

Establishment Of Ex Vivo Machine Perfusion Protocols For Vascularized Composite Allografts Across Multiple Preclinical Models

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Introduction

- Vascularized composite allotransplantation (VCA) is limited by ischemia–reperfusion injury (IRI) and the short preservation time of static cold storage (SCS)
- These limitations reduce graft viability, transport flexibility, clinical applicability and contribute to increase graft immunogenicity.
- Machine perfusion offers a promising approach to extend preservation and enable graft reconditioning
- Subnormothermic machine perfusion (SNMP) may better preserve tissue metabolism, vascular integrity, and immunological function
- Objective: develop and evaluate clinically relevant ex vivo SNMP protocols in large-animal VCA models

Methods

- SNMP was applied across 4 large-animal VCA models: the non-human primate (NHP) forearm model, the NHP partial face model, the swine uterus model, the swine whole eye transplant (WET) model (**Figure 1**)

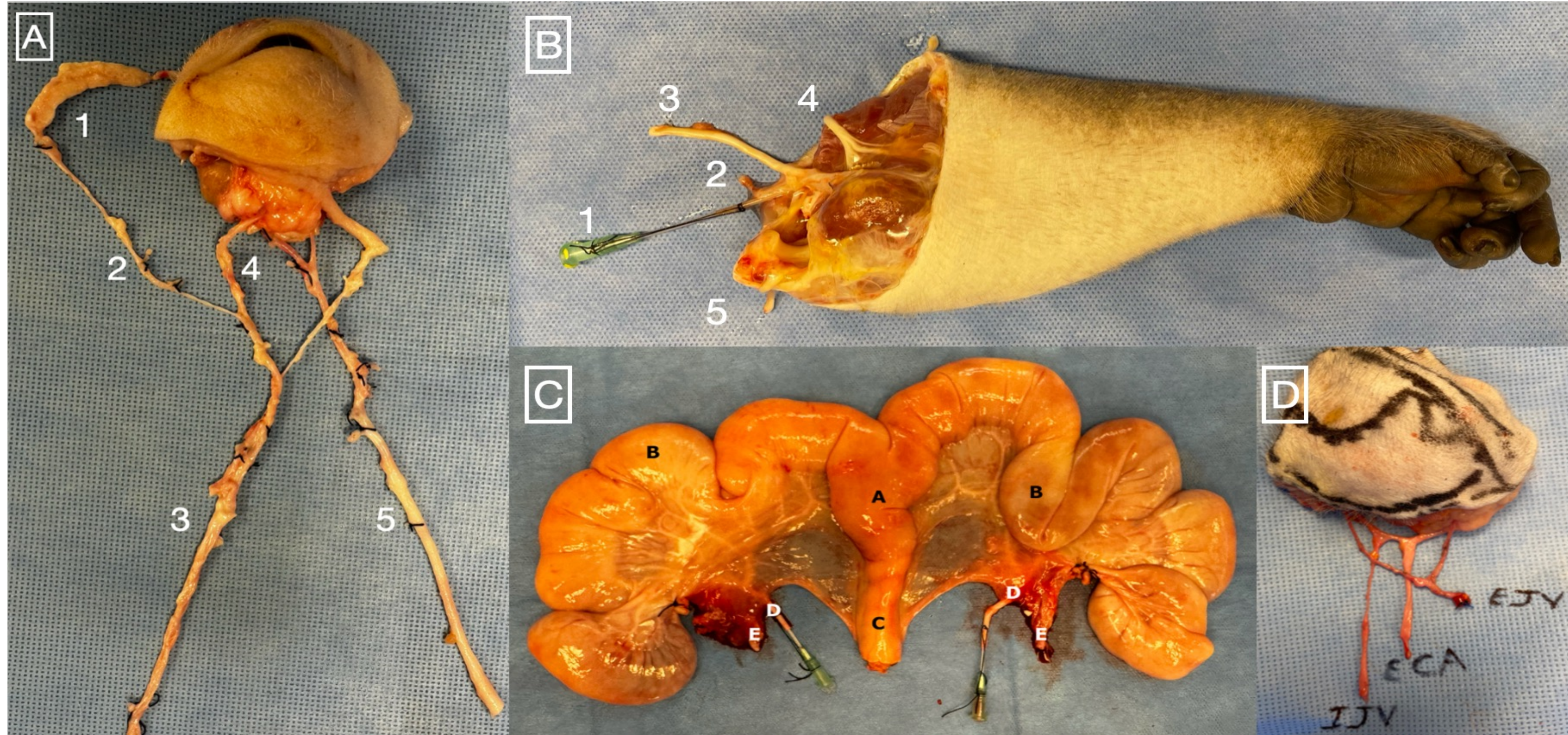


Figure 1: WET swine (A) with the frontal (1), facial (2) the external jugular (3) veins, the communicating branch (4) between the ophthalmic and the facial veins, the ophthalmic and external carotid arteries (5). NHP Forearm (B) with brachial artery (1) and veins (2), median, radial and ulnar nerves (3-5). Swine Uterus (C) with main body (a) and horns (b), cervix (c) and uterine arteries (d) and veins (e). NHP partial face VCA model (D) with external and internal jugular veins (IJV and EJV) and external carotid artery (ECA).

- Perfusion using albumin-enriched Steen+ solution under stable low-flow and room temperature (20°C) conditions for up to 24 hours (SNMP protocol optimized for VCA) using a custom-made perfusion system (**Figure 2**)

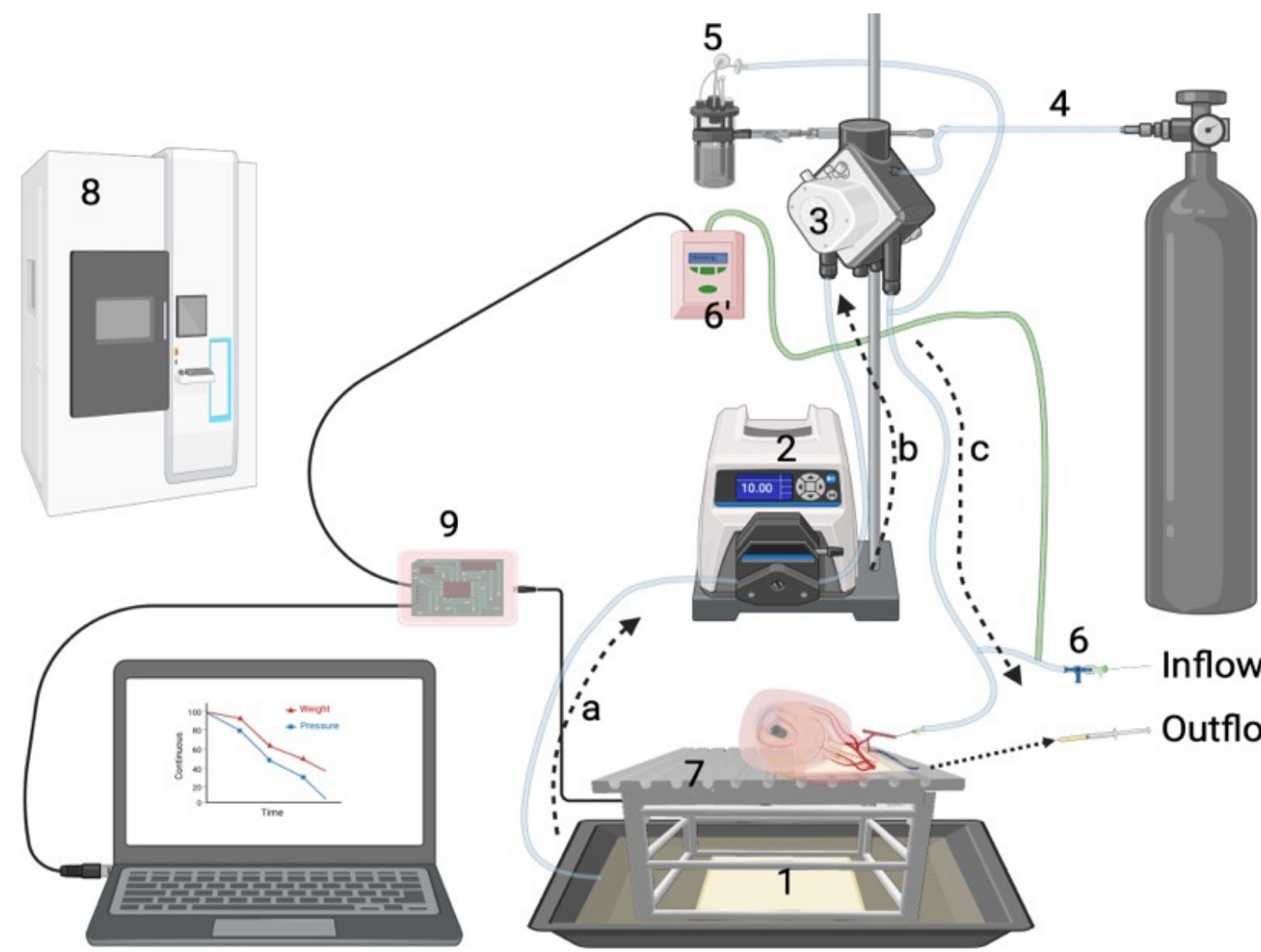


Figure 2. SNMP setup diagram showing the (1) perfusion solution reservoir, (2) roller pump, (3) oxygenator connected to a (4) carbogen tank, (5) bubble trap, (6, 6') sensors, (8) 3D-printed scale and perfusate analyzer, (9) microcontroller and laptop. The perfusate flow is indicated as (a) from the reservoir to the pump, (b) from the pump to the oxygenator, and (c) from the oxygenator to the graft.

Results

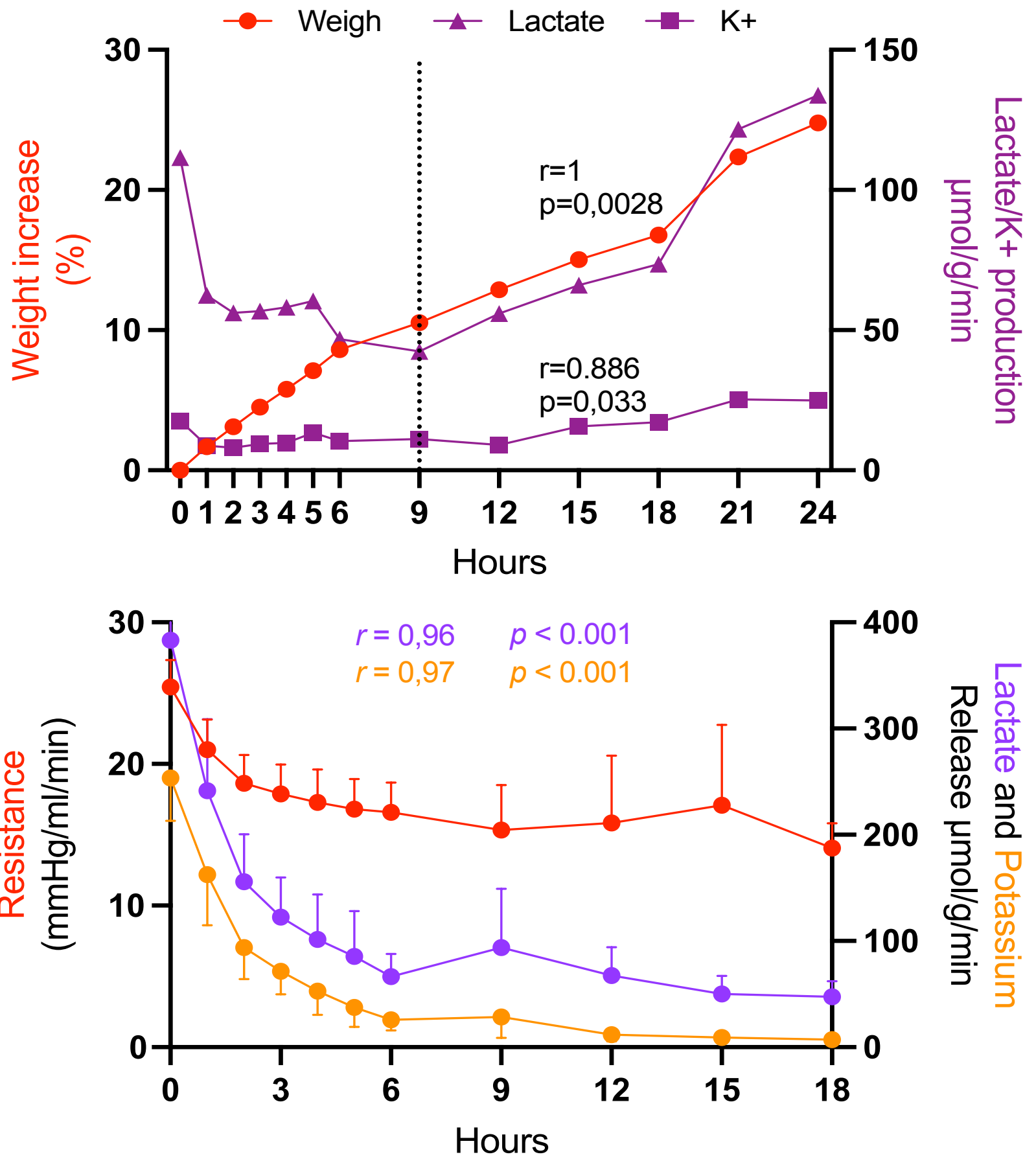


Figure 3. Weight gain correlation with potassium and lactate release revealing the onset of a compartment syndrome past a 10% weight gain (non optimized SNMP protocol) (upper). Main metabolic parameters during a WET perfusion (lower) showing a recovery phase (first 6 hours) and a preservation phase (remaining 12 hours) with stable metabolic state (optimized SNMP protocol).

- Continuous monitoring of hemodynamics: flow, pressure, vascular resistance, weight.
- Assessment of metabolic markers: O₂/glucose consumption, lactate, potassium, LDH
- Evaluation of outcomes: histology, tissue integrity, and functional recovery (muscle/organ-specific responses)
- Inflammation and immune compartment evaluation (cytokines, RNAseq, FACS)

- Metabolism and edema remain stable (**Figure 3**):
 - Two main phases: recovery (after a mean of 3h of WIT) followed by a preservation phase until EOS.
 - stable parameters across all 4 models when applying SNMP protocol developed for VCAs (high oncotic pressure and stable low flow perfusion regimen)
 - NHP forearm: highly sensitive to edema with compartment syndrome onset past 10% weight gain (clinical relevance).
- Muscular contractility can recover (tested on 3 different types of muscles):
 - mean muscle contraction of 2.4/5 ± 0.89 in the NHP forearms.
 - mydriasis and myometer contractility also recovered under SNMP, respectively after administration of atropine in the WET and oxytocin in the uterus model (**Figure 4**).
- Limited histology damage after perfusion (**Figure 5**)

Results

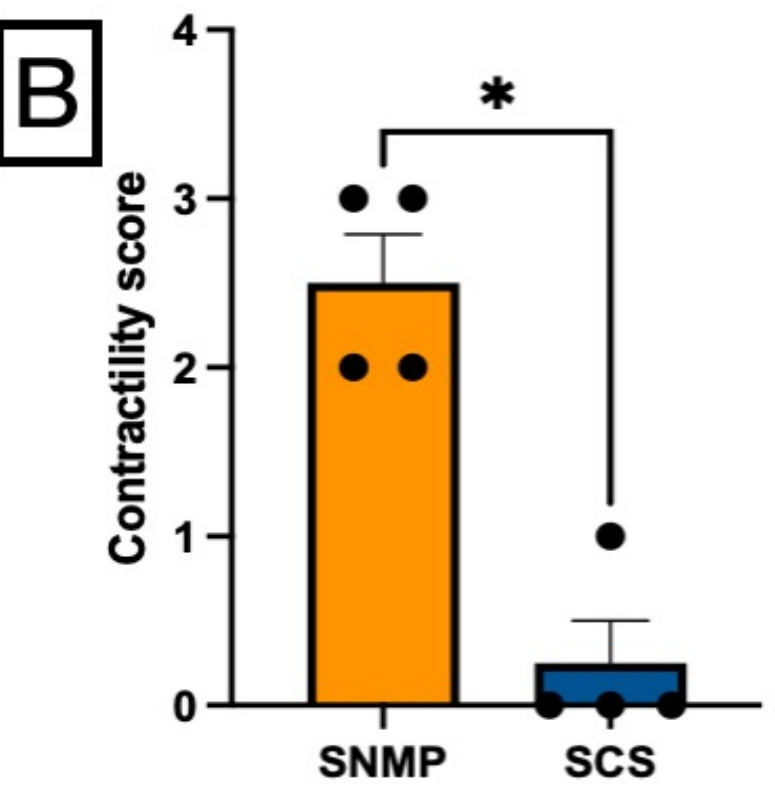


Figure 4. Mydriasis recovery following 18 hours of SNMP in the swine WET (atropine induced) (A); Myometer contraction recovery following oxytocin administration in the SNMP group. No control (either SCS of 3 hours WIT) showed signs of contraction upon stimulation.

- In the NHP partial face model:
 - the graft immune compartment, mostly contained in the skin is preserved, with leucocyte viability (FACS) and cytokine production preserved
 - proinflammatory profile (skin RNA-seq and outflow cytokine assessment) showing room for improvement although no structural damage is seen in histological analysis and metabolic and dynamic profiles are stable.

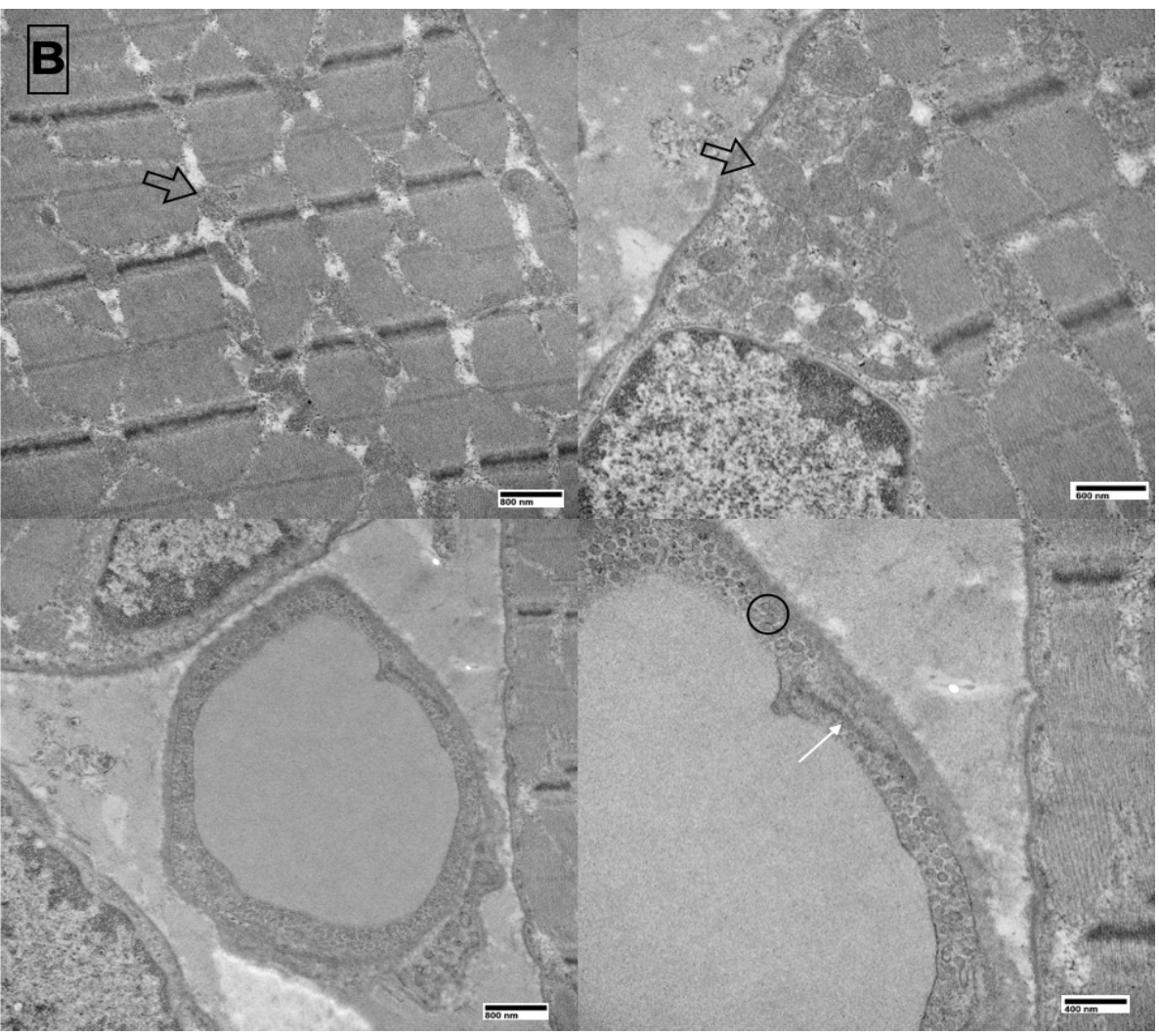
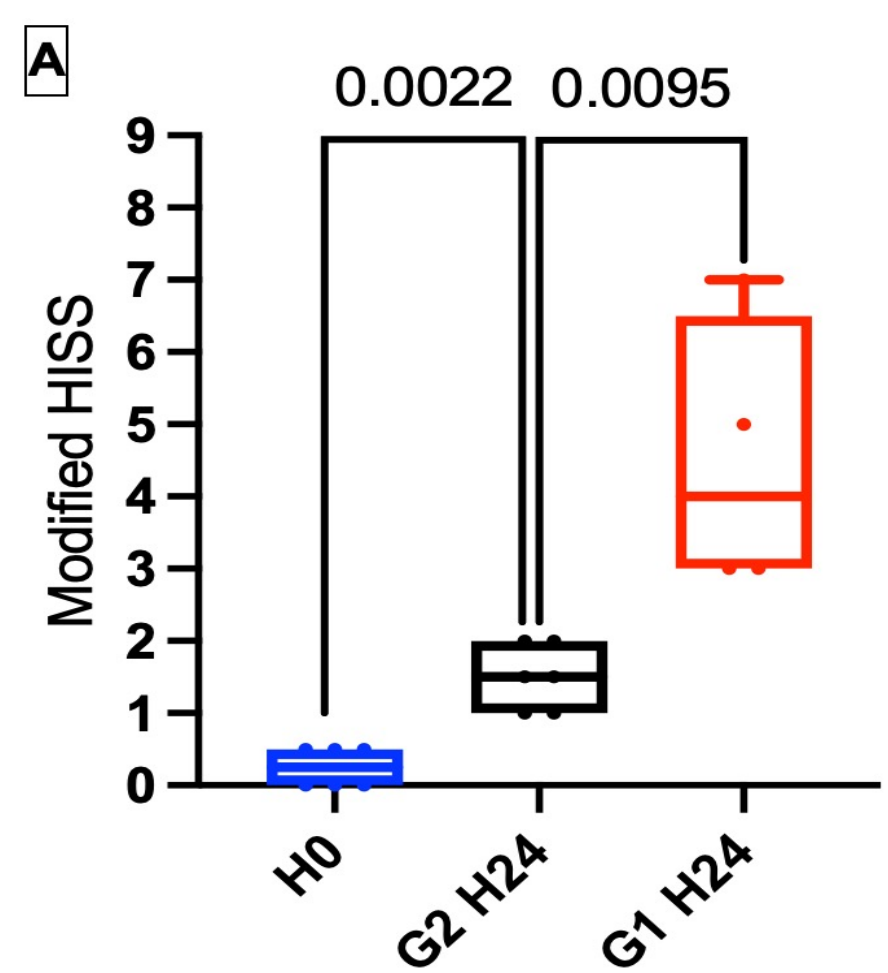


Figure 5. Muscle injury scores (A) and transmission electron microscopy (muscle and capillaries) (B) show low histological damage after 24 hours of optimized SNMP in the NHP forearm model. The same was demonstrated in the other models, including WET posterior segment.

Conclusions

- Each model has unique characteristics and relevance
- SNMP allows for preservation and reconditioning of the grafts and opens new possibilities for VCA preconditioning and diagnostic before replantation.

Main References

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Acknowledgments

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