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van Leeuwen, Leonie

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Chapter 7

Exploring porcine precision-cut kidney slices as a model for transplant-related ischemia-reperfusion injury

L.A. Furth
H.G.D. Leuvenink
L. Seras
I.A.M. de Graaf
P. Olinga
L.L. van Leeuwen

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Abstract
Marginal donor kidneys are more likely to develop ischemia-reperfusion injury (IRI), resulting in inferior long-term outcomes. Perfusion techniques are used to attenuate IRI, and improve graft quality. However, machine perfusion is still in its infancy, and more research is required for optimal conditions and potential repairing therapies. Experimental machine perfusion using porcine kidneys is a great way to investigate transplant-related IRI, but these experiments are costly and time-consuming. Therefore, an intermediate model to study IRI would be of great value. We developed a precision-cut kidney slice (PCKS) model that resembles ischemia-reperfusion and provides opportunities for studying multiple interventions simultaneously. Porcine kidneys were procured from a local slaughterhouse, exposed to 30min of warm ischemia, and cold preserved. Subsequently, PCKS were prepared and incubated under various conditions. Adenosine triphosphate (ATP) levels and histological tissue integrity were assessed for renal viability and injury. Slicing did not influence tissue viability, and PCKS remained viable up to 72h incubation with significantly increased ATP levels. Hypothermic and normothermic incubation led to significantly higher ATP levels than baseline. William’s medium E supplemented with Ciprofloxacin (and Amphotericin-B) provided the most beneficial condition for incubation of porcine PCKS. The porcine PCKS model can be used for studying transplant IRI.

Introduction
Due to the worldwide shortage of donor organs, marginal kidneys from extended criteria donors (ECD) and donation after circulatory death (DCD) donors are increasingly used for transplantation. These kidneys are exposed to more (ischemic) injury compared to kidneys from donation after brain death (DBD) donors and are therefore more likely to develop ischemia-reperfusion injury (IRI). Ischemia is characterized by the restricted blood supply, causing a shortage of oxygen delivery to the cells needed for adenosine triphosphate (ATP) production. Upon reperfusion, the blood supply is restored, which induces an inflammatory response. The re-introduction of oxygen and normalization of pH are detrimental to the previously ischemic cells, leading to increased reactive oxygen species (ROS) production and thus oxidative stress. Unfortunately, IRI’s underlying pathophysiological mechanisms can lead to transplant-related complications, such as the development of delayed graft function (DGF), early graft failure, chronic allograft nephropathy, and late graft failure. Therefore, diminishing the effects of IRI would be of great interest.

Machine perfusion (MP) is a novel technique widely used to attenuate IRI. It provides the unique opportunity to preserve and resuscitate the donor organ outside the body. MP can be performed at different temperatures and for various purposes. Hypothermic machine perfusion (HMP), preservation at 4°C, has been developed as an alternative to static cold storage (SCS) as it offers superior organ preservation, especially for grafts of suboptimal quality. HMP can be performed with or without oxygen; however, recent studies show that adding oxygen during HMP results in better preservation of the mitochondria and less post-transplant complications. Hypothermic and normothermic incubation led to significantly higher ATP levels than baseline. William’s medium E supplemented with Ciprofloxacin (and Amphotericin-B) provided the most beneficial condition for incubation of porcine PCKS. The porcine PCKS model can be used for studying transplant IRI.
Animal model
All experiments were carried out with porcine kidneys. These kidneys were retrieved from a local slaughterhouse after a highly standardized slaughtering process. Pigs were anesthetized using an electric shock followed by exsanguination. The kidney was selected based on color, arterial branching, and the absence of abnormalities. Each kidney was exposed to 30 minutes of warm ischemia time (WIT), thus exposed to the same extent of induced ischemic injury. This workflow was chosen to reflect the donation after circulatory death conditions.

Cold preservation
Kidneys were preserved and transported using SCS or HMP. All kidneys were flushed with 180 mL of 0.9% saline solution (Fresenius Kabi, Louviers France) before cold preservation. Kidneys preserved using SCS were submerged in 300 mL ice-cold University of Wisconsin (UW) cold storage (CS) solution (Bridge to Life Ltd., United Kingdom) and preserved on ice at 4°C for 24 hours before undergoing the slicing procedure.

Kidneys preserved using HMP were surgically prepared and connected to the Kidney Assist Portable (XVIVO, Gothenburg, Sweden) perfusion machine. HMP was performed for either 3 or 24 hours, at a set mean pressure of 25 mmHg using 330 mL oxygenated (100 mL/min) UW machine perfusion (MP) or CS solution at 3-5° C.

Precision-cut kidney slices
After cold preservation, kidneys were immediately flushed with 120 mL 0.9% saline solution to remove the UW solution from the vasculature. With a 10 mm blade, the renal capsule was carefully removed, and the cortex was cut off from the kidney. Cores were prepared from the cortex with a 6 mm biopsy punch and stored in ice-cold UW-CS solution.

PCKS were obtained using the Krumdieck tissue slicer (Alabama Research and Development, Munford, USA). The Krumdieck slicer was assembled and filled with ice-cold oxygenated Krebs-Henseleit buffer (25 mM NaHCO₃, 25 mM D-glucose (Merck, Darmstadt, Germany), and 10 mM HEPES (Merck, Darmstadt, Germany), pH: 7.4). Slices were collected and selected on round and intact macroscopic morphology. The slices weighed 4.5-5.5 mg with an estimated thickness of 300 μm. To ensure viability, the slices were preserved in ice-cold UW-CS solution for a maximum of one hour. Slices were then incubated for 24, 48 and 72 hours at 4°C or at 37°C with 80% O₂ & 5% CO₂ while gently shaking at a rate of 90 rpm. The medium composition for each experimental group is described in Table 1 and was refreshed every 24 hours.
Table 1  Incubation conditions.

| Experimental group | Medium composition |
|--------------------|--------------------|
| WME                | Williams Medium E (1X) with GlutaMAX (WME) (Gibco) + 10µg/mL ciprofloxacin (Fresenius Kabi, France) |
| WME + Glucose      | WME + 10µg/mL ciprofloxacin + extra added D(+)-glucose solution (Sigma-Aldrich, St Louis, USA) total end concentration 25 mM |
| WME + Dextran40    | WME + 10µg/mL ciprofloxacin + 25µg/mL Amphotericin B + 3.5% Dextran40 (Sigma-Aldrich, St Louis, USA) |
| WME + Fungi (0.25) | WME + 10µg/mL ciprofloxacin + 25µg/mL Amphotericin B (Fungizone) (Merck) |
| WME + Fungi (1)    | WME + 10µg/mL ciprofloxacin + 100µg/mL Amphotericin B |
| RPMI               | Roswell Park Memorial Institute 1640 (RPMI) (Gibco) + 10µg/mL ciprofloxacin + 11 mM D(+)-glucose solution |
| Blood              | Diluted blood NMP perfusate (Table S1) |

Evaluation of viability
Viability of PCKS were evaluated by assessing the ATP content as described before by De Graaf et al. A bioluminescence kit was used (Roche Diagnostics). Luminescence was measured using a luminometer (Packard LumiCount, Illinois, United States). The obtained ATP values were normalized against total protein content using the Pierce™ BCA protein assay kit. The final ATP content was expressed as pmol ATP/µg protein. Lactate dehydrogenase (LDH) and aspartate aminotransferase (ASAT) levels in the medium, as a marker for renal injury, were determined in a routine fashion at the lab of microbiology (UMCG).

Histological analysis
PCKS were fixed in 4% formalin, embedded in paraffin wax, and cut into sections of 4 µm. Sections were stained using a conventional HE staining to visualize morphological features. Sections were scanned with a C9600 NanoZoomer (Hamamatsu Photonics, Hamamatsu, Japan) to obtain high-resolution digital data. Semi-quantitative scores were assigned to HE-stained sections in a blinded manner by two individuals, marking glomerular dilatation and structure, tubular dilatation and acute tubular necrosis. Scores ranged from 0-2 with 0 representing no damage, 1 representing mild damage, and 2 representing severe damage.

Determination of fungal infection
The type of fungal infection was determined in a routine fashion at the lab of microbiology (UMCG).

Statistical analysis
Data were visualized and analyzed using GraphPad Prism 8.0 (GraphPad Software, USA). Values are shown as means with appropriate standard error of the mean (SEM) and as individual values. A Kruskal–Wallis analysis of variance combined with a Dunn’s multiple comparison test was performed to analyze statistical differences between experimental groups. The cut-off for statistical significance was set for p < 0.05.

Results
HMP-O₂ as cold preservation results in significant higher PCKS viability
We first analyzed which cold preservation technique resulted in the most viable slices after 48 hours of incubation (Figure 2A). After cold preservation and the slicing procedure, no differences in ATP levels were observed. After 48 hours of incubation in WME with glucose, PCKS showed significantly higher ATP levels after oxygenated HMP-O₂ compared to SCS preservation (p=0.0467). The tubular dilatation trended higher at T0, with more tubular dilatation in the HMP-O₂ group (Figure 2B) but was not significantly different. Acute tubular necrosis scores fluctuated over time but were similar in both groups after 48h incubation (Figure 2C and 2D). As the ATP levels were significantly higher in the HMP-O₂ group, oxygenated HMP was chosen as the standard cold preservation method for the respective experiments.

Porcine PCKS remain viable up to 72 hours
Next, we analyzed whether porcine PCKS can be incubated for up to 72 hours to observe biological processes for a more extended period. After 3 hours of oxygenated HMP with UW-MP, ATP levels significantly increased compared to after 30 minutes of WIT (p=0.0168) (Figure 3A). After the slicing process (T0), ATP levels remained similar to before slicing, and ATP levels were significantly higher compared to after 30 minutes of WIT (p=0.0118). Slices remained viable up to 72 hours of incubation with WME, as ATP levels were significantly higher after 24, 48, and 72 hours (p=0.0100, p=0.0374, and p=0.0197 respectively) of incubation with WME compared to T0. Tissue integrity by means of histological staining was studied next (Figure 3B). Tubular damage significantly increased over time, as shown by tubular dilatation and acute tubular necrosis (Figure 3C & 3D). Glomerular dilatation significantly decreased after 24 hours of incubation, no further differences were seen between the times of incubation. (Figure 3E).

WME provides the most beneficial incubation conditions
We then compared different medium compositions (Figure 4). After HMP-O₂ with UW-CS, ATP levels significantly increased compared to baseline when PCKS were incubated in WME, WME with added glucose, and RPMI (p<0.0001, p<0.0001, and p=0.0013, respectively) (Figure 4A).
Figure 2. Tissue viability during PCKS incubation after cold preservation. A. ATP levels of porcine kidneys that were preserved with oxygenated HMP (HMP-O₂) (●) (n=4) or static cold storage (SCS) (●) (n=4) for 24 hours. B. Histological tubular dilatation score of porcine kidneys. C. Histological acute tubular necrosis score of porcine kidneys preserved with HMP-O₂ or SCS. D. Histological images after 48h of incubation of PCKS. T0 represents ATP/protein levels directly after slicing and T24 and T48 after incubation for 24 hours and 48 hours, respectively. * p<0.05. SCS, static cold storage; HMP, hypothermic machine perfusion; ATP, adenosine triphosphate. The data are shown as mean±SEM.

After HMP-O₂ with UW-MP, ATP levels increased significantly when PCKS were incubated in WME (p=0.0005). When slices were incubated in a blood-based perfusate, ATP levels were significantly lower than in the WME group (p=0.0101). The addition of Dextran40 resulted in lower ATP levels compared to WME alone, however, this was not a significant difference (Figure 4B). PKCS incubated in RPMI showed significantly more tissue injury in terms of LDH and ASAT production compared to WME (p=0.0263 and p=0.0088, respectively) (Figure 4C & 4E). No differences in injury markers were observed when glucose was added to the WME. Incubation of PCKS in a blood-based perfusate led to significant more LDH production compared to WME (p=0.0219).

PKCS remain viable under both hypothermic and normothermic conditions

To observe the effect of hypothermic incubation on viability, we incubated PCKS at 4°C, 37°C, and a combination of 4°C and 37°C (Figure 5). When slices were incubated at a normothermic temperature, ATP levels increased significantly compared to baseline (p=0.0053). When PCKS were incubated under hypothermic conditions or when slices were incubated at 4°C for 24 hours and then rewarmed to 37°C for another 24 hours, no significant increase in ATP levels were observed compared to baseline.

Figure 3. Tissue viability after slicing and up to 72 hours of incubation. A) ATP/protein levels after 30 minutes of WIT (●) (n=6) porcine kidneys were preserved by means of oxygenated HMP (●) (n=6) for 3 hours, sliced (T0) (●) (n=7) and incubated up to 72 hours (●) (n=6). B) Histological images directly after slicing (T0) and after 72 hours of incubation. C) Histological tubular dilatation after incubation at different time points. D) Histological acute tubular necrosis after incubation. E) Histological glomerular dilatation. * p<0.05, *** p<0.001, **** p<0.0001. WIT, warm ischemia time; HMP, hypothermic machine perfusion; ATP, adenosine triphosphate. The data are shown as mean±SEM.

(Figure 4D): however, no significant differences in ASAT levels were observed (Figure 4E). The addition of Dextran40 did not lead to more LDH or ASAT production compared to WME alone (Figure 4D & 4F).
When comparing 37°C to 4°C or 4°C + 37°C, no significant differences in ATP levels were observed (Figure 5).

Amphotericin B is safe to add to prevent fungal infections

One of the obstacles during the incubation of the porcine PCKS was the occasional incidence of a fungal infection by *Candida guilliermondii* (Figure 6A). To prevent this, we analyzed whether Amphotericin B (fungizone) could be added to the medium without negatively affecting viability. WME and WME with added fungizone (25µG/mL and 100µG/mL) showed significant higher ATP levels compared to baseline (*p*<0.0093, *p*<0.0001 and *p*<0.0001 respectively) (Figure 6B). Furthermore, no significant differences were observed after 48 hours of incubation between the three experimental groups. Additionally, fungal infections in follow-up experiments were prevented.

![Figure 4: Viability and injury markers after 48 hours of incubation using different medium compositions.](image)

![Figure 5: Incubation of slices at different temperatures. ATP/protein levels of porcine kidneys that were preserved with oxygenated HMP sliced at baseline (●) (n=7) and incubated for 48 hours in WME at 4°C (●) (n=7) or 37°C (●) (n=7) or first 24h at 4°C and then rewarmed to 37°C for 24h (●) (n=3). ** *p*<0.01. ATP, adenosine triphosphate. The data are shown as mean±SEM.](image)
damaged the most. (Figure 3). This is in line with studies that showed that HMP preservation improves the viability of renal tissue after normothermic reperfusion\textsuperscript{13,33}.

**WME best supports the renal metabolism of porcine PCKS**

During NMP, a red blood cell-based perfusate is needed for optimal oxygen delivery to the tissue\textsuperscript{34}. However, incubating slices in a diluted blood-based perfusate did not result in enough support for renal metabolism (Figure 4B). This could be due to the hemolysis, reflected by high LDH levels that were observed during incubation (Figure 4D).

As kidneys need colloid osmotic pressure for their filtration processes, we added Dextran\textsubscript{40} to the incubation medium to test the effects on renal toxicity. Although these filtration processes are not active in PCKS, the adequate concentration could be translated to a perfusion model. The addition of a colloid during HMP has increased renal metabolic activity during reperfusion; however, we observed a decrease in PCKS viability with added Dextran\textsubscript{40} (Figure 4B). This decrease could be explained by a decrease in oxygen and nutrients diffusion due to the higher viscosity of the incubation medium\textsuperscript{35} or because the cells within the slices are more prone to stress caused by a higher colloid osmotic pressure as they lack a glycocalyx\textsuperscript{36}.

Furthermore, WME provided enough nutrients for ATP production, and extra glucose did not increase viability, whereas incubation in RPMI resulted in lower ATP levels (Figure 4A). These results are in line with De Graaf et al\textsuperscript{37}, who observed significantly higher cell proliferation in precision-cut liver slices incubated in WME compared to RPMI.

Porcine PCKS can be incubated under both hypo- and normothermic conditions

As proposed before, HMP is a commonly used technique for preserving the kidney graft before transplantation. Therefore, the incubation of slices under both hypothermic and normothermic temperatures was investigated. With cold incubation of slices, prolonged hypothermic preservation can be studied efficiently. Although there is no active perfusion during PCKS incubation, the medium is oxygenated and agitated, resulting in oxygen and nutrient delivery to the renal cells, just like during machine perfusion. To our knowledge, hypothermic incubation and rewarming (4°C and 37°C) have not been studied before in PCKS but are promising methods to further explore in follow-up studies.

**ATP as a biomarker for renal tissue viability**

Kidneys are big consumers of oxygen as renal cells require high energy levels due to their ATP-dependent functions like reabsorption and secretion\textsuperscript{38}. Therefore, the renal
metabolic state is an excellent representation of kidney health\textsuperscript{39}. To produce ATP and thus energy, the mitochondria need to work properly, and nutrients and oxygen are necessary to fuel these metabolic processes. It is also shown that renal ATP levels are inversely correlated to histological injury\textsuperscript{40}. Therefore, ATP levels provide a great biomarker for kidney tissue viability\textsuperscript{41}.

Advantages and disadvantages of porcine PCKS
As already mentioned before, PCKS provide a platform to study organ-specific cellular mechanisms as they maintain cellular heterogeneity and organ structure\textsuperscript{23}. PCKS have been widely implemented for kidney research as a model to study the metabolism, toxicity, and efficacy of drugs\textsuperscript{42–46}. The advantage is that multiple compounds or conditions using the same kidney can be studied, therefore keeping biological differences minimal\textsuperscript{23}. PCKS could provide a platform to investigate biopharmaceuticals that target IRI, inflammation, and fibrosis-related injury. These processes frequently lead to transplant-related complications, and no effective treatment is currently available.

As the kidney is not transplanted, it is challenging to mimic reperfusion using PCKS. During incubation, the tissue is rewarmed, and oxygen is reintroduced, presumably triggering cellular reperfusion injury. However, the strength of this model is to investigate ischemic damage caused by organ procurement and cold preservation.

Good kidney function is defined by good filtration and reabsorption\textsuperscript{38}. Unfortunately, the glomerular filtration rate and sodium reabsorption cannot be measured as PCKS do not produce urine. These parameters would have to be tested in follow-up studies using \textit{ex vivo} reperfusion studies. However, \textit{ex vivo} reperfusion for periods longer than 6 hours remains a challenge.

Because of the size of the porcine kidney and the relative simplicity of the method, hundreds of slices can be produced and used to test different treatments under a variety of conditions. Slaughterhouse kidneys are commonly used to research renal transplant-related questions in NMP setups\textsuperscript{48–54}. They provide an excellent alternative for laboratory animals and are more similar to human kidneys than any other species\textsuperscript{55}. A limitation of the use of slaughterhouse kidneys is that these kidneys cannot be obtained in a (semi) sterile manner and are therefore more prone to infections. Our only observed infection during our experiments was a \textit{candida guilliermondii} infection and adding Amphotericin B to the medium resolved this problem entirely, and no signs of nephrotoxicity were found.

Future perspectives porcine PCKS in translation to NMP
Although NMP is a hot topic in the renal transplant field, it is clinically only scarcely implemented. One main obstacle of NMP remains the uncertainty of the needs of an isolated kidney during NMP\textsuperscript{20,21}. PCKS could provide a platform to test different interventions like electrolyte compositions, nutrients, and antioxidant supplementation, and oxygen and carbogen concentrations simultaneously to better understand the underlying mechanisms of ischemia and how to repair this phenomenon, whereafter a translation to a perfusion study can be made. Furthermore, implementing human PCKS for transplant-related research would be of great interest to close the gap between interspecies differences.

Limitations
This pilot study consisted of small sample sizes, and the results are primarily based on viability analysis only. A larger sample size and more extensive analyses would be beneficial for follow-up studies. However, with these pilots experiments we were able to illustrate the value of porcine PCKS as an ischemia-reperfusion model for transplant related research, which could be optimized in future studies.
Perfusate composition.

| Component                                    | Quantity |
|----------------------------------------------|----------|
| Red blood cells                              | 360 mL   |
| Plasma                                       | 475 mL   |
| Amoxicillin / Clavulanic acid (1000 mg/200 mg) | 10 mg    |
| 8.4% Sodium Bicarbonate (B. Braun)           | 15.25 mL |
| 5% glucose (Baxter)                         | 12.50 mL |
| Dexamethasone (Centrafarm)                   | 8.3 mg   |
| Mannitol (Baxter)                            | 10 mg    |
| Creatinine (Merck)                           | 135 mg   |
| Sodium Nitroprusside (Merck)                 | 2.7 mg   |
| Aminoplasmal (B. Braun)                      | 90 mL    |
| Insulin (100 IU/ml) (Novorapid)              | 0.186 mL |
| 250 µg/mL Amfotericin B (Fungizone) (Merck)  | 1 mL     |

**Table S1**

**Supplementary information**

41. Bellini MR, Yiu J, Nozdrin M, Papalois V. The Effect of Preservation Temperature on Liver, Kidney, and Pancreas Tissue ATP in Animal and Preclinical Human Models. J Clin Med; 2019;8.

42. Stibrós EGD, Seelen MA, van Goor H, Olinga P, Mutsaers HAM. Murine Precision-Cut Kidney Slices as an ex vivo Model to Evaluate the Role of Transforming Growth Factor-β1 Signaling in the Onset of Renal Fibrosis. Frontiers in Physiology. Frontiers; 2017;8:1026.

43. Bigaeva E, Stibrós EGD, Mutsaers HAM, Piersma B, Leilveld AM, de Jong IJ, et al. Inhibition of tyrosine kinase receptor signaling attenuates fibrogenesis in an ex vivo model of human renal fibrosis. American Journal of Physiology-Renal Physiology. American Physiological Society; 2020;318:F117–34.

44. Bigaeva E, Cavanzo NF, Stibrós EGD, de Jong AJ, Bel C, Mutsaers HAM, et al. Predictive value of precision-cut kidney slices as an ex vivo screening platform for therapeutics in human renal fibrosis. Pharmaceutics. MDPI AG; 2020;12:459.

45. Tingkow SJ, Jensen MS, Pedersen CET, de Araujo IBBA, Mutsaers HAM, Nørregaard R. Tamoxifen attenuates renal fibrosis in human kidney slices and rats subjected to unilateral ureteral obstruction. Biomedicine and Pharmacotherapy. Elsevier Masson s.r.l.; 2021;133:111003.

46. Vaughan RH, Kresse J-C, Farmer LK, Thézénas ML, Kessler BM, Lindeman JHN, et al. Decreased donor kidney degadomics indicates cytoskeletal proteolytic alterations impacting post-transplant function. medRxiv. Cold Spring Harbor Laboratory Press; 2021;2021.06.10.21254305.

47. van Leeuwen LL, Leuvenink HGD, Olinga P, Ruigrok MJR. Shifting paradigms for suppressing fibrosis in kidney transplants: Supplementing perfusion solutions with antifibrotic drugs. Frontiers in Medicine. Frontiers; 2021;0:2917.

48. Grosse-Siestrup C, Unger V, Fehrenberg C, v Baeyer H, Fischer A, Schäper F, et al. A model of isolated autologously hemoperfused porcine slaughterhouse kidneys. Nephron. 2000;92:414–21.

49. Venema LH, Brat A, Moors C, Hart NA, Ploeg RJ, Hannoert P, et al. Effects of Oxygen During Long-term Hypothermic Machine Perfusion in a Porcine Model of Kidney Donation After Circulatory Death. Transplantation. 2019;108:2057–64.

50. KDW, Hendriks Brüggenwirth IMA, Maassen H, Gerding A, Bakker B, Porte RJ, et al. Renal temperature reduction progressively favors mitochondrial ROS production over respiration in hypothermic kidney preservation. Journal of Translational Medicine. BioMed Central; 2019;17:1–10.

51. Pool MBF, Hartveld L, Leuvenink HGD, Moers C. Normothermic machine perfusion of ischemically damaged porcine kidneys with autologous, allogeneic porcine and human red blood cells. PLoS ONE. Public Library of Science; 2020;15:e0239566.

52. Karuthu S, Blumberg EA. Common infections in kidney transplant recipients. Clin J Am Soc Nephrol. American Society of Nephrology; 2012;7:2058–70.

53. Blum MF, Liu Q, Soliman B, Dreher P, Okamoto T, Poggio ED, et al. Comparison of normothermic and hypothermic perfusion in porcine kidneys donated after cardiac death. Journal of Surgical Research. 2017;216:35–45.

54. Annick Van Furth L, Venema LH, Hendriks KDW, Vogelaar PC, Krenning G, Leuvenink HGD, et al. The Effects of 6-Chromanol SUL-138 during Hypothermic Machine Perfusion on Porcine Deceased Donor Kidneys. Transplantology 2021, Vol 2, Pages 304-314. Multidisciplinary Digital Publishing Institute; 2021;2:304–14.

55. Golriz M, Fosouni H, Nickholgh E, Hafezi M, Garoussi C, Mehrabi A. Pig kidney transplantation: an up-to-date guideline. European surgical research. Europaische chirurgische Forschung Eur Surg Res; 2012;49:121–9.