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Margaux Navez, Nicholas Gilbo, Morgan Vandermeulen, Pauline Epicum, Marc Gilbert Lagny, Clarisse Malisoux, Claire Guarin, Tiago Pinto Coelho, Gabriel Thierry, Chantal Lechanteur, Alexandra Briquet, Etienne Baudoux, Caroline Goff, Etienne Cavalier, Jo Caers, François Joutet & Olivier Detry

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Infusion of human mesenchymal stromal cells during normothermic machine perfusion of porcine kidneys: a randomized, blinded, preclinical study

Margaux Navez, MSc ^{1,2}, Nicholas Gilbo, MD, PhD ^{2,3}, Morgan Vandermeulen, MD, PhD ³, Pauline Erpicum, MD, PhD ⁴, Marc Gilbert Lagny, MSc, PhD ⁵, Clarisse Malisoux, MSc ⁵, Claire Guarin, MSc ⁵, Tiago Pinto Coelho, MSc ¹, Gabriel Thierry, MD ⁶, Chantal Lechanteur, Ph, PhD ⁷, Alexandra Briquet, MSc, PhD ⁷, Etienne Baudoux, MD, PhD ⁷, Caroline Le Goff, MSc, PhD ⁸, Etienne Cavalier, MD, PhD ⁸, Jo Caers, MD, PhD ⁹, François Jouret, MD, PhD ^{1,4} and Olivier Detry, MD, PhD ^{2,3}

¹ Laboratory of Translational Research in Nephrology, GIGA Institute, ULiege, Liege, Belgium

² Experimental Surgical Research Center, GIGA Institute, ULiege, Liege, Belgium

³ Department of Abdominal Surgery and Transplantation, CHU of Liege, Liege, Belgium

⁴ Division of Nephrology-Dialysis-Transplantation, CHU of Liege, Liege, Belgium

⁵ Department of Cardiovascular Surgery, University Hospital of Liege (ULiege CHU), Liege, Belgium

⁶ Department of Anaesthesia and Intensive Care, University Hospital of Liege (ULiege CHU), Liege, Belgium

⁷ Laboratory of Cell and Gene Therapy, CHU of Liege, Liege, Belgium.

⁸ Department of Clinical Chemistry, CHU of Liege, Center for Interdisciplinary Research on Medicines (CIRM), Liege, Belgium

⁹ Laboratory of Haematology, GIGA Institute, ULiege, Liege, Belgium

Corresponding author contact information:

Margaux Navez, Avenue de Hippocrate 11, 4000 Liege

+32(0)4/3662432

margaux.navez@uliege.be

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Abstract

Background: Kidney transplantation from donation after circulatory death (DCD) donors is increasingly used but remains associated with warm ischemic injury. Normothermic machine perfusion (NMP) enables functional assessment and therapeutic interventions. Mesenchymal stromal cells (MSCs) display immunomodulatory and regenerative properties, yet their translational efficacy during NMP remains unclear.

Methods: In a porcine model, six pairs of kidneys subjected to 30 minutes of warm ischemia followed by 3 hours of static cold storage underwent 6 hours of NMP. In each pair, one kidney randomly received an intra-arterial injection of placebo, while the contralateral kidney received 10 million clinical-grade human bone marrow-derived MSCs (hMSCs). Perfusion characteristics, glomerular filtration, tissue injury, and inflammatory markers were assessed.

Results: hMSC infusion during NMP was technically feasible and hemodynamically well tolerated, with no adverse effects on perfusion stability. Perfusion parameters, urine output, and creatinine/iohexol clearance showed no significant differences between groups. NGAL and cytokines levels increased during perfusion, but hMSCs did not alter their dynamics.

Conclusions: Intra-arterial delivery of clinical grade hMSCs during NMP was safe but did not improve renal function or reduce histological injury. These results highlight the challenges of achieving MSC engraftment during *ex situ* perfusion and highlight the need for refined strategies such as repeated dosing, prolonged perfusion, or extracellular vesicle therapy. Despite the negative findings, this study presents a highly translational large-animal model, supporting further investigation of MSC-based therapies in human kidneys discarded for transplantation.

1. Introduction

Kidney transplantation remains the optimal treatment for end-stage renal disease; however, its potential is constrained by a significant global shortfall of suitable organ donors.¹ This shortage contributes to a prolonged transplantation waiting lists, and has driven a growing reliance on grafts obtained from donation after circulatory death (DCD) donors.²⁻⁴ These DCD donors may increase organ availability for transplantation.⁵ Evidence suggests that kidneys from DCD donors can achieve short- and mid-term survival rates comparable to those obtained from donation after brain death (DBD) donors.

However, the higher incidence of primary graft dysfunction, coupled with the detrimental effects of the warm ischemia time inherent to DCD procurement, remains a significant limitation.^{6,7}

Machine perfusion is emerging as a valuable approach to kidney graft preservation, offering advantages over static cold storage (SCS), the current gold standard, by enabling both improvement and assessment of graft quality prior to transplantation.⁸⁻¹² In particular, normothermic machine perfusion (NMP) offers significant advantages to overcome current limitations in organ quality and preservation time. This technology provides enhanced preservation conditions, extends viable storage duration, enables kidney quality assessment, facilitates organ reconditioning, and allows for targeted therapeutic interventions.¹³⁻¹⁵ NMP holds promise for clinical benefits, potentially improving early graft function, reducing ischemia-reperfusion injury, and enabling viability assessment prior to transplantation.¹⁶⁻¹⁸

Recently, regenerative medicine has emerged within the field of organ transplantation, particularly in conjunction with machine perfusion techniques.^{19,20} A potentially therapeutic intervention during NMP is the administration of mesenchymal stromal cells (MSCs), known for their immunomodulatory, anti-inflammatory, and regenerative properties.²¹⁻²³ We and others have previously investigated the use of MSCs in the setting of liver and kidney transplantation in early-phase clinical studies, demonstrating their favorable tolerability profile.²⁴⁻²⁷ Although the feasibility of MSC delivery during kidney NMP has previously been explored, a recent systematic review

and meta-analysis of stem cell-based therapies during machine perfusion identified important methodological limitations across the existing preclinical literature, including inconsistent investigator blinding, unclear or absent randomization procedures, and variable use of clinical-grade cell products.²⁸ These gaps currently limit the translational relevance of available data and the ability to draw robust conclusions regarding efficacy.

Building on this background, the present study was therefore designed to directly assess these limitations and investigate the potential therapeutic effects of clinical-grade human mesenchymal stem cells (hMSCs) in a large animal model. Specifically, we examine hMSCs perfused into renal grafts during NMP with the goal of mitigating ischemia-reperfusion injury.²⁹ The primary objective was to assess the feasibility of delivering hMSCs to ischemic porcine kidneys during NMP. Secondary objectives included evaluating their impact on perfusion quality, severity of renal tissue injury, modulation of key inflammatory markers, and overall kidney function.

2. Material and Methods

2.1. Ethics and Animals

This study was designed and reported in accordance with the ARRIVE guidelines 2.0 for reporting animal research. Female laboratory pigs (30–60 kg), resulting from a Landrace and Pietrain cross, were sourced from the Walloon Agricultural Research Centre (CRA-W farm, Gembloux, Belgium). These animals were used for the collection of washed red blood cells and the procurement of kidneys. The study protocol was reviewed and approved by

the Animal Ethics Committee of the University of Liege (approval number 21-2447). No formal sample size calculation was performed; six animals were deemed sufficient to assess feasibility and biological effects in this randomized, blinded, preclinical large-animal model, in accordance with the principles of animal reduction and refinement. Animal care and handling complied with the guidelines set out in the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health and the National Academy of Sciences, ensuring ethical and humane treatment throughout the experimental process.

2.2. Study Design

Six pairs of kidneys were perfused simultaneously using NMP, following the protocol recently published by our group.³⁰ Following a 30-minute period of warm ischemia, kidneys underwent 3 hours of SCS, after which they were subjected to 6 hours of NMP. At 45 minutes of NMP, an intra-arterial injection was administered: kidneys were randomly assigned to receive either placebo (NMP group) or 10 million clinical-grade bone marrow-derived hMSCs (NMP + hMSC group). This dose was selected based on prior studies by Pool *et al.*, who evaluated a range of MSC doses (10^5 – 10^7 cells) during porcine kidney NMP.³¹ Perfusate and urine samples were collected every 30 minutes and every hour, respectively, during the 6-hour perfusion period. Renal tissue biopsies were obtained at the end of the NMP period (**Fig 1**).

2.3. MSCs and Placebo Preparation

Detailed methodologies for clinical-grade hMSC culture have been reported previously.²⁹ In summary, hMSCs used in this study were produced under clinical-grade conditions by the Laboratory of Cell and Gene Therapy (LTCG), a GMP-licensed of accredited facility at the University Hospital of Liege. hMSCs were isolated from bone marrow aspirates of healthy allogeneic donors and processed via automated Ficoll-based mononuclear cell isolation closed-system. Cells were expanded in culture up to passage 2 using serum-containing media supplemented with gamma-irradiated foetal bovine serum. At passage 2, cells were harvested, formulated in a cryopreservation medium containing saline, human albumin, and 10% dimethyl sulfoxide, then frozen using a controlled-rate protocol before storage in the vapor phase of liquid nitrogen. For this study, hMSCs were thawed and directly diluted with a saline-albumin solution prior to intra-arterial administration during NMP. The final cell product was perfused within one hour post-thawing to ensure stability and viability.²⁹ The placebo consisted of a 20 mL syringe containing saline, human albumin and DMSO, identical in appearance and volume to the syringe containing 10 million thawed hMSCs, prepared as described above.

2.4. Randomization

For each kidney pair, one kidney was randomly assigned to receive a placebo treatment (NMP group), while the contralateral kidney received the hMSC-based therapy (NMP + hMSC group). Randomization by coin flip (1:1 allocation ratio) was performed by the LTCG, which was responsible for preparing and supplying both the placebo and hMSC products. On the day of the experiment,

two identically prepared opaque syringes, labelled 'right' and 'left', were provided and administered to the corresponding kidneys in a blinded manner under standardized experimental conditions. To ensure blinded and unbiased evaluation, the surgical team, as well as all personnel involved in kidney graft procurement, NMP, sample collection, and data analysis, remained blinded to group allocation throughout the entire study.

2.5. Surgical Procedure

Operative procedures have been described previously.³⁰ Briefly, animals were sedated thirty minutes prior to surgery via intramuscular injection of tiletamine/zolazepam (4.4 mg/kg; Zoletil 100, Virbac, Leuven, Belgium). An auricular vein catheter was placed, followed by intravenous induction of anesthesia and orotracheal intubation. Anesthesia was maintained using continuous propofol infusion (2–4 mg/kg/h; Aspen Pharma, Dublin, Ireland), boluses of sufentanyl (0.2 µg/kg Viatris; Canonsburg, Pennsylvania, USA). Animals were mechanically ventilated to maintain PaO₂ >100 mmHg and PaCO₂ between 35–45 mmHg. A carotid arterial catheter was inserted for hemodynamic monitoring and blood sampling. After intravenous administration of 25,000 IU of heparin, the abdominal vessels were cannulated. Warm ischemia was induced by clamping the thoracic aorta and maintained for 30 minutes. Subsequently, the kidneys were flushed in situ via the aortic cannula with 2 L of cold (4°C) IGL-1 preservation solution (Institut Georges Lopez, Lissieu, France), and the abdominal cavity was cooled with 4°C saline and topical slush ice. Whole blood was collected via the vena cava

cannula during the flush and processed using a cell-saving system (Xtra autotransfusion system; LivaNova, Zaventem, Belgium) to obtain leukocyte-depleted, concentrated red blood cells. Animals underwent euthanasia by exsanguination, resulting in cardiac arrest under general anaesthesia. Following bilateral nephrectomy, the renal artery and the ureter were dissected and cannulated. Kidneys were then preserved by SCS in IGL-1 solution at 4°C for 3 hours. Prior to connection to the NMP circuit, each kidney was weighed and flushed on the back table with 500 mL of cold IGL-1 solution.

2.6. Normothermic Machine Perfusion

As described in a previous study by our group, both kidneys from the same pig underwent simultaneous 6-hour NMP using two identical and independent, pressure-controlled, pulsatile perfusion systems. These were custom-made devices adapted from two discontinued clinical cardiopulmonary extracorporeal circulation systems (Stockert GmbH, Freiburg, Germany).³⁰ Each NMP circuit consisted of a kidney receptacle, roller pump, membrane oxygenator (Dideco Perfusion Tubing Systems; LivaNova, Zaventem, Belgium), and heat exchanger (Stockert GmbH, Freiburg, Germany) connected to coated tubing (LivaNova; Zaventem, Belgium). The arterial pressure (WPI, Friedberg, Germany) and flow rates (Sonotec, Halle, Germany) were continuously measured. The renal artery was cannulated and connected to the NMP circuit, while the renal vein was left open to drain into the circuit for reperfusion. The ureter was cannulated for urine collection. The NMP circuits were primed with a solution of autologous red blood cells and isotonic

electrolyte solution (Plasmalyte; Baxter, Lessines, Belgium) to achieve a haematocrit of 20–30%. Flolan (Epoprostenol sodium, 7.5 µg/h; GSK, Wavre, Belgium) was continuously infused. Urine output was replaced 1:1 with Plasmalyte, and a parenteral nutrition solution (Aminomix 2 Novum; Fresenius Kabi, Willebroek, Belgium) was added to maintain glucose levels > 100 mg/dL. Sodium bicarbonate (10 mL, 8.4%; B Braun Melsungen, Germany) was used to maintain a pH in the range of 7.2 to 7.5. Perfusion temperature was maintained at 38 °C with an oxygen flow of 100 mL/min at FiO₂ 21%. During perfusion, arterial pressure was gradually increased to a target range of 60–80 mmHg. Intrarenal resistance (IRR) was calculated as the ratio of arterial pressure to flow and expressed in mmHg/mL/min/100 g of tissue.

2.7. Perfusate and Tissue Collection

A sampling line was connected to the arterial inflow cannula for perfusate collection. Samples of 2 mL were drawn into SST BD Microtainer tubes (BD, New Jersey, USA), centrifuged at 2000 × g for 5 minutes at 4 °C. Supernatant was aliquoted and stored at –80 °C for subsequent analysis. Biochemical parameters (pH, sodium, potassium) were measured in the arterial line perfusate using the Epoc Blood Analysis System (Siemens Healthcare NV, Dilbeek, Belgium). Renal function was assessed via clearance of predefined concentrations of creatinine (75mg/L) and iohexol (518 mg/L) added to the perfusate during circuit priming (GE Healthcare, Diegem, Belgium). Clearance of these markers over time was used to evaluate excretory performance. The glomerular filtration rate was measured based on creatinine clearance and

expressed as mL/6h/100g of kidney tissue. Perfusate and urine concentration of creatinine was determined at each sampling time point and cumulatively over the 6-hour NMP period to establish the filtration rate. All values were normalized to the kidney weight. At the end of perfusion, kidney biopsies were obtained for histological analysis. Tissue samples were fixed in 4% formalin, paraffin-embedded, and stained with haematoxylin-eosin and Periodic Acid-Schiff. Tubular injury was semi-quantitatively evaluated by an experienced nephropathologist (P.E.), blinded to group allocation, based on the percentage of tubular epithelium exhibiting brush border loss and epithelial thinning across the examined sections. All collected samples were analyzed, and no data points were excluded from the study.

In a separate experiment, one donor kidney pair was exclusively used for MSC tracking, to avoid any confounding effect on the primary experimental outcomes. Prior to infusion, hMSCs were prelabelled with CellTracker CMPTX Red fluorescent dye (C34552, Thermo Fisher Scientific, Massachusetts, United States) to enable subsequent cell tracking within the renal tissue. Renal biopsies were collected at 2 and 4 hours post-infusion and immediately embedded in Tissue-Tek OCT compound for cryopreservation. Cryosections of 5 μ m thickness were prepared and counterstained with DAPI (4',6-diamidino-2-phenylindole) to visualize cell nuclei. Fluorescence imaging was performed using ECHO Revolve (Discover Echo Inc., San Diego, CA, USA).

2.8. ELISA

Levels of neutrophil gelatinase-associated lipocalin (NGAL) were measured using commercially available enzyme-linked immunosorbent assay kits [human Lipocalin-2/NGAL Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA)]. A custom electrochemiluminescent Milliplex® assay was performed on perfusate samples to quantify cytokine expression. The 8-plex panel included IFN- γ , IL-2, IL-10, IL-1 α , IL-6, TNF- α , IL-1 β and IL-8 (PCYTMAg-23K; Millipore, Darmstadt, Germany). All assays were carried out according to the manufacturer's instructions.

2.9. Statistical Analysis

A total of six animals ($n = 6$) were selected to account for expected biological variability in large-animal models. Results are reported as mean $\pm$ standard deviation. Data visualization was performed using GraphPad Prism (version 10). For comparative analyses, the area under the curve (AUC) was determined, followed by a non-parametric Wilcoxon signed-rank test. A generalized linear mixed model was used to assess the effects of time, treatment group (kidney), and their interaction, using a compound symmetry covariance structure to account for repeated measures. Assumptions of normality and homoscedasticity were verified, and log transformation was applied where appropriate. For two specific parameters, beta regression was used due to the distribution of the data. Model fit was evaluated using Akaike and Bayesian Information Criteria (AIC/BIC), with lower values indicating better fit. Statistical significance was defined as $p < 0.05$. All statistical analyses were

conducted using SAS (version 9.4) and R (version 4.2.2), with the *Inormimp* package used for censored data imputation.

3. Results

3.1. Animal Characteristics

Six pigs (mean weight: 40.50 ± 9.77 kg) underwent the experimental protocol enabling the procurement of six pairs of kidneys (**Table 1**). In total, six kidneys were allocated to each group: the NMP group (1 right/5 left) and the NMP + hMSC group (5 right/1 left) (**Suppl. Table 1**). Baseline kidney weights were comparable between the control group (138.17 ± 32.46 g) and the treated group (134.83 ± 25.52 g; $p = 0.56$). At the end of perfusion, kidney weights had increased in both groups, with no significant difference observed in kidney weight at the end of perfusion (176.50 ± 45.92 g for NMP group vs. 184.33 ± 55.74 g for NMP + hMSC group; $p = 0.31$). Cold and warm ischemia times were identical between groups (CIT: 183.67 ± 7.61 min; WIT: 30 min). Baseline perfusate creatinine levels were also the same (1.04 ± 0.05 mg/dl in both groups). All kidneys were included in the final analysis.

3.2. Perfusion Characteristics

The administration of hMSCs did not significantly affect the majority of perfusion or biochemical parameters during the 6-hour perfusion period compared with controls (**Suppl. Table 2**). Electrolyte and acid-base homeostasis did not differ between groups, with comparable values for pH, sodium, potassium, $p\text{CO}_2$, and $p\text{O}_2$ (all $p > 0.1$) (**Fig. 2**). Similarly, no significant intergroup differences were detected in glucose, lactate, creatinine,

or iohexol clearance. In contrast, the NMP + hMSC group demonstrated significantly higher hematocrit (18.29 ± 2.73 vs. 17.40 ± 2.92 ; $p = 0.018$) and hemoglobin concentrations (6.21 ± 0.91 g/dl vs. 5.92 ± 0.98 g/dl; $p = 0.017$). In addition, mean perfusion pressure was greater in the NMP + hMSC group (53.65 ± 27.58 mmHg vs. 46.43 ± 16.51 mmHg; $p = 0.035$), while cumulative urine output was slightly lower (20.49 ± 15.68 ml vs. 21.70 ± 24.49 ml; $p = 0.033$). No significant difference in vascular resistance was observed between groups (3.28 ± 4.32 vs. 1.96 ± 2.24 ; $p = 0.20$). Although hMSC-treated kidneys exhibited a tendency towards greater weight gain (38.33 g $\pm$ 17.77 g for NMP group vs. 49.50 g $\pm$ 33.22 g for NMP + hMSC group), this difference was not statistically significant ($p = 0.44$) (**Fig 3**).

3.3. Renal Function and Histology

Clearance of creatinine and iohexol from the perfusate was used to evaluate glomerular filtration. Both analytes exhibited similar elimination profiles in the NMP + hMSC and NMP groups, with no significant differences observed in the calculated AUC. Only five kidneys ($n = 5$) were included in the GFR (Glomerular Filtration Rate) analysis, as one kidney pair from the same pig (pair 3) did not produce urine and was therefore excluded from this measurement. GFR followed a similar pattern, with slightly higher mean values in the NMP + hMSC group (108.30 ± 89.14 mL/6h/100g for NMP vs. 131.10 ± 74.12 mL/6h/100g for NMP + hMSC), though without statistical significance ($p > 0.1$). Histological evaluation of the kidney biopsies was conducted solely at the end of perfusion using PAS staining. Findings demonstrated features consistent with acute

tubular injury, including loss of the proximal tubular brush border, thinning of the tubular epithelium and vacuolization of tubular cells. Mild peritubular capillarity was consistently observed across all samples (**Fig 4**) The extent of tubular injury was semi-quantitatively estimated as the percentage of affected tubular epithelium per biopsy. Overall, tubular injury affected a substantial proportion of the epithelium in both groups, with mean estimates of approximately 79% in the MSC group and 69% in the NMP control group, without complete tubular denudation observed in any sample. Fluorescence microscopy revealed the presence of CMTPIX-labelled hMSCs (red) within the renal parenchyma at 2 and 4 hours of perfusion, indicating that infused hMSCs remained detectable in the kidney tissue throughout the NMP procedure (**Fig 5**).

3.4. NGAL Dynamics

Perfusate concentrations of NGAL increased significantly from baseline to 6 hours in both groups (linear mixed-effects model, time effect: $p < 0.0001$), consistent with progressive injury or stress during NMP. However, no significant intergroup differences were detected at any time point (Group $\times$ Time interaction: $p = 0.4$), indicating that hMSC treatment did not alter the temporal pattern of NGAL release. Urinary NGAL concentrations followed a similar pattern, with no significant difference between groups ($p = 0.7$) (**Fig 6**).

3.5. Cytokine Measurement

No significant differences were observed between the NMP + hMSC group and NMP group for any of the measured cytokines for which a statistical comparison could be performed (Group $\times$ Time interaction: TNF- α , $p = 0.98$; IL-10, $p = 0.99$; IL-1 α , $p = 1.00$). Nevertheless, a characteristic temporal pattern was noted across both groups: TNF- α concentrations varied significantly over time ($p = 0.001$), peaking at 3 hours before declining towards baseline, while IL-6 and IL-10 were initially nearly undetectable at the onset of perfusion and began to rise after 3 hours (IL-10 time effect, $p = 0.0024$). IL-1 α and IL-1 β showed increased levels only at the 6-hour mark of perfusion; owing to the near-absence of detectable values at earlier timepoints, the mixed-effects model could not be fitted for IL-6 and IL-1 β , and group comparisons for these two cytokines could therefore not be statistically tested. IL-2 remained below the detection threshold throughout the perfusion period in both groups and was likewise not statistically tested (**Fig 7**).

4. Discussion

In this porcine model of NMP, intra-arterial infusion of 10 million clinical-grade human bone marrow-derived MSCs was technically feasible and hemodynamically well tolerated, with no disruption of perfusion hemodynamic or adverse events. Despite seemingly efficient delivery, hMSC treatment failed to improve renal function (as assessed by creatinine clearance and urine output) nor did it reduce ischemia/reperfusion-induced histological injury when compared with controls.

These findings are broadly consistent with previous reports from other groups. Lohmann *et al.* administered a comparable dose of MSCs into porcine kidneys during 4h of NMP and observed no functional or histological improvement after transplantation, despite confirming the presence of MSCs in renal tissue.³² Pool *et al.* conducted two relevant studies: in 2020, they perfused ischemic pig kidneys with hMSCs for seven hours, resulting in reduced injury markers and increased growth factor release, yet without improvements in perfusion flow or excretory function ³³. In a 2019 study, the same group demonstrated that most MSCs rapidly disappeared from circulation, with only a limited number of cells retained in glomerular capillaries.³¹ In line with these observations, our additional cell-tracking experiment using CMTPIX-labelled hMSCs confirmed the presence of labelled cells within the renal parenchyma at 2 and 4 hours post-infusion, although these cells were sparse and heterogeneously distributed. A key methodological distinction between our study and that of Lohmann *et al.* lies in the preservation strategy: while their protocol incorporated a phase of cold oxygenated hypothermic perfusion (oxHMP) prior to NMP, our model utilized SCS followed immediately by NMP. Importantly, the warm and cold ischaemia times used in our study closely mirror those encountered in human DCD donor kidneys, suggesting that this may represent one of the more translationally relevant large-animal models described to date. However, none of these prior studies combined investigator blinding, formal randomization, and clinical-grade cell products within the same experimental design, a methodological gap explicitly identified in our

recent systematic review.²⁸ By addressing this gap, the present study provides a more rigorous and clinically translatable benchmark, even though the functional outcome itself converges with earlier reports.

The translational potential of MSC therapy in kidney transplantation has also been explored by our group in both preclinical and clinical settings, evaluating the anti-inflammatory, immunoregulatory, and reparative properties of MSCs in models of ischemia-reperfusion and transplantation.^{24,26} In a pilot clinical trial, Erpicum *et al.* administered allogeneic bone marrow-derived MSCs ($\sim 2 \times 10^6$ cells/kg) into kidney transplant recipients and observed improved early graft function and increased regulatory T cell populations, without any adverse events.²⁵ These findings confirmed the safety of hMSC administration in humans and highlighted the potential of MSC-based therapies to enhance post-transplant recovery, although their long-term effects remain to be defined. Notably, donor-specific anti-HLA antibodies (DSAs) were detected in these studies, highlighting the risk of recipient sensitization following direct MSC injection.^{25,34} In this context, NMP offers a strategy to deliver MSCs directly to the graft while theoretically minimizing the risk of recipient immunization.

A key strength of our study lies in the use of clinical grade, hMSCs, manufactured under validated and standardized conditions in accordance with International Society for Cellular Therapy (ISCT) criteria. Unlike other preclinical studies that have employed research-grade, we deliberately selected the same hMSCs that have already been administered to patients in

phase I/II clinical trials.²⁵ This strategy maximizes the reproducibility and translational relevance of our findings. Furthermore, with an experimental design that replicates the most recently developed preservation pathways (static cold storage followed by NMP), our study provides a unique and robust model for evaluating MSC-based interventions in a setting that most closely mirrors clinical reality.

Nevertheless, several limitations must be acknowledged. Although limited by a small sample size inherent to large-animal models, our design using paired kidneys minimized inter-individual variability. Moreover, the consistent absence of effects across functional, histological, and biomarker endpoints strongly suggests that a clinically meaningful benefit of a single MSCs dose during NMP is unlikely. In addition, broader mechanistic profiling, such as NF- κ B signaling or transcriptomic analysis, was not performed in this study. Only inflammatory mediators in the perfusate were assessed, providing limited insight into the biological response to MSC administration. Such analyses would be of interest in a more extensive future study. Finally, the lack of standardized protocols in the literature—regarding MSC source, dose, timing, and administration route—remains a major obstacle to inter-study comparisons and reproducibility. This variability underscores the urgent need for harmonized protocols to generate robust and comparable results.

In conclusion, this study confirms the safety and feasibility of hMSC delivery during NMP using a clinical-grade cell product, under ischemia and preservation conditions comparable to current clinical practice, representing

one of the most translationally relevant large-animal models reported to date. While hMSC administration did not confer a measurable functional or histological benefit under the conditions tested, these findings provide valuable guidance for refining future strategies — for instance through repeated or prolonged dosing, cell pre-conditioning, or the use of MSC-derived extracellular vesicles — helping to prioritize the most promising directions for further research.^{32,33,35,36} Harmonization of experimental protocols across centers will remain essential to enable direct comparability between studies and accelerate progress toward clinical application.

List of abbreviations

AUC, Area Under the Curve

DBD, Donation After Brain Death

DCD, Donation After Circulatory Death

GFR, Glomerular Filtration Rate

GMP, Good Manufacturing Practice

hMSCs, Human Mesenchymal Stromal Cells

IRR, Intrarenal Resistance

LTCG, Laboratory of Cell and Gene Therapy

MSCs, Mesenchymal Stromal Cells

NGAL, Neutrophil Gelatinase-Associated Lipocalin

NMP, Normothermic Machine Perfusion

SCS, Static Cold Storage

Declarations

Ethics approval: This study was reviewed and approved by the Animal Ethics Committee of the University of Liege (approval number 21-2447) under the approved project entitled “Effects of normothermic perfusion of porcine kidneys with porcine mesenchymal stromal cells (MSCs) on ischemia-reperfusion injury”, approved on March 28, 2022. Animal care and handling complied with the guidelines set out in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and the National Academy of Sciences, ensuring ethical and humane treatment throughout the experimental process. Human mesenchymal stromal cells were obtained from donors who provided written informed consent for the use of their biological samples for research purposes, in accordance with institutional and national regulations. The use of Human MSCs was reviewed and approved by the Hospital-Faculty Ethics Committee of the University of Liège under the approved project entitled “TB0202F11-Consent-collecte moelle allo MSC”, with approval reference number 2025-470, approved on November 7, 2024.

Consent for publication: Not applicable

Availability of data and materials: All data analysed during this study are included in this published article. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Authors' Contributions: M.N., F.J., and O.D. designed the study. M.N., N.G., M.V., P.E., T.P.C., M.G.L., C.M., C.G., G.T., C.L., A.B., E.B., C.L.G., E.C., and O.D. performed the surgeries and experiments. M.N., N.G., F.J., and O.D. contributed to the analysis and interpretation of results. M.N. conducted statistical analyses. M.N., N.G., F.J., and O.D. were involved in data discussion and revised the manuscript for intellectual content. M.N. and O.D. participated in the writing of the paper. M.N. prepared the figures and tables. All authors have reviewed the results and approved the final version of the manuscript.

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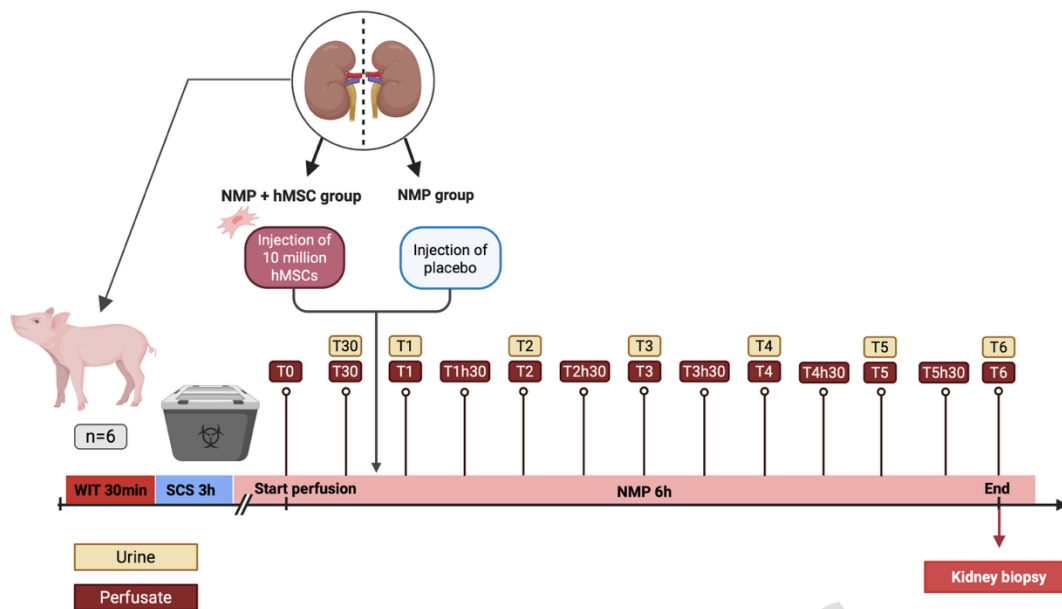


Figure 1. Experimental design and samplings at different timings. Samples of urine and perfusate were collected during NMP. Biopsies were performed at the end of NMP. NMP, normothermic machine perfusion; T, timing; WIT, warm ischemia time; SCS, static cold storage; hMSCs, human mesenchymal stromal cells.

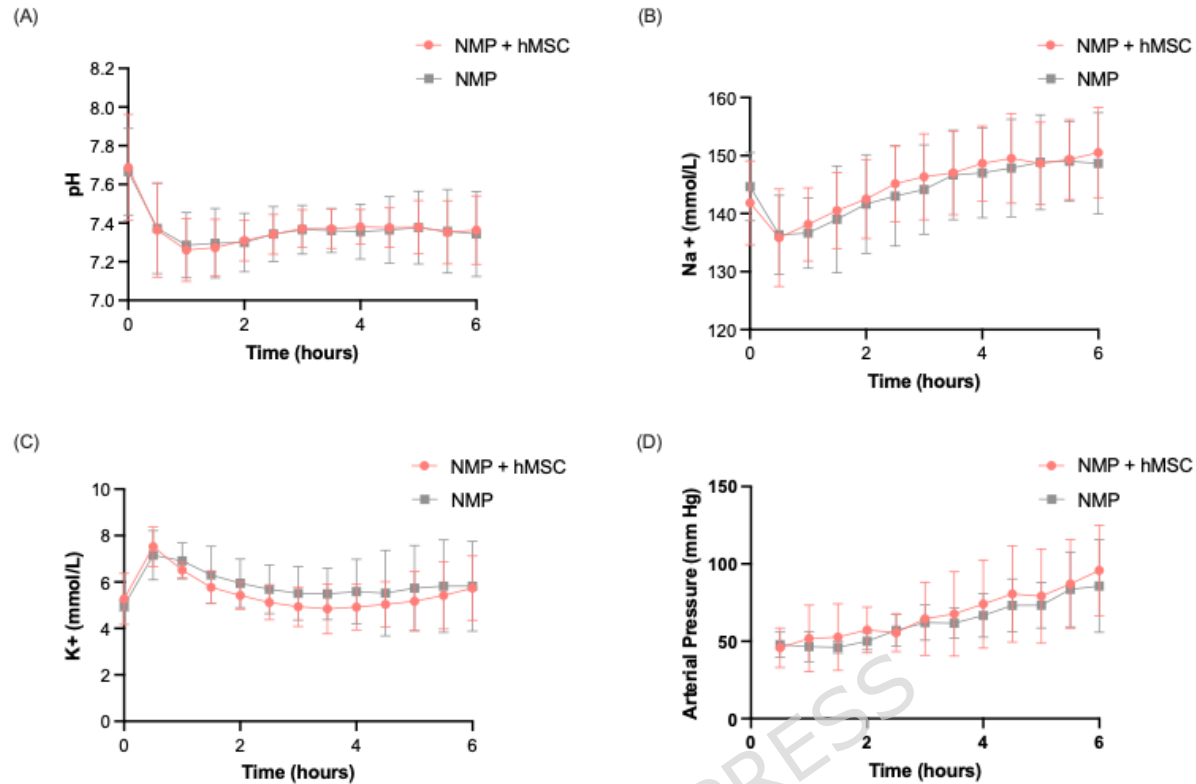


Figure 2. Perfusion characteristics during 6 hours of NMP with administration of placebo or 10 million hMSCs. (A) pH ;(B) sodium levels (mmol/l) ;(C) potassium levels (mmol/l) ;(D) arterial pressure (mmHg). Data presented as mean $\pm$ SD.

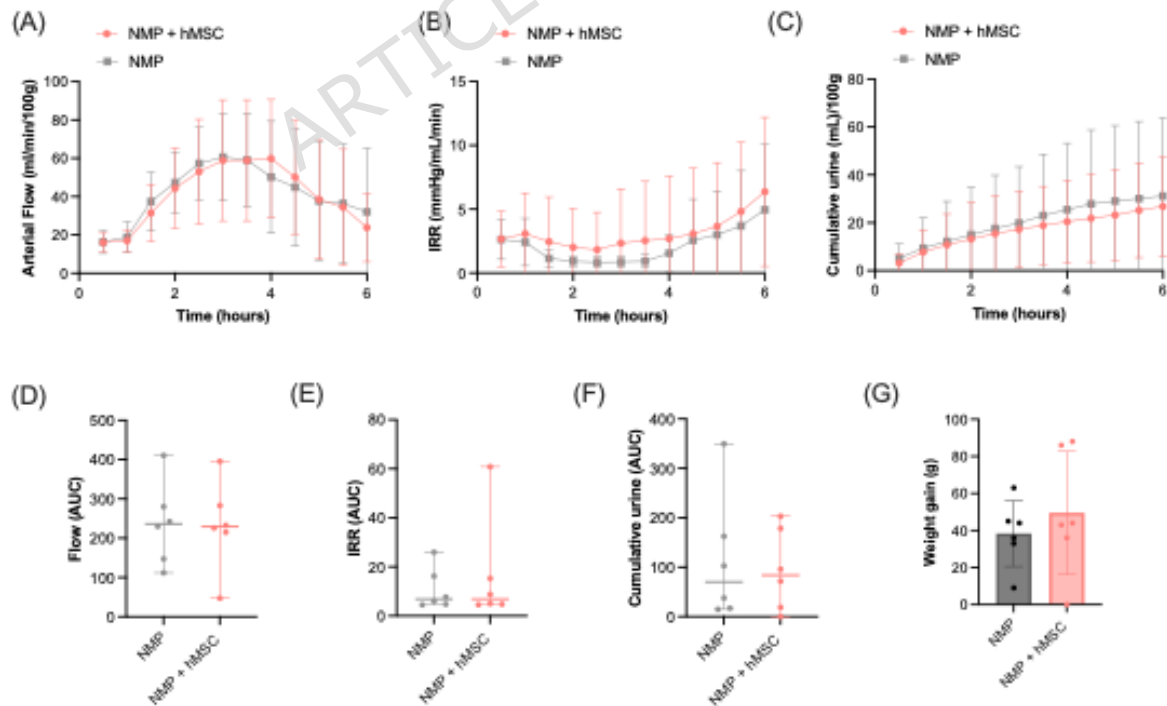


Figure 3. Perfusion characteristics during 6 hours of NMP with administration of placebo or 10 hMSCs. (A) Arterial flow (ml/min/100g) ;(B) Intrarenal Resistance (mmHg/ml/min) ;(C) Cumulative urine (ml/100g) ;(D) AUC analysis of flow; (E) AUC analysis of intrarenal resistance; (F) AUC analysis of cumulative urine and (G) weight gain (g). Data presented as mean $\pm$ SD.

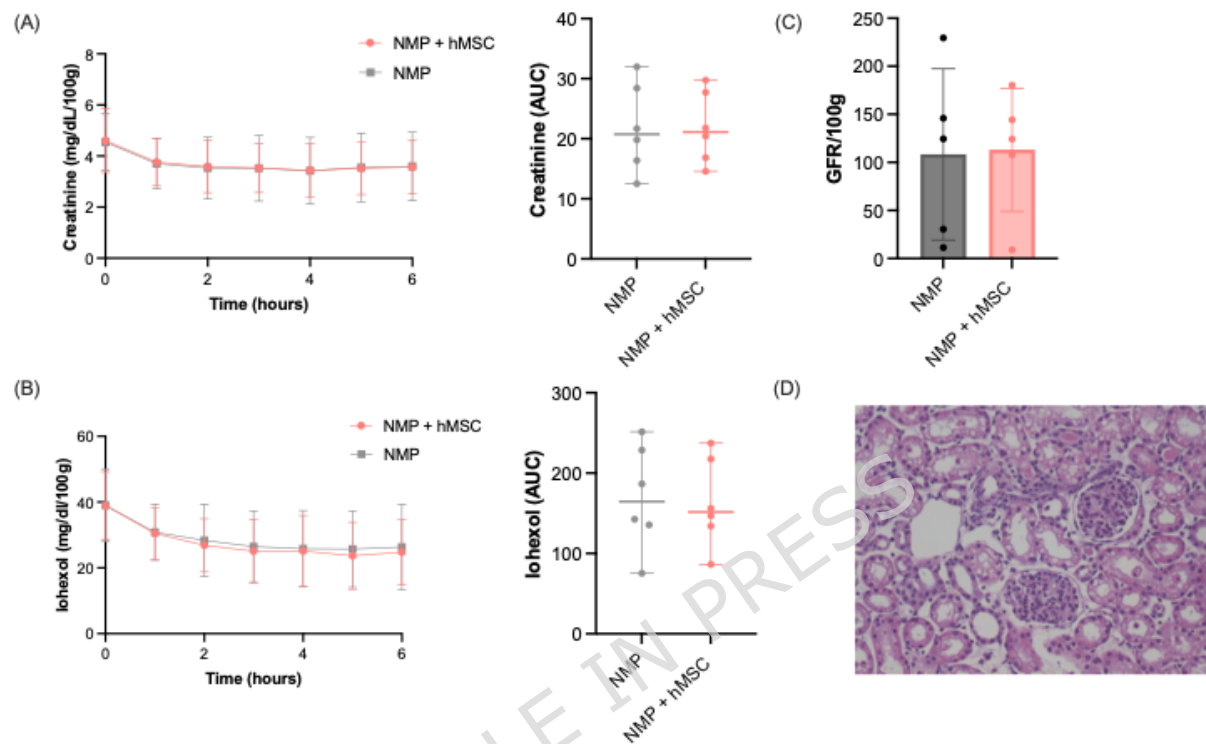


Figure 4. Assessment of renal function during 6 hours of NMP with administration of placebo or 10 million hMSCs. (A) Elimination of creatinine in perfusate (mg/dl/100g) ;(B) Elimination of iohexol in perfusate (mg/dl/100g). Left panels: kinetics and right panels: AUC analyses. (C) Glomerular filtration rate (ml/6h/100g). (D) Histological representation of kidney biopsies, original magnification x20, PAS staining. Data presented as mean $\pm$ SD.

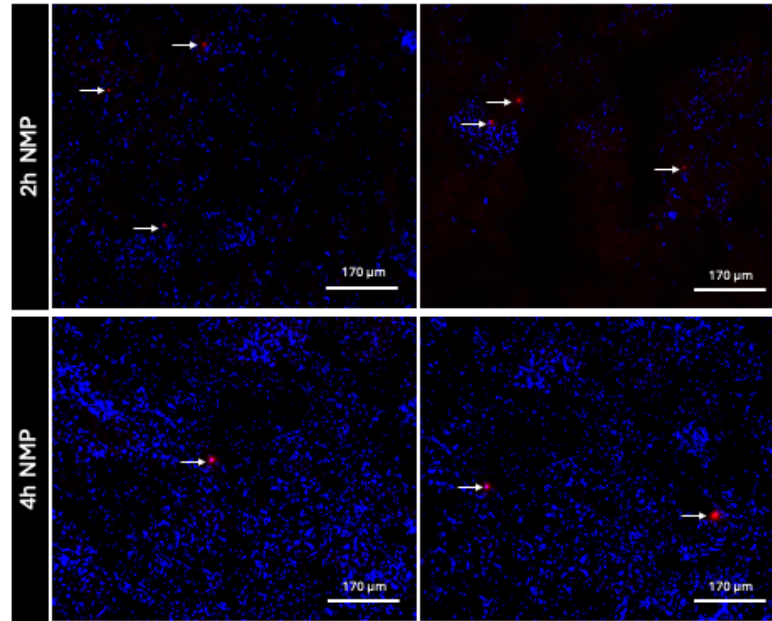


Figure 5. Detection of CMTX-labelled human mesenchymal stromal cells (hMSCs) within porcine renal tissue during normothermic machine perfusion (NMP). Representative immunofluorescence images of renal biopsies obtained at 2 hours (top panels) and 4 hours (bottom panels) following hMSC infusion. Cell nuclei are counterstained with DAPI (blue), and CMTX-labelled hMSCs are visualized in red (white arrows). Two representative fields are shown for each timepoint. Scale bar = 170 μ m.

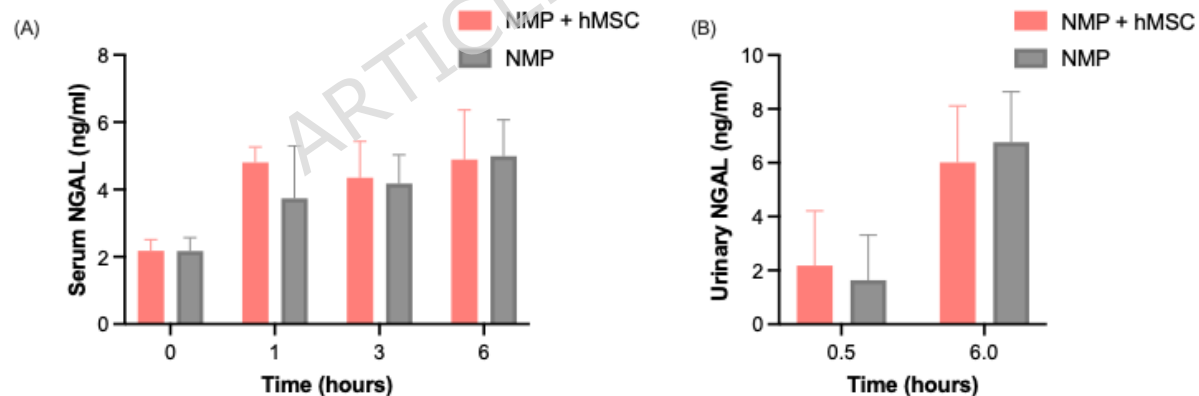


Figure 6. Assessment of kidney injuries during 6 hours of NMP with administration of placebo or 10 million hMSCs. (A) Concentration of NGAL in serum (ng/ml) ;(B) Concentration of NGAL in urine (ng/ml). Data presented as mean $\pm$ SD.

	NMP group		NMP + hMSCs group		p-value
	Mean	SD	Mean	SD	
Pig weight (kg)	40.50	9.77	40.50	9.77	-
Pig creatinine, baseline (mg/dl)	1.04	0.05	1.04	0,05	-
Kidney side (R/L)	1/5	-	5/1	-	-
Kidney weight, baseline (g)	138.17	32.46	134.83	25.52	0,56
Kidney weight, end perfusion (g)	176.50	45.92	184.33	55.74	0,31
CIT (min)	183.67	7,61	183.67	761	-
WIT (min)	30.00	-	30.00	-	-

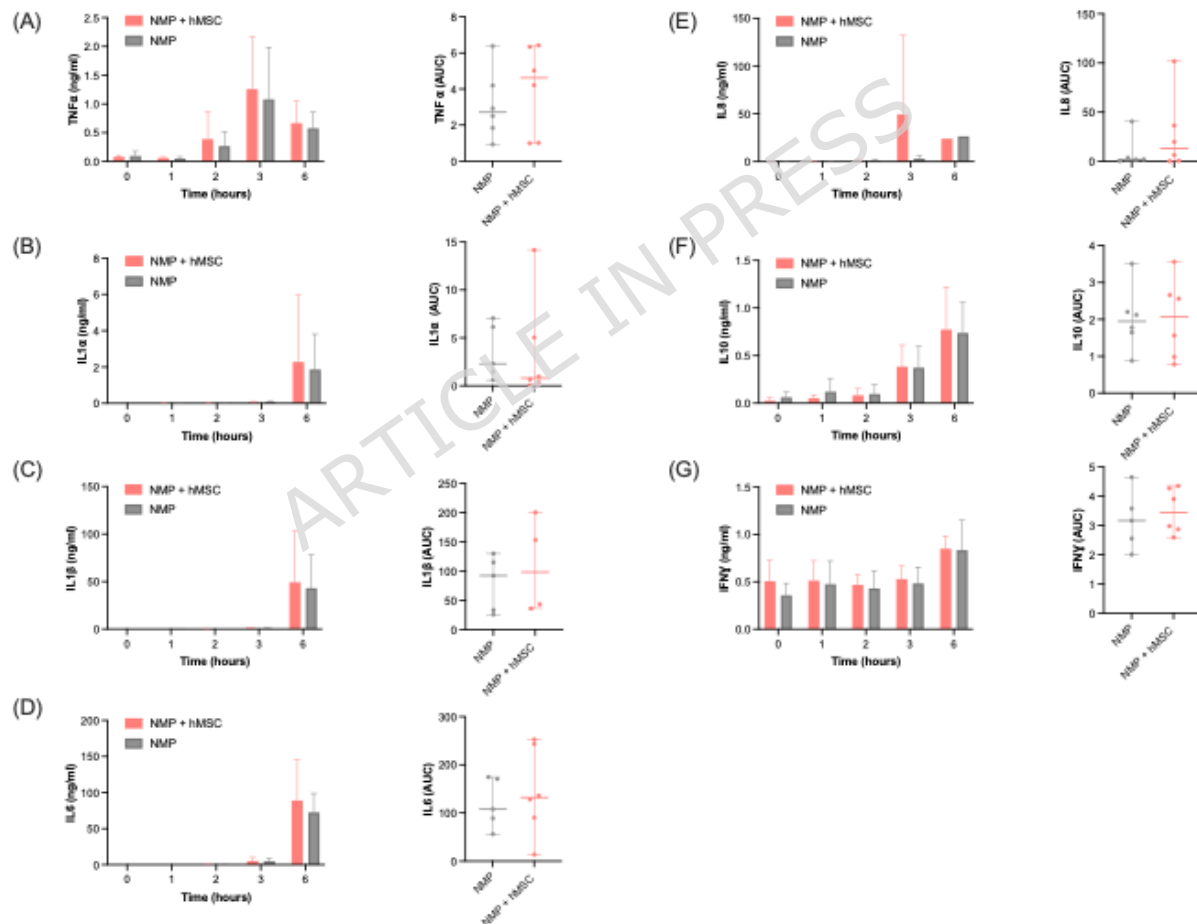


Figure 7. Concentration of cytokines in NMP perfusates: IFN γ , IL-10, IL-1 α , IL-6, TNF α , IL-1 β and IL-8. Left panels: kinetics and right panels: AUC analyses. Data presented as mean $\pm$ SD.

Table 1. Statistics of characteristics of paired kidneys included in the study (mean, SD and p-value)

Note : CIT, cold ischemia time ; WIT, warm ischemia time

Supplementary Data

Table S1. Characteristics of paired kidneys included in the study.

Note : CIT, cold ischemia time ; WIT, warm ischemia time

	NMP #1	NMP #2	NMP #3	NMP #4	NMP #5	NMP #6	NMP/hMSCs #1	NMP/hMSCs #2	NMP/hMSCs #3	NMP/hMSCs #4	NMP/hMSCs #5	NMP/hMSCs #6
Pig Weight (kg)	31	33	37	40	58	44	31	33	37	40	58	44
Pig creatinine, baseline (mg/dl)	0.99	1.06	1.12	1.04	1.02	0.99	0.99	1.06	1.12	1.04	1.02	0.99
Kidney side	Left	Left	Right	Left	Left	Left	Right	Right	Left	Right	Right	Right
Kidney weight, baseline (g)	102	137	107	170	182	131	116	134	100	150	173	136
Kidney Weight, end perfusion (g)	111	170	151	206	245	176	116	177	136	238	259	180
CIT (min)	170	190	180	189	185	188	170	190	180	189	185	188
WIT (min)	30	30	30	30	30	30	30	30	30	30	30	30

Table S2. Comparison of biochemical and hemodynamic parameters between the NMP group and the NMP + hMSCs Group over the entire 6-hour NMP period (mean, SD and p-value).

	NMP group		NMP + hMSCs group		p-value
	Mean	SD	Mean	SD	
pH	7.37	0.19	7.37	0.17	0.96
pCO₂ (mmHg)	42.84	11.61	43.30	11.59	0.79
pO₂ (mmHg)	106.63	39.93	105.36	40.80	0.81
Na⁺ (mmol/l)	144.14	8.44	144.89	7.96	0.37
K⁺ (mmol/l)	5.86	1.39	5.54	1.18	0.18
Hct (%)	17.40	2.92	18.29	2.73	0.018
Hb (g/dl)	5.92	0.98	6.21	0.91	0.017
Glucose (mg/dl)	129.22	33.88	121.13	27.70	0.14
Lactate (mmol/l)	13.77	4.77	15.48	3.94	0.17
Creatinine (mg/dl)	3.65	1.17	3.69	0.99	0.34
Iohexol (mg/dl)	28.71	11.30	28.09	10.21	0.39
Art. Press. (mmHg)	46.43	16.51	53.65	27.58	0.035
Flow (ml/min)	41.98	26.00	40.12	27.80	0.38
IRR mmHg/ml/min)	1.96	2.24	3.28	4.32	0.20
Temperature (°C)	34.60	3.38	33.87	3.48	0.26
Cum. urine vol. (ml)	21.70	24.49	20.49	15.68	0.033