombolytic drugs seemingly allows proper oxygenation of the ischemic tissue and effectively inhibits the deleterious effects of WIT and I/R-I. Apart from resolving some of the physiological hurdles of uDCD, ample time was offered for patient consent. We are presently looking into the possibility of an even longer period, beyond 4.5 h after circulatory arrest, to further simplify the donation and retrieval procedure. To minimize logistical problems, we plan to conduct a proof-of-concept study in our university clinic. We believe that most kidneys can be retrieved within 2 h, and if this is not possible, we will still have time to insert the ice slush within the first 2 h and retrieve within the 4-h limit. The crucial time point is to obtain donor consent in time, and we believe that this is possible within this time frame. The eligible patients for the proof-of-concept trial will have a travel distance of 4 h to the hospital, to be able to be part of the study. Based on the experimental data, we estimated an ischemia time of 14 h or less for the study to be within the scope of clinical feasibility.

Our experience showed a preference for resistance levels under 200 wood units, which is close to the values found in the kidneys of live donors. Renal flow should also be >100 mL/100 g tissue/min, consistent with a recent publication by Sandal et al.[16]

The United States Food and Drug Administration has issued qualifications for clinical safety biomarkers, such as NGAL and KIM-1/TIM1.[17] NGAL can be detected in urine following ischemic or nephrotoxic insults.[18] However, acute kidney injury is rarely triggered by ischemia, as observed in the present study. NGAL is produced by several tissues in different molecular forms and may be altered in patients with chronic kidney disease. Therefore, it is reliable only in patients with normal baseline function.[18] In clinical studies, the levels are in the nanogram range, whereas the data obtained in urine were measured in significantly lower amounts, from 600 to 800 picograms (Figure 5), compared with other workers.[18,19] The levels in the present study were very low, possibly because the recipient was never subjected to I/R-I and had a limited timeframe. NGAL in the urine usually reacts within hours of I/R-I, but we feel confident that no significant damage was present, acutely or chronically, since the study groups and the sham group had similar values, and after 3 mo, no difference was observed when compared with healthy untreated pigs (Figure 5).

KIM-1 is a type 1 transmembrane protein. The immunoglobulin and mucin domains of this protein are upregulated in the proximal tubule of the postischemic kidney.[20] Levels of KIM-1, measured by enzyme-linked ImmunoSorbent assay, are increased in patients with chronic kidney disease but are promptly increased in urine and blood in cases of ischemic kidney trauma.[20] We did not find suitable enzyme-linked ImmunoSorbent assay kits for pigs and were limited to the analysis of biopsies using IHC. From the data, we found the expression of KIM-1 in the early

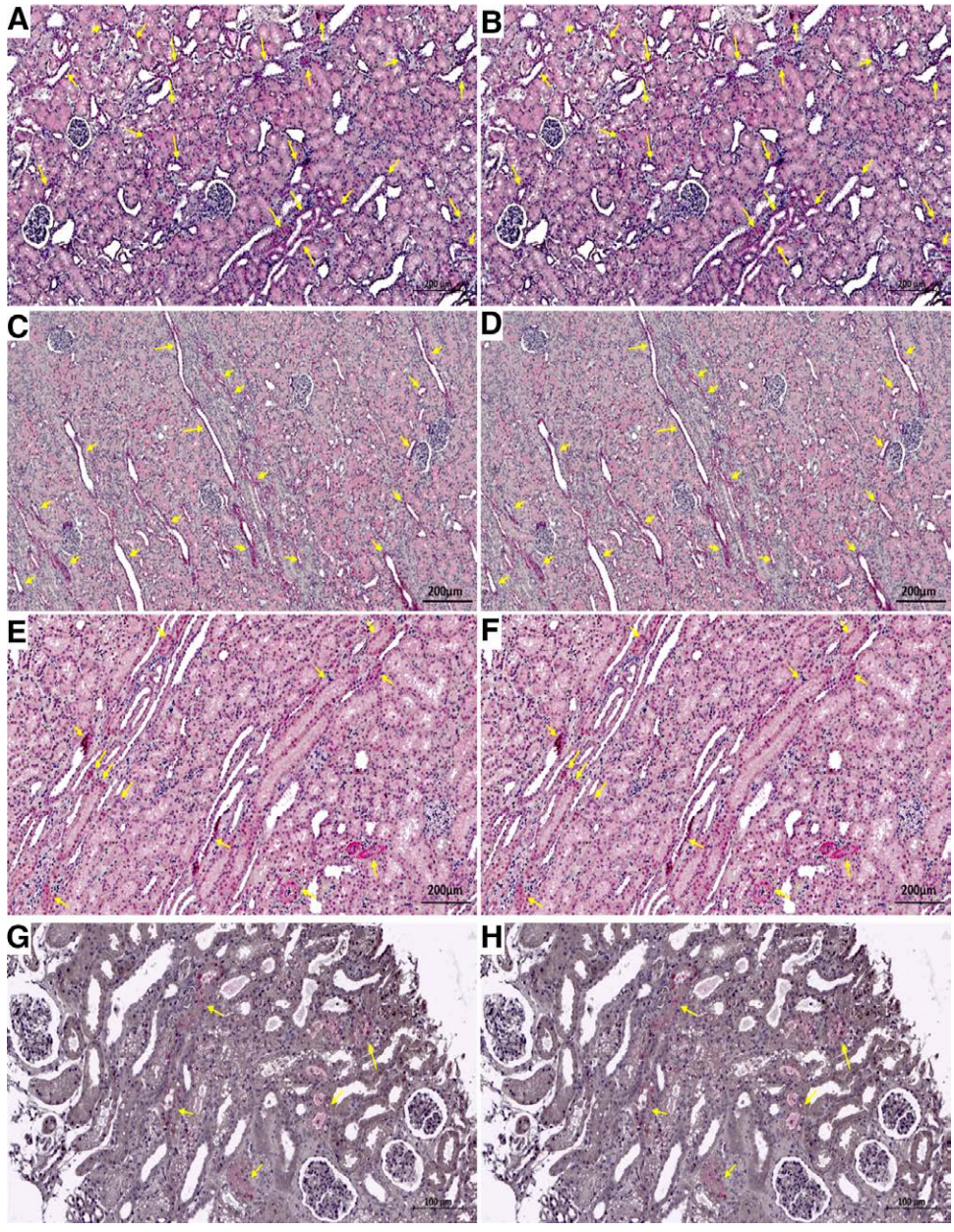

FIGURE 7. IHC staining on tissues taken from all 3 groups at the end of the 3 mo evaluation. (A) represents positive staining for KIM-1 and (B) shows no presence of KIM-1 in tissues taken from 2 different animals from the 3 mo study group. (C) shows positive staining for KIM-1 and (D) shows no presence of KIM-1 in tissues taken from 2 different animals of the live-donor operated group. Similarly, (E) represents positive staining for KIM-1 in the nephrectomized control group, whereas (F) shows no presence of KIM-1 in the same group. In (G) and (H) normal kidneys are seen. KIM-1 stains as red color and is pointed out with black arrows. HE stains the nucleus blue. All images are 10×. IHC, immunohistochemistry; KIM-1, Kidney Injury Marker-1

biopsies taken 90 min after reperfusion but only in 1 out of 4 animals, equally distributed between the study and sham-operated animals. Furthermore, biopsies taken after 3 mo revealed KIM-1 expression in all 3 groups as well as in normal untreated control kidneys. The living-donor animals showed the most pronounced changes, followed by the kidneys of the study group, suggesting that surgical trauma was more important than long-term ischemia in this experiment. However, due to the limited number of animals, it was not possible to draw any definite statistical conclusions from the results, although we believe that any detrimental effects of ischemia would probably have resulted in more severe changes in early biopsies, which was not the case.

Measures of iohexol clearance have provided a more precise picture of renal blood flow and function, although standardized protocols for iohexol clearance measurements in anesthetized pigs are lacking. The levels presented in this study were lower than those reported in humans using the present model of the elimination phase. This can partly be explained by the fact that the pigs in the study were growing at 3 mo of age at the beginning of the experiment, and almost doubling the weight during the 3 mo they were followed. Another factor is the possible influence of anesthesia. Because we used a living-donor group, as previously described,[14] we feel confident that the comparison of the clearance levels is valid, further supported by the fact that clearance levels seen in pigs with 2 kidneys also

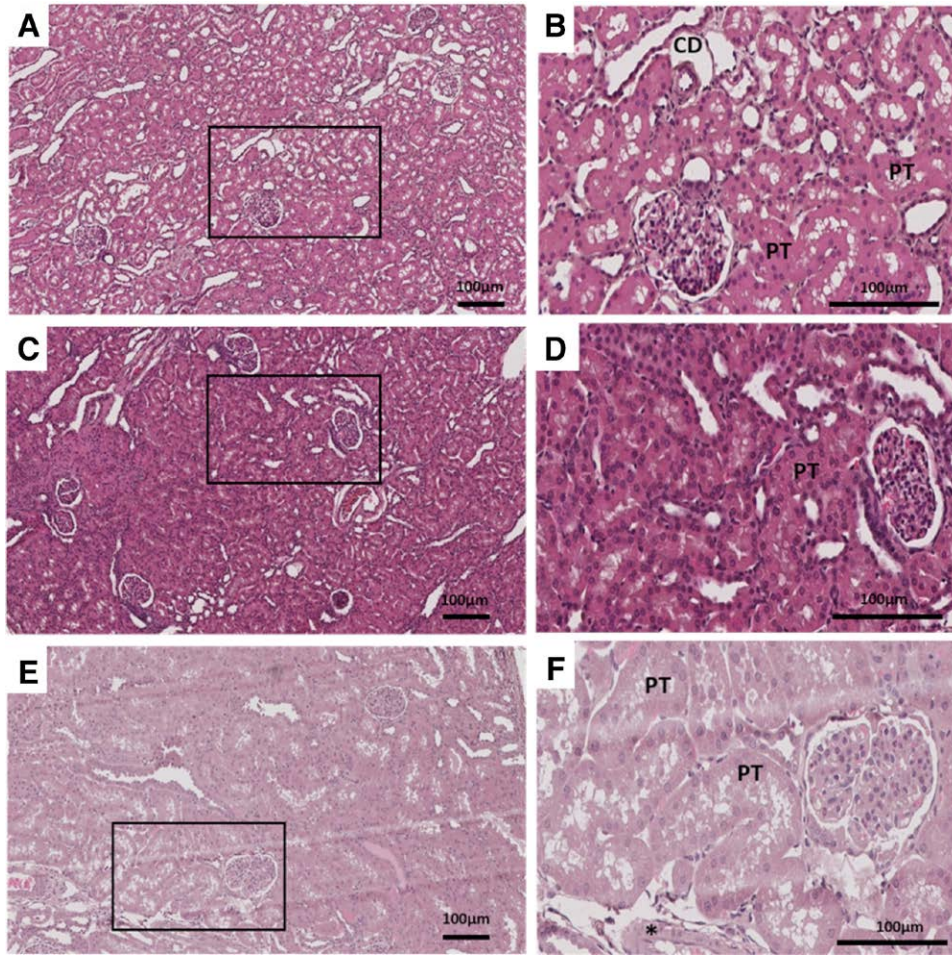

FIGURE 8. Histological evaluation of the kidney tissue (hematoxylin). (A) and (B) shows the renal morphology of the study group. No major histological changes apart from a slight focal simplification of the proximal tubular epithelium and slight swelling of the tubular epithelium. No fibrosis or inflammatory infiltrates. (C) and (D) show the morphology of the living-donor operated group. No distinct histological changes can be seen. (E) and (F) show the results from histological analysis of the normal untreated control kidney group. Again, no major histological aberrations can be seen apart from apical sloughing of the plasma membranes. No chronic changes. (A, C, and E) show ×10 magnification, whereas (B, D, and F), represent areas of higher magnification (×30). All images were stained by hematoxylin/eosin. CD, collecting duct. Scale bars are 100 µm; PT, proximal tubule.

had lower values than those normally observed in humans (Figure 4). The living-donor and the study group experienced a more extensive surgical trauma compared with the nephrectomized animals. We believe that this explains the lower creatine in these animals (Figure 2) at day 90. The kidneys undergo a decline in function during the first week; however, in most cases, this decline is reversible. Dialysis was not an experimental option for this model. Within the present series, we conclude that 3 pigs in the early learning curve and 4 in the latter part of the study survived 3 mo of transplantation within the permitted protocol.

Histological analysis of the kidneys from both the study and living-donor groups showed minimal changes, confirming the results of functional studies. The pathologist was also unable to observe any significant differences or systematic changes between these groups, such as fibrosis, which could be attributed to the ischemic challenges.

Thus, by removing the fibrin clots ex vivo, using Lys-plasminogen and tPA, and preventing fibrin reformation, using AT-III, abciximab, and argatroban, kidneys subjected to circulatory arrest despite extensive WIT can be reconditioned during a 6-h oxygenated perfusion cycle in an ex vivo device. Despite retrieving these donor organs after warm ischemia times, which are considered unsuitable for transplantation by most active transplantation surgeons, very promising results were achieved, both functionally (creatinine and iohexol) and morphologically, with early functional recovery already after 10 d.[9]

The implications of this novel technology in a clinical setting may be significant. Today, organ donation is performed at institutions in countries with highly advanced healthcare systems. In many parts of the world, no resources or expertise are available to establish organ donation and transplantation. Typically, a potential organ donor in Sweden requires several days of intensive care in an advanced ICU. Care includes interventional and conventional radiology, neurological consultations, and advanced operating room facilities. We believe that the proposed protocol could enable registration of a potential donor, examination of the donor, and acquisition of consent in institutions with limited healthcare resources. Subsequently, organs can be procured from the same location. Thrombolysis and oxygenation can be initiated before moving the organ to the transplantation site.

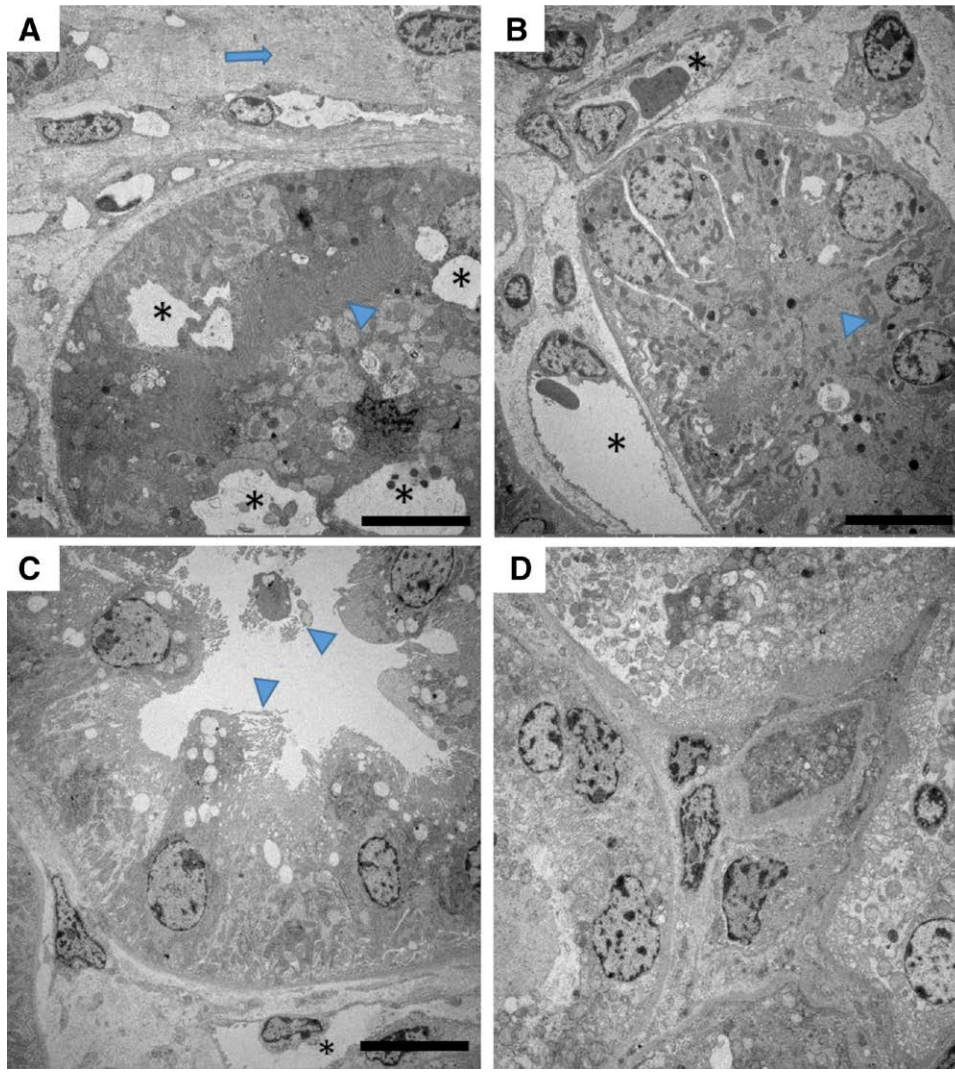

FIGURE 9. Ultrastructural evaluation of kidney tissue at the 3-mo evaluation by TEM. In the study group (A), the tubular epithelium is generally well preserved with most of the apical brush border membranes intact (arrowheads). Signs of intracytoplasmic vacuolization can be seen focally (*). Focal signs of minor interstitial fibrosis could be seen (arrow). In some tubular profiles of the study group (B), edematous swelling of the epithelium occluded the lumen of the tubules and the apical brush border was reduced (arrowhead). The peritubular capillaries were patent without thrombi (*). In the live-donor operated control group (C), a patent peritubular capillary is seen (*). The proximal tubular epithelium displays a relatively normal configuration, but also signs of apical blebbing (arrowhead) and focal reduction of brush border height. Morphology from a control case with focus on the tubulointerstitium is seen in (D). In the center a peritubular capillary can be seen (*), surrounded by 3 proximal tubular profiles. These have a high mitochondrial content and preserved apical brush border membrane. Scale bars 10 μm. TEM, transmission electron microscopy.

This study had several limitations. This is an animal study, using healthy young pigs, which contrasts with a clinical situation using elderly donors, often with marginal kidneys. Previous studies[21] have demonstrated that 120 min of WIT in a single kidney porcine model produced significant renal failure and mortality, and using our extended WIT without reconditioning, did not allow any pig surviving beyond 5 d.[9] A proof-of-concept study therefore must take into account the selection of suitable donors, to make conditions similar to the proposed model, and at least initially avoid marginal donors. Another limitation was that we did not test this in an allogeneic setting with long-term immunosuppression. We have transplanted allogeneic pigs without immunosuppression and know that they recover renal function in a similar way as seen in autologous kidneys, but we have not administered immunosuppression in any of our series so far. Nevertheless, since we did not see any difference between living-donor-operated and study pigs regarding early recovery or in late function, we feel encouraged by the outcome and its applicability in humans.

In summary, we describe a novel method to salvage kidneys from extended uDCD, enabling successful transplantation. These results support the procurement of uDCD organs subjected to prolonged warm ischemia and subsequent transplantation, in a clinically acceptable manner. This approach is similar to that clinically performed by Steen et al[22] for human uDCD lung transplantation.

As previously noted,[23] uDCD kidneys have the potential for excellent function and can constitute a valuable extension of the donor pool. We are currently preparing an ethical application for a "first in man" proof-of-concept study. The discussion of our work continues through open dialogue in the public domain and consultation within our profession to gain the acceptance of this new technology.

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