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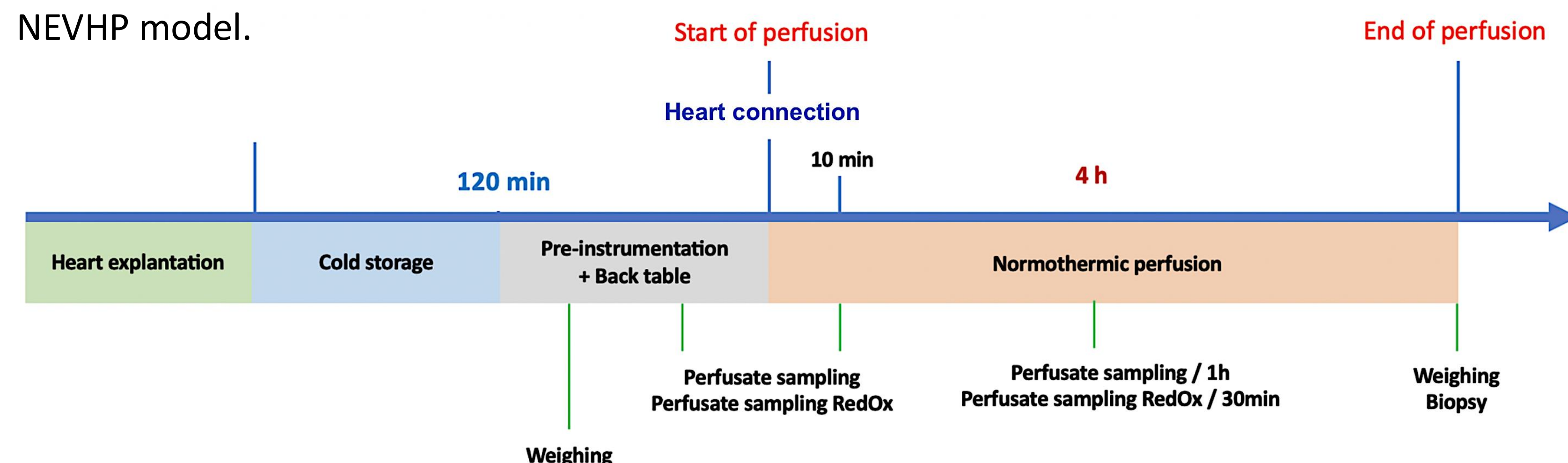
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## Introduction

Heart transplantation is limited by donor shortage, short static cold storage time, and primary graft dysfunction (PGD). Normothermic ex-vivo heart perfusion (NEVHP) was developed to reduce ischemia-reperfusion injury, prolong preservation, and expand donor criteria<sup>1</sup>. However, current assessment relies mainly on lactate kinetics<sup>2</sup>, without evaluation of additional biomarkers. Given the artificial surfaces and non-physiological flow of NEVHP, along with the increasing use of marginal and DCD donors<sup>3</sup>, assessing endothelial and coagulo-inflammatory responses—key PGD factors<sup>4</sup>—is highly relevant.

We conducted a monocentric pilot study on five brain-dead donor hearts at Rennes University Hospital<sup>5</sup>, revealing strong inflammation and some endothelial activation.

To explore these findings, we developed a translational porcine NEVHP model.



## Material and Methods

This study was conducted at INSERM U1241 and INRAE UE3P (Rennes, France) in compliance with national ethical guidelines<sup>6</sup>. To reduce animal use, it was combined with a uterine transplantation model simulating multi-organ procurement.

Post-pubertal Large White sows underwent beating-heart procurement. After heart explantation and collection of 1200 mL of donor blood, grafts were stored in cold ischemia for 2 hours to simulate average transport time in a Brittany regional cardiac hub, then perfused with a NEVHP for 4 hours.

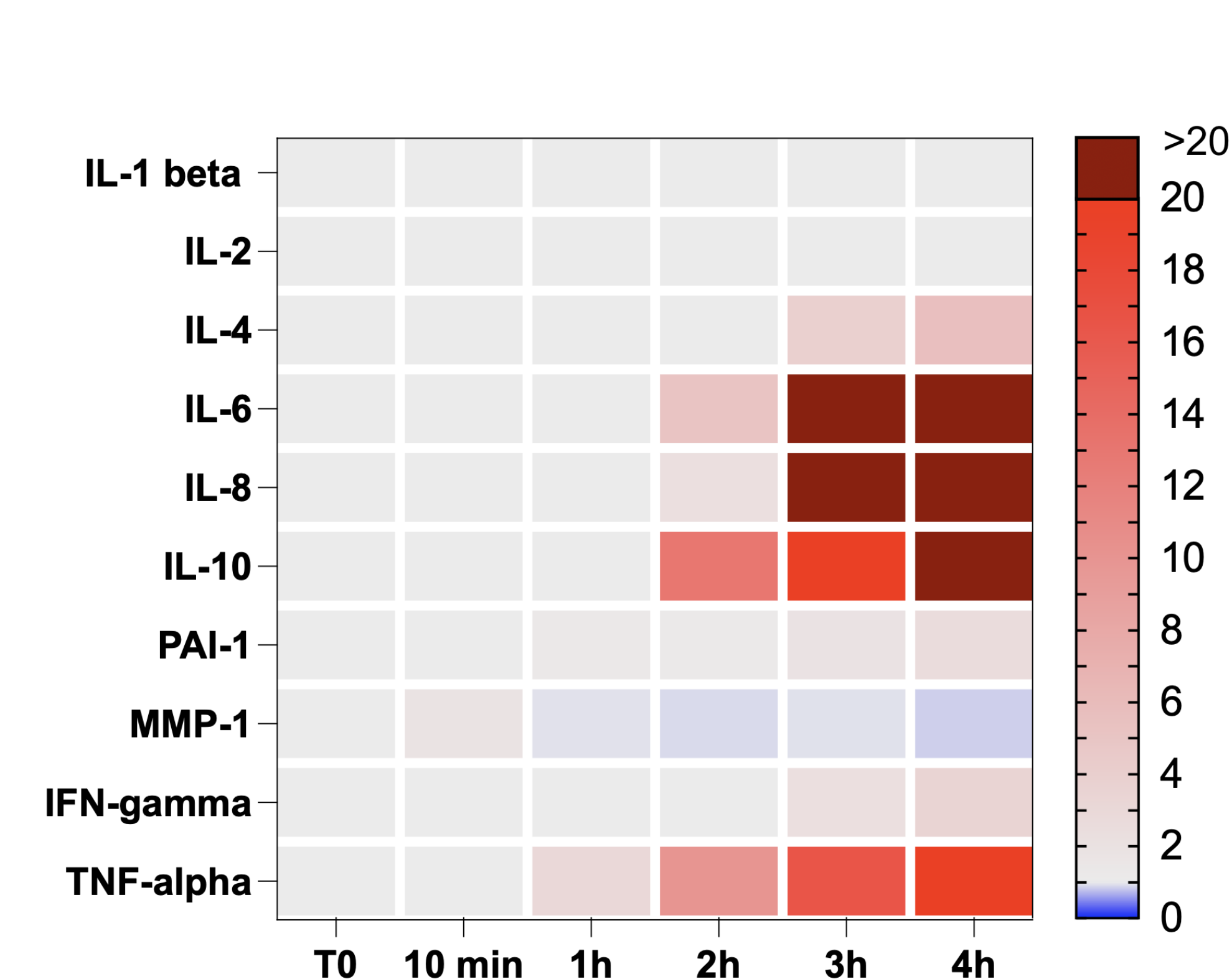
The circuit was primed with donor blood, unfractionated heparin, and a nutrient solution. Perfusion followed a standardized protocol based on clinical and OCS™ guidelines, with coronary flow maintained at 650–850 mL.min<sup>-1</sup> and lactate

levels monitored throughout. Perfusate samples were collected at T<sub>0</sub> (donor blood only), T<sub>10</sub> (10 minutes post-connection of the heart), and hourly for 4 hours.

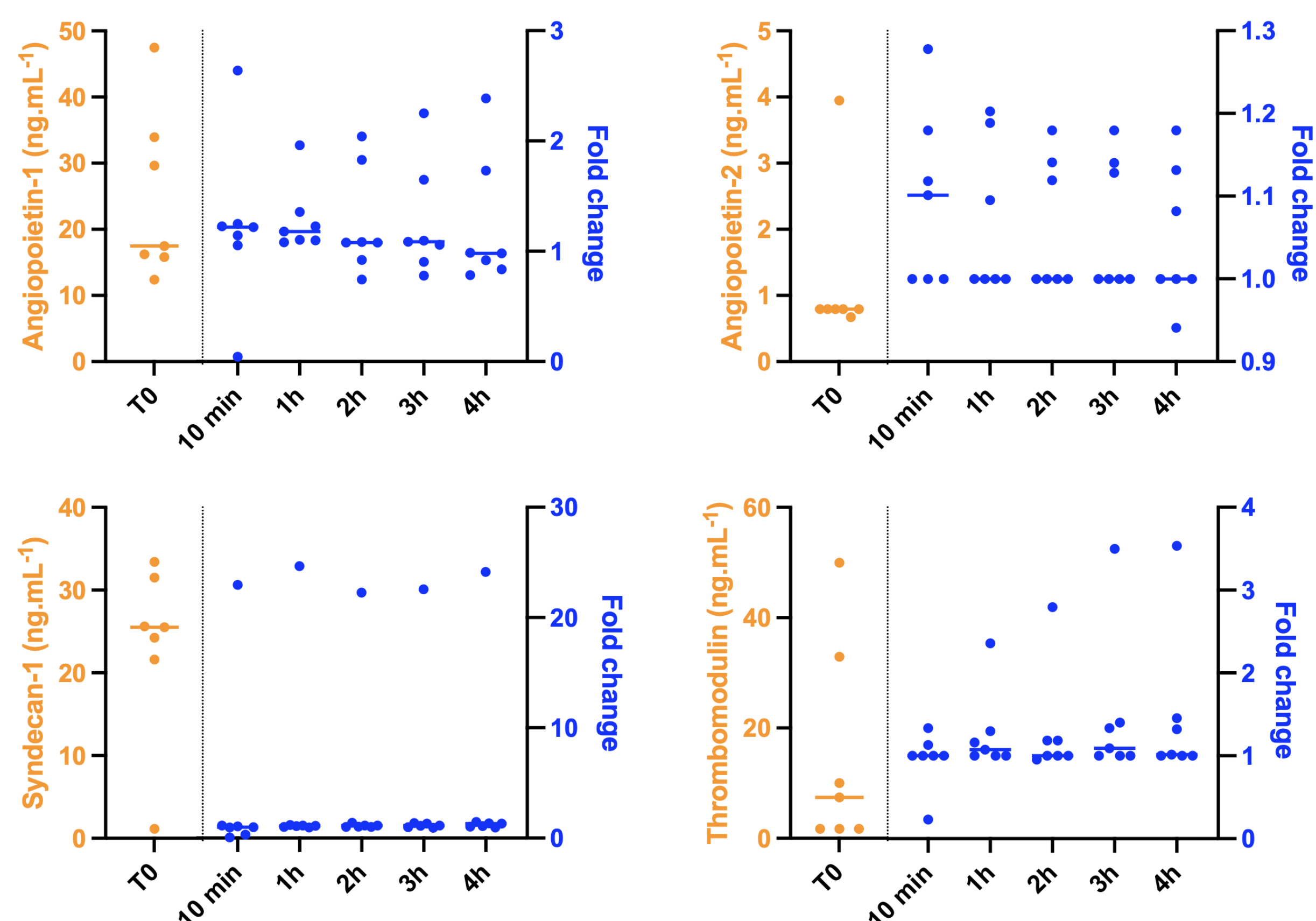
Biomarkers included inflammatory cytokines measured using Luminex® (R&D, Bio-Techne) and endothelial markers quantified by ELISA.

## Results

We performed seven procedures and successful myocardial resuscitation was confirmed by a mean coronary flow of 710 ± 180.3 mL/min, along with a significant decline in arterial lactate concentration from T<sub>10</sub> (4.45 [3.27–5.11] mmol/L) to T<sub>4h</sub> (2.12 [1.59–3.35] mmol/L). Changes in inflammatory markers are shown in Figure 1 and endothelial marker dynamics are presented in Figure 2.



**Figure 1. Evolution of inflammatory markers during normothermic ex-vivo heart perfusion.** Results are expressed as fold changes relative to T<sub>0</sub> (fold change = concentration at Tx / concentration at T<sub>0</sub>).



**Figure 2. Evolution of endothelial markers during normothermic ex-vivo heart perfusion.** Results at T<sub>0</sub> are expressed as raw concentrations. Results at subsequent time points are expressed as fold changes relative to T<sub>0</sub> (fold change = concentration at Tx / concentration at T<sub>0</sub>).

## Conclusion

This study is the first study to evaluate inflammatory and endothelial responses during ex-vivo normothermic heart perfusion (NEVHP) in a porcine model. Sequential biomarker analysis revealed both pre-perfusion injury and perfusion-related changes. Despite limitations such as small sample size and hemodilution, the study demonstrates the feasibility of dynamic biomarker monitoring and provides preliminary insights to guide future strategies for graft preservation and function optimization.



**Strong inflammatory response**



**No modulation of endothelial markers**