



Successful Extension of Vascularized Composite Allograft Perfusion Cold Storage to 24 Hours in a Rat Hind Limb Transplant Model



Joy Huang, BA^{1*}, Po'okela Ng, BS^{1*}, Dor Yoeli, MD^{1*}, Yuhuan Luo, MD¹, Yong Wang, MD¹, Bing Li, MD¹, Li Lu, MD¹, Zhaohui Wang, DVM¹, Paula Arrowsmith, BS, HT(ASCP)², Swati Jain, PhD¹, An-Jey A. Su, PhD¹, David M. Mathes, MD¹, Kia M. Washington, MD¹, Evan Farkash, MD, PhD², Alkesh Jani, MD^{1**}, Christene A. Huang, PhD^{1**}

¹University of Colorado Anschutz Medical Campus, 13001 E 17th Pl, Aurora, CO, 80045
²University of Michigan School of Medicine, 1301 Catherine St, Ann Arbor, MI 48109



Department of Surgery
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

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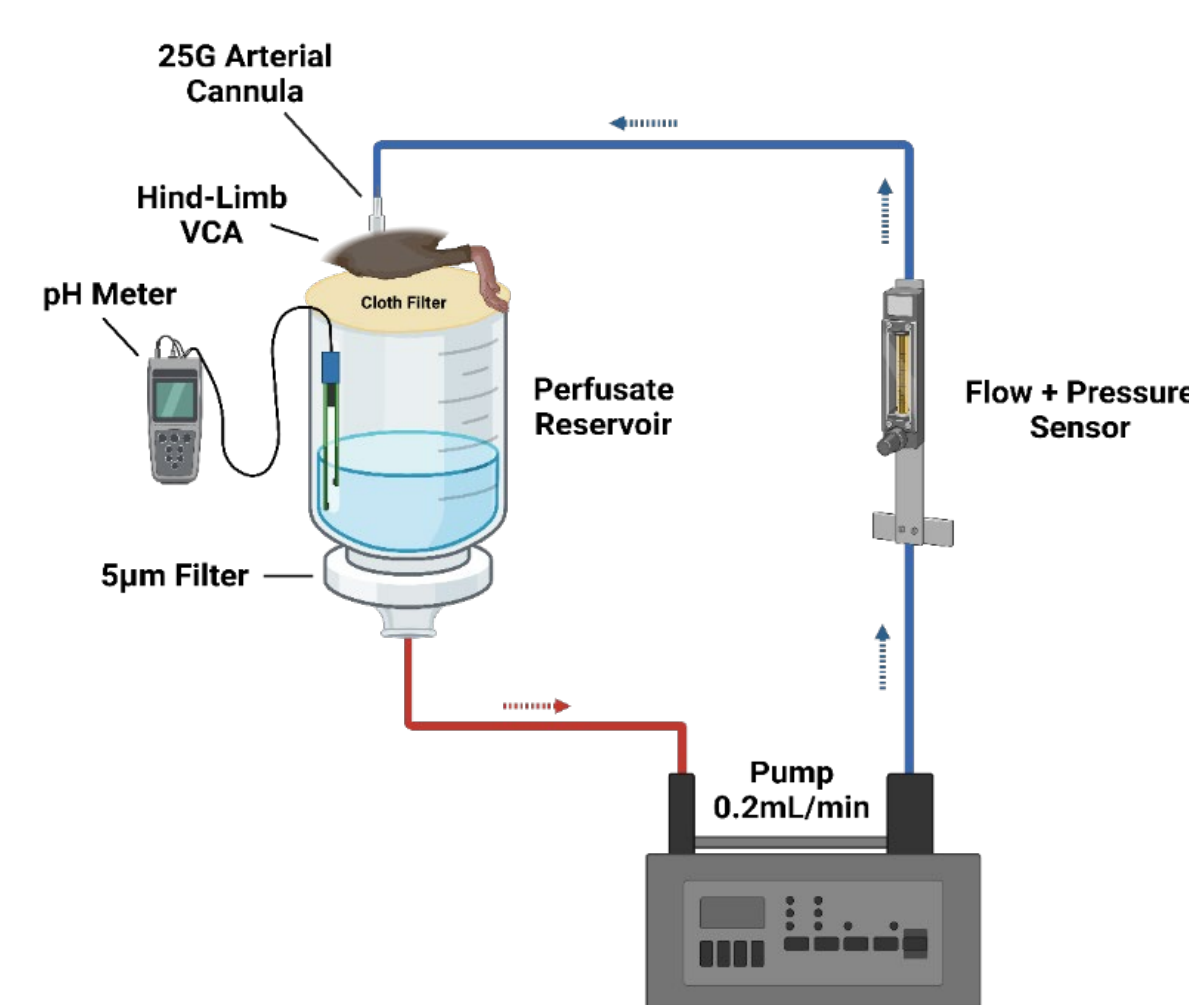
Background

- Vascularized Allograft (VCA) transplantation is a treatment option for complex tissue injuries.
- Strategies to extend VCA preservation times are required to improve VCA transplant outcomes and expand the geographical donor pool.
- Hypothermic machine perfusion (HMP) using acellular storage perfusate is a potential solution.

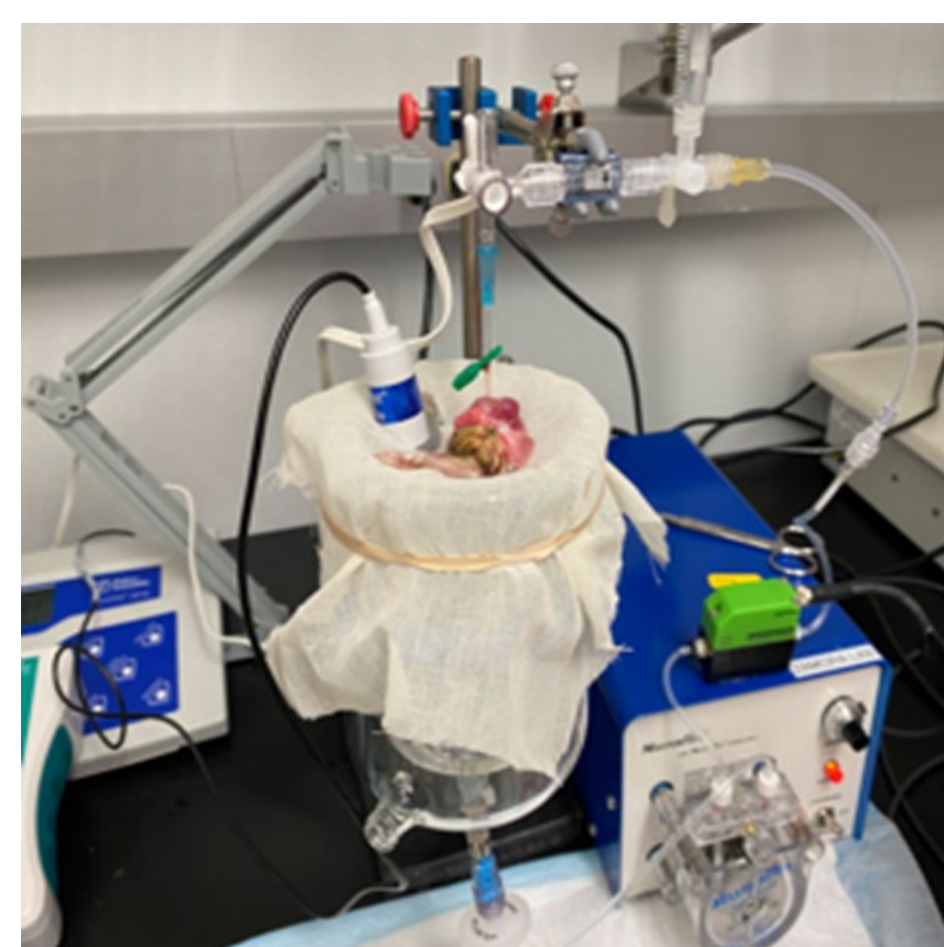
Objective

- Evaluate the use of University of Wisconsin (UW) Kidney Preservation Solution (KPS-1) to preserve donor rat hind limbs subjected to 24h of *ex-vivo* perfusion cold storage.
- Assess edema and muscle cell death following 24-hours of *ex vivo* limb perfusion using KPS-1 compared with normal saline (NS).

Methods



- Brown Norway hind limbs were subjected to 24-hour perfusion cold storage with heparinized KPS-1 (n=6) or heparinized NS (n=6).
- Limbs were weighed before and after perfusion cold storage to approximate the extent of edema.
- Muscle was collected for histological analysis of edema and apoptosis using H&E, TUNEL, and Cleaved Caspase-3 (CC3) staining.



Results

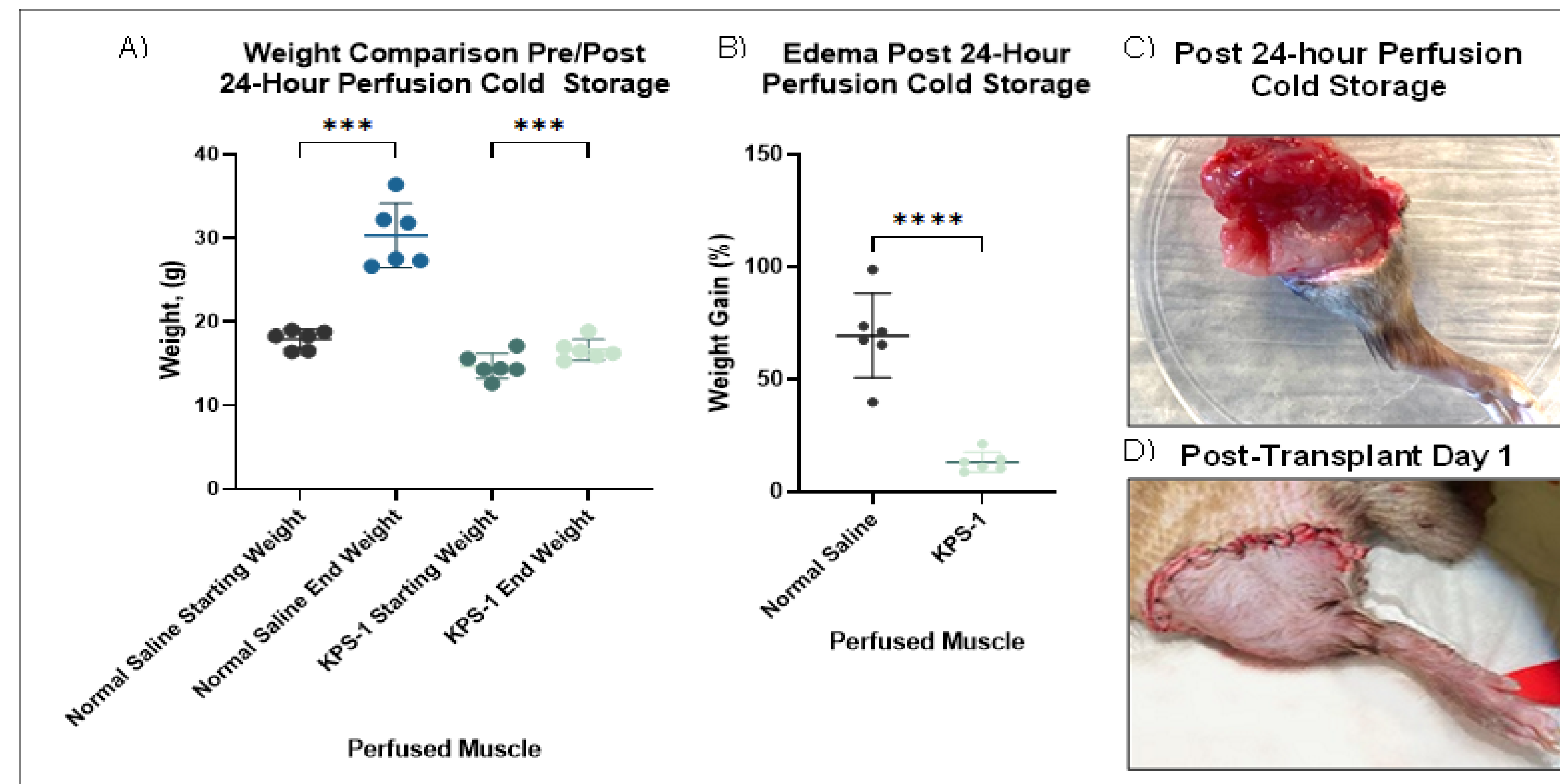


Figure 1: (A) Comparison of weight (in grams) before and after 24-hour perfusion with NS/KPS. (B) Quantification of weight gained displayed as a percentage of weight gained relative to starting weight of hind limbs (n=12). (C-D) Representative images of Brown Norway hind limbs that were subjected to 24 hours perfusion with KPS solution *ex vivo* (top), and *in vivo* (bottom) following successful orthotopic transplantation. Significant differences, *p < 0.05, **p < 0.01, *** p < 0.0001.

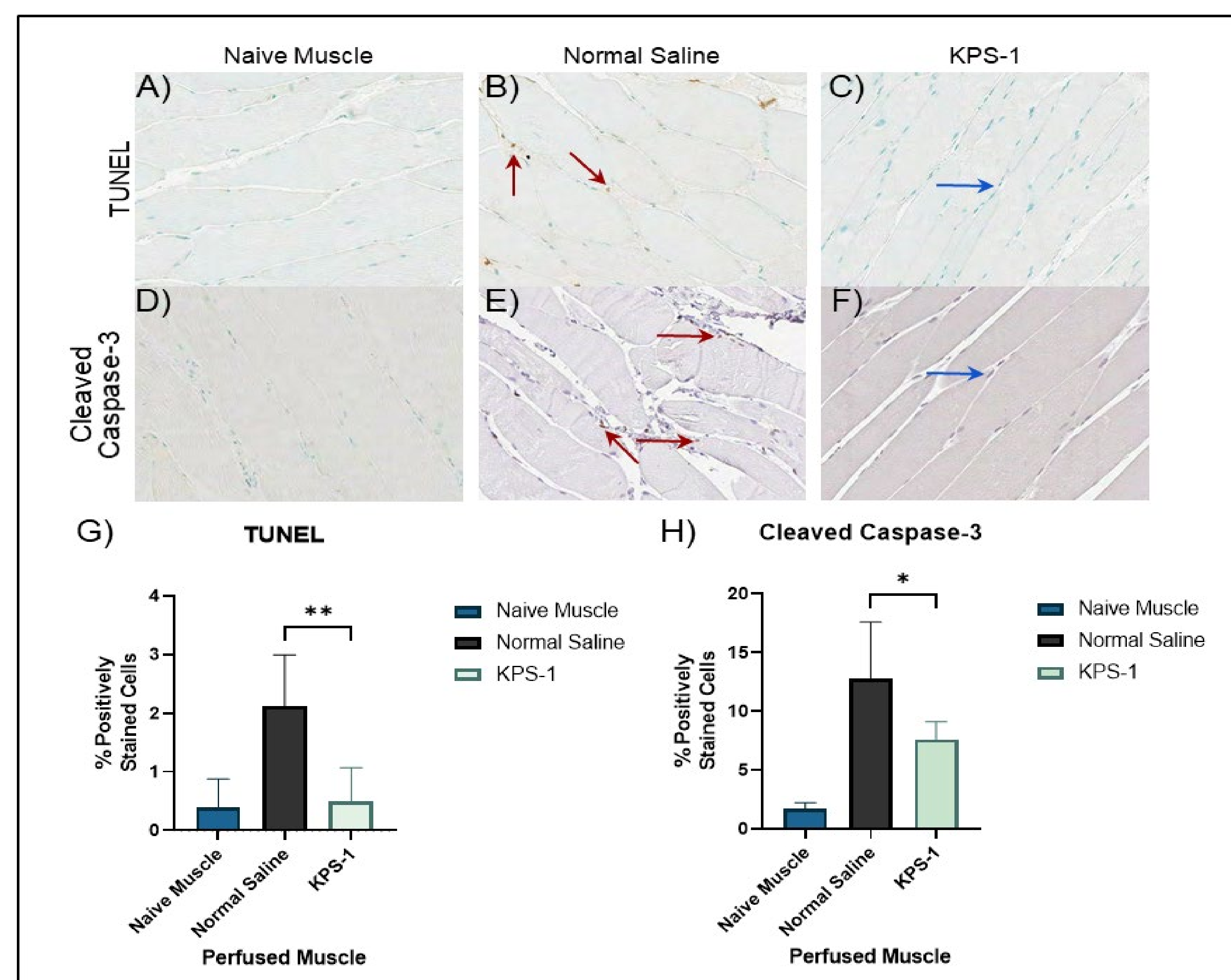


Figure 2: Representative 10x TUNEL, and 10x Cleaved Caspase-3 staining images of rat hind limb muscle, positive/negative staining indicated by red/blue arrows, respectively. TUNEL staining of hind limbs (A) naive muscle, (B) muscle perfused with NS, and (C) muscle perfused with KPS solution. Cleaved caspase-3 staining of hind limbs (D) naive muscle, (E) muscle perfused with normal saline, and (F) muscle perfused with KPS solution. Quantification of TUNEL (G), and Caspase staining (H). Significant differences, *p < 0.05, **p < 0.01, *** p < 0.0001.

Summary

- KPS-1-perfused rat hind limbs did not show significant muscle edema based on histological assessment of muscle interfascicular space.
- TUNEL staining showed that muscles perfused with KPS-1 had significantly less apoptosis than those perfused with NS.
- CC3 staining was also significantly decreased in KPS-1 perfused muscle tissue compared to NS perfused muscle consistent with decreased apoptosis.
- Orthotopic hind limb transplantation could successfully be performed with limbs subjected to 24 hours *ex vivo* perfusion cold storage using KPS-1, but not with NS-perfused limbs due to excessive edema.
- The use of heparinized NS as a perfusion solution proved to be damaging.

Conclusions

- 24-hour *ex-vivo* HMP with KPS-1 as a perfusion solution for VCAs is feasible in a rat hind limb model and may open doorways to future preservation strategies for VCAs.
- The use of KPS-1-perfused limbs can be successfully transplanted onto a recipient animal. However, future survival studies are needed.
- NS to prepare VCA and other allografts for transplantation should be reconsidered, especially if prolonged cold storage is required.

Acknowledgements

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- *JH, PK, and DY contributed equally
- ** AHJ and CAH contributed equally