# The Development of improved 12-hour porcine kidney preservation protocol

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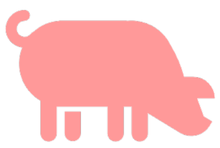
## Introduction

Currently, the gold standard of organ preservation remains Cold static storage. This 'gold standard' technique involves slowing down organ metabolism in an oxygen depleted environment, leading to increasing organ damage over time. Normothermic machine perfusion restores the organs physiological environments ex-vivo; warm, oxygenated blood is combined with essential nutrients and circulated throughout the kidneys vasculature. This restores the kidneys metabolic functions, maintain the organ in 'perfect health' prior to transplant. Marginal kidneys may also be reconditioned and assessed before transplantation.

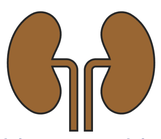
## Methods

### Organ Retrieval



All kidneys were retrieved en bloc from a local abattoir. Organs and autologous blood were collected immediately after exsanguination. Organs were stored on a bed of ice separated by a plastic membrane to lower core temperatures

### Organ Preparation



The kidney was identified, and the renal artery was isolated and cannulated. This was flushed with 1L preservation solution. During this time the vein and ureter were divided, with the ureter being cannulated and the vein remaing detached.

### Transport


Kidneys were stored in 500ml preservation solution before being submerged within an ice slurry. A standardised cold ischemic time of 3 hours was achieved within this study

### Circuit preparation

Autologous whole blood was cell saved to produce packed Red blood cells (RBCs), this was combined with a proprietary perfusate base solution (Pebble Biotechnology Laboratories) forming a blood-based solution.

### Ex-vivo Perfusion

The **LIVING-ORGAN** system was primed using the blood-based solution, and carbogen gas was attached to the system.
This used a close-loop system consisting of a central reservoir, oxygenator, and centrifugal levitating pump.
Prior to the organs being attached to the kidneys were flushed with 200ml cold Ringers solution.

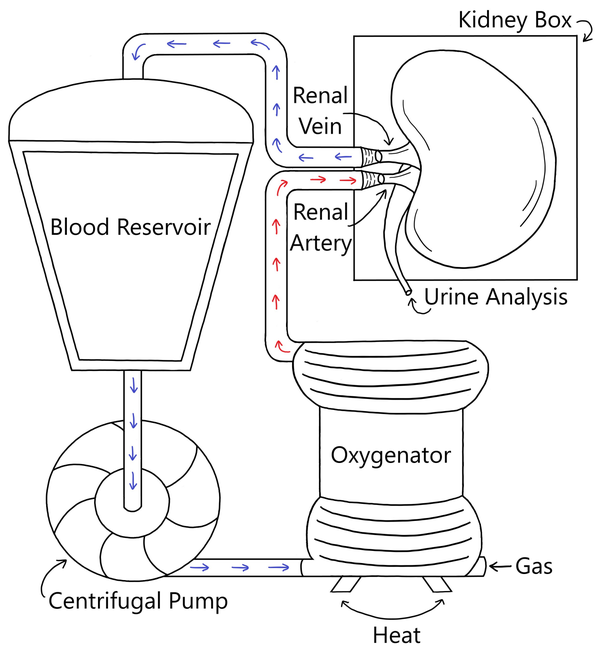



Figure 1 – A schematic diagram of the ex-vivo normothermic kidney preservation circuit. This is a closed-loop circuit; therefor blood is recirculated through the organ returning to a central reservoir.

This study consisted of n=20 kidneys, each kidney underwent 12 hours of perfusion following pebbles perfusion protocol. Continual renal haemodynamics, biochemistry and urine output were recorded and analysed. At the end of perfusion, kidneys were scored based on the clinical assessment score and their suitability for transplant determined. Histology was completed at Easter bush pathology (The University of Edinburgh).

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## Results

### ✓ Homogenous perfusion of tissue

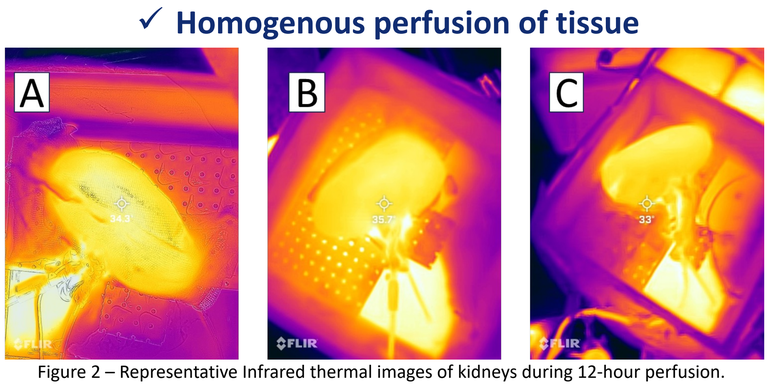


Figure 2 – Representative Infrared thermal images of kidneys during 12-hour perfusion.

### ✓ Tissue remains functional

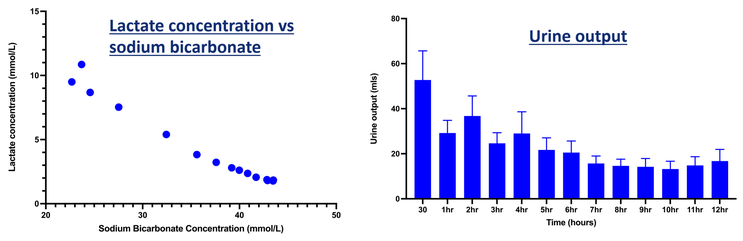


Figure 3 – Graphs showing functional parameters within the kidney. A) sodium bicarbonate – lactate concentration graph. B) Average Urine produced per hour over the 12-hours of perfusion

### ✓ Stable renal haemodynamics maintained

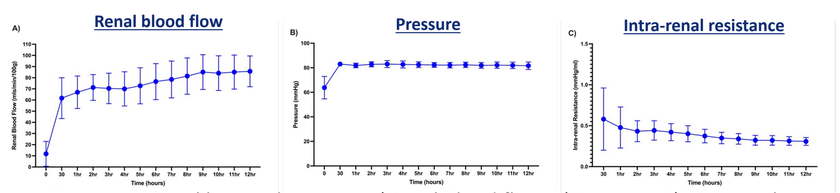


Figure 4 – Renal haemodynamics. A)Renal Blood flow. B)Pressure. C)Intra-renal resistance.

### ✓ blood biochemistry remains with physiological parameters

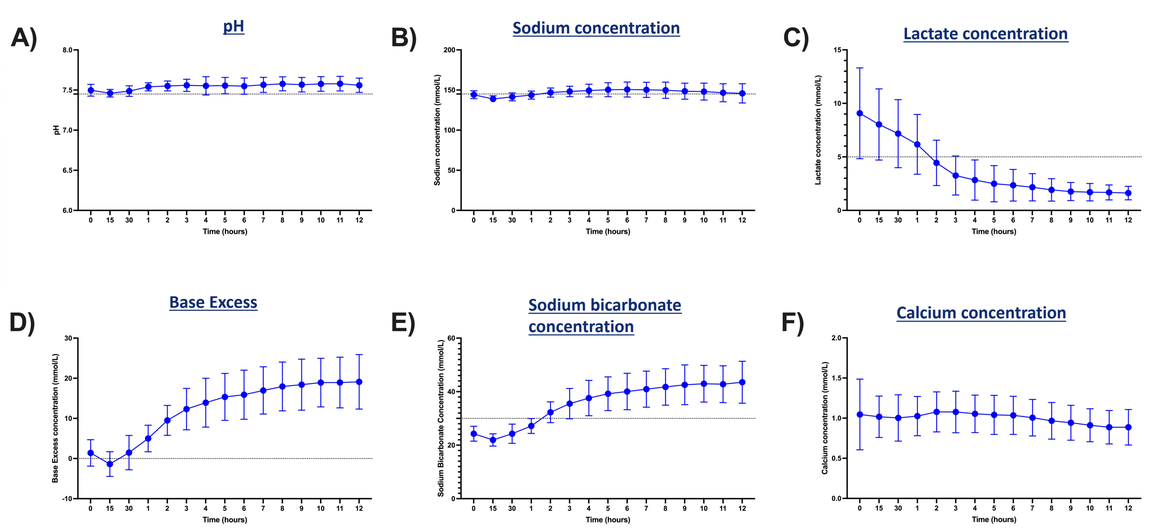


Figure 5 – Blood biochemistry. A) pH. B) Sodium concentration. C) Lactate concentration. D) Base Excess. E) Sodium bicarbonate concentration. F) Calcium concentration.

### ✓ Tissue integrity is preserved

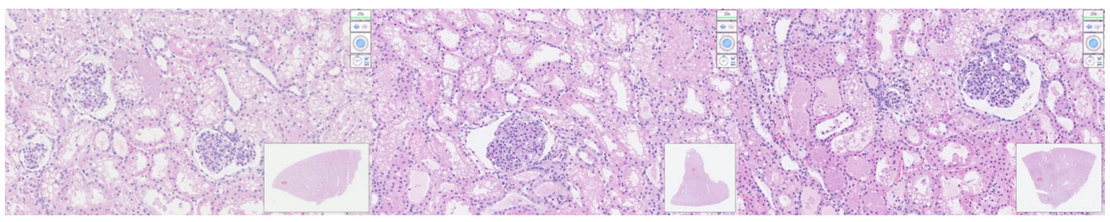

Figure 6– Representative histology images from 3 separate kidneys (H&E stain) , images taken at x20 magnification.

### ✓ Healthy tissue observed after 12 hours

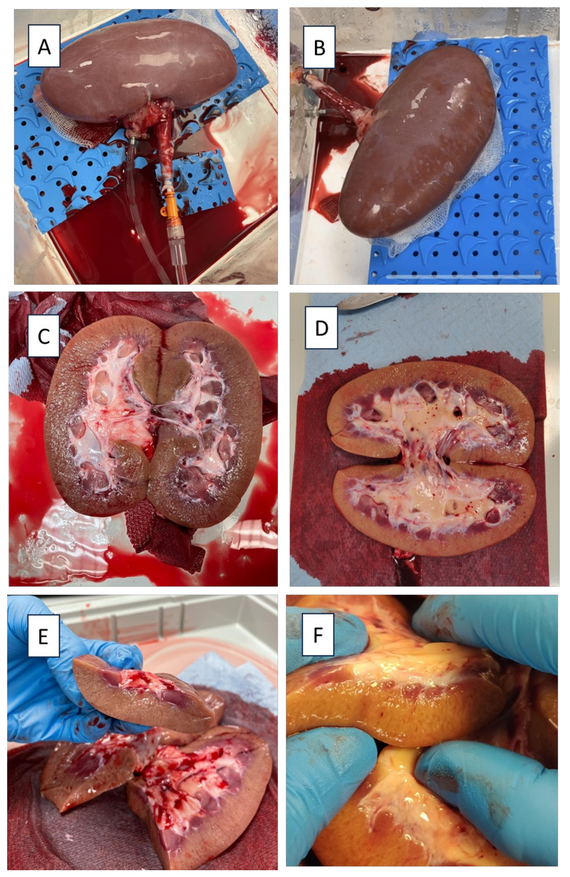


Figure 7 – Representative images taken from some of the 12 hours kidneys. A-B, kidneys attached to the LIVING-ORGAN system. C-D, images of sagittal dissection of kidneys, showing calyx, medulla, and cortex. E-F, transverse cut of the kidney showing calyx, medulla, and cortex.

## Conclusions and future perspectives

We have successfully developed a protocol that can maintain kidneys for 12 hours ex-vivo that has:

- ✓ **Improving renal haemodynamics –** Intra-renal resistance decreases over 12 hours with increasing flows and stable pressure .
- ✓ **Stable blood biochemistry -** Renal blood biochemistry remains with physiological parameters
- ✓ **Restoration of metabolic processes** – The kidney performs neoglucogensis and also actively converts lactate into bicarbonate ions
- ✓ **Healthy tissue -** Representative histology reported a Remuzzi score of 0 and a clinical assessment score of 0
- ✓ **Future work –** The next steps include testing the protocol on discarded human kidneys, and if successful progressing towards a clinical trial

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