In Vivo Safety of Enzyme-Converted O Whole Blood During Autologous Transfusion After Hemorrhage in Swine

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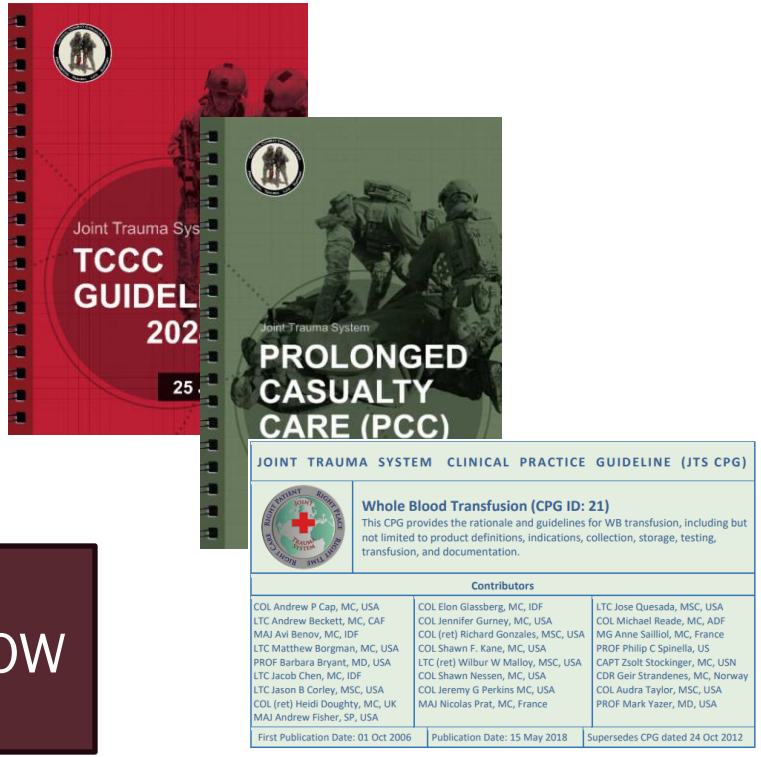
Conflicts of Interest: Craig Nowadly, Brian Casleton, Manuel Caballero, Hui Xia, and Jason Rall have no conflicts to report. Haisle Moon, Isis Ip, Spencer Macdonald, Karolina Jones, Peter Rahfeld, Jayachandran N. Kizhakkedathu, and Stephen G. Withers are employed by or have a financial interest in Avivo Biomedical, which holds the sole license on this technology.

Deployed Blood Operations

- Low-titer O whole blood (LTOWB) remains the primary blood product of Department of War (DOW) Combat Operations.
- Recent “Transfusion of Type A Whole Blood for the Role 3” expands availability of typed WB



Blood product shelf life and blood-type compatibility are primary constraints of DOW blood operations at scale.

UBC/Avivo Enzyme Removal Technology

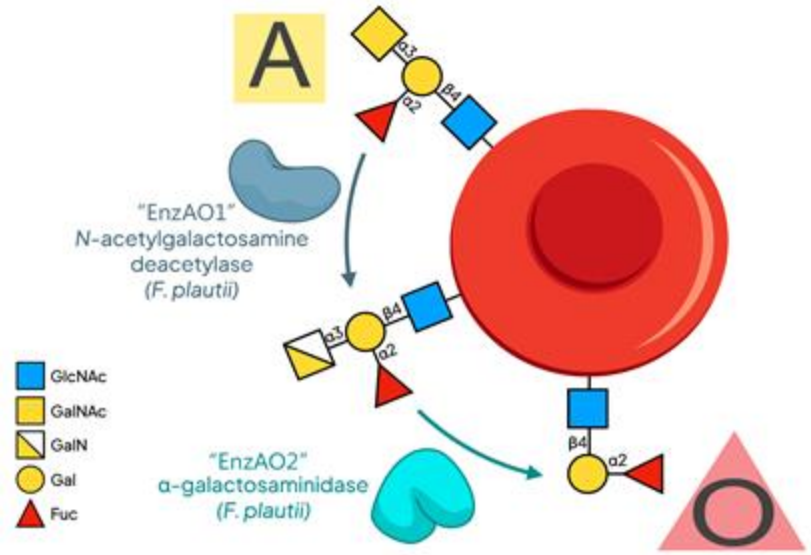


Figure 1: Avivo Enzyme Conversion Technology. Schematic representation of the enzymatic conversion of A antigen to H antigen on red blood cells. The two-step Avivo enzyme system, consisting of a deacetylase (EnzAO1) and a galactosaminidase (EnzAO2), removes the terminal α-N-acetylgalactosamine (α-GalNAc) residue from A antigens. This process converts A-type RBCs into universal donor O-type RBCs expressing the H antigen.

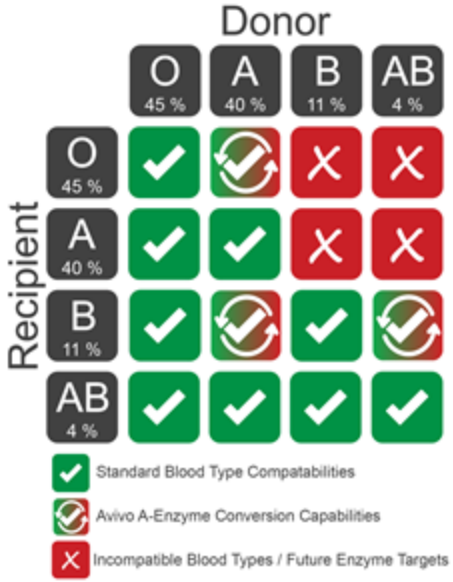


Figure 2: Compatible Blood Types. Figure shows transfusion compatibility between a donor (Top) and a recipient (Left) using the Avivo Biomedical Enzyme System.

Preliminary DOD Testing of Enzyme Technology

Preliminary 59th MDW data confirms rapid removal of all detectable A-antigen in less than 120 minutes from Type A swine whole blood. No evidence of clinically significant changes to enzyme-converted (ECO)-WB.

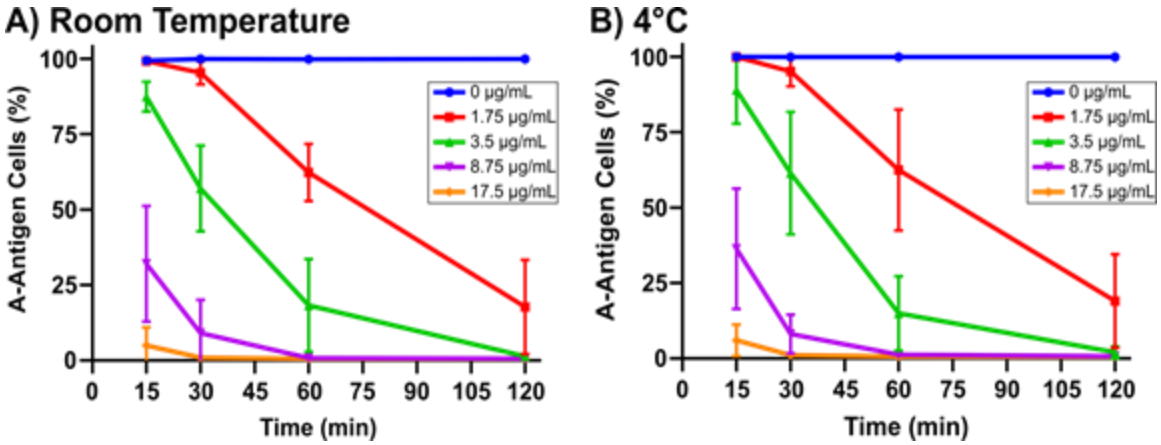


Figure 3. Flow Cytometry A Antigen Removal Curves. Curves display the proportion of red blood cells positive for A antigen at 15, 30, 60, and 120 minutes following enzymatic treatment at A) Room Temperature (22-23°C) and B) 4°C. Conditions include control (no enzyme) and four concentrations of EnzAO1 and EnzAO2: 1.75 µg/mL, 3.5 µg/mL, 8.77 µg/mL, and 17.5 µg/mL.

Nowadly et al. Generation of enzyme-converted type O whole blood from type A whole blood: A translational study using swine whole blood. *Transfusion*. 2026 Mar;66(3):568-578.

Current Research Objective

Do Type-A swine tolerate ECO-WB (and conversion enzyme) in the first 6 hours after hemorrhage and autologous transfusion?

Research Protocol

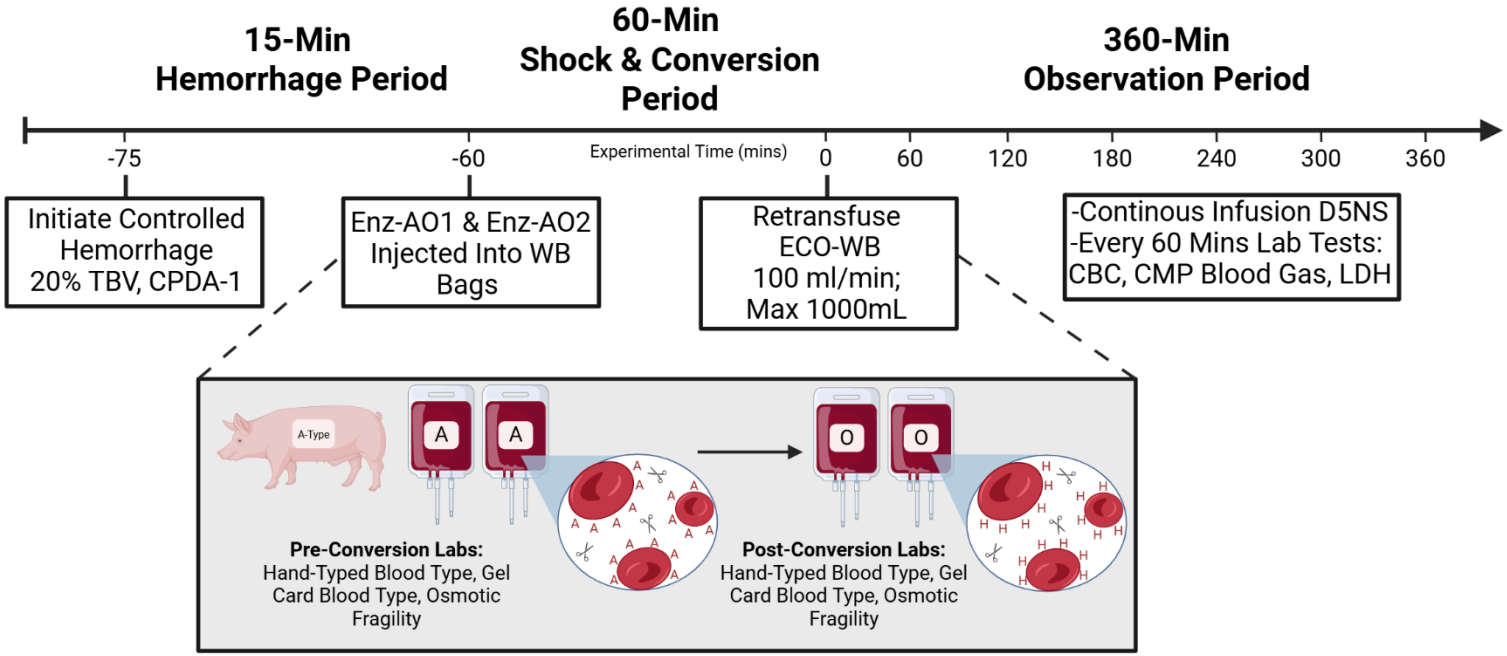


Figure 4. Research Protocol Overview. Created in https://BioRender.com

Model Development – Transient Pulmonary HTN

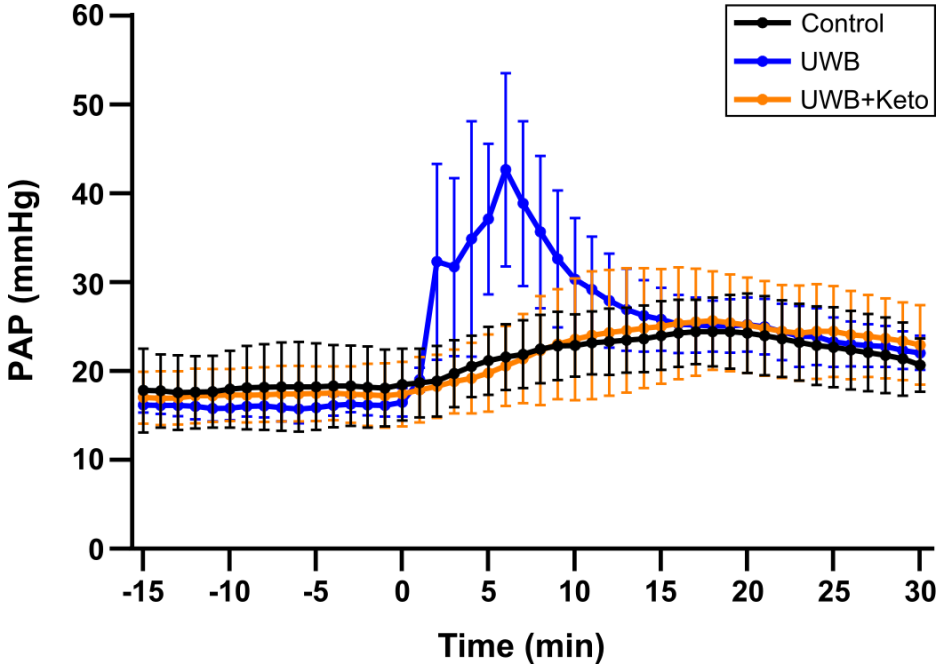


Figure 5. Experimental Pulmonary Arterial Pressure.

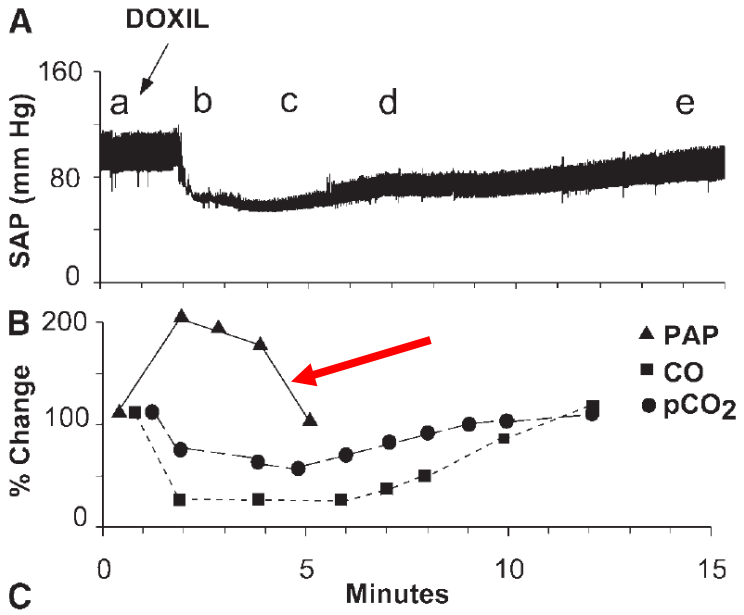


Figure 6. Complement activation-related cardiac anaphylaxis in pigs.
Figure modified from: Szebeni et al. *Am J Physiol Heart Circ Physiol.* 290: H1050 –H1058, 2006.

Research Protocol

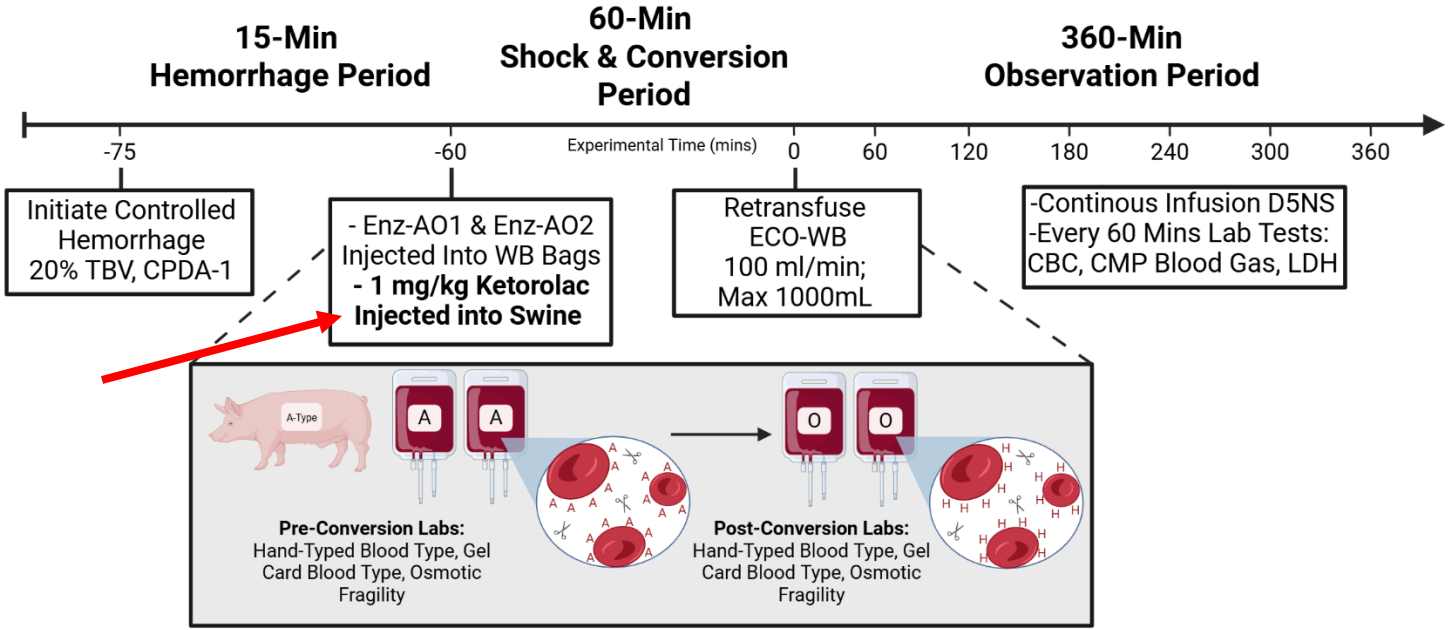
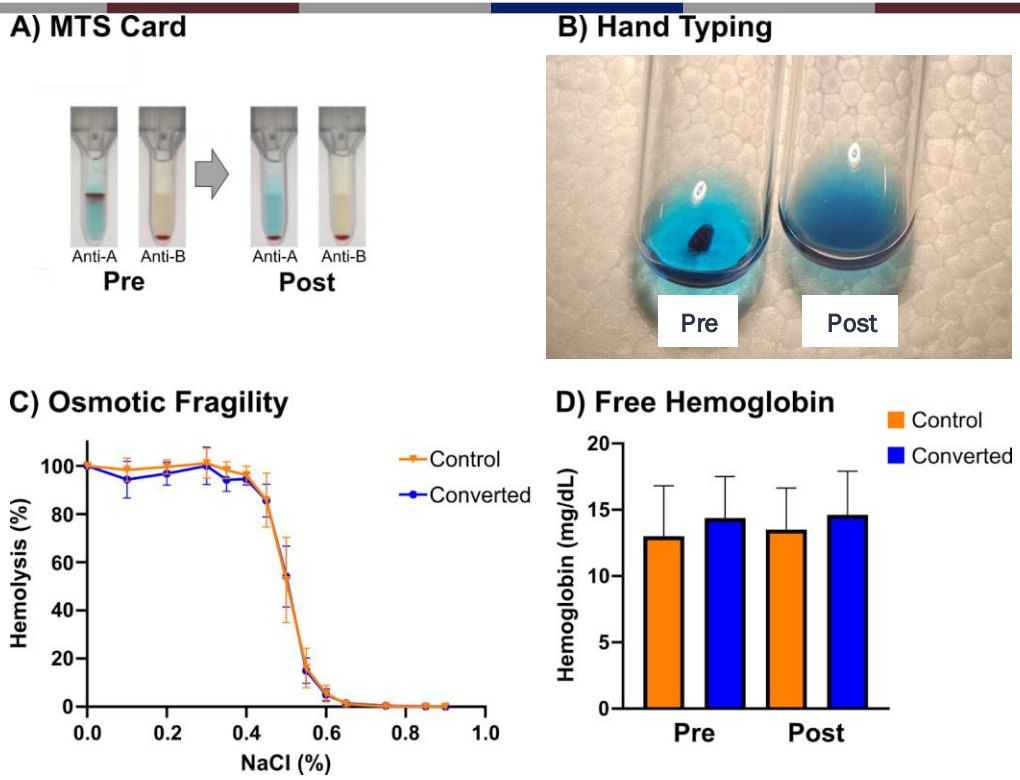


Figure 7. Modified Research Protocol Overview. Created in https://BioRender.com

Results - Conversion

Figure 8. Enzyme Converted Whole Blood (ECO-WB) Conversion Testing. A) MTS™ A/B/D Monoclonal and Reverse Grouping Card Typing. B) Hand Typing. C) Osmotic Fragility. D). Free Hemoglobin. All pre-conversion samples tested as A-Type pre-conversion and O-Type as post conversion. There were no differences in osmotic fragility or free hemoglobin between control (orange) or ECO-WB (blue).



Results - Hemodynamics

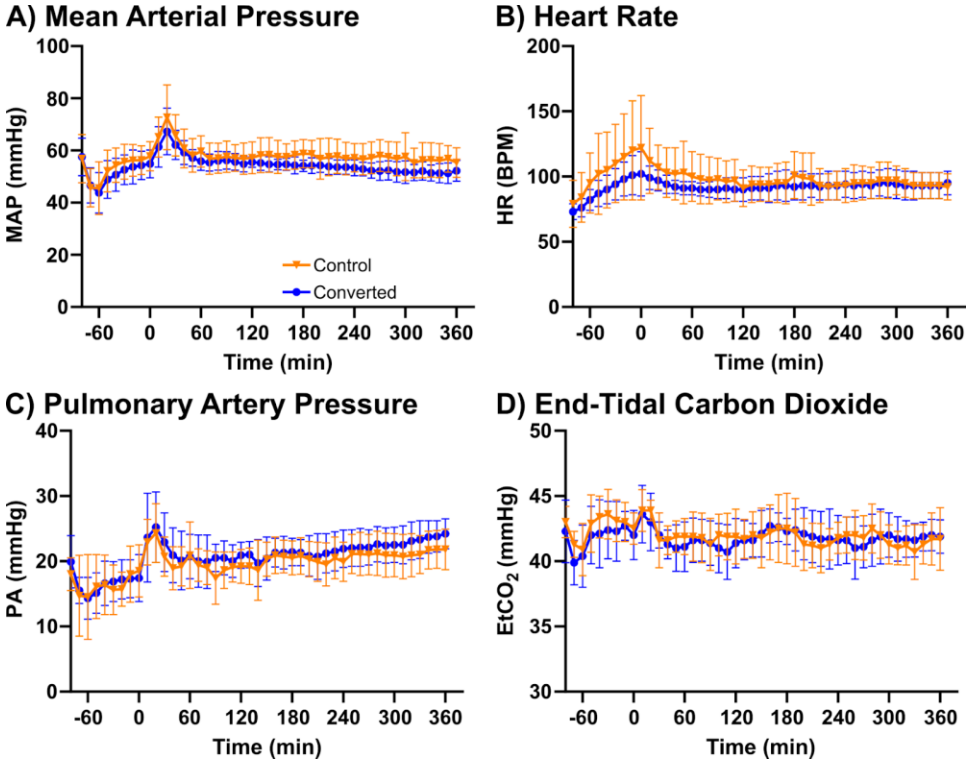


Figure 9. Hemodynamic results. A) Mean arterial pressure. B) Heart Rate. C) Pulmonary Artery Pressure. D). End-Tidal Carbon Dioxide. There were no statistically or clinically significant differences between control (orange) and ECO-WB (blue).

Results – ABG's

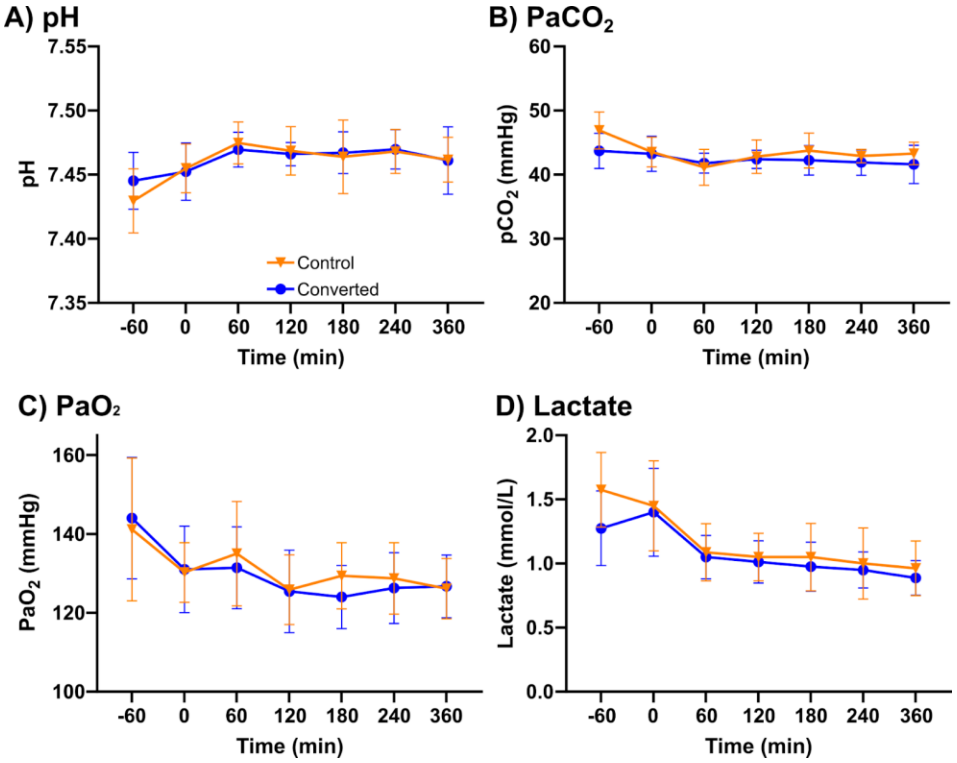
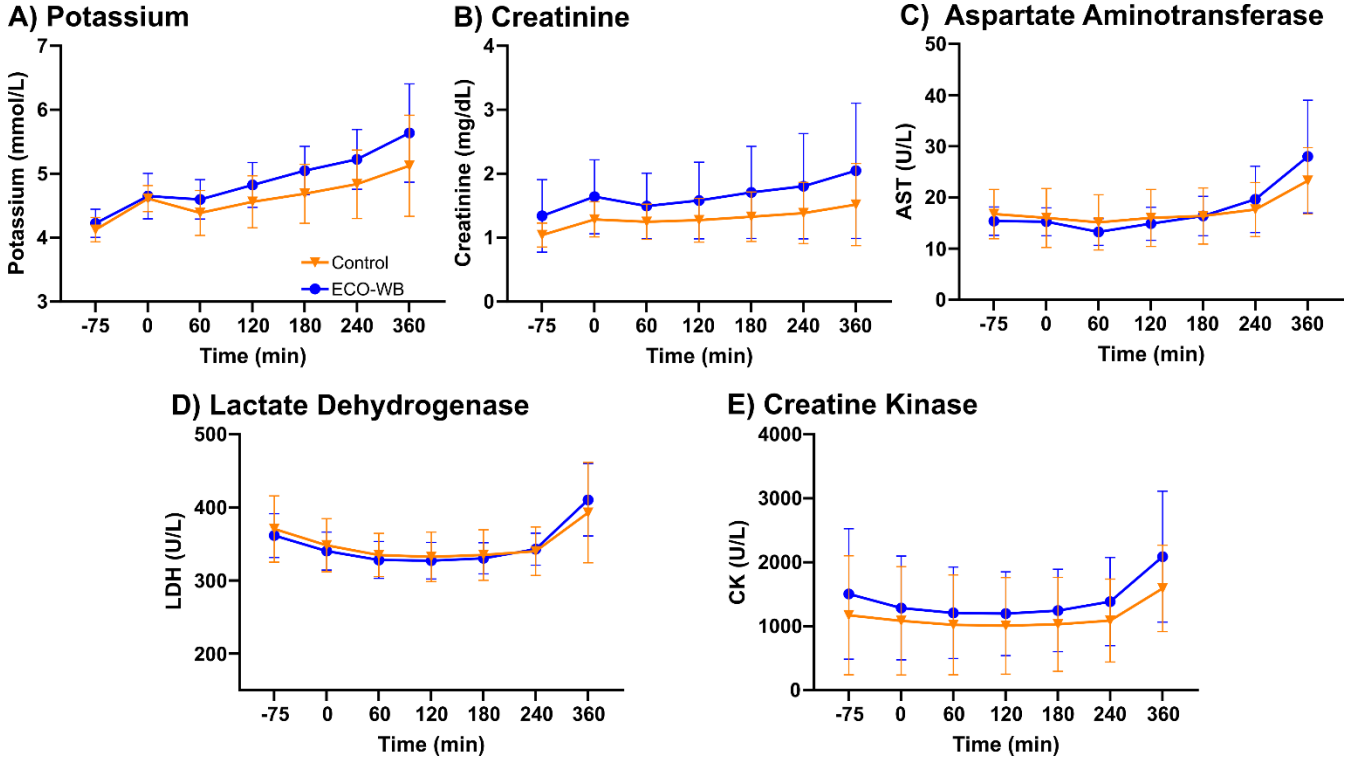


Figure 10. Arterial Blood Gas Results. A) pH. B) Partial Pressure of Carbon Dioxide. C) Partial Pressure of Oxygen. D). Lactate. There were no statistically or clinically significant differences between control (orange) and ECO-WB (blue).

Results – Indicators of Hemolysis

Figure 11. Indicators of hemolysis. A) Potassium. B) Creatinine, C) Aspartate Aminotransferase D) Lactate Dehydogenase. E) Creatine Kinase. There were no statistically or clinically significant differences between control (orange) and ECO-WB (blue).



Conclusions

1. After treatment with ketorolac, swine tolerated autologous transfusion of ECO-WB through a 6-hour observation period.
2. ECO-WB rapidly converted after 1-hour of treatment with enzyme.
3. No evidence of changes in ECO-WB, indicators of hemolysis, or hemodynamic changes compared to control animals.

Limitations

- Limited ability to contextualize CARPA-like reaction in this experiment
- Limited animal models for alternative testing (A-Type: Swine; B-Type: Great Apes)
- Swine A-Type is very similar structurally to humans, but there are physiologic differences (antigen density; loading onto surface of RBCs)
- Swine have poorly understood “non-A” blood types; blood reaction physiology

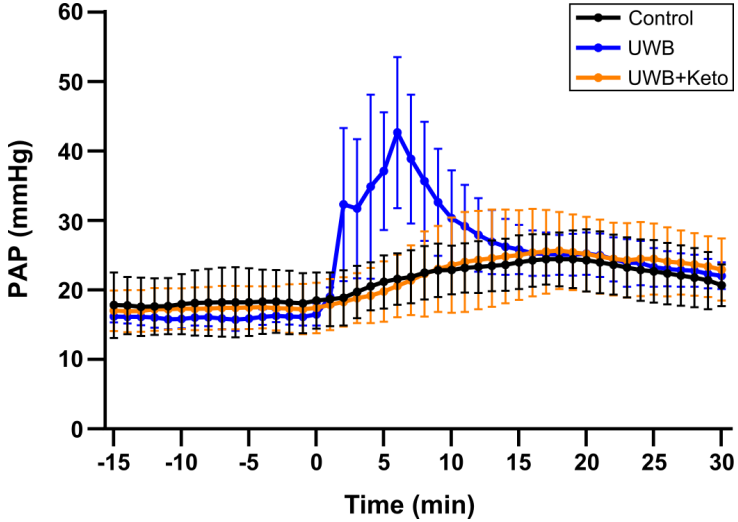


Figure 5. Experimental Pulmonary Arterial Pressure.

Next Steps

In Progress:

- Tolerance of swine to 28-days after autologous transfusion of ECO-WB (CDMRP)
- Pre-clinical testing of ECO-WB in Armed Services Blood Bank provided human Type-A WB and RBCs (DHA CCCRP)

Follow-On Projects FY27-29 (Joint with Avivo, ISR, NAMRU-SA):

- Heterologous transfusions, extensive safety/crossmatch testing, characterizing the CARPA-like reaction, etc.

Questions?

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