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"Successful Porcine Renal Transplantation after 60 Minutes of Donor Warm Ischemia: Extracorporeal Perfusion And Thrombolytics"

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Abstract

Donation from uncontrolled circulatory determination of death donors (uDCD) is impractical in America because of the time needed to organize procurement before irreversible organ damage. Salvaging organs after prolonged warm ischemic time (WIT) may address this limitation. We evaluated the combination of extracorporeal support (ECS) and thrombolytics in a porcine uDCD renal transplant model.

Non anti-coagulated uDCD sustained 60min of WIT, and two groups were studied. Rapid recovery **(*RR-uDCD*),** kidneys procured using rapid topical cooling; and ECS assisted donation **(*E-uDCD*),** 4hr ECS plus thrombolytics for *in-situ* perfusion prior to procurement. All kidneys were flushed and cold stored, followed by transplantation into healthy nephrectomized recipients without immunosuppression. Delayed graft function (DGF) was defined as creatinine>5.0mg/dL on any postoperative day.

Twelve kidneys in *E-uDCD* and 6 in RR-*uDCD* group were transplanted. All 12 *E-uDCD* recipients had urine production and adequate function in the first 48hr, but two grafts (16.7%) had DGF at 96hr. All 6 recipients from *RR-uDCD* group had DGF at 48hr and were euthanized. Creatinine and BUN levels were significantly lower in *E-uDCD* compared to *RR-uDCD* group at

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DISCLOSURE
The authors of this manuscript have no conflicts of interest to disclose as described by Transplantation Journal. The authors had full control of the design of the study, methods used, outcome parameters and results, analysis of data and production of the written report.

24hr (2.9±0.7mg/dL vs. 5.2±0.9mg/dL), and 48hr (3.2±0.9mg/dL vs. 7.2±1.0mg/dl); BUN levels at 24hr, (28.3±6.7mg/dL vs. 39.5±7.5mg/dL), and 48hr (23.9±5.0mg/dL vs. 46±12.9mg/dL) respectively.

ECS plus thrombolytics precondition organs *in-situ* yielding functional kidneys in a porcine model of uDCD with 60 minutes of WIT. This procurement method addresses logistical limitations for uDCD use in the US, and could have a major impact on the organ donor pool.

Keywords

kidney transplantation; extracorporeal support; organ perfusion; thrombolytics; donation after circulatory determination of death

INTRODUCTION

Organ donation after circulatory determination of cardiac death (DCD) could be a major source of organs for transplantation. However DCD is limited by the logistics of procuring organs immediately after death. Organs deteriorate quickly during the warm ischemic time (WIT) between withdrawal of care, determination of death and organ retrieval. In some decorticate (but not brain dead) patients, supportive care is electively withdrawn, the heart stops, and organs can be taken urgently after the heart stops (controlled DCD - cDCD), but the logistics are so difficult that cDCD accounts for less than 8% of all deceased donors in the United States.[1,2]

The logistical problems and the warm ischemic time can be minimized in cDCD by *in-situ* perfusion of warm oxygenated blood using extracorporeal support that allows reperfusion, conditioning and evaluation of organs before elective removal, this is called E-DCD.

We have reported the largest series of E-DCD [3,4]. This experience addressed many of the logistical problems resulting in elective organ retrieval and successful transplantation of kidneys, livers, and pancreas.

E-DCD results in routinely successful transplantation, but the number of potential donors is relatively small. A much larger source of DCD organs could be death after unexpected cardiac arrest (uncontrolled DCD - uDCD) but the logistics of establishing extracorporeal support within few minutes after death make E-DCD in unexpected, uncontrolled death difficult, if not impractical.

In an animal model we have studied the details of temperature, anticoagulation, pressure, flow, adjuvant medication, and warm ischemic time.[5-8] Our results had shown that room temperature extracorporeal support (ECS) is as effective as 37°C, and that ECS can be used during DCD donation in animals anticoagulated with heparin before or soon after cardiac arrest. This results in conditioning of kidneys, livers and lungs to transplantable status after 30 minutes of WIT. However, in these experiments, kidneys routinely presented poor urine output and low creatinine clearance after 30 min WIT, despite full pre-mortem heparinization of the donor when the “rapid recovery” technique was used. Renal grafts were successful condition and functional if ECS *in-situ* perfusion was used. In these

experiments we found that microvascular thrombosis during WIT uniformly caused kidney failure.[5] Intravascular thrombosis after death appears to limit tolerable WIT longer than 30 minutes, even with ECS and heparinization. This study was undertaken to evaluate the use of ECS with streptokinase (STK) as a thrombolytic, on kidney resuscitation and transplantation after 60 minutes of untreated WIT after cardiac death.

METHODS

Animals and Anesthesia

All animals were cared for by the standards of the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. Pigs with a weight of 25-30kg were used. Animals were anesthetized and hemodynamic monitoring lines were placed as previously described.[5]

Porcine Model of uncontrolled DCD (uDCD)—An uDCD model of organ donation with one hour of WIT was used for donor animals. After placement of catheters for hemodynamic monitoring, the left femoral artery (14-16Fr) and vein (18-20Fr) were dissected and prepared for cannulation. Next, a left-side thoracotomy was performed cephalic to the diaphragm and an umbilical tape was passed around the descending aorta immediately proximal to the diaphragmatic hiatus. Cardiac arrest was induced by apnea via ventilator withdrawal and paralytics were given as previously described. [5,7] No heparin was given prior to cardiac arrest and no external heat source was used, to mimic a clinical uDCD setting. Circulatory death was defined as a systolic carotid arterial pressure <20mmHg and a pulse pressure <10mmHg. The agonal period, defined as the time between vent withdrawal and circulatory arrest, was measured. The protocol is shown in Figure 1.

ECS Circuit—The Automated Perfusion System (APS) (MC3, Ann Arbor) is a prototype ECS circuit designed specifically for E-uDCD. The circuit consists of a peristaltic pump (M-pump), with compliant pump chamber which allows for Starling flow pump behavior and prevents cavitation, and a membrane oxygenator (MC3 Biolung). [9,10]

The APS prime included: 100mEq sodium bicarbonate, methylprednisolone 0.5gm, mannitol 12.5gm, heparin 10,000 Units, streptokinase (STK) 1 MIU. The priming volume was ~400ml. Another 1 MIU STK were diluted in 500ml of normal saline solution and given slowly throughout the perfusion.

Experimental Group (uncontrolled ECS assisted uDCD) - E-uDCD group—After the agonal period and circulatory arrest without pre-mortem anticoagulation, no intervention was made for 60 minutes in the donors (n=6 donors, n=12 kidneys). After 60 minutes of arrest veno-arterial (VA) perfusion via the femoral vessels was initiated. The distal thoracic aorta was occluded to simulate the intravascular aortic balloon occlude used in the clinical protocols. ECS assisted donation was performed for 4h at room temperature (28±2°C). Systemic arterial, device inlet, and device outlet blood gases were measured every 15-30min to monitor physiological changes during ECS. During the ECS run, target mean device outlet pressures and circuit flows were 65-75mmHg and >50ml/kg/min, respectively.

Intravenous (iv) fluids were administered as indicated for low pump filling pressures, and device flow was adjusted to meet the targets.

Kidney Procurement—After four hours of ECS, a midline laparotomy was performed and both kidneys were procured without interruption of perfusion. Both kidneys were then flushed with 120-150ml of cold Custodiol-HTK Solution. One kidney was transplanted directly (simulating living donor procurement). The other was stored at 5°C in an iced-cooler (simulating standard cold storage). The stored kidney was transplanted into a different recipient after 4 hours.

Control Group (uncontrolled Rapid Recovery DCD) Rapid Recovery Model - RR-uDCD—Kidneys were retrieved from similar uDCD subjects after 60 minutes of WIT, without pre-mortem anticoagulation. Donor animals (n=3 donors, n=6 kidneys) were anesthetized, instrumented, and euthanized as described above. A midline incision was made and the kidneys immediately procured for transplant without ECS or thrombolytics. After flushing with 120-150ml of cold HTK, one kidney was immediately transplanted and the other cold stored before transplant, as the experimental group. This control group simulates the technique of “rapid recovery (RR)”. Clinically RR is used only when the WIT is less than 10 minutes, so this model is well outside the range when RR would usually be used. Based on our prior experience we expected no renal function in the control group, so we limited the number of donors to three.

Renal Transplantation Model (nephrectomized healthy swine)—After carotid arterial and jugular venous catheters were placed and tunneled for chronic access in recipient animals, a midline laparotomy was made and both kidneys were nephrectomized prior to transplant. The distal aorta and inferior vena cava were dissected and prepared for anastomosis. 150units/kg of Sodium heparin were given prior to vessel clamping. Renal venous and arterial anastomoses were then completed and a ureteral stent was placed across the bladder anastomosis. 250mg of methylprednisolone were given prior to reperfusion of the kidney. No additional immunosuppression was given to recipient animals. Urine output at the time of ureteral anastomosis was recorded. In this model, rejection does not occur until 7 days (11), so normal renal function should occur immediately after transplant and continue for 4 days.

Postoperative Care—Recipient animals were allowed to recover after surgery and to eat and drink freely. IV fluid administration, medication administration, and blood draws were performed according to the schedule in Table 1. Blood was drawn daily for measurements of urea and creatinine. No immunosuppression was given to minimize any discussion about medications that may affect or improve immediate renal function. It has been described that porcine kidney allografts present with uniform rejection after 7 days without immunosuppression. (11)

Renal failure was defined as creatinine >5mg/dl for two consecutive days. Animals with renal failure were terminated. Animals with functioning grafts were terminated for evaluation at 2, 3, and 4 days post-transplant. Animals were euthanized with 7ml of Fatal-Plus solution, necropsy was performed, and kidneys removed for histology.

Histology—Kidney damage was assessed on H&E sides by an experienced and blinded veterinary pathologist. The extent of proximal tubule necrosis, degeneration, and regeneration was scored from 0 (normal) to 3 (severe). Tubulo-interstitial inflammation, interstitial fibrosis, and perivascular lymphoplasmocytic infiltration were also scored from 0 to 3 by the same criteria. Each slide was scored blindly on 2 consecutive days and rare differences were resolved by a 3rd evaluation.

Data Analysis

Independent sample Student's t-tests were performed to determine significant differences in postoperative serum creatinine and blood urea nitrogen (BUN) levels between experimental group (***E-uDCD***) and control group (***RR-uDCD***) animals. All analyses were performed using SPSS predictive analytic software (International Business Machines Corp., Armonk, New York). A $p<0.05$ was considered statistically significant.

RESULTS

Six donor animals were used for ***E-uDCD***, which resulted in 12 transplanted kidneys in this group. Three donor animals were used for 6 control kidneys, ***RR-uDCD***. For all donors, mean agonal time was 15.8±0.8min and mean WIT was 64.4±1.0 min (death to ECS, or to rapid organ procurement).

During ECS pH and partial pressure of carbon dioxide (pCO_2) reached target values within 15min in all donors. Over the 4h perfusion period, urine output increased from 1.3±0.6ml/h to 4.2±1.3ml/h. Lactate levels decreased slightly through the ECS run from 10.4 to 7.7 mg/dl. Measurements during perfusion are shown in Table 2.

Hemorrhagic complications during EuDCD: No major events were observed in this study. Initial and prior ECS hemoglobin values were 10±1.9g/dL at the end of 4hr of ECS hemoglobin dropped to 5.5±0.9 due to fluid resuscitation. However, in 2 animals hemoglobin dropped below 5.0g/d and excessive bleeding from surgical sites was observed.

All kidneys were successfully transplanted into healthy, nephrectomized recipients. Mean anastomotic times for the renal vein were 14.4±1.2min Vs 14.0±1.9, and for the renal artery 15.3±1.3min Vs 14.9±3.6min for the ***E-uDCD,*** and the ***RR-uDCD*** group respectively. All kidneys in the ***E-uDCD*** group made urine after vascular anastomosis. None of the control kidneys made urine during the retrieval or the transplant operation.

Postoperative Transplant Results

Initially, all 12 ***E-uDCD*** recipient animals had adequate renal function. On post-operative day #3, two animals were euthanized on day #3 (due to increase serum creatinine meeting criteria of renal failure), both organs came from the same donor. This ***E-uDCD*** donor had lower perfusion flows (<50mL/kg/min) and pressures (<60mmHg), than the target parameters set for E-uDCD. Additionally we had 2 complications associated with the management of the chronic arterial line (non-renal complications) that required the euthanasia of two animals in this group; specifically, on post-operative day #3 (arterial line bleeding) and post-operative day #4 (arterial line air embolism). All 6 ***RR-uDCD*** control

kidneys met renal failure criteria on post-operative day #2 and were euthanized. There was no urine in the bladder. In all transplanted kidneys, vascular anastomoses were patent. Kidneys were removed for histologic examination. Creatinine levels were significantly different between groups on post-operative day #1, 2.7±0.6mg/dl compared to 5.22±0.9mg/dl (p=5.5*10-6), and on post-operative day #2, 3.2±0.9mg/dl compared to 7.21±1.0 mg/dl (p=1.6*10-6), in ***the E-uDCD*** group and the ***RR-uDCD*** control group respectively.(Figure 2)

Similar results were observed with BUN between groups on post-operative day #1, 28.3±6.7mg/dl compared to 39.5±7.1mg/dl (p=0.0098), and on post-operative day #2, 23.9±5.0mg/dl compared to 46±12.9mg/dl (p=0.0003), in the ***E-uDCD*** group and the ***RR-uDCD***control group respectively (Figure 3).

Renal Pathology (Figure 4)

Control kidneys were removed after criteria for renal failure on day 2. Histology of the control grafts showed moderate to severe multifocal extensive acute tubular necrosis, tubular proteinosis and moderate subacute interstitial nephritis. The ***E-uDCD*** kidneys were removed on days 2, 3, and 4. The histology on day 4 showed normal blood vessels and glomeruli with mild nephrosis and interstitial nephritis. In both groups the lesions reported by the pathologist were reversible. No difference was observed in the amount of perivascular lymphocytes, TIN, and the regeneration of proximal tubules for the ***E-uDCD*** (1.32±0.2, 1.39±0.2, and 0.64±0.1) and the ***RR-uDCD*** (1.13±0.4, 1.63±0.1, and 0.50±0.3) groups respectively. However, proximal tubules necrosis was significant higher in the ***RR-uDCD*** group 1.50±0.6 Vs 0.18±0.1 (p=0.0042), and ***E-uDCD*** group. Proximal tubule degeneration was also significantly higher in the ***RR-uDCD*** group 2.13±0.4 compared to 0.86±0.2 in the E-uDCD group (p=0.012). Figure 4.

DISCUSSION

The United Network of Organ Sharing (UNOS) has reported that over 108,000 candidates are awaiting a kidney as of June 2014.[1] The shortage is compounded by a plateau in the number of living donors and a decrease in the number of donors after neurological determination of death (DND) as preventive measures decrease the severity of neurologic injury. In 2007, the Joint Commission, based on recommendations from the Institute of Medicine, mandated the use of DCD organs at all United States transplant centers as a means for increasing the organ supply; however, DCDs currently comprise <8% of all donors.[1,2]

These DCDs are almost only controlled (Maastricht types III and IV) in hospitalized patients in which death is expected and preparations can be made for organ procurement in advance. Most of the cDCD organs are recovered using the “rapid recovery” technique in which kidneys are removed within a few minutes of cardiac arrest, making application to uncontrolled arrested donors impractical.

However, by a conservative estimate, the inclusion of uDCD – primarily out of hospital cardiac arrests – could increase the number of donors by 22,000 each year, resulting in up to

44,000 additional kidney transplants if this potential is fully realized.[2] Urgently removing organs within a few minutes of death (rapid recovery -RR technique) is impractical in the emergency room setting. However ECS is has been successful in salvaging kidneys in E-uDCD.

E-uDCD has been used clinically in Spain because of state policy on intervention after death. In Spain DCD donation follows an “opt-out” organ donation system where consent for donation is implied unless otherwise specified.[12] With an ECS machine primed and available, cannulation and ECS can be instituted by ER staff within 5 minutes of patient arrival. With warm ECS underway, there is ample time to contact the transplant team and family. The results of successful transplantation are very good in witnessed arrest because the WIT is known, and short. The results of ECS with out-of hospital arrest of longer or unknown WIT are still acceptable, because the function of perfused kidneys can be measured, and only functional kidneys are taken for transplantation.[13-15] This approach requires cannulation and management of the ECS system by trained emergency room staff. It has rarely been reported in the United States, primarily because of policies that require family consent before any interventions are instituted on dead body (the opt-in donation system). Until and unless the United States adopt a opt-out policy, the use of uDCD donors depends on extending the tolerable WIT, preferably to at least one hour.

However, even in Spain, cannulation and perfusion must be initiated within a few minutes of death. If kidneys could be procured after an hour or more of WIT the limitations of uDCD could be solved. In this animal model of uDCD we demonstrated that the combination of ECS and thrombolytics can result in routinely functional kidneys after 60 minutes of WIT.

From past experience we knew that the controls would routinely fail, which is why we conducted only 6 controls. It is possible that some might have recovered function after a period on dialysis, but our experiment was designed to evaluate immediate renal function. From past experience we knew that ECS alone would not restore kidney function after 60 minutes of WIT. However we found that ECS plus thrombolytics was routinely successful.

Several groups have studied prolonged WIT (>45min) in porcine and canine models of renal transplantation. Brasile et al, has studied prolonged WIT (2h) in a canine auto-transplant model after *ex-situ* perfusion of renal grafts using a novel acellular perfusate. Her technique focuses in the delivery of fibroblast growth factors during 24hr ex-situ perfusion at 32°C with the goal to trigger cellular pathways that induce cellular recovery after prolonged warm ischemia injury. [16,17] This group works focused on immunomodulation of vascular endothelium during the warm *ex-situ* perfusion, limited by the need of a oxygen carrier agent in the perfusate with approval for human use. Snoeijs, et al reported the use of porcine renal grafts after 0, 30 and 45 minutes of WIT followed by cold machine perfusion (4°C) for 24hr with follow up for 10 days. Their results were primarily to describe surgical methods for autologous renal transplant in pigs.[18] Their post-operative results of serum creatinie and BUN are similar to the ones observed in our studies. Both values peaked to a maximum level on post-operative day #2-#3, with a return to normal values on post-operative day #10. Due to the UCUCA protocol approved by our institution, and in order to minimize animal

suffering, we selected to stop the experiment and euthanize animals with serum Creatinine >5mg/dL.

We have presented some of this work at the international Non-Heart Beating Donor (NHBD) meeting. Based partly on these presentations and discussion, Oleg Resnik of St Petersberg initiated a clinical program of ECS and thrombolytics in uDCD in humans. He gave heparin soon after declaration of death, and streptokinase at the time of ECS. The abdominal aorta was occluded with a balloon and the temperature was room temperature. His circuit included an open reservoir and was primed with Custodiol-HTK,solumedrol, perflurocarbon as an oxygen carrier. Dr. Reznik reported the application of this protocol in 22 human cadaveric donors with an average warm ischemia time of 61.4 min. The average VA-ECS perfusion time before kidney procurement was of 2.5h.[18,19] All 44 kidneys were transplanted. Delayed graft function (DGF) occurred in 70% requiring dialysis , but only one never functioned (primary graft failure, PGF).This study proves that kidneys can be conditioned to transplantable status using warm in-situ ECS with thrombolytics in humans. Longer perfusion, as in our lab study, may decrease the incidence of DGF.

Our study design has limitations compared to clinical uDCD. We induced cardiac arrest via apnea and did not attempt any resuscitative measures after cardiac death (Maastricht Type II), simulating out of hospital arrest. In future experiments we will evaluate ECS after failed CPR, and longer WIT times. The animal model requires surgical incisions that are not performed in the clinical setting (access to monitoring lines in the neck and clamping of the abdominal aorta via a low thoracotomy), the extent of these incisions were associated with bleeding complications in two animals. We believe that in the clinical setting the use of modified Seldinger's technique for cannulation with ultrasound guidance will limit the use of an open approach.

CONCLUSION

In a porcine model of kidney transplantation, after 1 hour of WIT, the use of 4 hours of room temperature extracorporeal circulation (ECS) resulted in immediate renal function, compared to no renal function in controls without ECS. ECS with an automatic perfusion system, and the addition of thrombolytics were significant factors. Applied to clinical practice, extending the tolerable warm arrest time to 60min could make uncontrolled DCD feasible in any Emergency Room.

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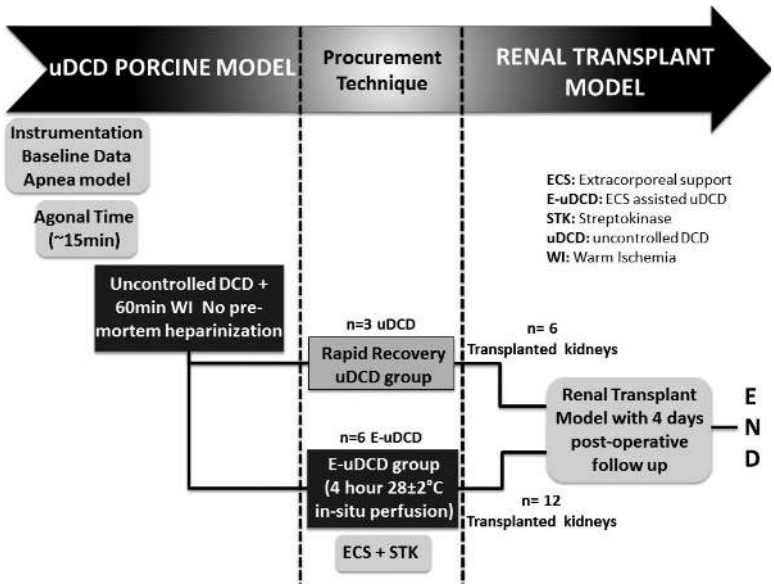

Figure 1.
Laboratory uDCD Porcine Animal Model with Renal Transplantation

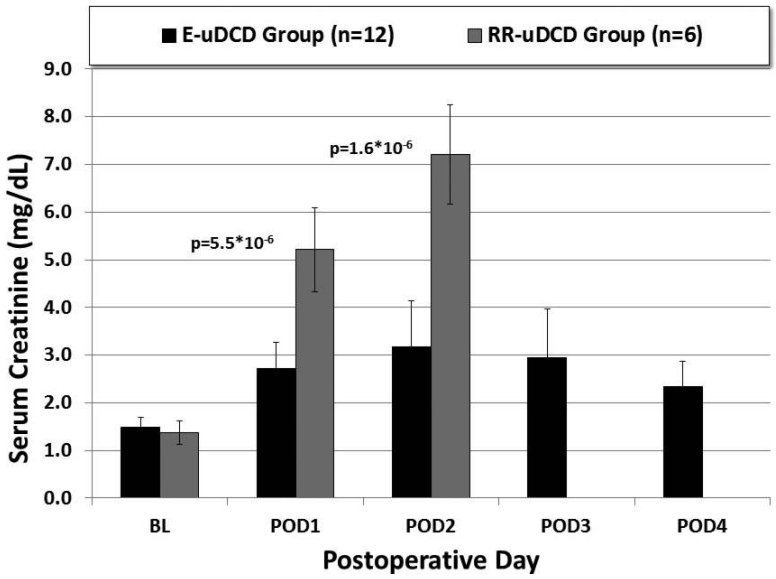

Figure 2.
Daily Post-Transplant Serum Creatinine

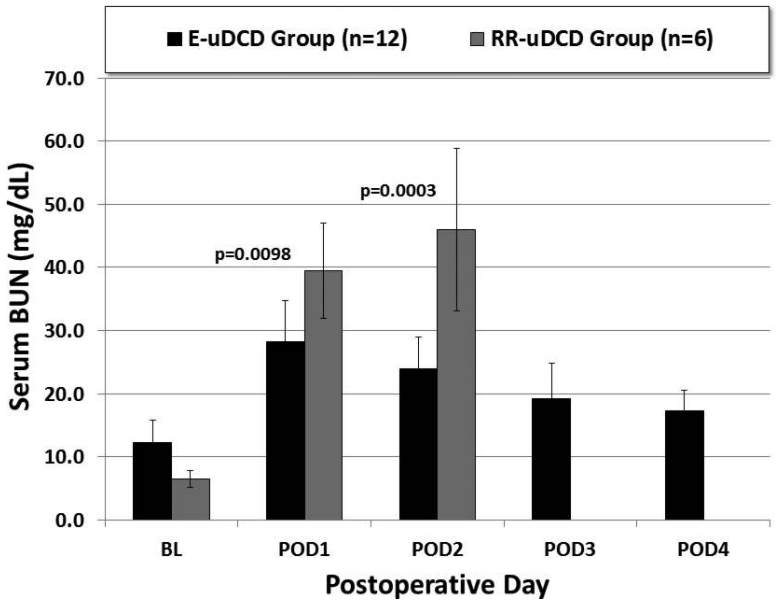

Figure 3.
Post-operative Serum BUN values

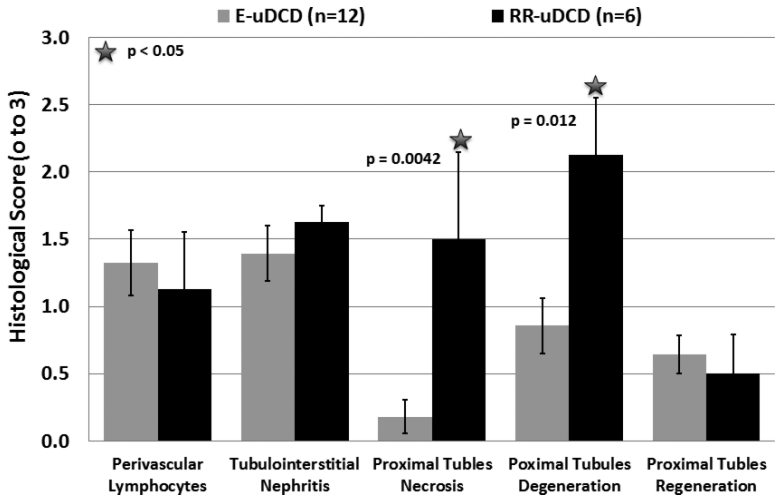

Figure 4.
Recipient Histopathology

Table 1

uDCD Renal Recipients Postoperative Care (n=18)

Postoperative day #	IV fluid + Medications (AM and PM)	Buprenorphine (IM)	Blood Draws (AM*)
1	1L of 0.9% NSS + 0.5g Nafcillin	0.3mg, every 6h	Serum: Cr and BUN
2	0.75L of 0.9% NSS + 0.5g Nafcillin		
3	0.5L of 0.9% NSS + 0.5g Nafcillin	0.3mg, as needed	
4	No IV fluids	0.3mg, as needed	

IV: intravenous; IM: intramuscular; AM: morning: PM: afternoon

NSS: Normal Saline Solution; Cr: Creatinine; BUN: Blood Urea Nitrogen

Table 2

Hemodynamic and perfusion parameters during E-uDCD (n=6)

Recipient	Baseline	ECS ON	30 min	60 min	120 min	180 min	240 min
Flow (mL/Kg/min)	N/A	43.1	47.2	54.9	59.8	60.2	57.8
Pressure (mmHg)	N/A	57.8	57.8	70	69.8	72.3	71.7
Sweep gas (L/min)	N/A	3.8	3.2	2.7	1.8	2.0	1.6
pH	7.43	7.03	7.25	7.23	7.25	7.3	7.23
pCO_2 (mmHg)	33.3	110.8	45.1	45.1	51.5	47.4	44.6
Lactate (mg/dL)	1.7	10.4	11.7	11.4	9.9	8.5	7.7
UO (mL/Kg/hr)	3.7	0	0	1.3	2.1	2.8	3.8