

AWARD NUMBER: W81XWH-20-1-0832

TITLE: Subnormothermic Ex Situ Limb Perfusion Using Red Blood Cells and Plasma: Critical Component Analysis

PRINCIPAL INVESTIGATOR: Kagan Ozer, MD

CONTRACTING ORGANIZATION: University of Michigan, Ann Arbor, MI

REPORT DATE: October 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE*Form Approved*
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1. REPORT DATE : October 2021	2. REPORT TYPE: Annual	3. DATES COVERED 01Sep2020-31Aug2021
4. TITLE AND SUBTITLE: Subnormothermic Ex Situ Limb Perfusion Using Red Blood Cells and Plasma: Critical Component Analysis		5a. CONTRACT NUMBER W81XWH-20-1-0832
		5b. GRANT NUMBER RT190045
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Kagan Ozer, MD E-Mail: kozer@umich.edu		5d. PROJECT NUMBER
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Regents of the University of Michigan 3003 S. State St., Ann Arbor, MI 48109-1274		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick. Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)
		11. SPONSOR/MONITOR'S NUMBER(S)
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		
13. SUPPLEMENTARY NOTES		

14. ABSTRACT: The long-term objective of this application is to keep vascularized composite tissues viable at or beyond 48 hours using ex-vivo perfusion systems. In this project, we used rat hind limb allograft transplantation model along with a miniaturized version of our custom-made ex-vivo perfusion system and tested red blood cell based perfusate solutions. Under SA1, we tested the efficacy of three plasma components in maintaining on muscle force generation on limb allografts after 8 hours of SNT ex-vivo perfusion/transplantation. These components (full, high molecular weight, and low molecular weight plasma) mixed with red blood cells were used to perfuse limbs, which were then transplanted to another recipient orthotopically to observe limb survival as well as the long-term outcomes of muscle and nerve regeneration 3 months following trasnplantation. Outcome measures included analysis of the EMG data, muscle and nerve histology, and proteomic analysis of the perfusate. At the end of first year, we completed all transplantation surgeries in control and experimental groups. In addition, we also completed EMG analysis of all control groups, and 80% of the animals in experimental groups. Muscle and nerve histology analysis are still ongoing. Our preliminary results indicate that the best outcomes are achieved when full plasma is included in the perfusate and when by-products of the perfusion are effectively filtered. Having said that, these results are still preliminary and needs further analysis once muscle and nerve histology data are available. Although we just passed the first-year mark since the official start date, we were unable to start experiments on time due to COVID restrictions which resulted in 3 months of delay. We are currently right on track to meet our deadlines as stated in SOW document for 9 months.

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 27	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER <i>(include area code)</i>

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1. Introduction:

The long-term objective of this application is to keep vascularized composite tissues viable at or beyond 48 hours using ex-vivo perfusion systems. To achieve this goal, we use ex-vivo perfusion systems to deliver oxygen and other nutrients to amputated vascularized composite tissue parts to maintain their survival and function. In this application, we propose to use various components of the human blood under sub-normothermic (21-24°C) temperatures using established methods of pulsatile ex-vivo perfusion systems. There is no doubt that full blood is the best perfusate perfected after billions of years of evolution. Red blood cells are essential to carry the oxygen, and the plasma contains many proteins and growth hormones essential for endothelial stability. Both red blood cells and plasma are vital for oxygenation, energy production, and survival of tissues. The critical components of the plasma needed for survival of the tissue are not identified previously. Identification of these critical components will have the potential for indefinite survival of tissues, not only for VCAs, but also for other solid organs. Proposed research has a strong potential to be quickly translated into clinical practice as the machine perfusion systems and plasma separation technologies are already in place.

2. Keywords:

CMN: Coagulative Myonecrosis

ESP: Ex-situ perfusion

EVP: Ex-vivo perfusion

HMW: High molecular weight

HT: Hypothermic (10-15°C)

HTK: Histidine-Tryptophan-Ketoglutarate

IED: improvised explosive device

IR: Ischemia-reperfusion

LMW: Low molecular weight

NNT: Near-normothermic (32-35°C)

OEF: Operation Enduring Freedom

OIF: Operation Iraqi Freedom

RBC: Red Blood Cells

SCS: Static Cold Storage

SNT: Sub-normothermic (21-24°C)

VCA: Vascularized composite tissue allograft

3. Accomplishments:

What were the major goals of the project?

SA1: To define the critical molecular weight proteins in plasma that maintains muscle force generation on limb allografts after 24 hours on SNT ex-vivo perfusion and transplantation.

What was accomplished under these goals?

To achieve this goal, we prepared 3 different perfusate solutions mixed with RBCs as the oxygen carrier, and perfused limbs using ex-situ perfusion system. These solutions included perfusate with full plasma, low molecular weight plasma and high molecular weight plasma. After 8 hours of subnormothermic perfusion at 21C, we transplanted limbs to another recipient. At the end of the first year, we completed all control groups (sciatic nerve transection/repair, immediate transplantation, static cold storage after 8 hours, RBC only perfusion) along with experimental groups. Last set group of recipients are waiting to complete nerve regeneration at 3 months following surgery. Preliminary results show superior results in the group perfused with full molecular weight plasma (please see appendix with preliminary SPSS output/analysis and graphs).

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

We are going complete functional assessment in group and begin to execute experimental groups under SA2. Funds for SA2 has just been released.

4. Impact:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/Problems:

- Changes in approach and reasons for change
- Actual or anticipated problems or delays and actions or plans to resolve them
We are not anticipating any issues.

- Changes that had a significant impact on expenditures
None.

- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: N/A
- Significant changes in use or care of human subjects: N/A

- Significant changes in use or care of vertebrate animals: None
- Significant changes in use of biohazards and/or select agents: None

6. Products: Nothing to report this point.

7. Participants and Other Collaborating Organizations:

What individuals have worked on the project?

Name: Kagan Ozer
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0001-7397-3696
Nearest person month worked: 3
Contribution to Project: : Overseaw all aspect of project execution, trained the postgrad student to perform experiments, analyzed preliminary results.

Name: Amir Dehdastian
Project Role: research fellow
Nearest Person month worked: 6
Contribution to the project: no change

Name: Erin Guy
Project Role: research tech
Nearest Person month worked: 6
Contribution to the project: No change

Name: Steve Kemp
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-5608-3584
Nearest person month worked: 3
Contribution to Project: : Oversees and analyzes EMG nad nerve histology data,

Name: : Jana Moon
Project Role: : Research tech
Nearest person month worked: 3
Contribution to Project: : Performs EMG testing, helps to process nerve samples

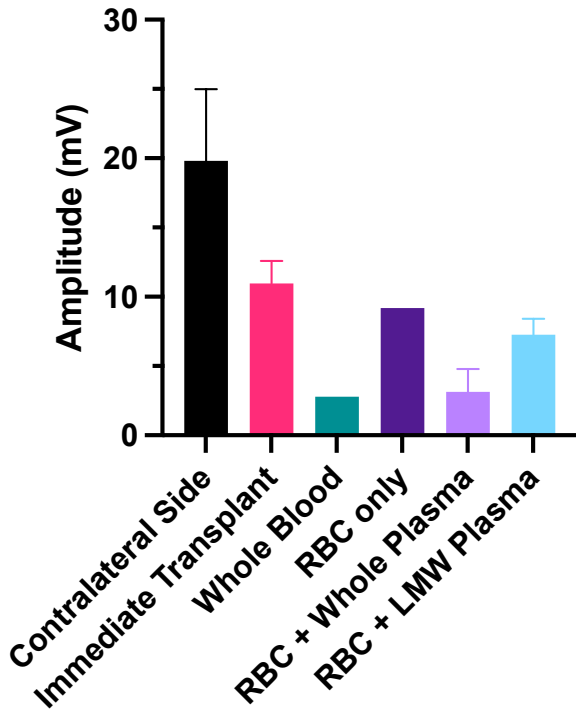
Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
 Nothing to report

What other organizations were involved as partners?
 Nothing to report

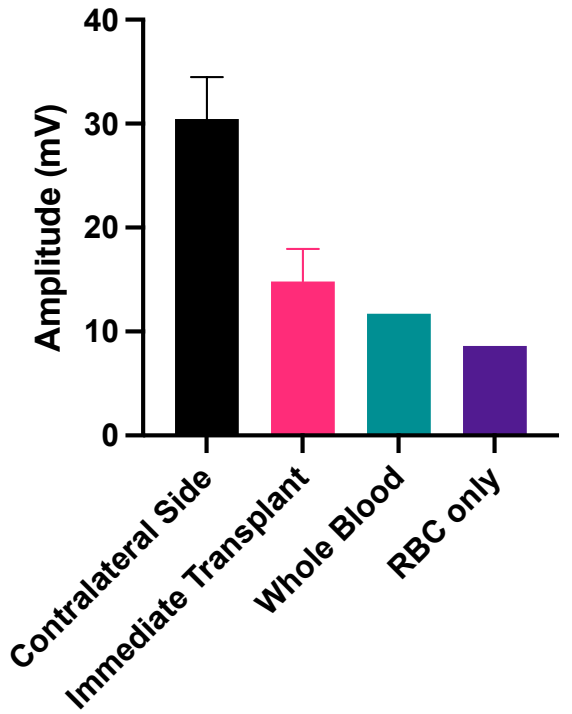
8. Special reporting requirements
 Nothing to report

9. Appendices
 Nothing to report at this point

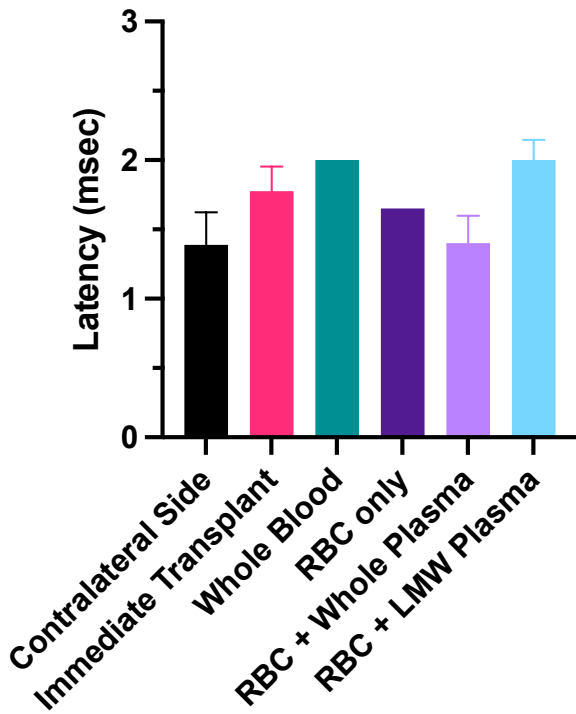
EDL CMAP



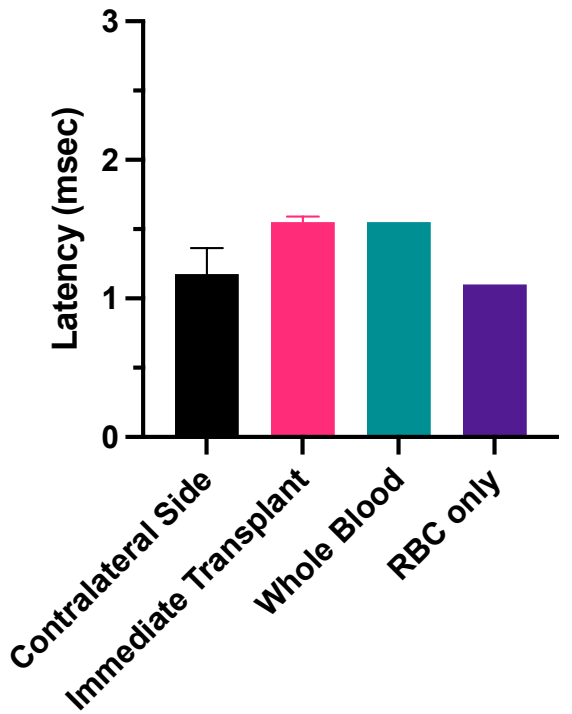
Gastrocnemius CMAP



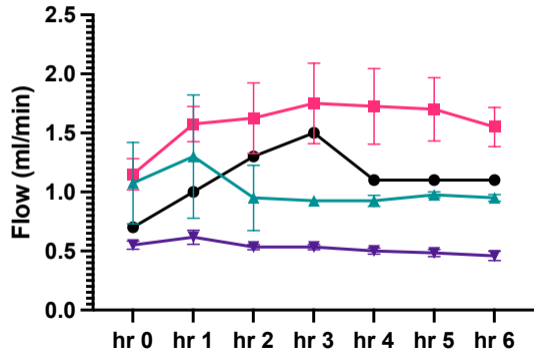
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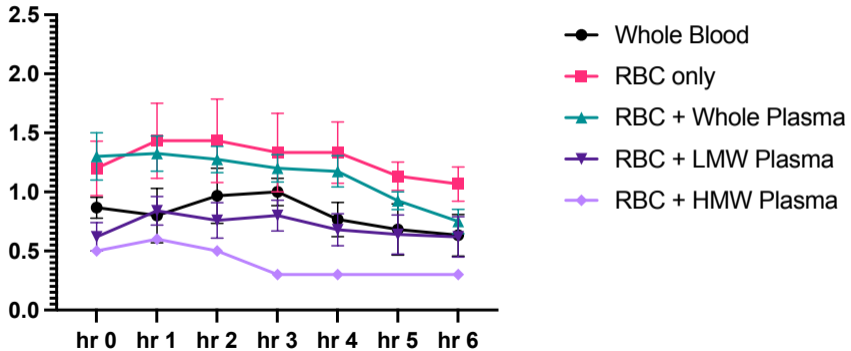
Gastrocnemius Latency



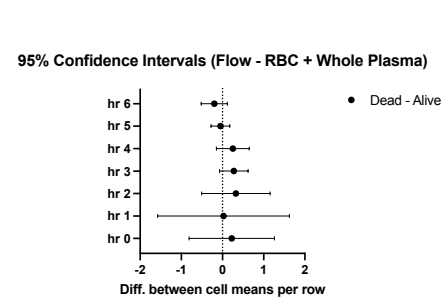
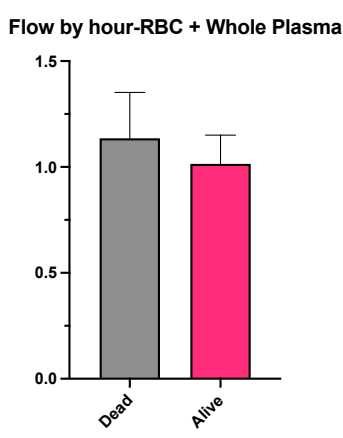
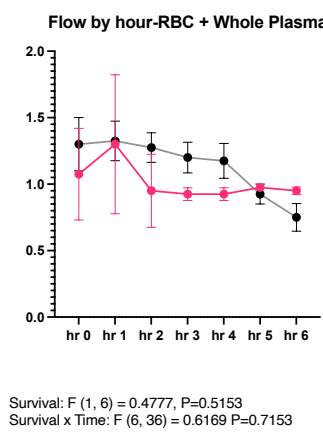
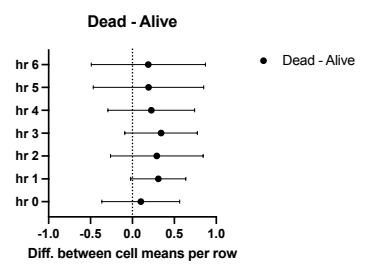
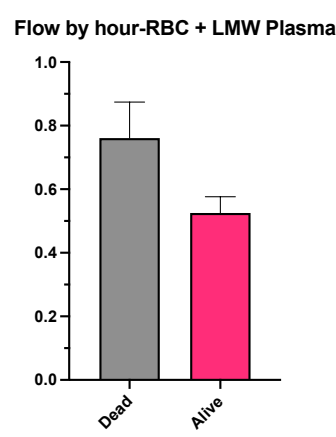
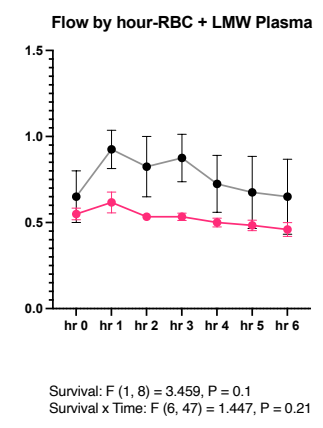
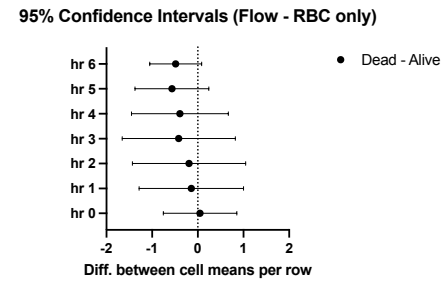
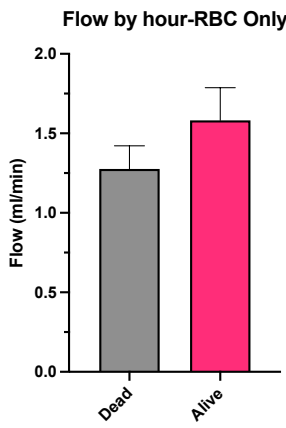
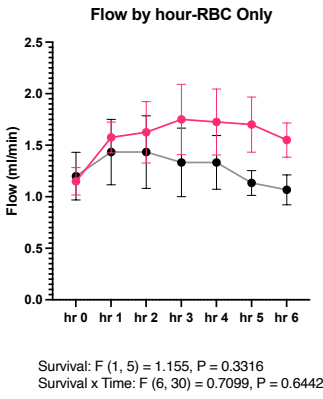
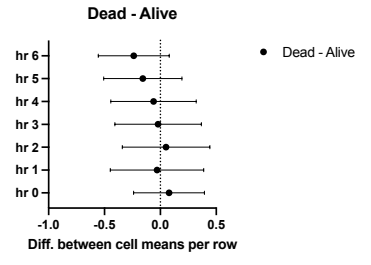
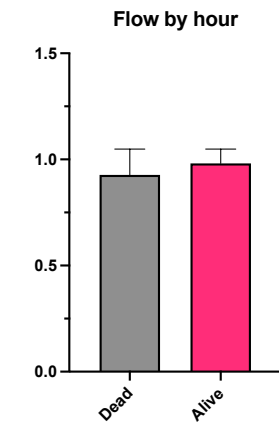
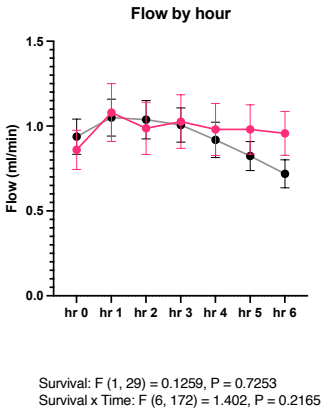
Flow by hour in Groups (Survived)



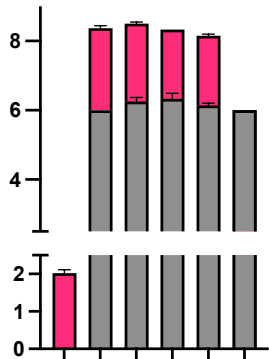
Flow by hour in Groups (Dead)



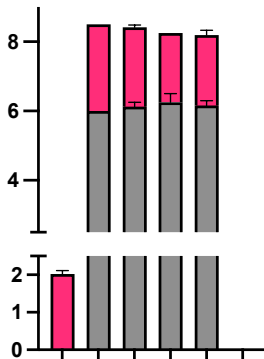
- Whole Blood
- RBC only
- ▲ RBC + Whole Plasma
- ▼ RBC + LMW Plasma
- ◆ RBC + HMW Plasma



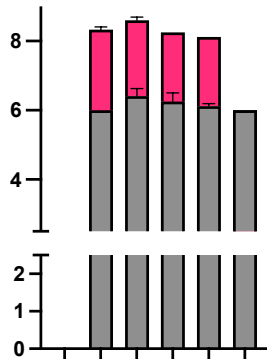
Ischemia



Ischemia in Survived



Ischemia in Dead



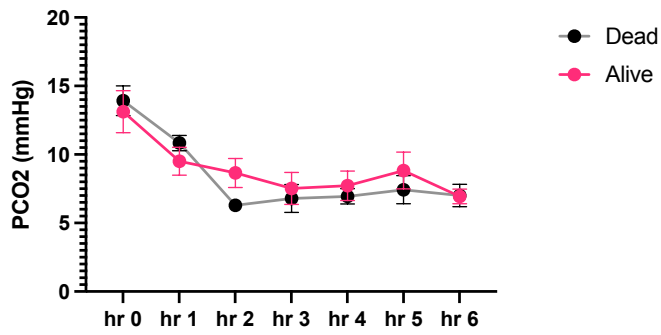
■ Perfusion time
■ Absolute ischemia

Immediate Transplant
Whole Blood
RBC only
RBC + Whole Plasma
RBC + LMW Plasma
RBC + HMW Plasma

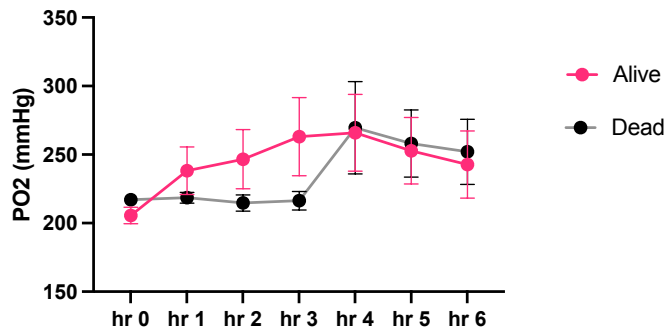
Immediate Transplant
Whole Blood
RBC only
RBC + Whole Plasma
RBC + LMW Plasma
RBC + HMW Plasma

Immediate Transplant
Whole Blood
RBC only
RBC + Whole Plasma
RBC + LMW Plasma
RBC + HMW Plasma

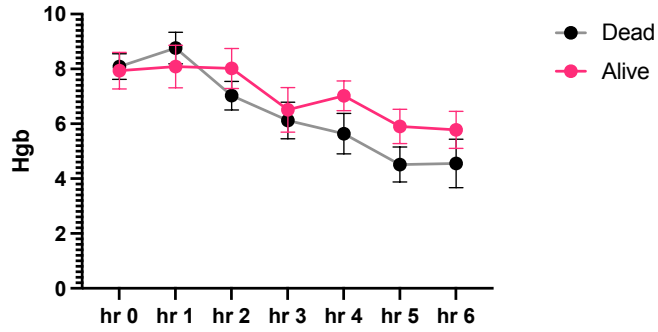
PCO2 by hour



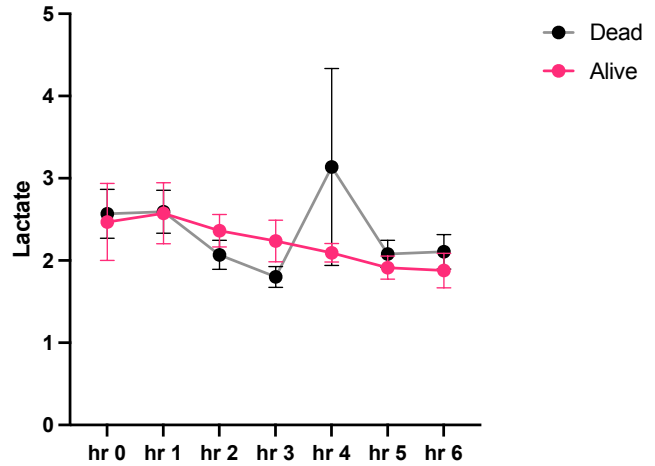
PO2 by Hour



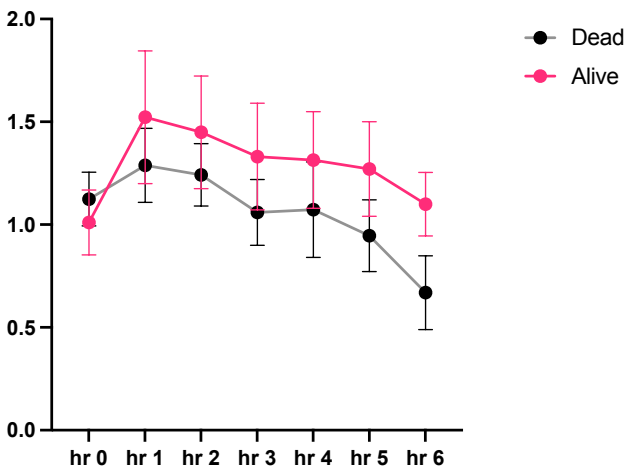
Hgb by Hour



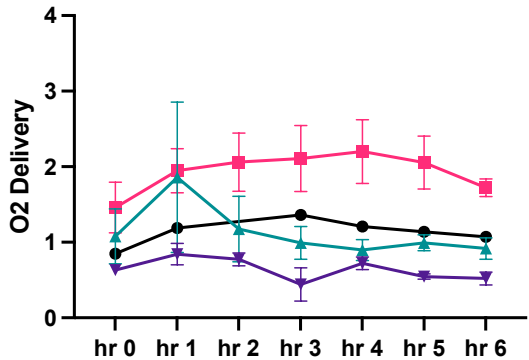
Lactate by Hour



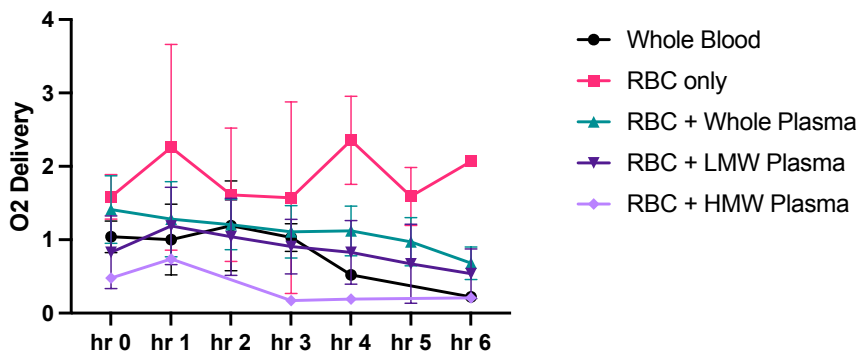
O2 Delivery by Hour



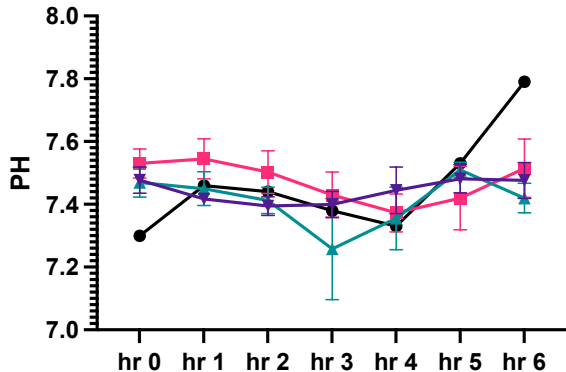
O2 Delivery by hour in Groups (Survived)



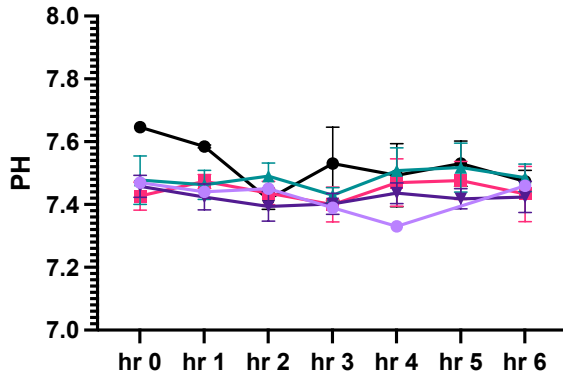
O2 Delivery by hour in Groups (Dead)



PH by hour in Groups (Survived)

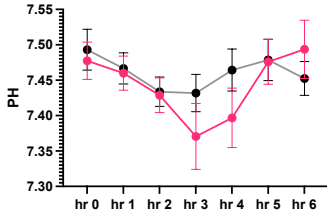


PH by hour in Groups (Dead)



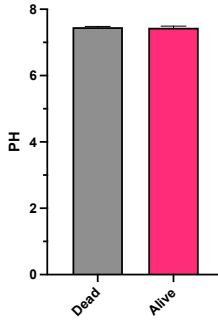
- Whole Blood
- RBC only
- ▲ RBC + Whole Plasma
- ▼ RBC + LMW Plasma
- RBC + HMW Plasma

PH by hour

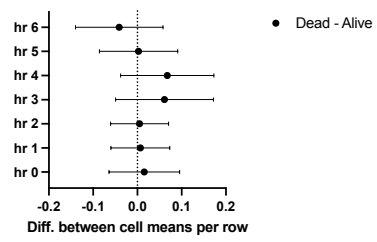


● Dead
● Alive

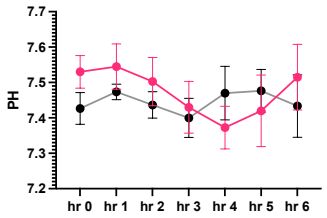
PH by hour



95% Confidence Intervals (PH)

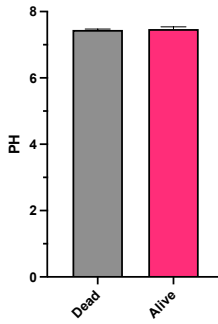


PH by hour-RBC Only

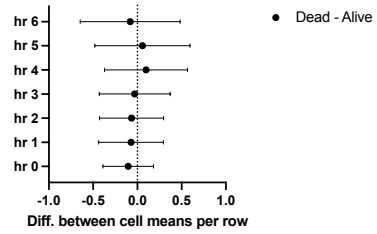


● Dead
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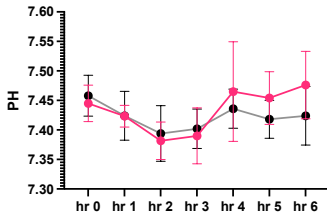
PH by hour-RBC Only



Dead - Alive

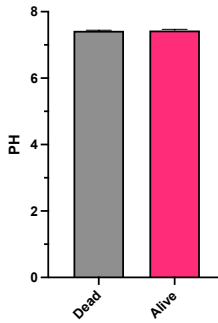


PH by hour-RBC + LMW Plasma

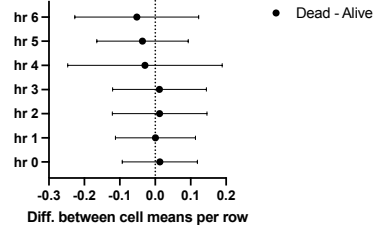


● Dead
● Alive

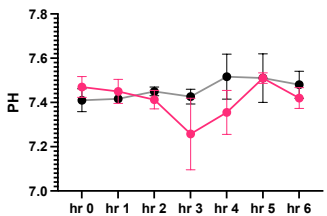
PH by hour-RBC + LMW Plasma



Dead - Alive

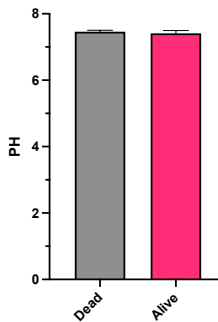


PH by hour-RBC + Whole Plasma

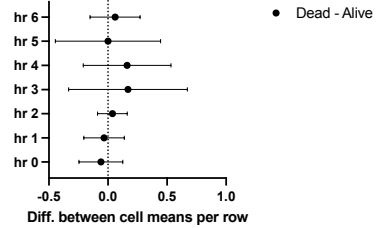


● Dead
● Alive

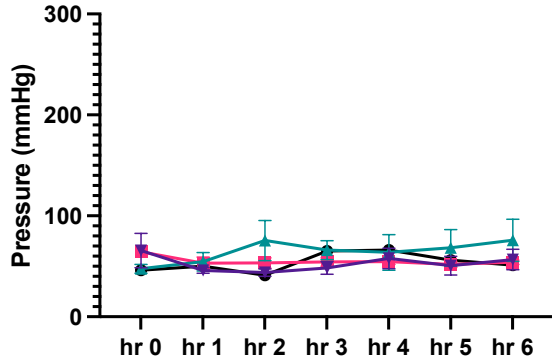
PH by hour-RBC + Whole Plasma



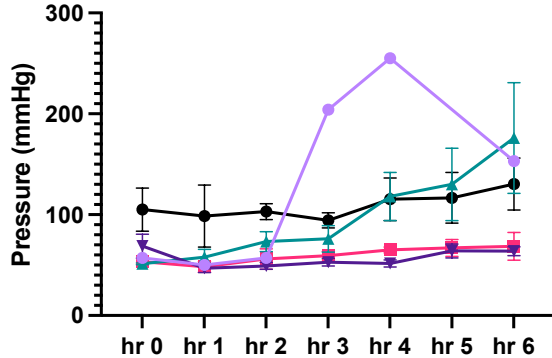
Dead - Alive



Pressure by hour in Groups (Survived)

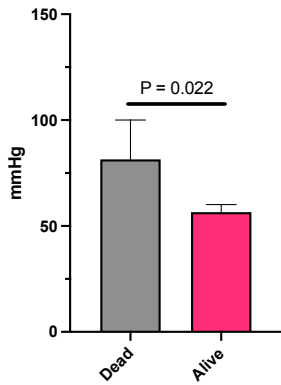
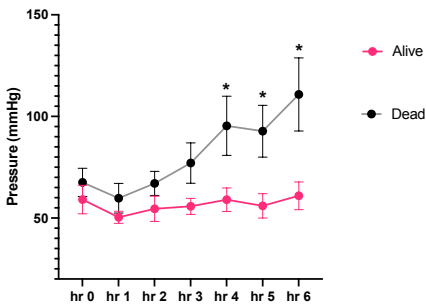


Pressure by hour in Groups (Dead)

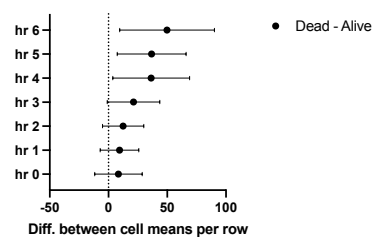


- Whole Blood
- RBC only
- ▲ RBC + Whole Plasma
- ▼ RBC + LMW Plasma
- RBC + HMW Plasma

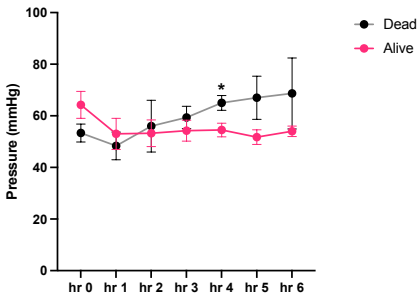
Pressure by hour (Mean effect)



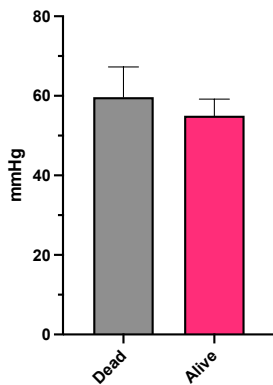
95% Confidence Intervals (Pressure)



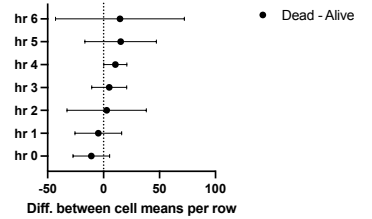
Pressure by hour-RBC Only



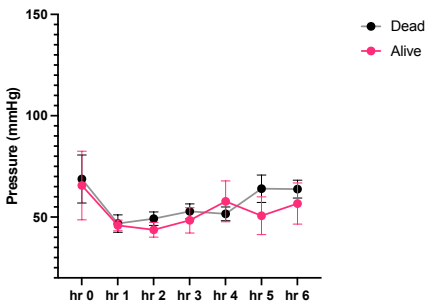
Pressure by hour-RBC Only



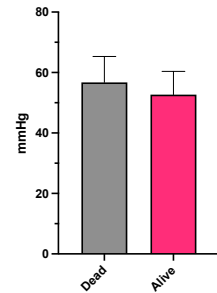
Dead - Alive



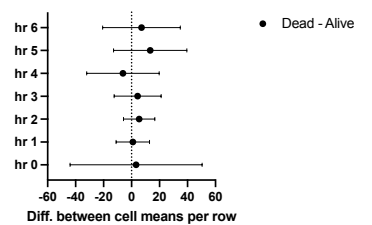
Pressure by hour-RBC + LMW Plasma



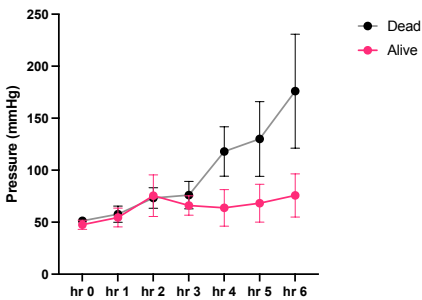
Pressure by hour-RBC + LMW Plasma



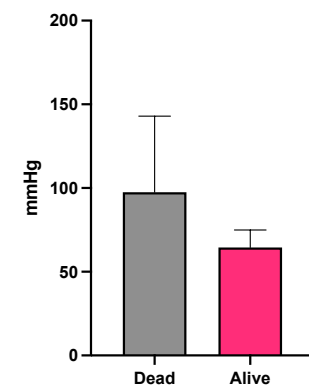
Dead - Alive



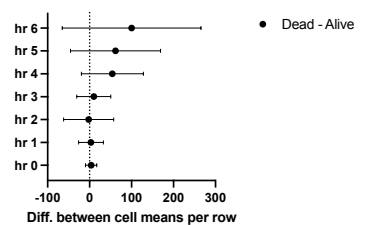
Pressure by hour-RBC + Whole Plasma



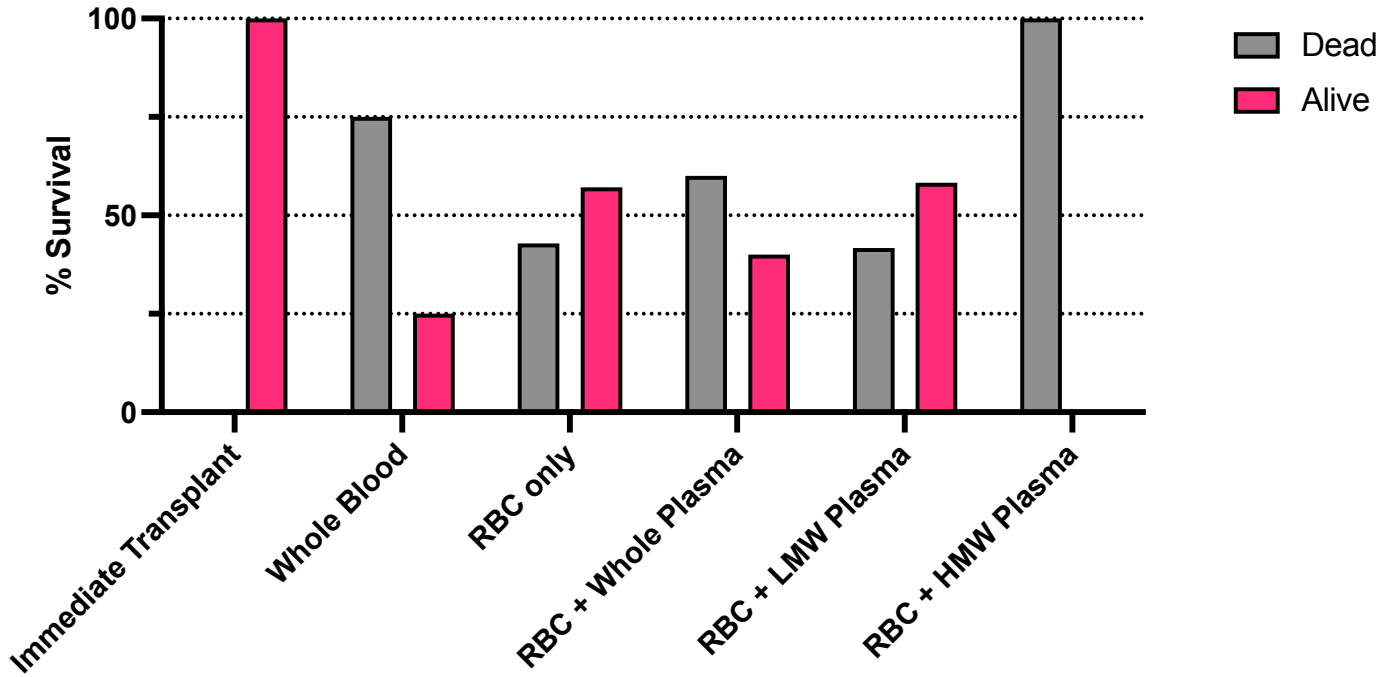
Pressure by hour-RBC + Whole Plasma



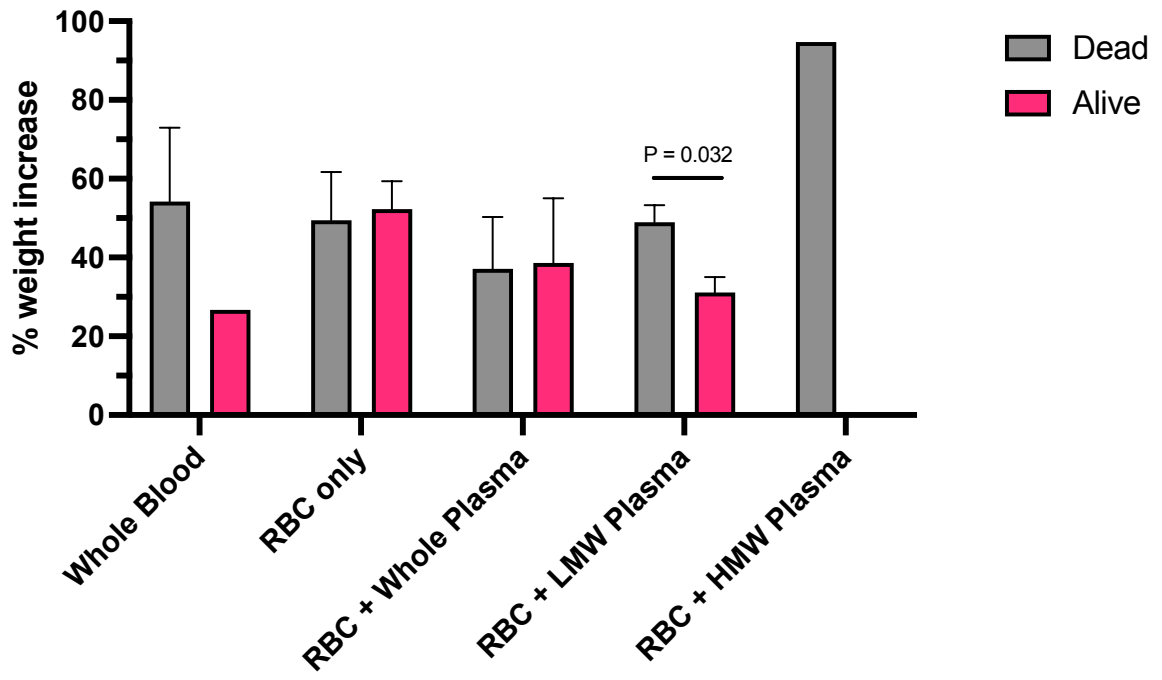
Dead - Alive



Survival

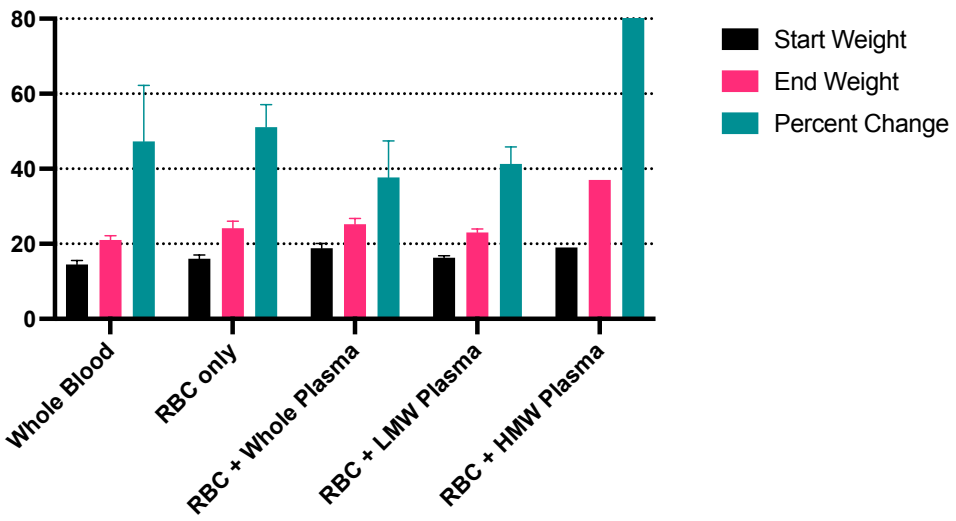


% weight increase

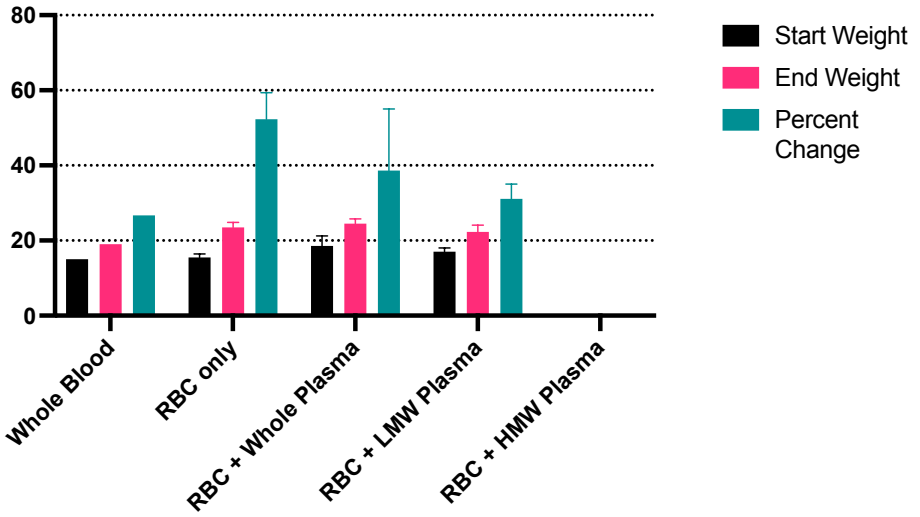


Note: % Weight increase has no correlation with survival:
Spearman = -0.159 , P = 0.410

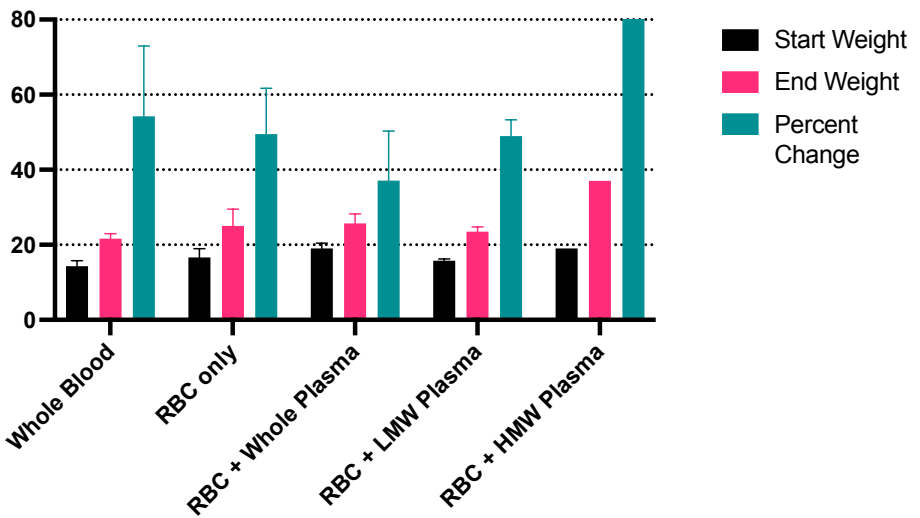
Weight

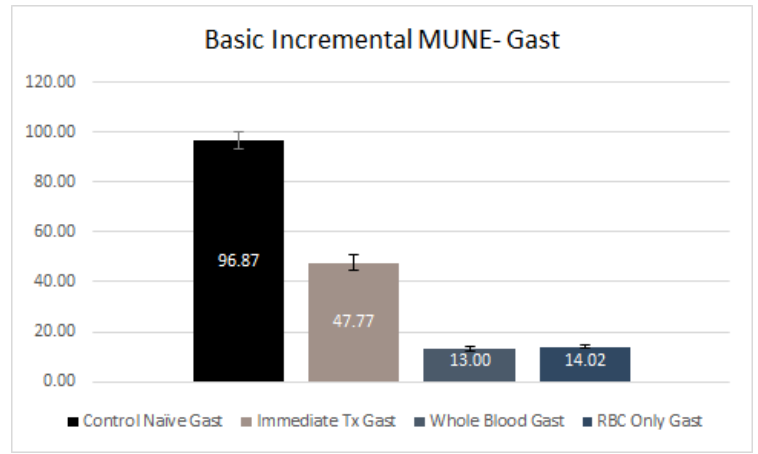
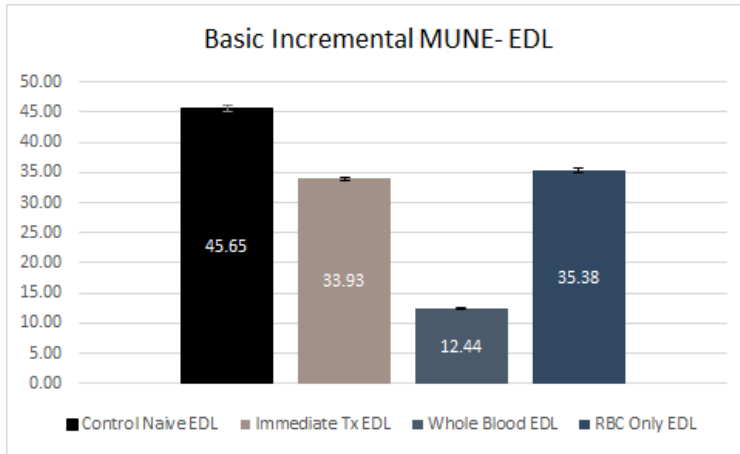


Weight in Survived



Weight in Dead

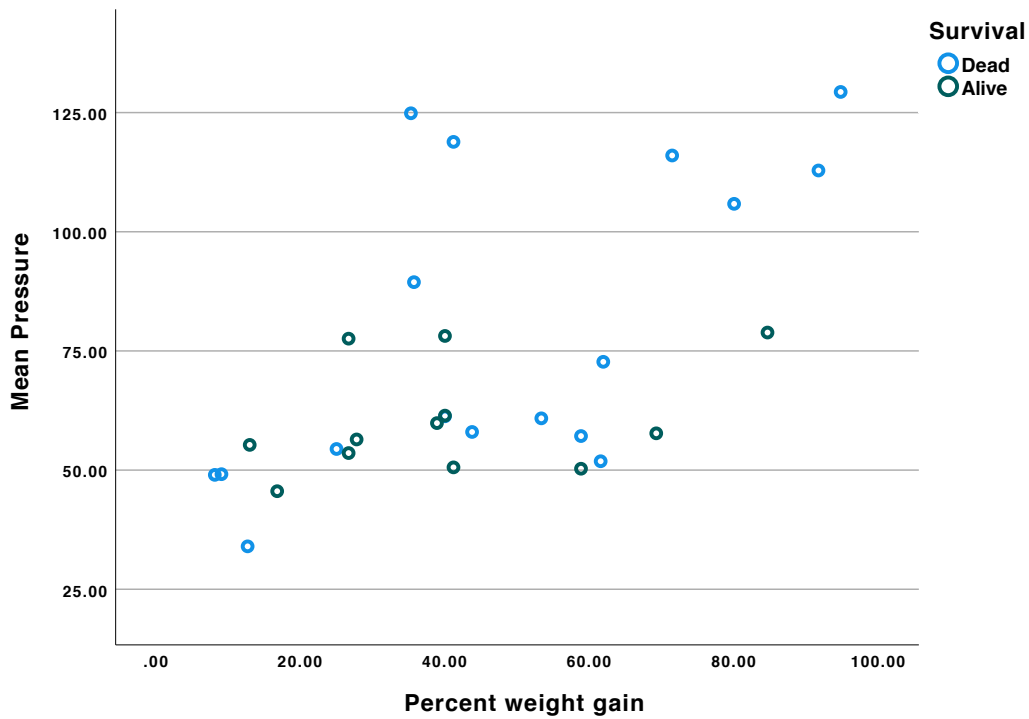




Graph

Notes

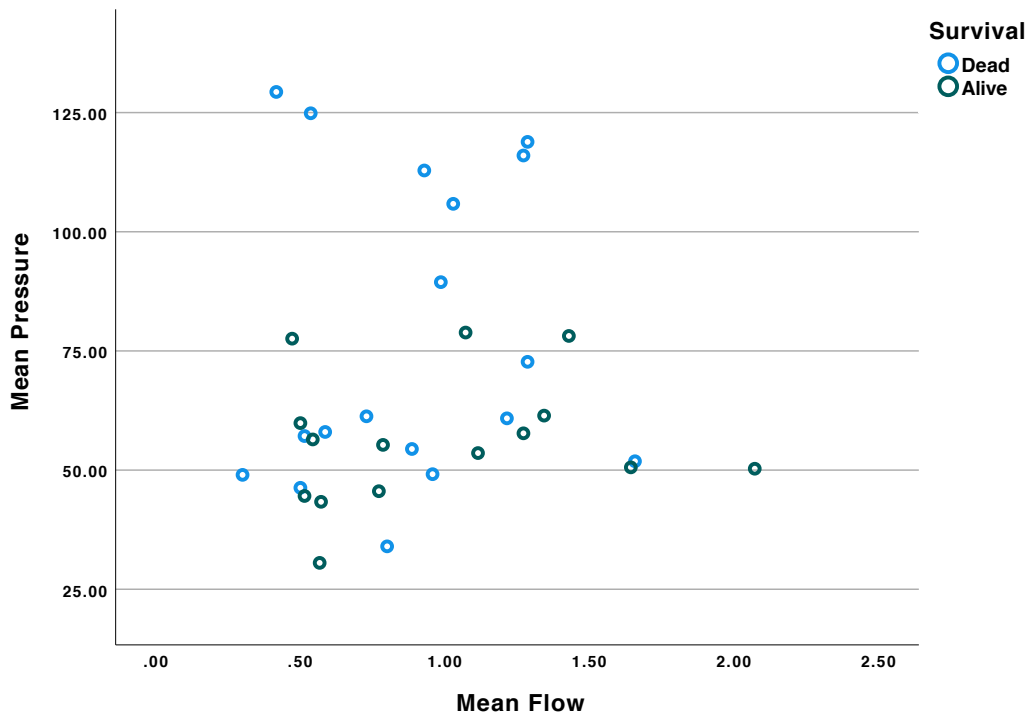
Output Created		02-SEP-2021 21:53:33
Comments		
Input	Data	/Users/amirdehdashtian /Dropbox (University of Michigan)/Limb Perfusion- Ozer/Data. sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	38
Syntax		GRAPH /SCATTERPLOT(BIVAR) =W_Change WITH Mean_Pressure BY Survival /MISSING=LISTWISE.
Resources	Processor Time	00:00:00.19
	Elapsed Time	00:00:00.00



Graph

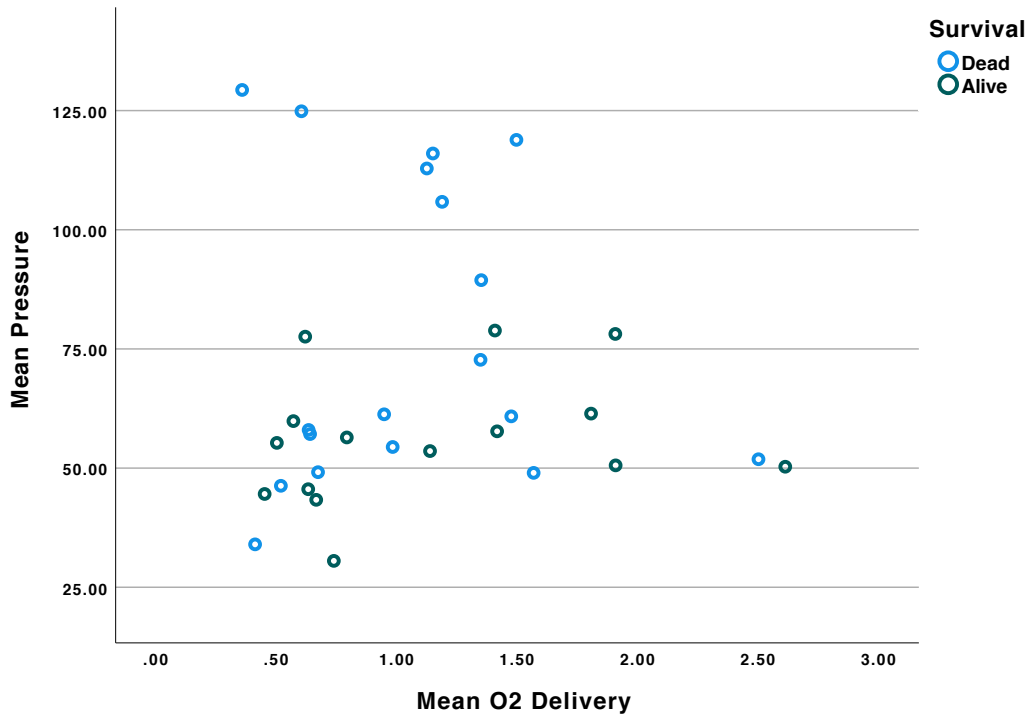
Notes

Output Created		02-SEP-2021 21:56:28
Comments		
Input	Data	/Users/amirdehdashtian /Dropbox (University of Michigan)/Limb Perfusion- Ozer/Data. sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	38
Syntax		GRAPH /SCATTERPLOT(BIVAR) =Mean_Flow WITH Mean_Pressure BY Survival /MISSING=LISTWISE.
Resources	Processor Time	00:00:00.16
	Elapsed Time	00:00:00.00



Notes

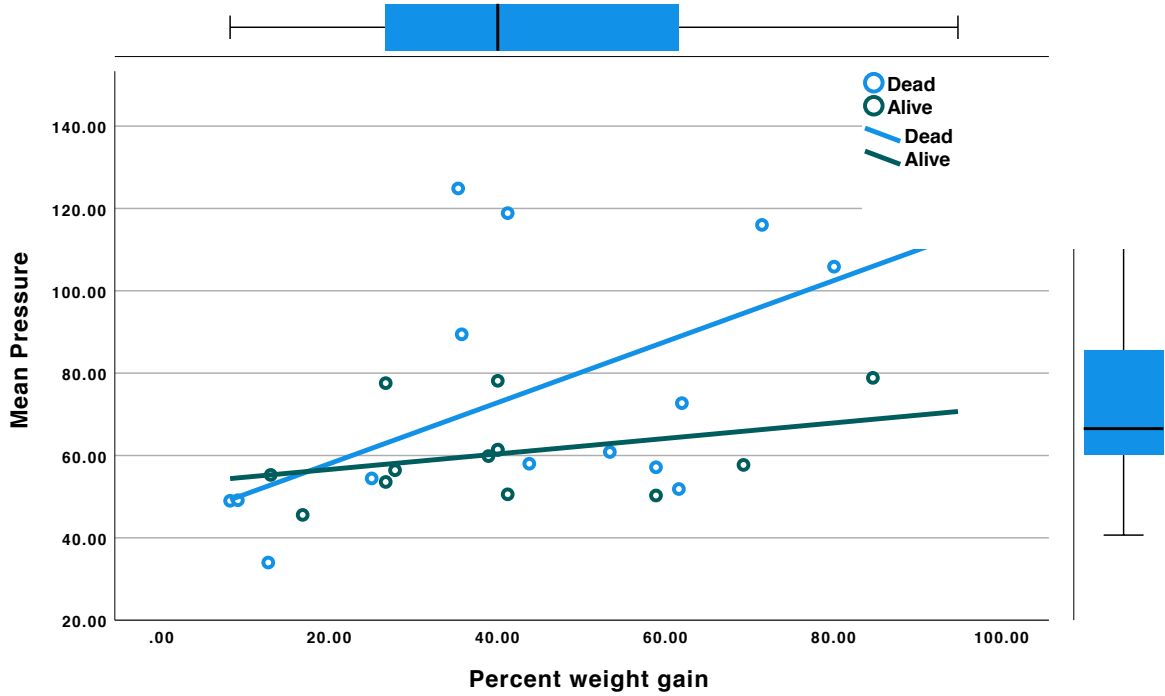
Output Created		02-SEP-2021 21:58:06
Comments		
Input	Data	/Users/amirdehdashtian /Dropbox (University of Michigan)/Limb Perfusion- Ozer/Data. sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	38
Syntax		GRAPH /SCATTERPLOT(BIVAR) =Mean_O2_Delivery WITH Mean_Pressure BY Survival /MISSING=LISTWISE.
Resources	Processor Time	00:00:00.18
	Elapsed Time	00:00:01.00



STATS REGRESS

Notes

Resources	Processor Time	00:00:00.23
	Elapsed Time	00:00:00.00

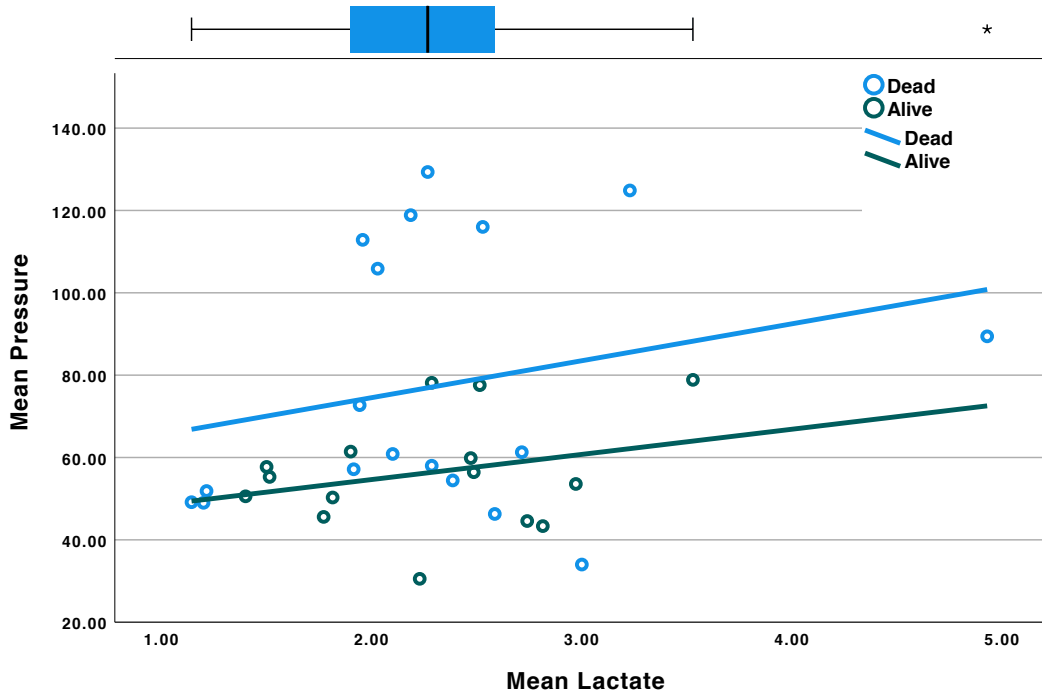


Notes

Output Created		02-SEP-2021 22:02:33
Comments		
Input	Data	/Users/amirdehdashtian /Dropbox (University of Michigan)/Limb Perfusion- Ozer/Data. sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	38
Syntax		BEGIN PROGRAM PYTHON3.
Resources	Processor Time	00:00:00.01
	Elapsed Time	00:00:00.00

Notes

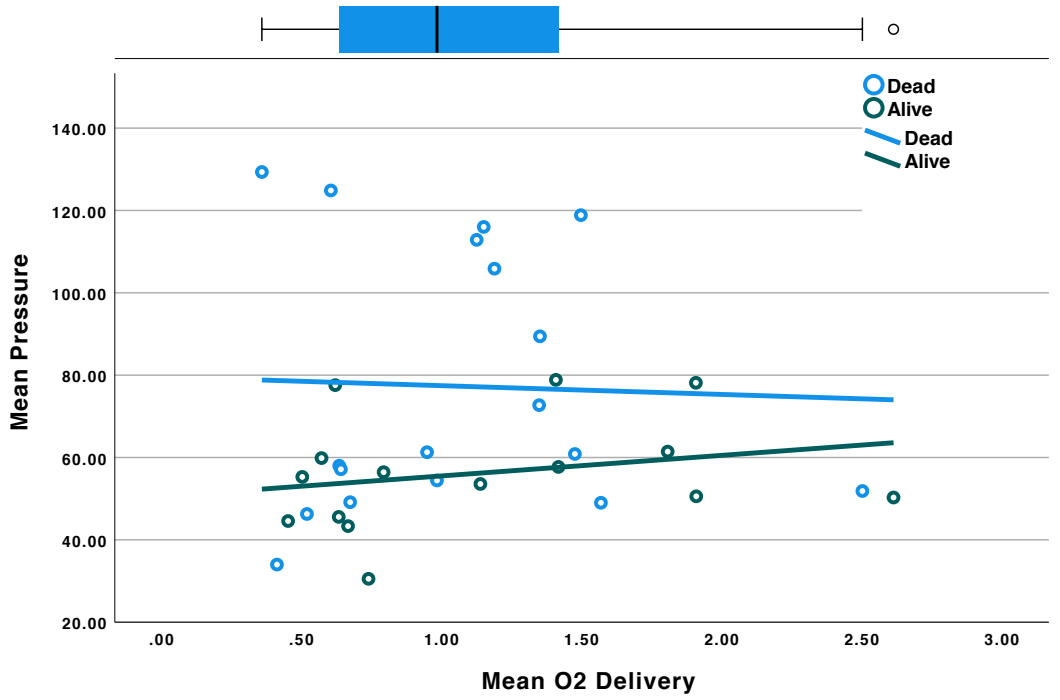
Resources	Processor Time	00:00:00.21
	Elapsed Time	00:00:00.00



STATS REGRESS

Notes

Resources	Processor Time	00:00:00.25
	Elapsed Time	00:00:00.00



STATS REGRESS

Notes

Resources	Processor Time	00:00:00.30
	Elapsed Time	00:00:00.00

