AWARD NUMBER: W81XWH-19-1-0440

TITLE: Supercooled Ex-Vivo Porcine VCA Preservation to Extend the Timeline Between Procurement and Transplantation and Enable Tolerance Induction to Eliminate Immunotherapy Needs and Risks

PRINCIPAL INVESTIGATOR: Korkut Uygun PhD

CONTRACTING ORGANIZATION: Massachusetts General Hospital

Boston MA 02114

REPORT DATE: AUGUST 2020

TYPE OF REPORT: Annual report

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
AUGUST 2020	Annual	8/1/19-7/31/20

4. TITLE AND SUBTITLE

Supercooled Ex-Vivo Porcine VCA Preservation to Extend the Timeline Between Procurement and Transplantation and Enable Tolerance Induction to Eliminate Immunotherapy Needs and Risks

5a. CONTRACT NUMBER
W81XWH-19-1-0437

5b. GRANT NUMBER
RT180063P1

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Korkut Uygun, Phd

E-Mail: KUYGUN@mgh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General
Hospital
51 Blossom St Boston MA 02114

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The project aims to develop a novel technology to preserve vascular composite allografts for extended periods. This project uses a porcine model.

In the first year, the focus was on scaling up the prior rat limb perfusion modality to porcine scale.

15. SUBJECT TERMS
Organ Preservation, VCA transplantation, limb transplantation, supercooled storage

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

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Enabling prolonged preservation of vascularized composite allografts (VCA) is critical to enable their use in a practical manner clinically. Machine perfusion technologies have enabled dynamic organ storage for many organs, in stark contrast to the current gold standard of static cold storage. Supercooling technology, which builds on machine perfusion, has been shown to further extend preservation, allowing the increase of viable preservation time to 27 hours for human livers, 3 times the clinical average. This project aims to translate these exciting results in livers to VCA, also leveraging prior studies in rats.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

VCA, preservation, supercooling, cryopreservation, transplantation, machine perfusion, Ischemia Reperfusion Injury

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. *Milestone # 1 ACURO approval obtained*. **100% complete** (February 2020)
2. *Milestone #2 Complete evaluation of Machine perfusion on VCA viability.* **30% Complete**
3. *Milestone #3 Develop a method to extend preservation duration for porcine limbs.* **15% Complete**
4. *Milestone #4 Develop a method to enable using mixed chimerism for VCA transplantation.* **Nothing to report**

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We started the project by scaling up from our prior experience in rats to a large animal limb ex vivo perfusion system and performed initial experiments.

1) Ex vivo subnormothermic machine perfusion (SNMP): Two partial hind limbs (swine, see Figure 1) were procured from a Yorkshire pig during a terminal procedure in the Knight surgery operative

room, with about 20minutes warm ischemia during recovery. One limb was perfused in a clinical perfusion device at a subnormothermic temperature (21°C) (Figure 2). The other limb was flushed with 100ml of heparine saline at room temperature and with 100ml of cold HTK, and then stored on ice in HTK.

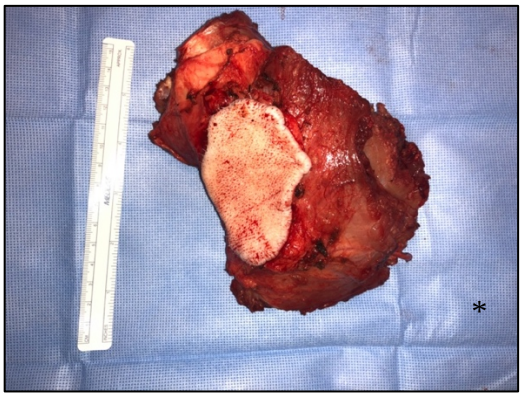

Figure 1. Picture of a swine partial hind limb. The VCA includes: skin paddle, thigh and leg muscles, proximal tibia, knee joint and distal femur, supported by the femoral vascular pedicule (star).

Specific objectives were to assess feasibility of the partial swine hindlimb perfusion for 24 hours and obtain insights for optimal perfusion parameters as well as perfusate composition. The key outcome was edema as measured by weight gain, and perfusion parameters relevant to viability, ischemia and cell injury such as resistance, lactate and potassium as compared with static cold storage.

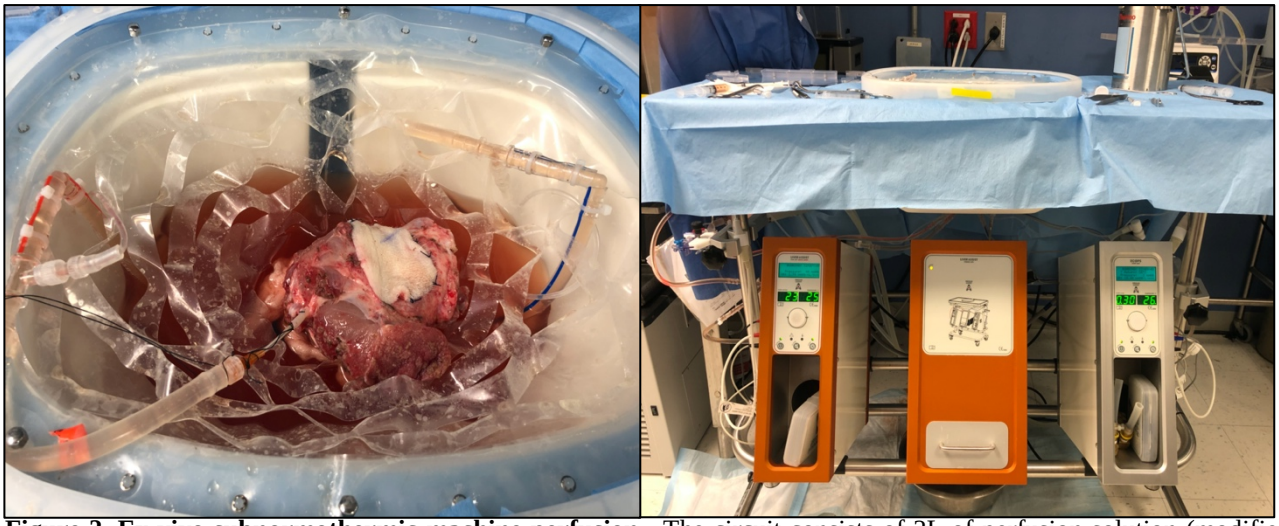

Figure 2. Ex vivo subnormothermic machine perfusion . The circuit consists of 2L of perfusion solution (modified Steen solution) that is poured in the VCA basin and goes through a pulsatile perfusion circuit at a subnormothermic temperature (21°C). The target pressure is set to 40mmHg. The flowrate is automatically adjusted subsequently. Inflow samples are taken fron the inflow valve and outflow is collected directly from the femoral vein.

2 liters of perfusate were used. The perfusate was made with a Steen solution mixed with BSA and 35kDa PEG. Pressure target was 40mmHg, flowrate was subsequently adapted automatically on perfusion device and varied between 15-40ml/min to accommodate for pressure target. Vascular resistances decreased after an hour of perfusion, plateaued between 1 and 1.5mmHg/ml/min until the 18th hour and then increased to 2.5mmHg/ml/min through the end of the experiment (Figure 3). Weight gain was greater in the perfused limb than the static cold storage control (+25.83% vs -2.9%).

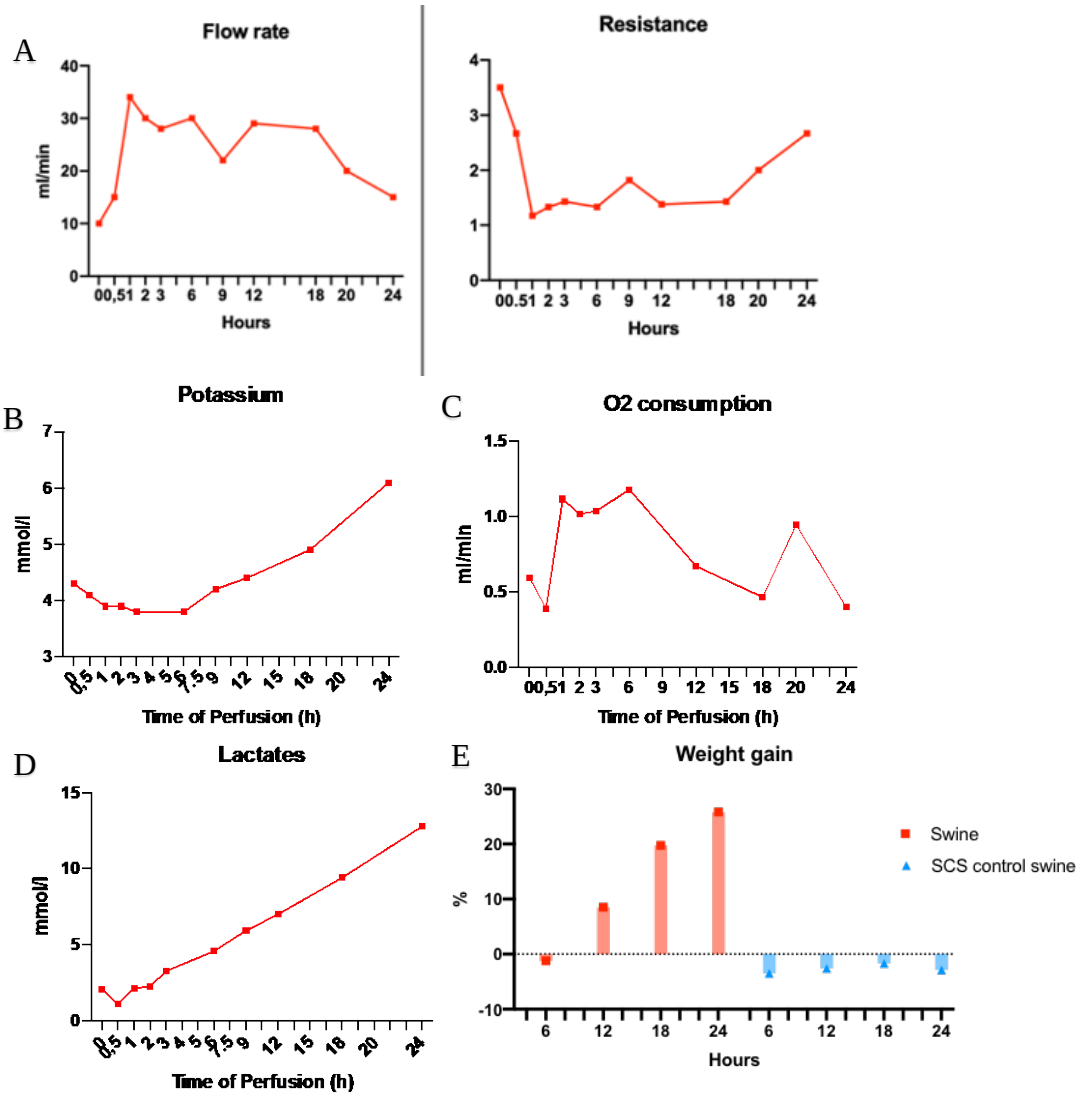

Figure 3. Overview of perfusion parameters. Panel A shows high vascular resistances at the beginning of the perfusion, then a plateau ending in an increase from 18 hours of perfusion until the 24th hour. Panel B shows stable potassium levels, a marker of cellular injury, in the outflow perfusate until 6 hours and then a linear increase. Panel C shows an increase in oxygen comsumption during the first 6 hours of perfusion. Panel D shows a linear increase in lactate levels, indicating sustained hypoxia. Panel E demonstrates an increase in edema in the perfused limb throughout the experiment compared to essentially no changes in the control SCS limb.

Deep and superficial muscle biopsies and perfusate samples were taken at T0, T6h, T9h, T12h, T18h, T24h. Analysis are still pending due to lab shutdown during the COVID 19 crisis. With the preliminary findings from this first swine partial hindlimb perfusion, we determined the necessity of replacing some of the recirculating perfusate to remove accumulating electrolytes (K+ and lactate), and we moved to a system that is easier to customize for supercooling studies.

For the next experiment, swine partial hind limbs were procured during a terminal procedure in the Knight surgery operative room, with about 29 minutes of ischemia during recovery. Limbs were flushed with 100ml of heparinized saline at room temperature through a 14G angiocatheter in the femoral artery. Experiment limb was placed in the perfusion system (Figure 4) that had previously been primed with 500ml of perfusate. The other limb was flushed with 100ml of cold HTK and stored at 4°C as a static cold storage control.

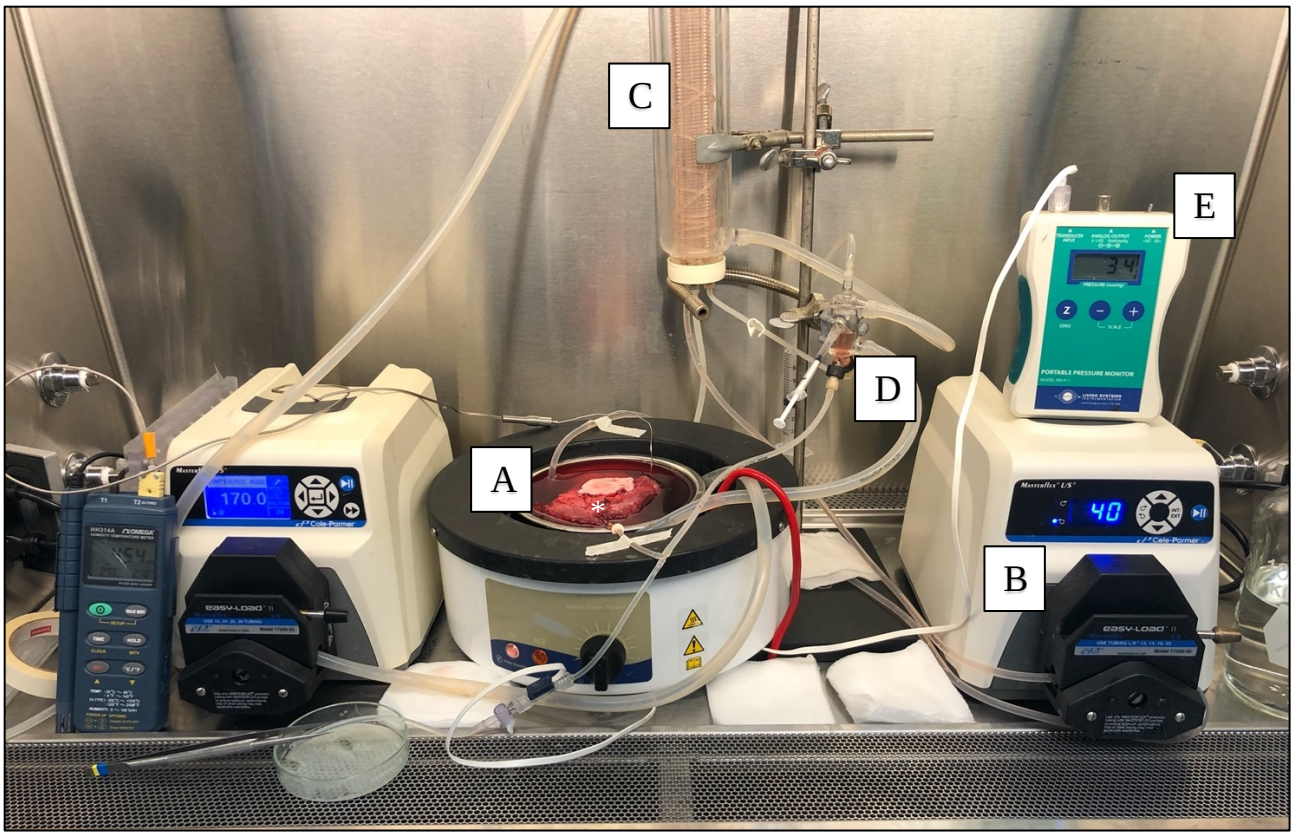

Figure 4. Continuous flow SNMP system. The perfusion system used for this experiment includes a basin (A), perfusion pump (B), an oxygenator (C), a bubble trap (D) and a pressure sensor (E). Temperature was monitored to remain at 21°C in the basin. The limb is attached to the inlet tubing through a 14G angiocatheter in the fermoral artery (white *). The venous outflow drains in the basin and the perfusate recirculates in the closed circuit.

The experiment was terminated at 18 hours for high levels of lactate and potassium and low pH despite perfusate exchanges at 2.5h and 6h. Data from both the continuous and pulsatile flow experiments are shown in Figure 5. Deep and superficial muscle biopsies and perfusate samples were taken at T0, T6h, T9h, T12h, T18h, analyses pending.

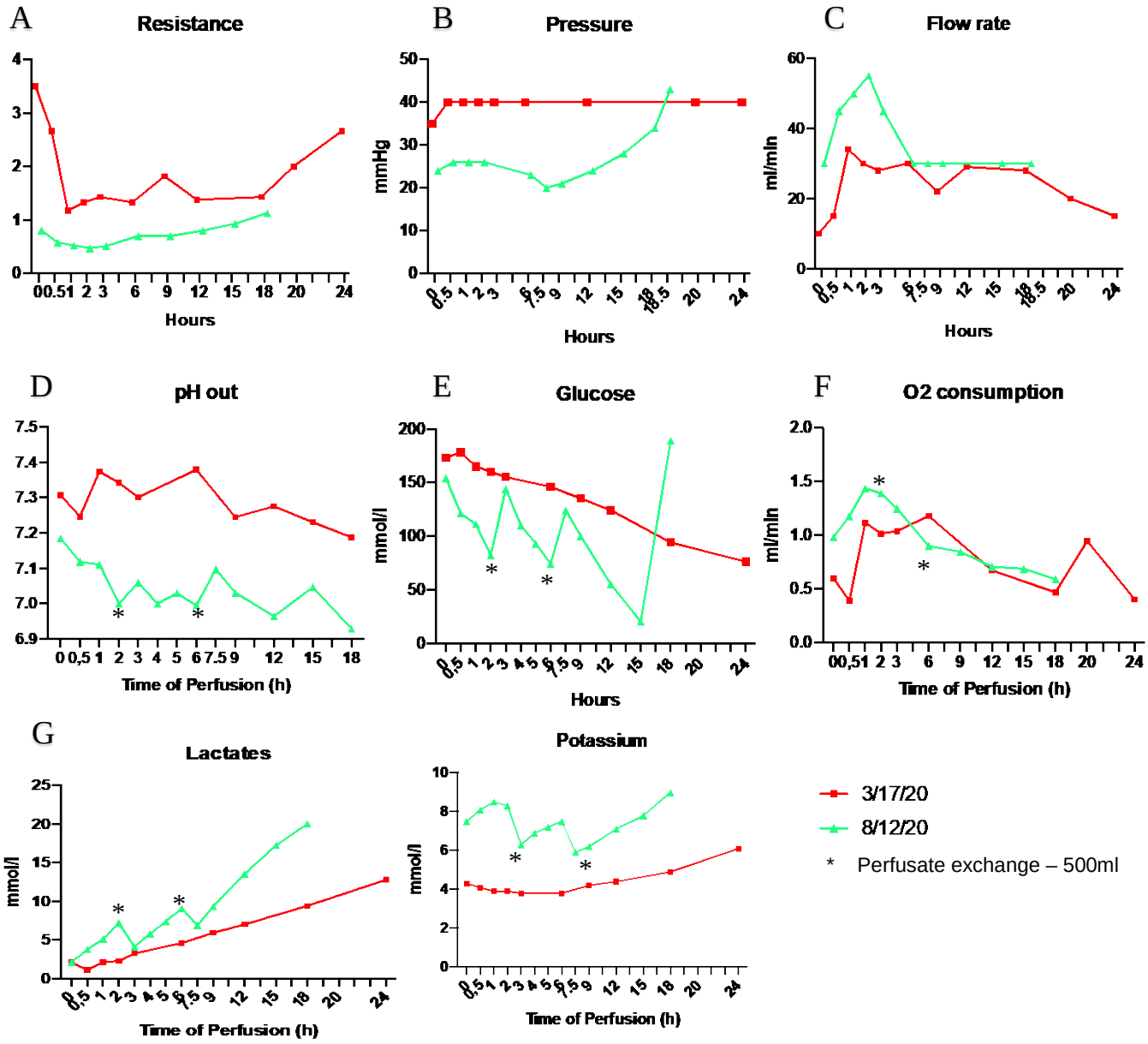

Figure 5. Overview of perfusion parameters. Panel A-C shows lower and more stable vascular resistances on the 24h SNMP on 8/12/20. Panel D shows an instable pH in the outflow perfusate that peaked after perfusate exchange. Panel E represents the high glucose consumption. Panel F shows higher O_2 consumption accordingly to higher flowrates in the beginning of the perfusion. Panel G shows increasing measures of lactates and potassium levels in the graft outflow.

The second system resolved resistance issues which was our main target. Some issues were identified; the main issue os thought to be due to a longer warm ischemia time during recovery and we are modifying the recovery protocol to add a cold flush. Increased rate of lactate and potassium accumulation is likely due to the smaller amount of perfusate used, which are not corrected for in the data presented; going forward we will increase the volume of perfusate and normalize to allow for

comparison. Finally, increasing lactate indicates insufficient oxygenation and we will reintroduce an oxygen carrier in the perfusate which we had experimented with in the rat system previously.

2) Supercooling experiments: Partial hind limbs were procured in a sterile manner from deceased pigs. The limbs were flushed with 70ml of Heparin saline and then 70ml of HTK. They were then preserved in HTK in a sterile bag where air was carefully removed. They were then placed in the chiller at -4°C. The limbs remained unfrozen for about 60 hours at -4°C (see Figure 6). However, decreasing the chiller temperature to -5°C led to the freezing of the HTK solution and the swine limb. Next experiments will include a cryopreservant loading phase with the machine perfusion.

Figure 6. VCA supercooling. Swine partial hind limb stored in a sterile bag in HTK. Chiller set at -4C.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

One post-doctoral research fellow, one PhD candidate and three technicians were trained. Training topics included surgical techniques of partial hind limb harvest in a swine model (attending plastic surgeon Dr Lellouch and Vice Chair of MGH IACUC Mark Randolph), machine perfusion, supercooling and applied thermodynamics, as well as scientific writing, experimental design, and various data analysis techniques.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Due to the Covid19 situation: nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Due to Covid-19 situation survival transplants in large animals is extremely problematic; we will therefore focus on nonsurvival studies, with the key targe of identifying a rationally optimized perfusion protocol for swine VCA tissues. In parallel we will optimize cryopreservative agents (CPA) for swine VCA tissues and identify the limits of supercooling. We will then evaluate the limits of supercooled storage of swine VCAs, likely in an ex vivo setting.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The key accomplishment is the scale up of machine perfusion to swine VCA, although further improvements are necessary. We have also demonstrated successful supercooling of swine limbs, which is key since different specied can have proteins (especially membrane lipids) that can unexpectedly act as freezing nucleating agents.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

As an interdisciplinary project, the results are expected to have impact on the fields of plastic surgery, transplantation, biopreservation and medical systems engineering.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Licensing of patents previously developed in project W81XWH-17-1-0680, precursor to this project, are in discussion. We also expect new IP may result from this work, or alternatively data supporting prior patent application would be obtained.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to the COVID-19 crisis, the laboratory was shut down for 3 months. This led to delays in experimentations and delays are still expected in research animal and supplies shipping. In particular survival transplants remain unfeasible to perform at the moment. To accommodate them, we plan to use nonsurvival models until the situation normalizes.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing additional to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.
Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report yet.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report yet.

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

A novel protocol for limb machine perfusion was developed.

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: *Korkut Uygun*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: *Co-led the project*

Name: *Mehmet Toner*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: *Co-led the project*

Name: *Shannon Tessier*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: *Led optimization of perfusate additives and supercooling protocol*

Name: *Stephanie Cronin*
Project Role: *Research technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: *Assisted in perfusion monitoring and supplies ordering*

Name: *Peony Banik*
Project Role: *Research technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *4*
Contribution to Project: *Assisted in graft recovery and perfusion monitoring*

Name: *Casie Pendexter*
Project Role: *Research technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3*
Contribution to Project: *Lead Perfusionist*

Name: *Sinan Ozer*
Project Role: *Research technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: *Assisted in building of large scale perfusion devices*

Name: *Lynne Stubbefield*
Project Role: *Staff*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: *Supply and small equipment management*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

See attached Other support documentation. No effects on the effort in this project.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

See attached quad chart.

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

No additional document to report.