Hypothermic and Subnormothermic Ex-Situ Heart Perfusion: Examining Diverse Strategies and Their Effects on Heart Function Preservation

by

Guilherme Mainardi Aguiar da Silva

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Department of Surgery

University of Alberta

ABSTRACT

The number of people suffering from heart failure grows annually. For those people who have advanced heart failure and are eligible for a heart transplant, this is the gold standard procedure to treat them. Allowing not only an improvement in quality of life but also an increase in survival.

However, the number of heart transplants performed per year in Canada and around the world is smaller than the number of people on the waiting list for a donated organ. In addition, one of the determining factors for this deficit between donated organs and transplants performed is the current method of organ preservation.

Ex-situ heart perfusion (ESHP) has proven to be a more effective method than the current one, static cold storage (SCS). However, there are different types of ESHP, being the hypothermic and subnormothermic the least complex in relation to normothermic for several reasons that will be addressed in this thesis.

The creation of protocols to make ESHP uniform are essential for the dissemination of this method of organ preservation. Since this method can extend the preservation time prior to transplantation, it can improve preservation and hence increase the number of organs available for transplantation.

The first study presented evaluated the ideal temperature to be applied during hypothermic ESHP using UW machine perfusion solution (UWMPS). It was seen that the lowest temperature studied was the one that obtained better heart function preservation after 12 hours of perfusion. The results were compared to the current preservation method.

The second study sought to optimize UWMPS with the aim of enhancing heart function preservation. Two modifications were applied in the perfusate studied, the addition of amino acids and the addition of some ions (as these components are presented in Somah). The results obtained in this study were compared with UWMPS as a control.

The results presented in these two studies can be used to guide new studies and build new protocols for ESHP. This strengthens the use of ESHP as a gold standard method and increases the number of heart transplants performed.

PREFACE

This thesis is an original work by Guilherme Mainardi Aguiar da Silva. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, AUP:00000943. The research shown in this thesis is intended to be submitted in peer reviewed journals as described below.

Chapter 2: **EVALUATION OF TARGET TEMPERATURE ON EFFECTIVENESS OF MYOCARDIAL PRESERVATION DURING HYPOTHERMIC MACHINE PERFUSION** - Guilherme Mainardi Aguiar da Silva; Mitchell J. Wagner; Sanaz Hatami; Parham Hassanzadeh; Xiuhua Wang; Benjamin A. Adam; Jayan Nagendran; Darren H. Freed.

Chapter 3: **EVALUATION OF MODIFIED UNIVERSITY OF WISCONSIN MACHINE PERFUSION SOLUTIONS FOR SUBNORMOTHERMIC EX-SITU HEART PERFUSION: A COMPARATIVE STUDY** - Guilherme Mainardi Aguiar da Silva; Mitchell J. Wagner; Sanaz Hatami; Parham Hassanzadeh; Xiuhua Wang; Jayan Nagendran; Darren H. Freed.

ACKNOWLEDGMENTS

First of all, I would like to thank my supervisor Dr. Darren H. Freed who supported and guided me during these two years of research. His admirable mentorship and enthusiasm for training other students to achieve their best, it is absolutely captivating. I am extremely grateful for his dedication in my medical career. I've learned a lot from his expertise in heart transplant, and ex-situ heart perfusion

I would like to thank my committee members Dr. Jayan Nagendran and Dr. Gopinath Sutendra, for all their support and feedback on my master's research. I'd also thank Dr Benjamin Adam for his considerate collaboration in the lab's research.

Thank all my colleagues who helped me during the years dedicated to this master's thesis. Including the laboratory manager Dr. Xiuhua Wang, Dr. Sanaz Hatami, Dr. Sayed Himmat, Mitchell Wagner, Parham Hassanzadeh and many others who have contributed to my work and progress over these years.

I am thankful to the University of Alberta, especially to the Department of Surgery and Ray Rajotte Surgical Medical Research Institute (RRSMRI). I'd like to thank Katie-Marie Buswell-Zuk and Ryan Edgar from RRSMRI. I also thank the Canadian Institutes of Health Research, the University Hospital Foundation and Natural Sciences and Engineering Research Council of Canada for their financial support that allowed me to undertake this work.

Finally, I would like to thank my wife, Mariane Mainardi, and my son, Gabriel Mainardi, for supporting me patiently during these years. I also dedicate this work to them and to my parents who have supported me during my medical studies and in my educational achievements.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF ABBREVIATIONS

ACC: American College of Cardiology

AF: atrial fibrillation

AHA: American Heart Association

ANOVA: Analysis of Variance

Ao: Aorta artery

AoP: Aortic pressure

CAD: Coronary artery disease

CBF: Coronary Blood Flow

CI: Cardiac Index

CS: Coronary Sinus

CT: computed tomography

CVR: Coronary Vascular Resistance

DCD: Donation after circulatory death

dP/dt max: Maximum dP/dt

dP/dt min: Minimum dP/dt

ESHP: *Ex-Situ* Heart Perfusion

ESC: European Society of Cardiology

HF: Heart failure

HFSA: Heart Failure Society of America

HOP: Hypothermic oxygenated perfusion

HNOP: Hypothermic non-oxygenated perfusion

HT: Heart transplant

IVC: Inferior vena cava

LAP: Left Atrial Pressure

LAF: Left Atrial Flow

LVAD: Left ventricular assist device

LVEF: Left ventricular ejection fraction

LVSW: Left Ventricular Stroke Work

MCS: mechanical circulatory support

NDD: Neurologic determination of death

NOP: Normothermic oxygenated perfusion

NYHA: New York Heart Association

PET: Positron emission tomography

RPM: Rotations per minute

SCS: Static Cold Storage

SNOP: Subnormothermic oxygenated perfusion

SVC: Superior vena cava

T0, T1....T11, T12: Preservation Time in hours.

UWMPS: University of Wisconsin Machine Perfusion solution

UW: University of Wisconsin solution

VAD: Ventricular assist device

WIT: Warm ischemia time

CHAPTER 1- INTRODUCTION

1.1 HEART FAILURE

Heart failure (HF) is a complex end stage clinical syndrome arising from any structural or functional impairment of blood ejection (Systolic HF) or ventricular filling (Diastolic HF), following the heart's incapacity to pump enough blood to supply organs. It is characterized by different signs and symptoms resulting from cardiac output reduction and/or high filling pressures during exertion or rest, for example, fatigue, dyspnea, renal failure and others[1,2].

There are different HF classifications and they vary according to the patient's symptoms, the symptoms' settlement time (abrupt or progressive) and the left ventricular ejection fraction (LVEF). Classifying correctly the HF stage is extremely important for choosing the appropriate treatment. There is a variety of therapeutic interventions from medicines to more complex ones, for instance left ventricular assist devices.

As presented in the 2022 HF guideline AHA/ACC[2-4] HF can be classified in four categories, according to the disease progression. Including: Patient at risk to develop HF (Stage A); patient with structural cardiac disease or evidence for increased filling pressures or risk factors, without symptoms (Stage B: pre-HF); patient with structural heart disease and previous or current symptoms (Stage C: Symptomatic HF); patient with symptoms that restrict with daily life activities and with recurrent hospitalizations (Stage D: Advanced HF) (Figure 1.1)[4].

The New York Heart Association (NYHA) classifies patients according to their symptoms and functional capacity. NYHA class I: patient with no limitation during ordinary physical activity. NYHA class II: patient with slight limitation during ordinary physical

activity (fatigue, palpitation, dyspnea) and comfortable at rest. NYHA class III: patient comfortable at rest, however, presenting marked limitations during less than ordinary physical activity. NYHA class IV: patient is unable to carry on any physical activity without discomfort. Patients NYHA class III and NYHA class IV presenting worsening clinical conditions and symptoms, more frequent hospitalizations and a higher mortality risk than NYHA I and II (Figure 1.1)[4].

Despite the HF therapeutic improvements, this syndrome is an increasing health and economic burden for the world, and heart transplantation is still the gold-standard treatment for those patients with advanced end-stage HF[5-7]. In the United States (USA), the total number of deaths caused by HF rose from 275,000 in 2009 to 310,000 in 2014, showing that HF is still remains as a severe pathological condition affecting, according to the AHA, 6 million Americans ≥20 years old from 2015 to 2018, with an estimated total cost of 30.7 billion dollars[8,9]. It is estimated that >8 million people ≥18 years of age will be affected, rising by 46% from 2012 to 2030, with a projection to increase from 2.4% in 2012 to 3.0% in 2030 the total number of people with HF. In a population over 55 years of age, the incidence of HF was around 1 million people in 2014, hitting more women than men[9].

In Canada, HF affects 750,000 people and it is responsible for the leading cause of hospitalization. More than 100,000 people are diagnosed each year with HF and approximately $2.8 billion are spent per year on HF health care. The readmission rates have increased 20%, while the mortality rate has increased 30%, and these numbers expect to escalate as the population ages[10-12].

1.2 HEART TRANSPLANT

In 1905, at the University of Chicago, Carrel[13] and Guthrie performed the first heterotopic cardiac transplantation in dogs. And until 1933 little more was done, Mann[14] and colleagues performed a successful transplantation of the heart into the neck of dogs. In the mid 1950's, Demikhov[15] and Sinitisin (from Russia) placed the donor heart in the chest, but still in a heterotopic locus.

After that, Dr Shumway and Lower, at Stanford, started to perform orthotopic heart transplantation in dogs with cardiopulmonary bypass. The preservation applied to the donor heart was by immersion for 5 minutes in 4ºC saline, and they reported their experience in 1960 with a 6-21 days of animal survival without immunosuppression. In 1965, a pivotal contribution of Lower and colleagues showed that a combination of azathioprine and methylprednisolone achieved a survival of 250 days[14,16].

On December 3 of 1967, the first human-to-human heart transplant was performed in Cape Town - South Africa, by Dr Christiaan Barnard[2,17]. Not long after that, other surgeons around the world also performed a heart transplant procedure.

Although heart transplantation is the gold-standard procedure, it remains limited by the shortage of donor organs. One of the reasons for that is the current organ preservation method, prior to transplantation, the SCS. This method limits the long-distance organ transportation, once it allows up to 4-6h of preservation[18,19].

According to the International Society for Heart and Lung Transplantation (ISHLT) and United Network of Organ Sharing (UNOS), it is estimated that the survival of patients undergoing heart transplantation is greater than 12 years, while the survival of patients in advanced stages of HF is less than 2 years[2].

In order to increase the donor pool, many innovations have been done in organ procurement and organ preservation[20], and also advancements in left ventricular assist devices. These devices are only applicable as bridge-to-transplant or bridge-to-decision or as destination therapy. The reason for that is the reduced survival of patients with those devices which after 3 to 4 years begins to be lower than the risk of death[2].

1.3 TYPES OF DONATIONS

1.3.1 NEUROLOGIC DETERMINATION OF DEATH (NDD):

The donation after neurologic determination of death (NDD) is determined by irreversible loss of brain functions[21]. For instance, traumatic head injury, stroke and intracerebral hemorrhage. In response to trauma, it occurs edema and intracranial pressure increase, increase vascular resistance, then reduce blood flow getting to the brain. So, decreasing cerebral perfusion which can result in transtentorial herniation with brainstem compression and determining brain death[21-23].

The majority of heart donations are retrieved from brain death donors, which is the only accepted in most countries. The United Kingdom, Australia, the United States of America, the Netherlands and other countries have been accepting donations after circulatory death (DCD) transplantation. However, those DCD hearts are still the minority of HT cases in those countries[24-26].

In Canada, HT relies exclusively on NDD. Because it is reliable and cheaper to do it, when compared to DCD hearts that need to be preserved in perfusion devices. In contrast, there are still many patients on the waiting list for a heart transplant in Canada, and some patients who die waiting for a HT. Therefore, it is important to balance the cost effectiveness of using the perfusion devices to expand the donor pool and to reduce the

disparities between supply and demand, diminishing the number of deaths on a HT waiting list around the world[27].

According to the Canadian Organ Replacement Register (CORR), in 2022 there were 153 HT performed across the country. While in Alberta, a total of 28 single HT (7: Pediatric and 21: Adult), and 3 combined HT (2: Heart-liver and 1: Heart-kidney)[27]. Remaining in the national waiting list 133 patients and 8 of them died waiting for a donated organ (Figure 1.2)[27]. All of those HT were using NDD heart donors[27].

1.3.2 DONATION AFTER CIRCULATORY DEATH (DCD):

The DCD term refers to donation of organs after permanent circulatory arrest. Which differentiates from NDD where neurologic parameters are assessed to determine the death. The DCD is based only on cardiopulmonary criteria[24-26].

The DCD has been advocated as an alternative to increase the availability of organs donated for transplantation in order to reduce the imbalance between supply and demand. However, there are some obstacles for its worldwide implementation, and to be compared and accepted as much as NDD[24-26].

A standoff period or warm ischemic time (WIT) is mandatory for DCD and it varies among different centers. There are side effects related to this WIT on cardiac function, increasing the primary graft dysfunction, challenge in heart procurement, resuscitation, preservation and transportation prior to a HT[23,28-31].

The DCD can be divided into controlled and uncontrolled. The categories I, II and V are uncontrolled DCD, which means the warm ischemic injury is already established when the potential donor is acknowledged. Consequently, this type of DCD is reserved

to kidney transplantation, closer or within transplant centers. While the categories III and IV are controlled DCD that means patients are usually those who do not fill the neurological criteria for brain death, however, they have complex and severe brain injuries that justify withdrawal of life-sustaining cardiorespiratory treatments after carefully discussing it with the patient's family (Table 1.1)[30].

1.3.3 MARGINAL DONORS

Certainly, the most desirable goal is to have only optimal donors, however, it is not the reality. Optimal heart donors are considered the donors younger than 65 years old, no previous heart disease (angina pectoris, myocardial infarction, prior cardiac surgeries, moderate or severe valve disease, cardiomyopathy and important arrhythmias), absence of chest trauma, cold ischemia time less than 2 hours, low or none doses of inotropes[32,33].

Owing to shortage of heart donors in optimal conditions limiting the number of HT done around the world, it has been suggested having marginal donors. They should help to suppress the demand for organs. These marginal donors are defined with the following criteria: age over than 60 years, reduced LV function (Ejection fraction around 40-50%), LV hypertrophy (septal thickness greater than 14 mm on echocardiogram evaluation), focal lesion of the coronary artery, and significant heart valve disease[32,34].

Although hearts from older donors present an earlier incidence of coronary disease than hearts from younger donors at 3 years of follow-up, after 5 years there is no difference between them. It has been seen that donor hearts with moderate coronary arteries do not affect survival after transplantation. However, when heart donors have 2 or more coronary arteries compromised it seems to be related with higher early graft

failure. It has been seen that donor hearts with moderate coronary arteries do not affect survival after transplantation[32-34].

Additionally, heart valve disease was considered an exclusion criteria for HT before. Some valve diseases can be easily repaired before the organ implantation. Nevertheless, it should be individualized, because some heart valve diseases are considered an absolute contraindication for heart transplantation, aortic stenosis with significant left ventricular hypertrophy, for instance[32-35].

Another point to consider is the use of marginal donors, is the development of organ perfusion devices. These devices allow not only heart preservation for a longer period of time than SCS but also enables recovery of those borderline hearts. Therefore, it offers plenty of time to evaluate which medical center should receive this marginal heart after recovering them properly[34-36].

1.3.4 XENOTRANSPLANTATION

The first-in-human heart xenotransplant occurred in 1964 at the University of Mississippi, when a man received a chimpanzee heart[37]. The man did not survive the surgery. The next one was performed at Loma Linda University in 1984, because there was not any human heart available matching size, and the patient lived for only 20 days. Recently, on January 7, 2022, in Baltimore, at the University of Maryland, the patient died 2 months later[38,39].

There are still many questions to be answered prior to xenotransplantation implementation on a large scale, clinical and legal challenges. For instance, immune

responses[37,40], which patients will receive those hearts and if patients should decide between human or animal organ[39-41].

1.4. TYPES OF HEART PRESERVATION

1.4.1 STATIC COLD STORAGE (SCS)

Static cold storage (SCS) is a widely used method for preserving donor organs, including hearts, prior to transplantation. It involves cooling the heart to low temperatures (usually around 4 degrees Celsius) to slow down cellular metabolism and reduce the demand for oxygen, thereby extending the viability of the organ outside the body[42-45].

In heart transplantation, the process typically involves removing the donor heart from the donor's body, flushing it with a cardioplegic solution to remove blood and introduce a protective solution, and then placing it in a sterile container filled with a cold preservation solution. This container is then placed in an ice bath or a refrigerated chamber for transportation to the recipient hospital[42-45].

A successful HT depends on many important steps, one of them is the heart preservation. The SCS gives usually up to 4-6h of preservation from the moment the donor aorta is clamped to the heart implantation. Therefore, transplants that are performed longer than 6h between the procurement and the implantation, they are related to higher early primary graft dysfunction[46,47].

Recently developed devices were proposed for heart preservation improvement in SCS, SherpaPak for instance. It is a portable organ preservation system designed to maintain the viability of donor hearts during transport from the donor to the recipient. This

system has demonstrated reduction in cold injury which occurs in SCS by the uneven cooling of the organ[42,48].

The SherpaPak device helps minimize ischemic injury to the donor heart by maintaining the organ at a constant low temperature, without fluctuations or avoiding dropping temperature below 4ºC, which can cause cold ischemic injury. Although it has reduced the risk of severe primary graft dysfunction compared to SCS, it did not prolonged the organ preservation time[49-53].

Therefore, the SCS has been the standard method for heart preservation for many years due to its simplicity, cost-effectiveness, and proven efficacy in maintaining organ viability during transport. However, it does have limitations, such as a relatively short preservation time compared to other methods like machine perfusion, which provides continuous oxygenation and nutrient delivery to the organ. As a result, research continues to explore ways to improve organ preservation techniques to further extend preservation times and enhance transplant outcomes[42-45].

In static cold storage (SCS) for organ preservation, including heart transplantation, cardioplegic solutions play a crucial role in maintaining the viability and functionality of the organ during storage and transport. These solutions are specially designed to provide the necessary nutrients, electrolytes, and buffers while minimizing cellular damage caused by ischemia (lack of blood flow) and reperfusion (restoration of blood flow)[42-45].

There are different solutions and many components in each preservation solution used for SCS. For instance, electrolytes (including sodium, potassium and calcium ions: essential for maintaining cellular membrane potential and various cellular functions), buffers (bicarbonate and phosphate which maintain the pH of the solution within a

physiological range, critical for preserving cellular function), colloids (albumin and hydroxyethyl starch maintain the oncotic pressure and prevent edema formation within the organ), osmolytes (mannitol or lactobionate regulating cell volume and reduce cellular swelling during cold storage), antioxidants (glutathione and superoxide dismutase reducing oxidative stress and preserving cellular integrity), energy substrates (adenosine triphosphate - ATP, glucose or pyruvate providing energy sources for cellular metabolism during storage), preservation agents (like in Celsior or University of Wisconsin solution: these two solutions are specifically formulated to minimize cellular damage during cold storage and have been extensively studied and validated for organ preservation)[54,55].

The exact composition of perfusate solutions may vary depending on factors such as the type of organ being preserved, the specific requirements of the transplant center, and ongoing research and development in the field. Optimizing the composition of perfusate solutions is an active area of research aimed at improving organ preservation techniques and transplant outcomes[54,55].

Several preservation solutions have been developed and optimized for use in SCS, each with its unique formulation and rationale aimed at minimizing ischemia-reperfusion injury and preserving organ viability. There are different preservation solutions used in heart transplantation with distinct transplant outcomes.

The University of Wisconsin (UW) Solution is one of the most widely used preservation solutions for heart transplantation. It contains a combination of electrolytes, colloid (hydroxyethyl starch), impermeants (lactobionate, raffinose), and antioxidants (glutathione, allopurinol). These components help to maintain cellular integrity, minimize

edema, scavenge free radicals, and reduce inflammatory responses during cold storage[54,55].

The UW solution has demonstrated good outcomes in heart transplantation, with high graft survival rates and relatively low rates of primary graft dysfunction (PGD). Studies have shown comparable or slightly superior outcomes compared to other preservation solutions[54,55].

Celsior solution is another commonly used preservation solution for heart transplantation which also contains electrolytes, colloids (hydroxyethyl starch), impermeants (lactobionate, mannitol). However, it has lower potassium content compared to the UW solution. The Celsior solution aims to provide effective cellular protection and reduce oxidative stress during cold storage[54,55].

Studies comparing Celsior solution with UW solution have shown similar post-transplant outcomes in terms of graft survival and incidence of PGD. Some studies have suggested a potential advantage of Celsior solution in reducing myocardial injury and improving early graft function[54,55].

The Histidine-Tryptophan-Ketoglutarate (HTK) solution is an alternative preservation solution used in heart transplantation. It contains electrolytes, amino acids (histidine, tryptophan), and ketone bodies (ketoglutarate). HTK solution aims to provide intracellular protection, maintain energy metabolism, and reduce cellular swelling during cold storage[54-57].

The HTK gives up to 4 hours of ischemic time. Studies comparing HTK solution with UW solution or Celsior solution have shown comparable outcomes in terms of graft

survival and incidence of PGD. The HTK may have advantages in terms of ease of use and lower cost[54-57].

Euro-Collins Solution was one of the earliest preservation solutions developed for organ transplantation containing electrolytes and high concentrations of glucose. This solution has been associated with higher rates of PGD and inferior post-transplant outcomes compared to newer preservation solutions. It was most used for abdominal organs transplantation[54,55].

Independently of the preservation solution used in SCS, the limited period of time to complete the HT, limits organ transportation to remote places, narrows the possibilities to find a compatible recipient. For long distant safe transportation it is crucial to have an ideal organ preservation which maintains the organ's viability from its retrieval, during storage and transportation, to transplantation into the recipient. Therefore, because of the many possibilities for organ preservation improvement, studies have been done developing dynamic organ perfusion[47].

1.4.2 EX-SITU HEART PERFUSION (ESHP)

There are many advantages of ESHP over SCS. The ESHP provides nutrient-rich solution, oxygen and removes metabolic waste from the organ. Additionally, it allows organ function assessment and repair marginal or DCD hearts. Improving the out-of-body preservation period, consequently, facilitates and makes possible the organ transportation to remote locations, giving enough time for better match between donated organ and the recipient[41,47,58,59].

This concept was first described in 1886 by Ludwig and Cyon. Langendorff developed a system capable of reanimating the heart of mammals in an isolated way through cannulation of the ascending aorta, perfusing the coronary arteries. Katz was the one who modified and facilitated the realization of a series of studies[44,47].

In 1967, Neely and Morgan developed a working heart model in which both ventricles were filled and pumped perfusate. The modified Langendorff and working heart models' concepts were incorporated into modern ESHP machines, which were made to preserve, evaluate and even recover donated hearts from the moment of retrieval until the moment of implantation[47,60-62].

This preservation method can be divided into different types according to the temperatures applied during perfusion. In the normothermic ESHP method, the hearts are preserved at 37ºC, while in the hypothermic method the temperature varies and usually the organ is preserved in a range from 4-12°C. Additionally, there is a subnormothermic ESHP method that preserves hearts at room temperature (around 22°C). Each one of these methods have their own advantages and disadvantages[47,63].

1.4.2.1 NORMOTHERMIC OXYGENATED PERFUSION (NOP):

In this ESHP method, the heart remains beating during the entire preservation period. The Transmedics Organ Care System is the only device used in clinic practice for NOP of the donor heart. It was associated with excellent patient and graft survival as well as significantly better patient survival at six months post-transplantation[43-45].

The NOP permits organ preservation closer to the physiological state of the heart. Which leads to the biggest advantage of normothermic oxygenated perfusion (NOP), because it allows heart function assessment during perfusion[47,64].

Another advantage over hypothermic ESHP is that it avoids cold ischemia injury related to lower preservation temperatures. Furthermore, there are other advantages but only when compared to SCS preservation methods. Once ESHP provides oxygenated perfusate and substrates to energy production, it also removes metabolic waste[47,63]. Being the normothermic ESHP method more appropriate to be used in DCD hearts, compared to hypothermic ESHP. This is because it allows organ recovery, facilitates the application of therapies in the donated hearts[47,63,64].

The disadvantages of NOP are almost the same for every ESHP method. The ESHP is expensive, complex and needs additional training to be done. Especially for NOP which needs a perfusate with blood that can be hard to obtain[47,63,64].

The dispute, between the procurement teams, for blood from the donor to run a normothermic ESHP is something that needs to be taken into consideration. Once there will be higher demand for a sufficient quantity of blood to prime the circuit, also there is no guarantee there will be blood from the donor or from the blood bank. Another point is to the blood quality to be used in the normothermic ESHP machine, once it can affect the organ preservation results[47,63-65].

Consequently, the NOP system has been used to facilitate clinical distant procurement of DCD heart transplantation programs, with excellent short-term outcomes, comparable to those of NDD transplantation. However, much still needs to be learned

about the optimal perfusion condition for NOP, because some experiments demonstrated that cardiac function declined during ESHP[47,63-67].

1.4.2.2 SUBNORMOTHERMIC OXYGENATED PERFUSION (SNOP):

Subnormothermic oxygenated perfusion (SNOP) is an emerging technique in heart transplantation where the donor heart is preserved and assessed outside of the body at temperatures below normal physiological levels. A middle ground between HOP and NOP (20-34°C), the subnormothermic method (SNOP) provides longer preservation periods and lower cellular metabolism at low temperatures. The goal of SNOP is to maintain organ viability while reducing metabolic demand, thereby extending the preservation time and allowing for thorough assessment and optimization of donor hearts prior to transplantation[44,47,68].

While SCS and HOP expose the organs to a rapid temperature decrease during storage and a quick increase during organ reimplantation. The SNOP benefits from a lower metabolic demand, while it can remove oxygen carriers from the system, simplifying the ESHP procedure[44,63,69].

Some studies have shown different perfusates composition, with and without blood addition. Oxygenated blood-based solutions have also been used in SNOP. These solutions aim to provide physiological oxygen levels to the organ during perfusion, potentially enhancing cellular metabolism and preserving viability[44,63,70].

The Steen solution is an example of extracellular perfusate which is commonly used in ex-situ organ perfusion and contains electrolytes, colloid (dextran), and albumin. It aims to provide optimal conditions for organ preservation by maintaining oncotic

pressure and supporting cellular metabolism during perfusion. The UW solution which is used in SCS and HOP, has also been investigated for use in SNOP[44,71].

A novel experimental cardioprotective solution called Somah was created specifically to satisfy the energy needs of coronary endothelium and cardiomyocytes. Previous research by Lowalekar and colleagues showed that using Somah during static storage at subnormothermic conditions (21˚C) improved functional recovery in experimental NDD and DCD heart models. Nevertheless, this solution had not been used in complete transplantation studies[72-74].

The Somah solution represents a promising advancement in organ preservation techniques, particularly for SNOP in heart transplantation. By providing oxygenated perfusion at subnormothermic temperatures, Somah solution may offer advantages over traditional static cold storage or hypothermic perfusion methods by allowing extended and improved donor heart preservation prior to transplantation[72-74].

The presence of creatine in Somah solution may have multiple effects on the heart. It functions as an antioxidant, prevents heart failure, and is involved in energy metabolism, protein synthesis, membrane stabilization, and intracellular buffering. To aid in the long-term production of ATP, creatine in the orotate form is present[72-74].

Orotic acid is a crucial component of phospholipids and RNA, which are the building blocks of tissue repair and synthesis. In addition to being ergogenic, orotic acid increases the uptake of glucose and the synthesis of ATP by acting as a pyrimidine intermediate in the pentose-phosphate shunt[75-77].

Carnosine, which is made up of l-histidine and β-alanine, acts as an antioxidant, antiglycating, aldehyde-quenching, and metal-chelating agent in addition to providing

cardioprotection through intracellular buffering and calcium regulation. Levocarnitine, also known as l-carnitine, is an amino acid that promotes the translocation of fatty acids across the mitochondria, improves the metabolism of glucose and carbohydrates in hypoxia and ischemia, stimulates the generation of anaerobic ATP necessary for maintaining membrane function during organ storage, and functions as an antioxidant during reperfusion[75-77].

Nitric oxide and urea cycles are mediated by citrulline, when combined with malate, it has been demonstrated to decrease fatigue and enhance muscle performance through increased ATP production in antiasthenic treatment. Additionally, it can enhance ATP and creatine phosphate recovery while lowering ammonia and lactic acid. The components of Somah also have additional organ-specific, synergistic effects, such as breaking down and/or synthesizing carnosine, citrulline malate, and creatine orotate into β-alanine, histidine, malate, and fumarate, which then enter the Krebs cycle to produce more ATP. The nitric oxide cycle is subsequently enhanced by the conversion of histidine to glutamine, which chelates ammonia (from the transaminase reactions) to form carbamoyl phosphate[75-77].

Precursor of ATP synthesis, adenosine in Somah solution preconditions by activating protein kinase C and mitochondrial potassium channels, and vasodilates blood vessels while antagonistically acting on catecholamines. Moreover, adenosine keeps the heart polarized and/or hyperpolarized, which lessens ionic imbalance and the corresponding degenerative changes in the stored heart[75-77].

Selecting the best perfusate solution for SNOP involves considering various factors such as its ability to support organ function, minimize ischemia-reperfusion injury,

and preserve viability during the perfusion process. While there isn't a single universally agreed-upon "best" perfusate solution for SNOP, several options have been investigated[44,63,70].

1.4.2.3 HYPOTHERMIC OXYGENATED PERFUSION (HOP):

Hypothermic oxygenated perfusion (HOP) is an advanced organ preservation technique used in transplantation to maintain organs outside of the body at low temperatures while continuously perfusing them with a preservation solution. This method helps to mitigate ischemia-reperfusion injury and improve organ function and outcomes post-transplantation[47,68].

Studies vary from 4°C-12°C and have shown promising results. However, this method has not been the main focus of most research groups due to the lack of heart function assessment during the preservation period. Therefore, new studies were looking into ways of testing myocardial viability[47,58,63,78].

Throughout HOP, myocardial viability can be assessed through metabolic parameters, such as coronary vascular resistance (CVR), myocardial oxygen consumption (MVO2), oxygen extraction (OER), lactate metabolism and glucose extraction. Nonetheless, only the former two are associated with myocardial heart function preservation[58,63,78-80].

Similar to SCS, hearts preserved in the HOP method stay in a non-beating state. Although SCS minimizes the heart's oxygen and energy needs by preventing contraction and reducing temperature, HOP corrects a fundamental problem in SCS preservation by providing nutrient-rich, oxygenated perfusate. Consequently, fixing the ongoing metabolic

activities which force cells in the myocardium to adopt anaerobic respiration, leading to intracellular acidosis and energy stress[47,63].

The University of Wisconsin machine perfusion solution (UWMPS) is a perfusion solution used for HOP in various organs, including the liver, kidneys, and pancreas. The rationale behind UWMPS formulation lies in its ability to provide optimal conditions for organ preservation during machine perfusion[47,54,55,78].

It provides electrolytes which are essential for maintaining cellular function and osmotic balance; colloids (such as hydroxyethyl starch, help to maintain oncotic pressure and prevent cellular edema); impermeants (lactobionate and raffinose, help to reduce cellular swelling and stabilize cellular membranes); antioxidants (glutathione and allopurinol, scavenge free radicals and reduce oxidative stress, thus protecting cellular integrity); buffers (bicarbonate and phosphate buffers maintain physiological pH, crucial for preserving cellular function); energy substrates (adenosine, inosine, and ATP precursors, provide a source of energy for cellular metabolism during perfusion[54,55,78].

The continuous perfusion process allows oxygenation and better nutrient delivery to the organ compared to SCS, thereby potentially improving preservation outcomes. The UWMPS aims to minimize ischemia-reperfusion injury, reduce inflammation, and maintain organ viability during the preservation period[54,55,78].

Evidence supporting the use of UWMPS in various organs is robust. Studies have demonstrated its effectiveness in improving organ function, reducing rates of primary graft dysfunction, and enhancing transplant outcomes compared to SCS alone. For example, in liver transplantation, UWMPS has been shown to reduce rates of early allograft dysfunction and improve graft survival compared to SCS; in kidney transplantation, it has

been associated with better graft function and reduced delayed graft function compared to SCS; in pancreas transplantation, UWMPS has been shown to improve islet cell viability and function, leading to better outcomes post-transplantation[47,54,55,78].

Overall, UWMPS has become an integral part of organ preservation strategies, offering significant benefits in terms of organ function and transplant outcomes across various organs. Its widespread adoption underscores its effectiveness in improving the success of transplantation procedures[47,54,55,78].

1.5 SUMMARY AND OBJECTIVES

The ESHP can expand the donor pool once it extends the organ preservation period when compared to SCS. Once it provides nutrient-rich perfusate, it is able to recover marginal donor and DCD hearts; it is more appropriate for long distance transportation. Consequently, reducing the imbalance between organ supply and demand, also the time and the number of deaths on the waiting list.

That is the reason ESHP has been studied, but it needs to be improved in order to be more effective, standardized and easily reproducible to other centers. The HOP and SNOP are easier to be performed compared to NOP. During HOP and SNOP is needed a few or none intervention during the preservation period, a person with minimal training would be able to take care of the organ transportation between centers.

In this thesis we have evaluated the heart function after an extended period of ESHP (12 hours) in low temperatures. We have also studied the effects of temperature, oxygen supplementation and modifications in the perfusate solution in heart function preservation. The specific objectives for each chapter of this thesis are described below.

The aim of the first chapter of this thesis was to evaluate what is the best temperature for hypothermic ESHP in preserving heart function. Porcine hearts were perfused for 12 hours and then transferred to a custom made normothermic ESHP device to assess heart function.

Additionally, in the same chapter, we aimed to assess the importance of oxygen supplementation for hypothermic EHSP in preserving heart function. Also, comparing these heart function parameters results with SCS for 6 hours, hence examining if even without oxygen ESHP would be more effective than SCS.

The second chapter aimed to improve perfusate for subnormothermic ESHP, with the prime objective to make a perfusate as beneficial as Somah for this temperature method. Studies have been conducted using Somah as an ideal perfusate for this preservation method in preserving heart function. In this study, UWMPS was modified twice, the first time with amino acids, and the second with ions. All the results found were compared to Somah heart function parameters results.

Optimization of protocols and perfusates are crucial for ESHP development. In hypothermic and subnormothermic, there are a great number of questions to be answered for those methods' improvement.

The objectives of this thesis are summarized below:

1. Assess the differences between the temperatures for heart function preservation in 12 hours of Hypothermic ESHP compared to SCS for 6 hours.
2. Demonstrate the importance of oxygen supplementation in 12 hours of Hypothermic ESHP, and its impact in heart function preservation

3. Evaluate the effects of the amino acids and antioxidants addition into University of Wisconsin machine perfusion solution in heart function preservation during Subnormothermic ex-situ heart perfusion.
4. Assess the impact of ionic components addition into University of Wisconsin machine perfusion solution in heart function preservation during Subnormothermic ex-situ heart perfusion.

1.6 TABLES AND FIGURES

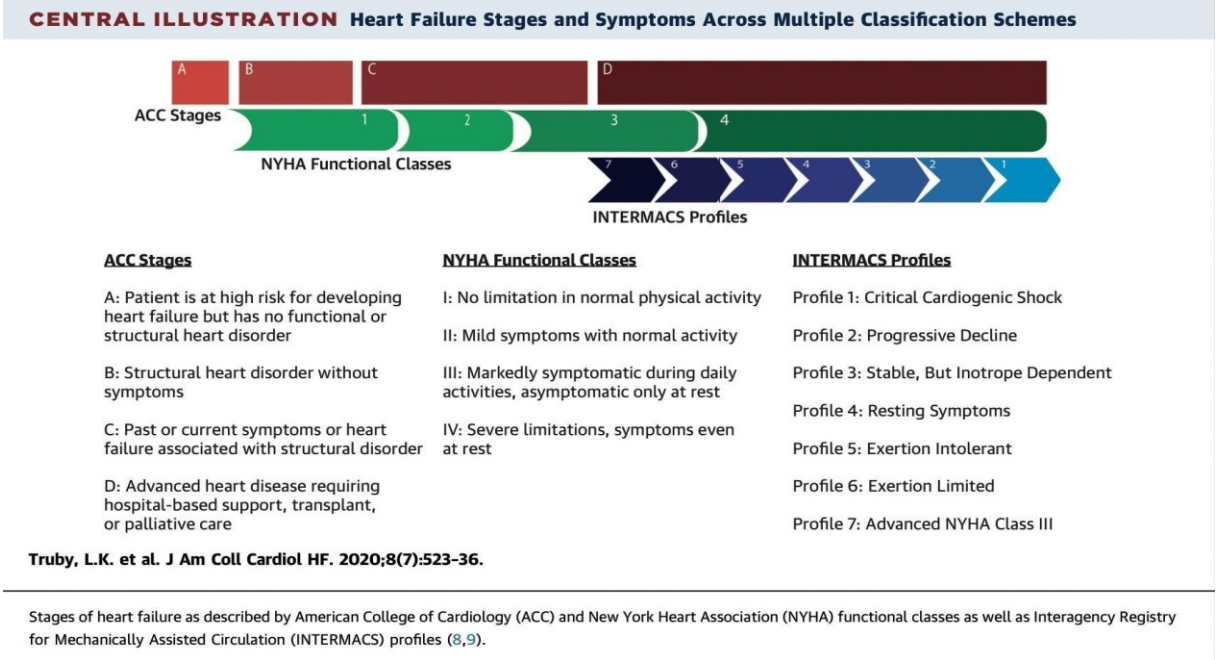


FIGURE 1.1: Heart Failure Stages and Symptoms Across Multiple Classification Schemes[4].

FIGURE 1.2: Heart transplants performed in Canada in 2022[27].

TABLE 1.1: Types of DCD - Modified Maastricht classification of DCD[30].

Category	Description	Type of DCD
I	Dead on arrival	Uncontrolled
II	Unsuccessful resuscitation	Uncontrolled
III	Anticipated cardiac arrest	Controlled
IV	Cardiac arrest in a brain-dead donor	Controlled
V	Unexpected arrest in ICU patient	Uncontrolled

CHAPTER 2

EVALUATION OF TARGET TEMPERATURE ON EFFECTIVENESS OF MYOCARDIAL PRESERVATION DURING HYPOTHERMIC MACHINE PERFUSION

Running Title: 12-hour Hypothermic Heart Preservation in a Pig model

Authors: Guilherme Mainardi Aguiar da Silva, MD[1]; Mitchell J. Wagner[1]; Sanaz Hatami, MD/PhD[1]; Parham Hassanzadeh[1]; Xiuhua Wang, PhD[1]; Benjamin A. Adam, MD[6]; Jayan Nagendran MD/ PhD[2,3,4,5]; Darren H. Freed, MD/PhD[2,3,4,5]

1 Department of Surgery, Faculty of Medicine, University of Alberta

2 Division of Cardiac Surgery, Department of Surgery, Faculty of Medicine, University of Alberta

3 Mazankowski Alberta Heart Institute, Edmonton, AB, Canada

4 Alberta Transplant Institute, Edmonton, AB, Canada

5 Canadian Donation and Transplantation Research Program, Edmonton, AB, Canada

6 Department of Laboratory Medicine and Pathology, University of Alberta

Corresponding Author's Complete Contact Information:

Each author's ORCID#

Darren H. Freed 0000-0002-6239-1444
Jayan Nagendran 0000-0001-5827-9823
Guilherme Mainardi - 0009-0006-3293-0976
Mitchell J. Wagner - 0000-0002-5883-6340
Sanaz Hatami 0000-0002-6792-6620
Parham Hassanzadeh - no ORCID ID
Xiuhua Wang- no ORCID ID
Benjamin A. Adam – 0000-0003-1908-1739

ABSTRACT

BACKGROUND: Ex-situ heart perfusion (ESHP) has been proposed as an optimal method for preserving donated hearts prior to transplantation. Hypothermic oxygenated perfusion (HOP) is a simple method from a device design perspective, with enhanced safety compared to normothermic perfusion in the event of device failure. However, the optimal temperature for cardiac HOP has yet to be determined. We evaluated the effectiveness of 12-hour HOP using University of Wisconsin Machine Perfusion Solution (UWMPS) in different temperatures compared to static cold storage (SCS) for 6 hours followed by simulated transplantation. Additionally, we sought to determine the impact of oxygen supplementation in hypothermic ESHP in the heart function preservation.

METHODS: Hearts were procured from Yorkshire pigs (n=35) randomized into 3 preservation therapies: 6h-SCS; 12h-HOP and 12h hypothermic non-oxygenated perfusion (HNOP-without oxygen supplementation). For either HOP or HNOP groups, three temperatures were tested (5°C; 10°C; 15°C). After the preservation period, hearts had their function assessed in a normothermic perfusion machine capable of working mode, simulating transplantation.

RESULTS: All perfusion parameters were stable throughout (mean±SD): aortic flow 65±5.57 mL/min, aortic pressure: 11.51±3.17 mmHg. All HOP hearts presented a better cardiac index than SCS ($p<0.05$). The HNOP hearts presented similar cardiac function results compared to SCS.

CONCLUSIONS: HOP for 12 hours had better heart function preservation than SCS for 6h. Even HNOP had similar results compared to SCS. Greater edema formation in ESHP

hearts did not affect heart function. Hypothermic ESHP safely enhances function preservation compared to SCS.

INTRODUCTION

Heart transplantation is the gold-standard treatment for end-stage heart failure (HF)[2,81]. Despite all the advancements in organ preservation and post-operative care, the number of transplants performed worldwide aren't sufficient to supply the demand for donor organs. This number remains limited by several reasons, including the shortage of donor organs[20,27,82].

The current standard preservation method, static cold storage (SCS), limits up to 4-6 hours of heart preservation prior to transplantation due to the sustained damage over the period of cold ischemia[18,19]. This restricts the donor-recipient matching, and places constraints if logistical challenges arise[19]. Thus, extending the organ preservation time will increase the number of hearts available for transplantation by providing enough time for improved patient selection and safer long-distance transport.

Ex-situ heart perfusion (ESHP) has been proposed to be an optimal preservation method. It continuously provides oxygenated, nutrient-rich solution to the lone organ prior to transplantation, extending organ preservation time. There are different perfusion methods: normothermic, subnormothermic and hypothermic[19].

Hypothermic ESHP has some advantages over the other two perfusion methods[18,19,66]. It is a simple method from a device design perspective, and also has enhanced safety in the event of device failure[65,66]. Different perfusates have been used for hypothermic ESHP[83-87].

The perfusate tested in this study was the University of Wisconsin machine perfusion solution (UWMPS). This solution has been evaluated in the preclinical context in donor hearts, and with clinical success in other organs (kidney, liver, pancreas)[88-90].

There appears to be a lack of study investigating what the optimal temperature is for hypothermic ESHP, which has yet to be determined[91-93].

We hypothesized that the lowest temperature is the best for heart function preservation in hypothermic ESHP. Therefore, the first aim of this study is to test what is the appropriate temperature for preserving heart function in a 12-hour hypothermic ESHP compared to the current standard preservation method SCS for 6 hours. Secondly, we aimed to test the impact on heart function preservation when there is no supplementation of oxygen. Consequently, the heart remains in anoxia during preservation similar to SCS.

METHODS

The University of Alberta Animal Care and Use Committee approved the experimental protocol AUP00000943. For this study, hearts from 35 female domestic pigs (39.4-55kg) were procured and were allocated to 1 of 3 methods: SCS for 6 hours, and 2 types of ESHP: 12-hour hypothermic oxygenated perfusion (HOP) and 12-hour hypothermic non-oxygenated perfusion (HNOP). Each perfused group (HOP and HNOP) were divided in three different temperatures (5°C, 10°C and 15°C) with a sample size of 5 animals per group (Figure 2.1).

Sternotomy was performed in anesthetized animals, then ~800 mL of heparinized blood was collected to be used as part of the perfusate during heart function assessment. The blood was stored at 4°C during preservation time (6h of SCS, 12h of HOP/HNOP). Prior to excision, donor hearts were flushed with ~500 mL of University of Wisconsin solution (UW) as a cardioplegia. The hearts were weighed post-explant (T0), after

preservation (T6: SCS - T12: HOP/HNOP) and after heart function assessment in order to determine edema formation along the entire procedure (Figure 2.1)[94,95].

Hearts in HOP and HNOP were perfused with UWMPS for 12 hours, while SCS hearts were stored in UW in an ice box for 6 hours. The HOP perfusate was supplemented with oxygen (pO2~700 mmHg) while the HNOP were kept perfused without oxygen supplementation (room air - pO2 expected ~159 mmHg)[78,96].

During hypothermic ESHP, physiologic parameters were continuously assessed, including aortic flow (AoF:0.3 mL/min/g_tissue), aortic pressure (AoP), coronary vascular resistance (CVR), temperature of the heart and of the perfusate circulating through the device. Blood gas analysis was performed every two hours of perfusion (T2, T4, T6, T8, T10 and T12)[94,96,97].

Those samples were collected from the arterial port and from the catheter introduced in the coronary sinus. This procedure allowed us to measure the myocardial viability parameters in hypothermic ESHP such as myocardial oxygen consumption (MVO2), oxygen extraction ratio (OER), lactate metabolism and glucose utilization.

Those parameters were measured accordingly: Myocardial lactate metabolism was determined by the difference between the lactate production (collected from the coronary sinus) and lactate consumption (collected from the aorta). A positive value means production and a negative means extraction; the CVR was continuously presented on the ESHP screen device, and it is the result from the difference between aortic and pulmonary pressure divided by the coronary blood flow and the heart weight; MVO2 was calculated according to the difference between the arterial and the venous content of oxygen multiplied by the coronary blood flow and divided by the heart weight; the OER is

the ratio between venous and arterial content of oxygen; glucose utilization was obtained from the difference between two time points in the beginning (T4-T2), in the middle (T8-T6) and in the end of the perfusion period (T12-T10), divided by the time in hours[66,80,98].

After preservation, hearts were transferred to a custom normothermic device. The perfusate used was composed of equal parts donor whole blood stored (~800 mL) and Krebs-Henseleit with albumin (~800 mL)[75,94].

Functional status was assessed at left atrial pressure of 6 mmHg: Cardiac Index (CI), maximum dP/dt, minimum dP/dt, left ventricular stroke work (LVSW), coronary blood flow (CBF). Blood gas samples were collected accordingly to check lactate accumulation, potassium, glucose, calcium and pH level[94,99]. Additionally, three hearts were procured and transferred directly to the normothermic perfusion device (Figure 2.1), to establish a control for heart functional parameters.

Given that myocardial edema formation might affect the coronary bed perfusion, particularly in the dependent regions of the heart, quality of perfusion was assessed with fluorescent microspheres. Fluorescent microspheres (Invitrogen by Thermo Fisher Scientific - FluoSpheres™ F8888) were added in the aortic port at the end of perfusion and waited 2-3 minutes and sample tissues were taken to be analyzed[100,101].

Our histological analysis was performed by a blinded pathologist. The myocardial tissue was randomly collected from the left ventricle after preservation and function assessment. Tissue samples were fixed in paraformaldehyde solution (10%), then stored in 70% ethanol and embedded in paraffin. These paraffin blocks were cut and stained with hematoxylin-eosin. Myocardial injury was assessed based on the presence of

hemorrhage, edema formation, endothelial swelling, inflammation and presence of contraction bands[102,103].

STATISTICAL ANALYSIS

Shapiro-Wilk test was used to assess normality. Kruskal-Wallis were performed on non-normally distributed data, and Dunn's test for multiple comparisons. One-Way ANOVA and Two-Way ANOVA were performed to compare normally distributed continuous variables between groups. Dunnett's and Tukey post-hoc tests were performed. Results are expressed as mean$\pm$standard deviation or as median and interquartile range. A $p<0.05$ was considered statistically significant.

RESULTS

1. METABOLIC PARAMETERS

There was no statistical difference in mean donor animal weight, heart weight, AoF and glucose utilization (Figure 2.2). However, the AoP (mmHg) was significantly higher in 5HOP hearts compared to 15HOP in different time points: T2 (12.6±2.1 5HOP vs. 8.46±2.3 15HOP, $p<0.01$); T4 (12.22±1.51 5HOP vs. 7.74±2.16 15HOP, $p<0.01$); T6 (11.88±1.18 5HOP vs. 8.14±2.53 15HOP, $p<0.05$); T8 (11.8±1.05 5HOP vs. 7.98±2.37 15HOP, $p<0.05$); T10 (11.78±1.08 5HOP vs. 8.36±1.67 15HOP, $p<0.05$); T12 (12.6±2.1 5HOP vs. 8.46±2.3 15HOP, $p<0.05$) (Figure 2.3). The PaO2 (mmHg) was significantly lower in 15HOP than 10HOP at T2 (752.2±40.18 10HOP vs. 594.4±117.87 15HOP, $p<0.01$), and significantly higher in HOP hearts compared to HNOP, as expected (Figure 2.4).

All the formulas used for myocardial parameters calculation are described in figure 2.13. Comparing the three temperatures, there was a significant difference at T4 in myocardial lactate metabolism. The 10HOP hearts had a higher lactate extraction (mmol/L) at T4 compared to 5HOP (10HOP: -0.22±0.18 vs. 5HOP: 0±0 mmol/L; $p<0.05$) and 15HOP (vs. 15HOP: 0.02±0.11; $p<0.05$) (Figure 2.5A). While comparing between HOP and HNOP hearts, there was a significant difference in the hearts perfused at 10°C at T2 (10HNOP: 0.2±0.14 vs.10HOP: -0.05±0.06; $p<0.01$) and at T4 (10HNOP: 0.2±0.14 vs. 10HOP: -0.22±0.18; $p<0.01$) (Figure 2.5B).

CVR was continuously presented on the ESHP screen device. Analyzing the different temperatures, CVR (mmHg*min/mL*100g) was significantly lower in 15HOP hearts compared to 5HOP and 10HOP at different time points: T2 (15HOP: 0.13±0.04 vs. 5HOP: 0.19±0.03, $p<0.05$ / 15HOP: 0.13±0.04 vs. 10HOP: 0.19± 0.04, $p<0.01$); T4 (15HOP: 0.12±0.03 vs. 5HOP: 0.18±0.01, $p<0.05$ / 15HOP: 0.12±0.03 vs. 10HOP: 0.18±0.04, $p<0.05$); T6 (15HOP: 0.12±0.04 vs. 5HOP: 0.18±0.01, $p<0.05$); T8 (15HOP: 0.13±0.04 vs. 5HOP: 0.18±0.01, $p<0.05$); T12 (15HOP: 0.13±0.03 vs. 5HOP: 0.18±0.01, $p<0.05$) (Figure 2.6A). There was no significant difference between the HOP and HNOP hearts within each temperature (Figure 2.6B).

MVO2 was calculated according to the difference between the arterial and the venous content of oxygen multiplied by the coronary blood flow and divided by the heart weight (CBF*[CaO2-CvO2]/100g heart weight). MVO2 (mL/min/100g) was significantly lower in 5HOP hearts compared to 10HOP and 15HOP at T2 and T4 (Figure 2.7A): T2 (5HOP: 12.69±1.31 vs. 10HOP: 22.44±6.89, $p<0.01$ / vs. 15HOP: 21.53±4.01, $p<0.01$);

T4 (5HOP: 10.43±4.26 vs. 10HOP: 19.85±5.45, p<0.01 / vs. 15HOP: 19.14±3.15, p<0.01).

There was a significant difference in MVO2 (mL/min/100g) between HOP vs HNOP at 10°C and 15°C at T2 and T4 (Figure 2.7B): T2 (10HOP: 22.44±6.89 vs. 10HNOP: 11.82±3.27, p<0.0001 / 15HOP: 21.53±4.01 vs. 15HNOP: 10.33±0.78, p<0.0001); T4 (10HOP: 19.85±5.45 vs. 10HNOP: 11.81±2.7, p<0.01 / 15HOP: 19.14±3.15 vs. 15HNOP: 9.23±1.11, p<0.0001).

The 5HOP had a significantly lower OER (%) than 10HOP and 15HOP at T2 and T4 (Figure 2.8A): T2 (5HOP: 19.85±5.15 vs. 10HOP: 31.5±10.49, p<0.05 / vs. 15HOP: 38.66±6.5, p<0.001); T4 (5HOP: 16.09±6.54 vs. 10HOP: 28.4±8.25, p<0.05 / vs. 15HOP: 30.5±4.94, p<0.01). HOP had significantly lower OER than those HNOP hearts during perfusion (p<0.0001). (Figure 2.8B)

2. EDEMA FORMATION AFTER PRESERVATION

Edema formation was significantly higher in perfused hearts compared to the SCS hearts. As illustrated in figure 2.9A and 2.9B, there was no significant difference between the HOP and HNOP, nor between the different temperatures.

3. FUNCTIONAL RESULTS

Heart function parameters from all hearts studied are summarized in table 2.1. The 12 hours of HOP was more efficient in preserving heart function than SCS and HNOP. There was a significantly higher CI (mL/min/g_tissue) in HOP compared to SCS (5.77±2.44 / vs. 5HOP: 13.53±3.18; p<0.05 / vs. 10HOP: 13.32±5.42; p<0.05 / vs. 15HOP:

12.58±4.78; p<0.05) (Figure 2.10A). There is no statistically significant difference between HOP and SCS in LVSW, maximum dP/dT, minimum dP/dT and coronary blood flow (Figure 2.10B).

We have encountered a strong correlation (r=0.8513) between HOP and improved cardiac index preservation in our study (Figure 2.14A). There was a significant difference between HOP and HNOP in CI (mL/min/g_tissue) (5HOP: 13.53±3.18 vs. 5HNOP: 2.91±1.5; p<0.001 / 10HOP: 13.32±5.42 vs. 10HNOP: 2.67±1.71; p<0.001 / 15HOP: 12.58±4.78 vs. 15HNOP: 3.65±1.86; p<0.01) (Figure 2.10E), LVSW (mmHg*mL) (5HOP: 1337±262.2 vs. 5HNOP: 296±274.3; p<0.01 / 15HOP: 1235±879.7 vs. 15HNOP: 302±153.7; p<0.05) (Figure 2.10F) and minimum dP/dT (mmHg/s) (5HOP: -1374±412 vs. -747.4±366; p<0.05) (Figure 2.10G).

4. HISTOLOGICAL RESULTS

In the sample tissues assessed, there was no inflammation, hemorrhage or endothelial swelling. However, all of those hearts presented edema formation in different levels, and some of those presented different levels of contraction bands in the myocardium[102,103]. Even the *in vivo* sample hearts and those placed, straight after retrieval, on the normothermic machine to have the normal function assessed, had edema and contraction bands. Figure 2.11 shows histological analysis of some edema formation and contraction bands in sample tissues collected.

DISCUSSION

Defining the best temperature for heart function preservation in hypothermic ESHP is essential to optimize the method. Many studies have been successfully conducted comparing hypothermic ESHP to the current gold standard method (SCS), which is known to be effective in preserving heart function up to 4-6h[18,19,104]. In addition, previous experimental preservation studies that exceeded 6h of SCS presented a significant decline in preserving heart function[105].

Previous studies were done testing hypothermic ESHP from 4°C-12°C and from 6-48h[18,19,92,93,106]. However, there is evidence that at 15°C hearts can be safely maintained in an aerobic state with UWMPS[70,96,107]. Therefore, 3 different temperatures (5°C/10°C/15°C) were selected to be tested with or without continuous oxygen supplementation.

We determined our methods according to previous studies (AoF, pO2 and the rationale behind the non-oxygenated ESHP is based on cooling the whole heart equally, without temperature fluctuations, and avoiding temperature dropping below 4°C, which has been seen in other devices such as SherpaPak.

Oxygen utilization is significantly reduced in the myocardium at low temperature, but not completely eliminated. We observed a beneficial effect of oxygen supplementation on cardiac output after simulated transplantation (Figure 2.10E). Even the HNOP hearts could be preserved for 12 hours with similar heart function preservation compared to 6 hours of SCS. This may be related to the constant control of temperature during HNOP, making it more effective in extending the preservation time even in the absence of oxygen supplementation.

These similar results in heart function preservation between HNOP and SCS might be related to this constant control of temperature during HNOP, also seen in the device above mentioned, making it more effective in extending the preservation time even in the absence of oxygen supplementation. SherpaPak has also shown reduction in cold injury caused by the uneven cooling of the organ, decreasing the risk of severe primary graft dysfunction compared to SCS[49,50,108]. However, it has not yet demonstrated a significant increase in organ preservation time[51].

We have demonstrated that hypothermic ESHP can safely preserve pig hearts for 12 hours. Reaffirming that reduced flow[78,92,96,97] and pressure are able to preserve heart function better than the current standard preservation method, SCS for 6 hours. Moreover, a higher perfusion flow would increase edema formation[70,104] and it might affect heart function preservation[52].

Edema formation is still a concern for ESHP. The compressive effect of tissue weight in a ESHP method where the myocardial mass is supported rather than suspended might result in insufficient tissue perfusion with low-flow and low-pressure. In order to check if this was affecting coronary bed perfusion, we added fluorescent microspheres in 3 additional hearts at the end of 12 hours of perfusion[80].

Our goal was to see microspheres in each tissue sample collected after preservation time, thereby ruling out coronary blood flow obstruction[100,101]. Tissue samples were randomly taken from 7 different parts of the heart: left ventricle (LV) apex, LV anterior wall, LV posterior wall, right ventricle anterior and posterior wall, left and right atria. As can be seen in figure 2.12, fluorescence was observed in all the heart tissues collected, suggesting that perfusion flow was adequate in all regions.

A recent study preserving human hearts in a hypothermic ESHP device for 4h, 6h and 8h, have shown a lower weight gain compared to ours results[53]. However, they have applied an even lower flow-rate compared to our project, and shorter preservation period. Although further analyzes must be done to determine whether the lower flow, or the shorter preservation time contributes reducing edema formation, we believe that the lower flow had benefited to lower edema because we presented a higher weight gain per hour during preservation.

Although we did not observe a statistical difference between the temperatures studied, we tend to believe that the lower temperature is more suitable for hypothermic ESHP. Since in a possible device failure event, the team responsible for transporting the organ has more time to act, whether to repair the equipment or even switch to the conventional preservation method. Thus, keeping the heart within the temperature range (5°-10°C) recommended by the International Society for Heart and Lung Transplantation (ISHLT)[109].

LIMITATIONS

We have measured and compared MVO2 and OER, however, our Hypothermic perfusion device was not sealed. Which may have affected the myocardial oxygen consumption measurement, as well as OER. They might not be as accurate as we expected it to be.

We have examined the effect of temperature and oxygen supplementation on myocardial preservation over an extended interval. We evaluated the quality of preservation utilizing a simulated transplant procedure with a normothermic working

mode perfusion device, rather than actual transplantation into a recipient animal. It may be that our observations would differ with transplant into an actual recipient animal, however this would increase cost considerably.

UWMPS was developed for kidney preservation, not myocardial preservation. It may be that an alternate perfusate may result in a different outcome. However, UWMPS is currently a commercially available product, making it attractive to evaluate[110,111].

We have not evaluated the effects of different storage time for the donor animal's heparinized blood collected during surgery and stored[75] for 6h and 12h (SCS and hypothermic ESHP groups respectively). This storage time difference might affect perfusion results, since increased blood storage times affects red blood cell quality[112].

CONCLUSIONS

Although HOP had higher functional status compared to SCS, there was no significant difference between the temperatures. However, a trend towards better results was observed in the cardiac function parameters analyzed in the 5HOP group. Furthermore, 12 hours of HNOP method had similar results compared to SCS.

DISCLOSURE STATEMENT: Dr. Darren Freed and Dr. Jayan Nagendran are co-founders of Tevosol Inc., a Bridge-to-Life company. None of the remaining authors have any financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

FUNDING STATEMENT: This study was supported by grants from the Canadian Institutes for Health Research - Canadian National Transplant Research Program (CIHR-CNTRP), the University Hospital Foundation (UHF), and in part by the Natural Sciences and Engineering Research Council of Canada (NSERC-CRSNG).

TABLES & FIGURES

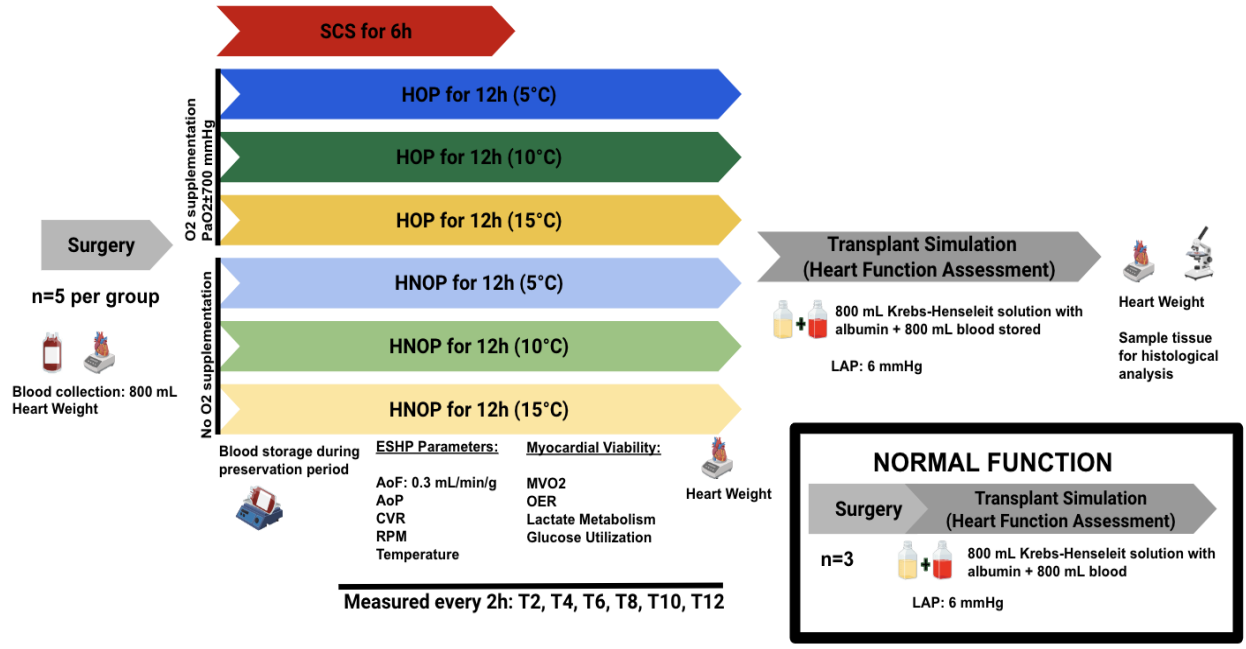


FIGURE 2.1: Summary of the procedures performed for the entire experiment

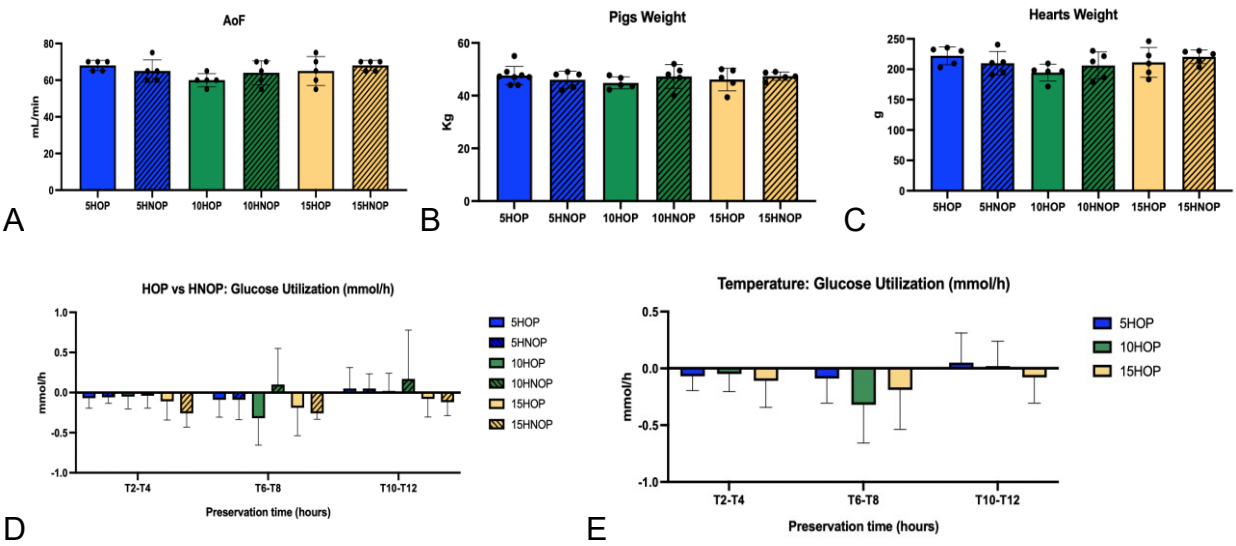
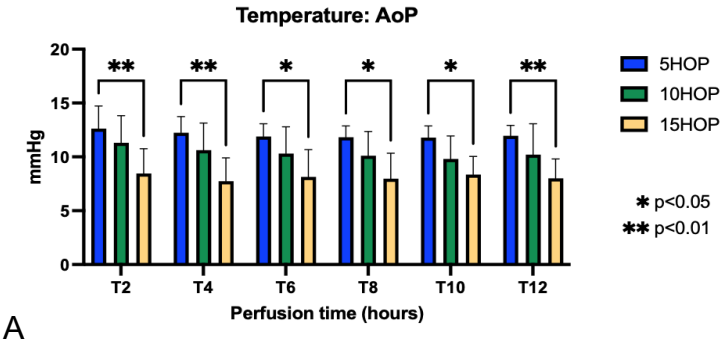
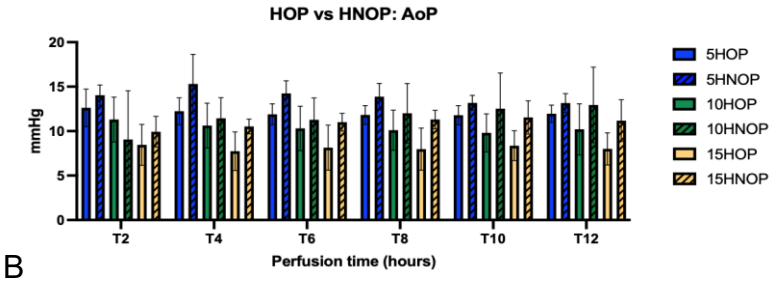


FIGURE 2.2: Perfusion parameters - Aortic flow (AoF), Pigs and hearts weight mean and glucose utilization per group.

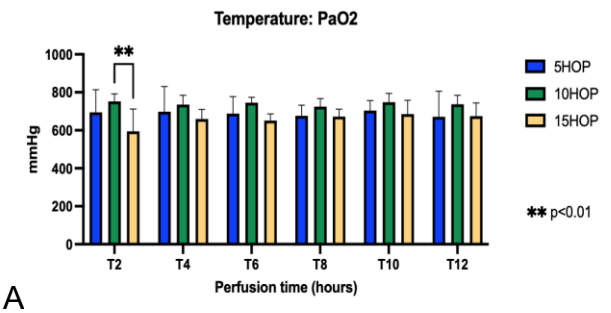


A

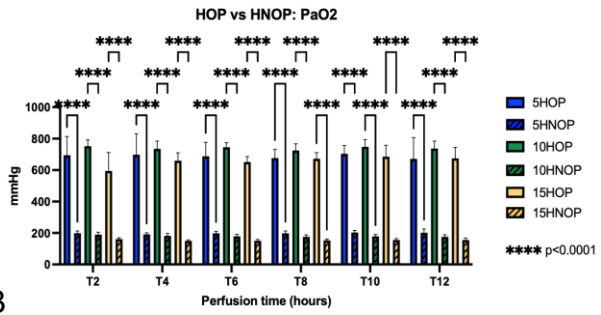


B

FIGURE 2.3: Aortic pressure compared between temperatures and HOP vs HNOP.



A



B

FIGURE 2.4: Arterial partial pressure of oxygen (PaO2) compared between temperatures and HOP vs HNOP.

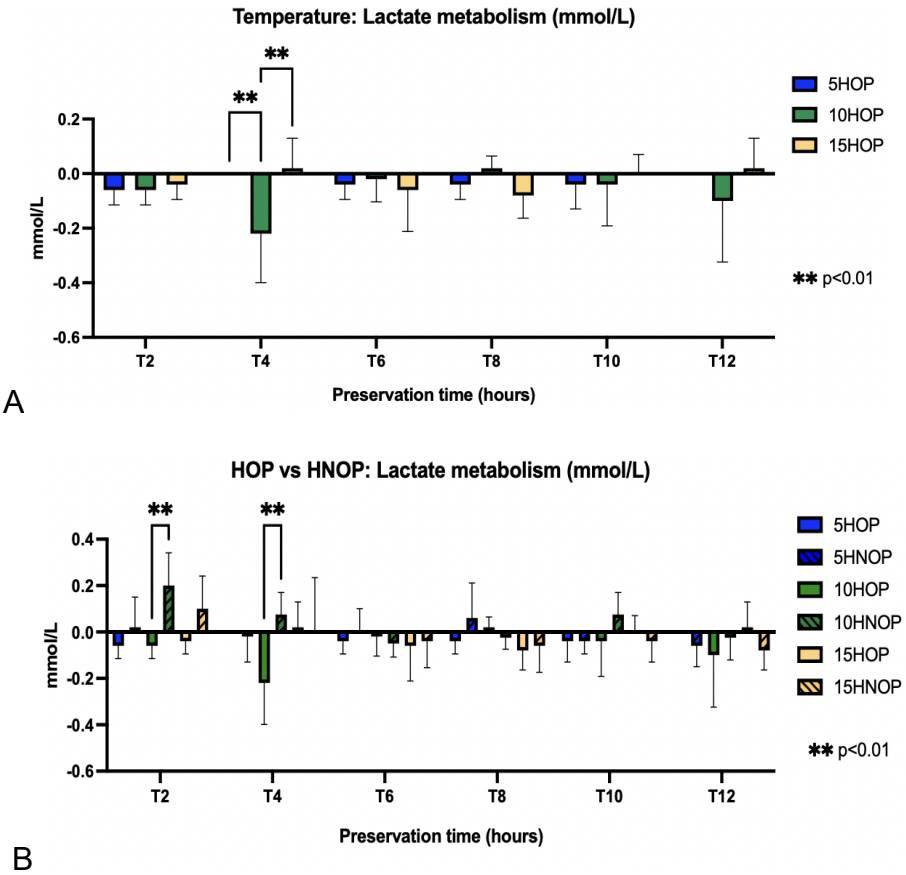
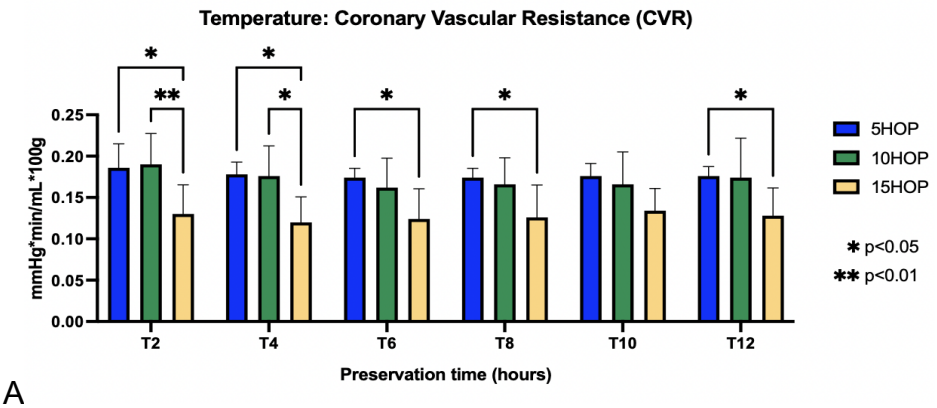
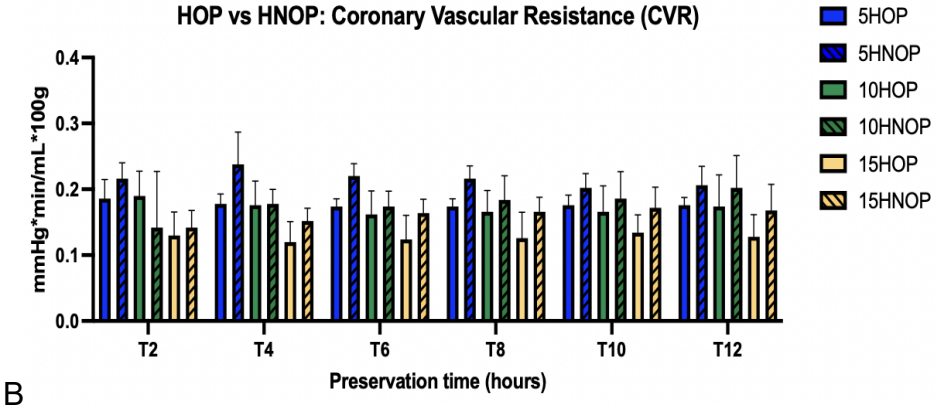


FIGURE 2.5: Lactate metabolism compared between temperatures and HOP vs HNOP.

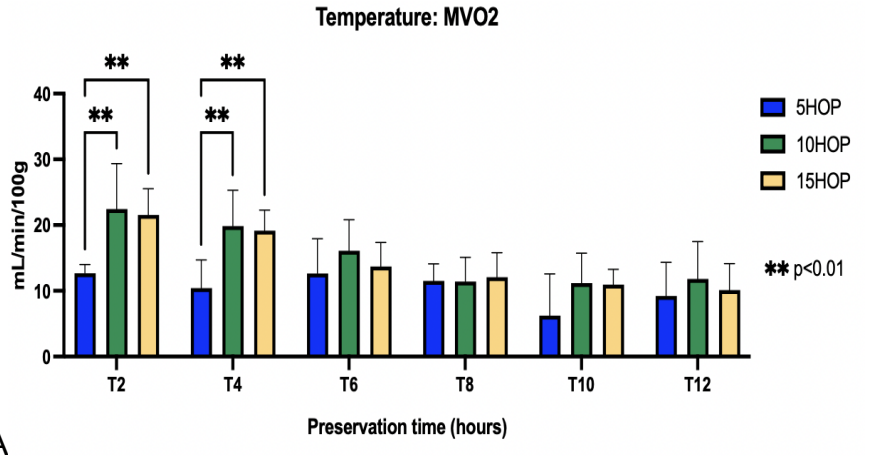


A

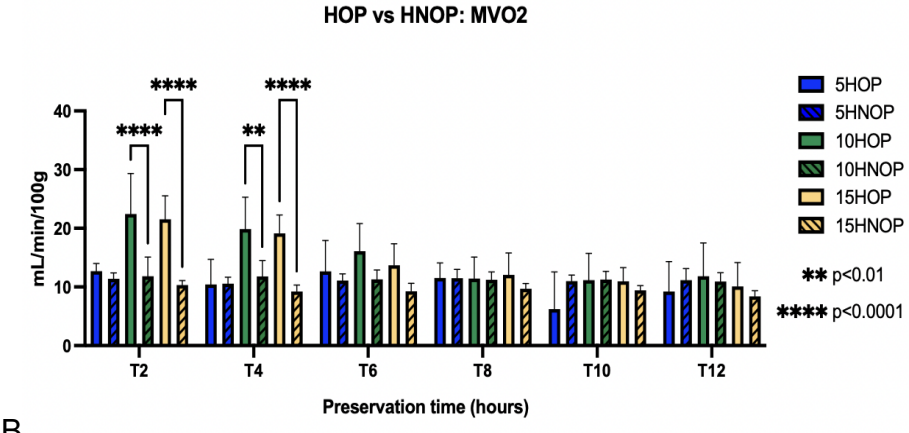


B

FIGURE 2.6: Coronary vascular resistance compared between temperatures and HOP vs HNOP.

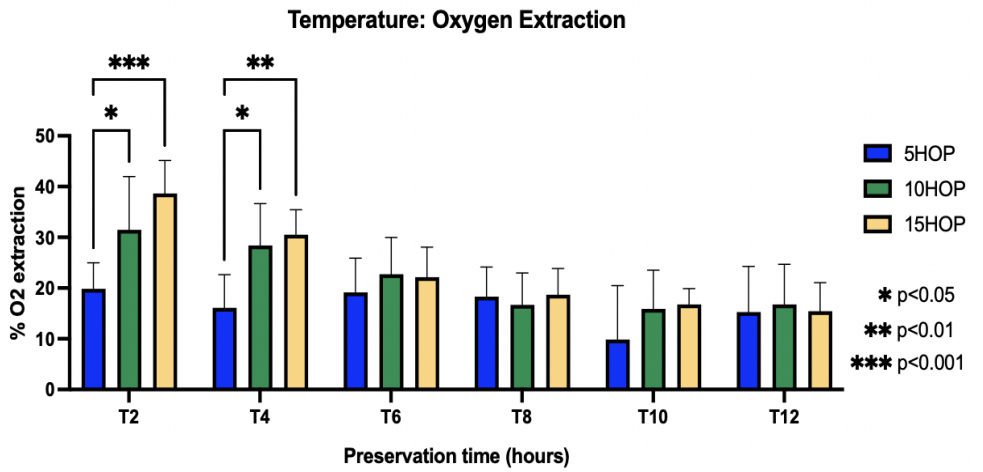


A

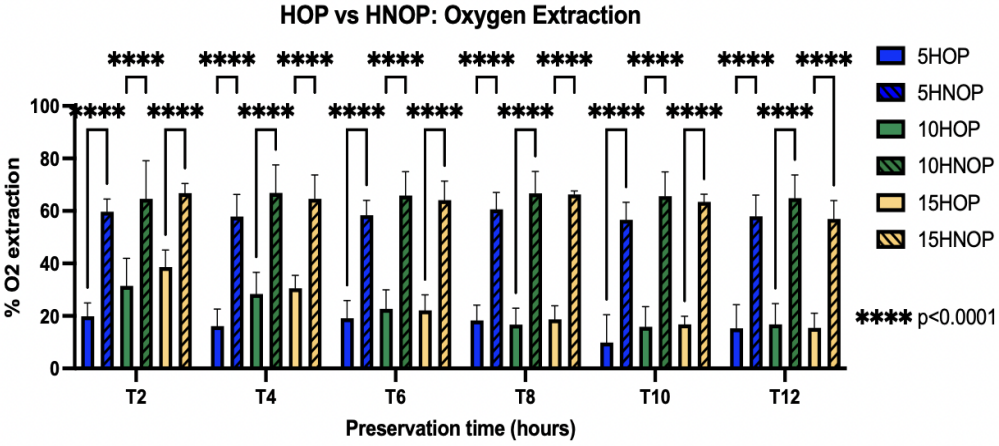


B

FIGURE 2.7: Myocardial oxygen consumption compared between temperatures and HOP vs HNOP.

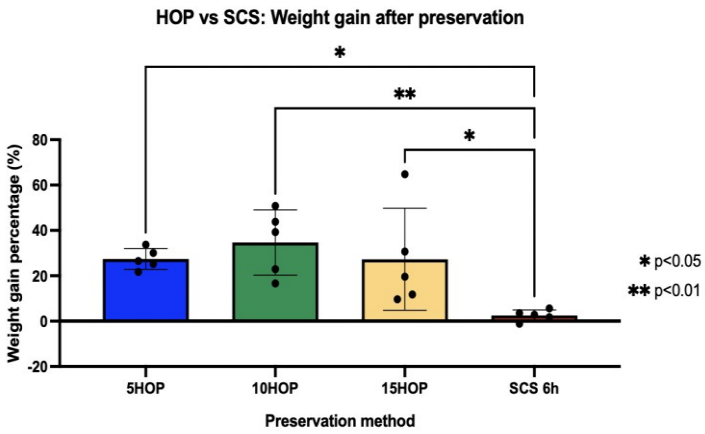


A

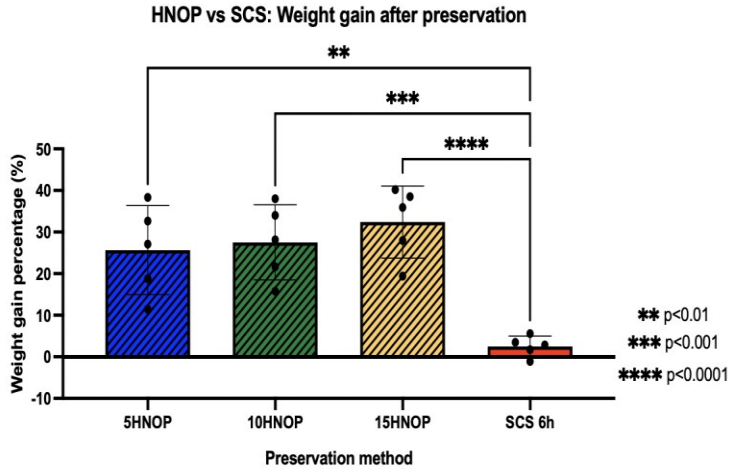


B

FIGURE 2.8: Oxygen extraction ratio compared between temperatures and HOP vs HNOP.

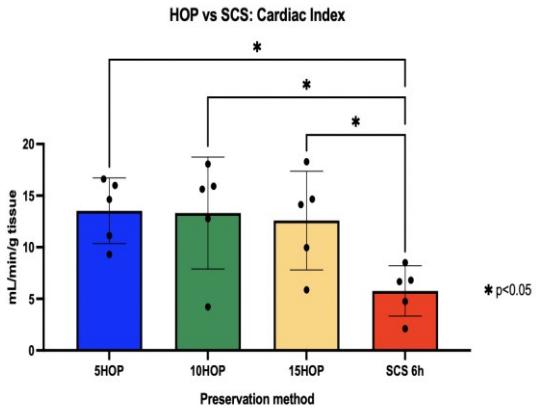


A

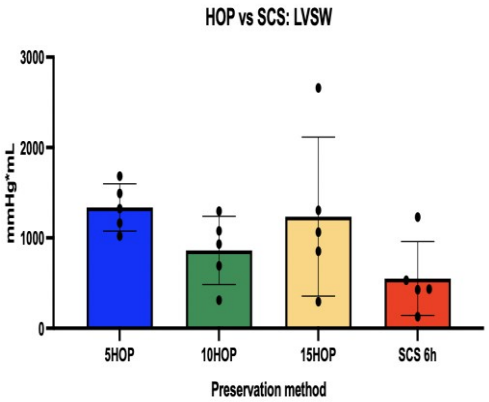


B

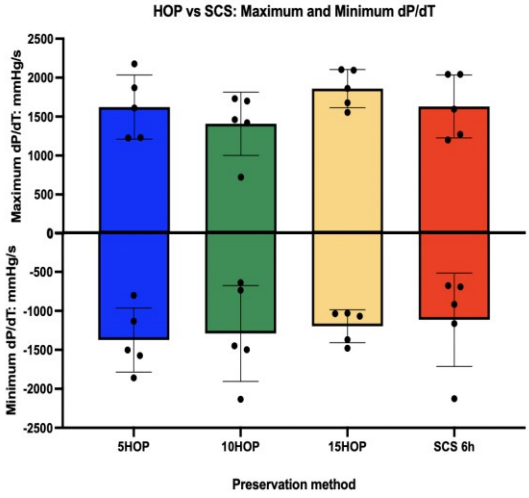
FIGURE 2.9: Edema formation for each group compared to SCS


HOP vs SCS: Cardiac Index
mL/min/g tissue
20
15
10
5
0
*
*
*
* p<0.05
5HOP
10HOP
15HOP
SCS 6h
Preservation method

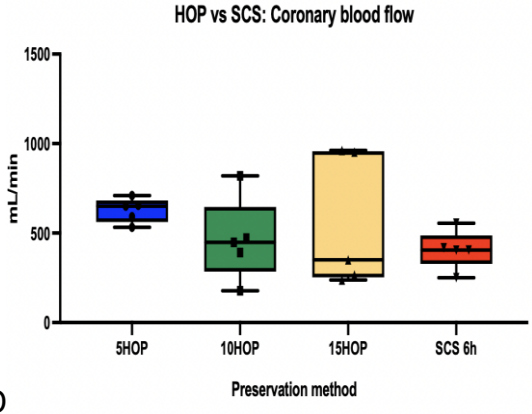
A


HOP vs SCS: LVSW
mmHg*mL
3000
2000
1000
0
5HOP
10HOP
15HOP
SCS 6h
Preservation method

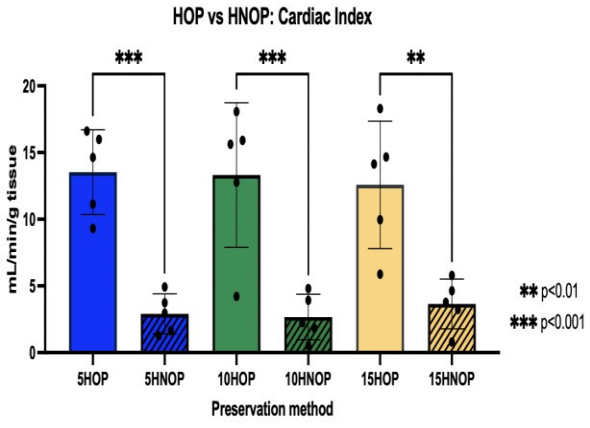
B


HOP vs SCS: Maximum and Minimum dP/dT
Maximum dP/dT: mmHg/s
Minimum dP/dT: mmHg/s
2500
2000
1500
1000
500
0
-500
-1000
-1500
-2000
-2500
5HOP
10HOP
15HOP
SCS 6h
Preservation method

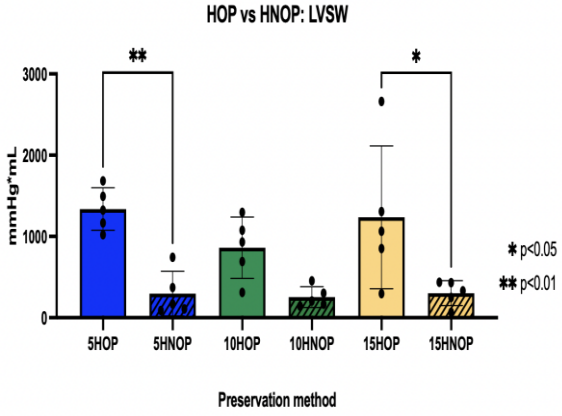
C


HOP vs SCS: Coronary blood flow
mL/min
1500
1000
500
0
5HOP
10HOP
15HOP
SCS 6h
Preservation method

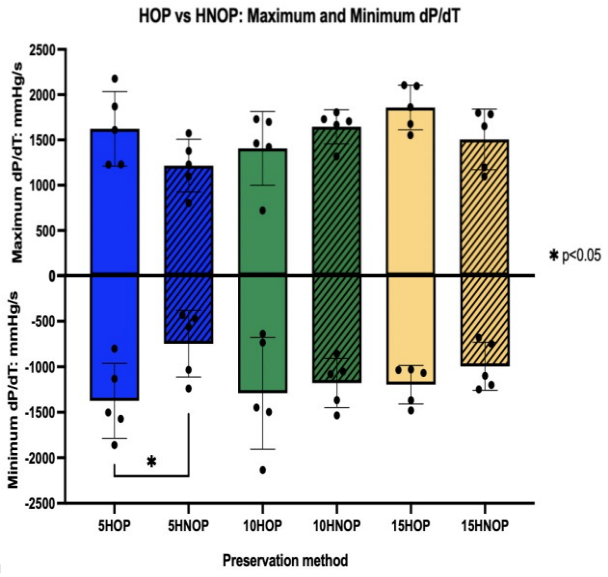
D


HOP vs HNOP: Cardiac Index
mL/min/g tissue
20
15
10
5
0
***
***
**
** p<0.01
*** p<0.001
5HOP
5HNOP
10HOP
10HNOP
15HOP
15HNOP
Preservation method

E


HOP vs HNOP: LVSW
mmHg*mL
3000
2000
1000
0
**
*
* p<0.05
** p<0.01
5HOP
5HNOP
10HOP
10HNOP
15HOP
15HNOP
Preservation method

F



G

FIGURE 2.10: Heart function parameters analyzed after preservation period (Cardiac Index- CI; LVSW- Left Ventricular Stroke Work; Maximum and Minimum dP/dT; Coronary blood flow- CBF)

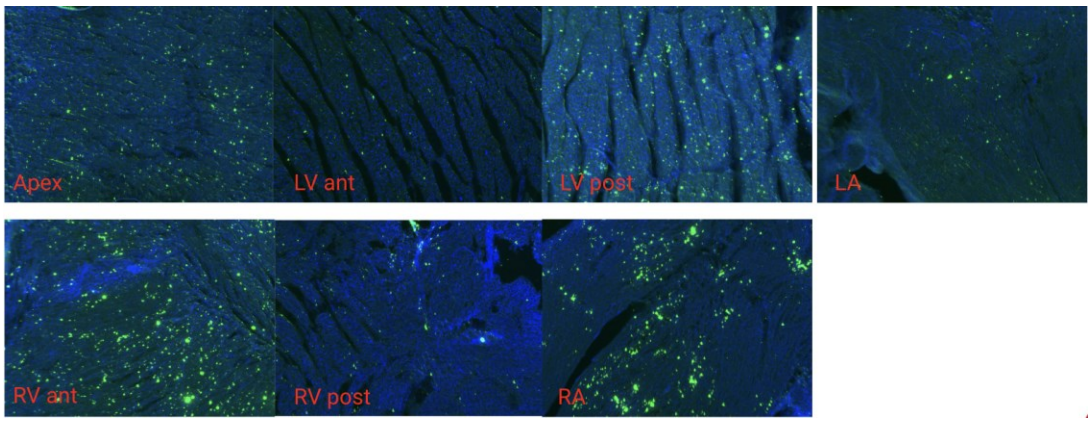


FIGURE 2.11: Fluorescent Microspheres in each myocardial sample: The fluorescence was seen in every tissue sample collected.

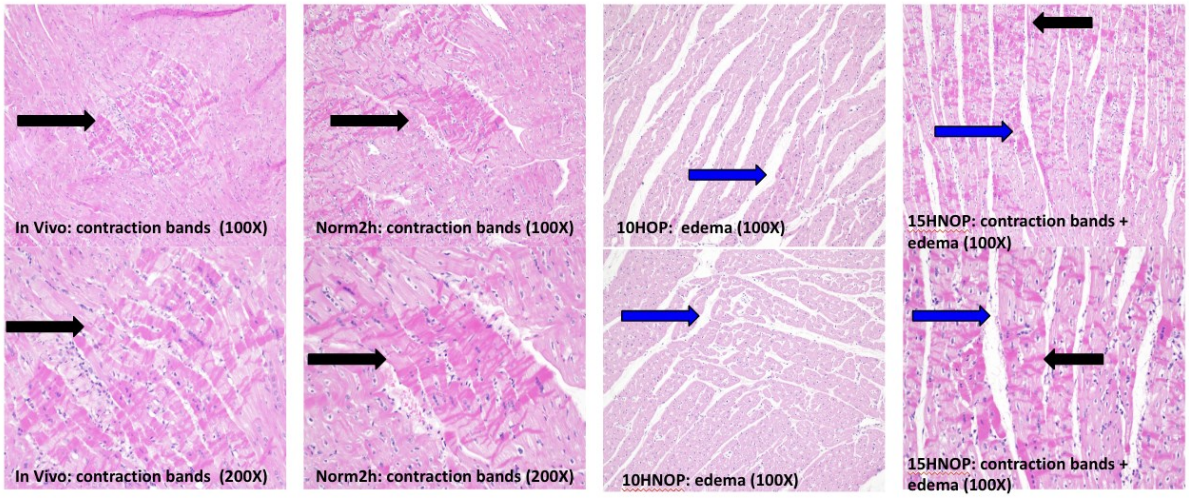


FIGURE 2.12: Histological Analysis of myocardial samples preserved. There were edema (blue arrow) and contraction bands (black arrow) in almost every sample tissue analyzed. Even the *In Vivo* samples had contraction bands in the myocardium.

- Myocardial lactate metabolism (mmol/L) = Coronary sinus lactate – Aortic lactate

  OBS: A negative result means lactate extraction and a positive result means production

- $MVO2\ (mL/min/100g) = \frac{CBF \times (CaO2 - CvO2)}{100g\ heart\ weight}$

- $CaO2\ (mL/dL) = (1.34 \times Hemoglobin\ concentration \times SaO2) + (0.0031 \times PaO2)$

- $CvO2\ (mL/dL) = (1.34 \times Hemoglobin\ concentration \times SaO2) + (0.0031 \times PvO2)$

- $CVR\ (mmHg*min/mL*100g) = \frac{(Mean\ aortic\ pressure - Mean\ right\ atrial\ pressure)}{CBF \times 100g\ heart\ weight}$

- $OER\ (\%extraction) = 1 - \frac{(CvO2)}{CaO2} \times 100$

- Glucose Utilization (mmol/h) (beginning of perfusion) $= \frac{T2\ venous\ glucose\ level - T4\ venous\ glucose\ level}{2\ hours}$

- Glucose Utilization (mmol/h) (middle of perfusion) $= \frac{T6\ venous\ glucose\ level - T8\ venous\ glucose\ level}{2\ hours}$

- Glucose Utilization (mmol/h) (end of perfusion) $= \frac{T10\ venous\ glucose\ level - T12\ venous\ glucose\ level}{2\ hours}$

FIGURE 2.13: Formulas used for myocardial viability parameters calculation.

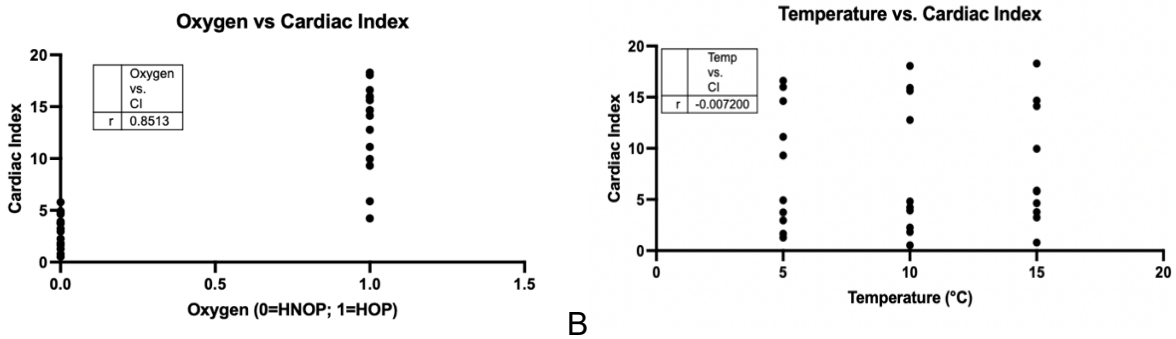


FIGURE 2.14: Correlation of Cardiac Index and Oxygen (A) and Temperature (B). Figure 2.14A demonstrates there is a strong correlation between HOP and better Cardiac index preservation.

TABLE 2.1: Heart function parameters of each preservation method

	Cardiac Index (mL/min/g_tissue) *Mean±SD	**LVSW (mmHg*mL) *Mean±SD**	**dP/dT max (mmHg/s) *Mean±SD**	**dP/dT min (mmHg/s) *Mean±SD**	**Coronary Flow (mL/min) *Median±SEM**
5HOP	13.53±3.18	1337±262.2	1623±412.1	-1374±412	650±30.37
5HNOP	2.91±1.5	296±274.3	1217±291.7	-747.4±366	352±52.55
10HOP	13.32±5.42	861.2±378.6	1407±407	-1290±615.1	448±103.5
10HNOP	2.67±1.71	253.8±127.5	1645±189.3	-1178±269.6	326±64.62
15HOP	12.58±4.78	1235±879.7	1859±246.1	-1196±211.9	350±165.8
15HNOP	3.65±1.86	302±153.7	1506±335.3	-995.2±264	420±56.16
SCS 6h	5.77±2.44	550.4±408.8	1630±404.8	-1115±599.2	405±48.15
Normal Function	16.10±2.44	2189±861.5	2026±612.3	-1269±93.13	455±25.51

CHAPTER 3

EVALUATION OF MODIFIED UNIVERSITY OF WISCONSIN MACHINE PERFUSION SOLUTIONS FOR SUBNORMOTHERMIC EX-SITU HEART PERFUSION: A COMPARATIVE STUDY

Authors: Guilherme Mainardi Aguiar da Silva, MD[1]; Mitchell J. Wagner[1]; Sanaz Hatami, MD/PhD[1]; Parham Hassanzadeh[1]; Xiuhua Wang, PhD[1]; Jayan Nagendran MD/ PhD[2,3,4,5]; Darren H. Freed, MD/PhD[2,3,4,5]

1 Department of Surgery, Faculty of Medicine, University of Alberta

2 Division of Cardiac Surgery, Department of Surgery, Faculty of Medicine, University of Alberta

3 Mazankowski Alberta Heart Institute, Edmonton, AB, Canada

4 Alberta Transplant Institute, Edmonton, AB, Canada

5 Canadian Donation and Transplantation Research Program, Edmonton, AB, Canada

Corresponding Author's Complete Contact Information:

Each author's ORCID#

Darren H. Freed 0000-0002-6239-1444

Jayan Nagendran 0000-0001-5827-9823

Guilherme Mainardi - 0009-0006-3293-0976

Mitchell J. Wagner - 0000-0002-5883-6340

Sanaz Hatami 0000-0002-6792-6620

Parham Hassanzadeh - no ORCID ID

Xiuhua Wang- no ORCID ID

ABSTRACT

BACKGROUND: Ex-situ heart perfusion (ESHP) has been proposed to increase the number of organs available for transplantation because it safely prolongs the preservation time prior to the organ implant. ESHP reduces the deleterious consequences of prolonged cold ischemia. The subnormothermic ESHP is a simple and adequate method with a variety of perfusate compositions, including Somah and University of Wisconsin machine perfusion solution (UWMPS). However, the optimal perfusate for subnormothermic ESHP has not been investigated yet. Here we investigated the effects of UWMPS and its modified forms in cardioprotection during subnormothermic ESHP. For those modified UWMPS we added some key elements from Somah (amino acids and ionic components).

METHODS: Hearts were procured from ~40-50Kg Yorkshire pigs (n=9) randomized into 3 perfusates: UWMPS, UWMPSa (UWMPS+amino acids) and UWMPSi (UWMPS+ionic components). After the 12-hour preservation period, the preserved hearts were transferred to a normothermic perfusion device to have their function assessed in working-mode. Additionally, 3 hearts were procured and preserved with Somah for 12h to serve as controls.

RESULTS: There was no significant difference in heart function preservation with the modified UWMPS. When compared to the Somah solution, the UWMPSi had a significantly lower functional status: Cardiac Index (CI:mL/min/g_tissue): (14.86±5.14 Somah vs. 0.16±0.15 UWMPSi, $p<0.05$); Left ventricular stroke work (LVSW:mmHg*mL): (1414.33±640.23 Somah vs. 8.67±7.09 UWMPSi, $p<0.05$); maximum dP/dT (mmHg/s): (1664.3±296.77 Somah vs. 176±117.46 UWMPSi, $p<0.001$); minimum dP/dT (mmHg/s) (-947±384.26 Somah vs. -141±70.34 UWMPSi, $p<0.05$); coronary blood flow (CBF:

mL/min) (696.33±182.94 Somah vs. 83.33±57.84 UWMPSi, $p<0.01$). The UWMPSa had a statistically lower coronary flow (696.33±182.94 Somah vs. 343±108.76 UWMPSa, $p<0.05$). Edema formation was not statistically different compared to UWMPS, but it showed a trend to be higher in the modified UWMPS groups (UWMPS:46.49±21.34%; UWMPSa:69.85±10.95%; UWMPSi:81.19±20.45%). The hearts preserved with Somah had a significantly higher edema formation compared to UWMPS (104.6% ± 12.96% of Somah vs. 46.49% ± 21.34% of UWMPS, $p<0.05$).

CONCLUSIONS: Modified UWMPS may not have additional cardioprotective effects during subnormothermic ESHP. More studies are warranted to improve these solutions for the purpose of superior cardiac viability and functional preservation in this setting.

INTRODUCTION

The number of people suffering with end-stage heart failure (HF) has increased over decades[10]. Although HF treatments have advanced, from medicines to ventricular assist devices (VADs), heart transplantation is the gold-standard treatment for those patients with end-stage Heart Failure (HF)[11,81].

While the estimated survival of patients with advanced end-stage of HF is only 2 years, and patients with VADs 3-4 years, people who undergo heart transplant are greater than 12 years[11]. Despite all the advancements in organ preservation, the number of transplants performed remains limited by several reasons, including the shortage of donor organs[20,27,82].

Additionally, the current standard preservation method, static cold storage (SCS), contributes negatively to the number of heart transplants performed. Due to the sustained damage over the cold ischemia period, SCS restricts the preservation time up to 4-6 hours before transplantation[18,19]. Then, limiting donor-recipient matching and logistical constraints[19].

Given that the ex-situ heart perfusion (ESHP) provides oxygenated, nutrient-rich solution continuously, it has been suggested as an optimal method for extended preservation period. This method can be performed in different temperatures (normothermic, subnormothermic and hypothermic) and with different perfusates[19].

Subnormothermic ESHP has some advantages over the other two methods, for example, lower energy device consumption. The heart preservation in hypothermic or normothermic ESHP places a significantly higher energy demand on the device. Furthermore, as well as in the hypothermic, in the subnormothermic ESHP none or

minimal intervention are needed throughout perfusion. Hence, reducing the ESHP machine size, increasing its portability[113].

However, since the heart remains arrested the entire preservation time in the subnormothermic method, heart function cannot be assessed during perfusion[86,114]. Therefore, metabolic parameters have been used to check myocardial viability, such as, lactate metabolism, oxygen extraction (OER), myocardial oxygen consumption (MVO2) and coronary vascular resistance (CVR). These parameters are associated with myocardial performance[80,87].

Different from normothermic ESHP in which the primary choice of perfusate is blood-based, in order to facilitate oxygen delivery to the heart, in the subnormothermic ESHP it can be performed with or without blood. Many perfusates have been studied and tested[83-85,106,115] such as Somah and University of Wisconsin machine perfusion solution (UWMPS).

Somah solution has been used and it has effectively preserved some organs such as heart, kidney and live in an EHSP. This novel solution is a physiological salt solution which was made aiming for synthesis of high-energy phosphates and maintaining cellular homeostasis to extend storage independent of the perfusion temperature[72,73].

This perfusate was developed to meet the energy requirements of cardiomyocytes and endothelium by priming the organ with substrates to facilitate metabolic and mechanical functional recovery upon reperfusion. Previous studies used Somah for the SCS of DCD hearts at room temperature[74]. These hearts had improved contractile recovery and endothelial function when compared to other standard hypothermic and hyperkalemic preservation solutions[72,75].

UWMPS is a hyperkalemic solution which was first used for hypothermic organ preservation, such as kidney, liver and heart[75]. However, when it is used in higher temperatures it decreases the preservation effect in hearts[76,77]. We planned to check that our two proposed UWMPS modifications would increase this solution preservation efficacy in higher temperatures[74,75,111,116].

In this study, we hypothesized that a modified UWMPS can increase heart function preservation in a 12-hour subnormothermic ESHP. Therefore, in this study we first checked that giving an additional energy substrate and antioxidants (UWMPSa), matching the amino acids and vitamin C presented in Somah content, increases UWMPS heart function preservation. Secondly, we evaluated that the addition of some other ionic components to UWMPS (UWMPSi), in order to meet the ions content in Somah, will improve heart function preservation (calcium chloride, sodium chloride, sodium phosphate, sodium bicarbonate and glucose).

METHODS

The University of Alberta Animal Care and Use Committee approved the experimental protocol AUP00000943. In this study, hearts from 9 female Yorkshire pigs (45.83±5.16Kg) were retrieved and were sorted in 3 different perfusate groups: UWMPS, UWMPSa and UWMPSi. The sample size of each group was three. Additionally, hearts (n=3) were procured and preserved in Somah, as a control, to evaluate whether our modifications had a positive impact in heart function preservation. (FIGURE 3.1)

Sternotomy was performed in anesthetized pigs, ~800 mL of heparinized blood was collected and stored for 12 hours to be used as part of the perfusate during the heart

function assessment period in our simulated transplant. All the hearts procured received ~500 mL of cardioplegia University of Wisconsin solution (UW). Immediately after explanted the hearts were weighted (T0), then they were weighted after preservation (T12) and then after heart function assessment in order to have their weight gain along the entire procedure[95,96]. (FIGURE 3.1)

During the preservation period, the hearts were perfused with 500 mL of the specific perfusate for each group: UWMPS, UWMPSa and UWMPSi for 12 hours. The study assessed various physiologic parameters, including aortic flow (AoF:0.3mL/min/g_tissue), aortic pressure (AoP), CVR, temperature of the heart and of the perfusate circulating through the device. Other parameters were assessed and analyzed every two hours, through blood gas samples in our ABG-machine[78,92,98].

Those samples were collected from the arterial port and from the catheter introduced in the coronary sinus. This procedure allows measurement of the myocardial viability parameters in ESHP. For instance, MVO2, OER, lactate metabolism, CVR, glucose utilization[75,80]. (FIGURE 3.1)

Those parameters were measured following the aforementioned formulas described: Myocardial lactate metabolism was determined by the difference between the lactate production (collected from the coronary sinus) and lactate consumption (collected from the aorta). A positive value means production and a negative means extraction; the CVR was continuously presented on the ESHP screen device, and it is the result from the difference between aortic and pulmonary pressure divided by the coronary blood flow and the heart weight; MVO2 was calculated according to the difference between the arterial and the venous content of oxygen multiplied by the coronary blood flow and divided by

the heart weight; the OER is the ratio between venous and arterial content of oxygen; glucose utilization was obtained from the difference between two time points in the beginning (T4-T2), in the middle (T8-T6) and in the end of the perfusion period (T12-T10), divided by the time in hours.

The hearts were perfused for 12 hours in the subnormothermic ESHP machine with the heater-cooler kept turned off. After that period, the hearts were transferred to the custom made normothermic ESHP machine, permitting a simulating transplantation in a working-mode perfusion.

We followed the same protocol already described by our research team in previous studies to assess heart function in the normothermic ESHP device. We used a perfusate made of blood (~800 mL) and Krebs-Henseleit with albumin (~800 mL)[95,99,100]. (FIGURE 3.1) We assessed the following heart function parameters at left atrial pressure of 6 mmHg: Cardiac Index (CI), maximum dP/dt, minimum dP/dt, left ventricular stroke work (LVSW), coronary blood flow (CBF). Lactate accumulation, potassium, glucose, calcium and pH level were checked through blood gas samples collected[95,100].

STATISTICAL ANALYSIS

Normality was assessed using the Shapiro-Wilk test. One-Way ANOVA and Two-Way ANOVA were performed to compare normally distributed continuous variables between groups. Dunnett's and Tukey post-hoc tests were performed. A p-value< 0.05 was considered statistically significant.

RESULTS

1. METABOLIC PARAMETERS

There was no significant difference in pigs' weight, hearts weight and AoF between the groups. There was a lower PaO2 (mmHg) levels in UWMPSi hearts compared to the UWMPS in different time points. At T4: UWMPSi (409±140.8) lower than UWMPS (590.33±39.88), $p<0.01$; at T8: UWMPSi (421.67±56.54) lower than UWMPS (599±13) $p<0.01$; at T10: UWMPSi (374.67±124.18) lower than UWMPS (621±3), $p<0.001$; at T12: UWMPSi (346.67±65.25) lower than UWMPS (547.67±21.03), $p<0.01$. (FIGURE 3.2)

The AoP increased steadily in those hearts preserved with UWMPSi. There was a significantly higher AoP (mmHg) in the UWMPSi hearts compared to the UWMPS at the end of the perfusion. At T10: UWMPSi (16.3±4.11) lower than UWMPS (10.53±2.65), $p<0.001$; at T12: UWMPSi (19.67±5.85) lower than UWMPS (11.47±4.17), $p<0.01$. (FIGURE 3.3)

There was no significant difference in myocardial oxygen consumption between the groups. The UWMPS hearts had a significantly higher lactate production (mmol/L) at T2 compared to UWMPSa (0.13±0.35 UWMPS vs. -0.13±0.06 UWMPSa; $p<0.05$). (FIGURE 3.4A) The UWMPSi had a significantly higher oxygen extraction (%) at T12 compared to UWMPS (86.72±0.08 UWMPSi vs. 54.83±0.18 UWMPS; $p<0.05$). (FIGURE 3.4B)

There was a significantly higher glucose consumption (mmol/h) in UWMPSi compared to UWMPS in the end of the perfusion (-1.57±0.55 UWMPSi vs. -0.23±0.15 UWMPS; $p<0.05$). (FIGURE 3.4C) The CVR (mmHg*min/mL*100g) was significantly lower in UWMPS compared to the UWMPSi at at T10 (0.15±0.05 UWMPS and 0.25±0.06

UWMPSi; $p<0.05$) and at T12 (0.17±0.08 UWMPS and 0.3±0.09 UWMPSi; $p<0.05$). (FIGURE 3.4D)

2. IONS LEVELS

The commercialized UWMPS was used as a based solution and the components were added accordingly to each UWMPS modification proposed in this study. Therefore, the recipes differ from one to another and the final concentration of each are described in table 3.1. The potassium level was significantly lower in Somah compared to the other solutions ($p<0.05$; UWMPS, UWMPSa and UWMPSi). The calcium level was significantly lower in UWMPS and UWMPSa compared to Somah, and there was no significant difference between UWMPSi and Somah. The sodium level was significantly different in UWMPSa compared to Somah.

3. WEIGHT GAIN AFTER PRESERVATION

Somah hearts had statistically significant higher weight gain percentage (%) after 12 hours of ESHP compared to between UWMPS (104.6±12.96 of Somah vs. 46.49±21.34 of UWMPS, $p<0.05$). There was no significant difference between the other groups, the two modified UWMPS and UWMPS. (FIGURE 3.5)

4. HEART FUNCTION

All the heart function parameters were assessed at LAP of 6 mmHg and those values are described in Table 3.2. Somah was the most efficient in preserving heart function than UWMPS and the two modifications made on UWMPS. There were no

significant differences between UWMPS and the two modified UWMPS in any of those heart function parameters analyzed.

The hearts preserved with Somah had all functional parameters significantly higher when compared to UWMPSi. CI(mL/min/g_tissue): (14.86±5.14 Somah vs. 0.16±0.15 UWMPSi, $p<0.05$) (Figure 3.6A); LVSW(mmHg*mL): (1414.33±640.23 Somah vs. 8.67±7.09 UWMPSi, $p<0.05$) (Figure 3.6B); dP/dt max(mmHg/s): (1664.3±296.77 Somah vs. 176±117.46 UWMPSi, $p<0.001$) (Figure 3.6C); dP/dt min(mmHg/s) (-947±384.26 Somah vs. -141±70.34 UWMPSi, $p<0.05$) (Figure 3.6C); CBF(mL/min) (696.33±182.94 Somah vs. 83.33±57.84 UWMPSi, $p<0.01$) (Figure 3.6D) and (vs. 343±108.76 UWMPSa, $p<0.05$) (Figure 3.6D).

DISCUSSION

The ESHP resolves the cold ischemic injury caused by the uneven cooling ischemia caused by SCS. Giving nutrient-rich solution and oxygen to the donor heart during preservation, it allows a longer preservation period prior to transplantation. Despite all advancements in ESHP, the best perfusate for preservation has not been investigated yet.

The UWMPS has shown effective preservation of different organs in hypothermic ESHP. However, in subnormothermic ESHP it has not demonstrated to be the most appropriate perfusate. The Somah solution has been proposed as an optimal perfusate for subnormothermic ESHP. We modified UWMPS in order to improve heart function preservation for subnormothermic ESHP implementation.

Somah is a cardioprotective solution combining different energy substrates, free radical scavengers, antioxidants, intracellular and extracellular hydrogen chelators, and a physiological concentration of calcium[100]. Which targets synthesis of high-energy phosphates and maintains cellular homeostasis to extend preservation[73]. Somah is related to improved heart function preservation, with less reperfusion injury and better metabolic and functional recovery, also has been demonstrated effectiveness in preserving DCD rat hearts for 24h in room temperature[73].

In this study we modified UWMPS with different additives present in Somah. The first one, we intended to give more energy substrate by adding amino acids and vitamin C. The second one, we added sodium bicarbonate, calcium chloride, sodium chloride, and sodium phosphate. After preservation all the hearts preserved were tested in a working mode normothermic machine using a blood-based perfusate with Krebs-Henseleit and albumin at left atrial pressure (LAP) of 6 mmHg[66].

Myocardial viability was assessed during subnormothermic ESHP. There were significant differences in OER, glucose utilization, CVR and lactate metabolism. Additionally, despite the increase of oxygen supplementation and the control of it during the entire perfusion according to the blood gas sample results, the PaO2 levels were significantly different between Somah and UWMPSi. The oxygen solubility might have been affected with the ion's addition in UWMPS[117].

Somah had a significantly higher functional status compared to UWMPSi. Despite the UWMPSi had the lowest heart function parameters, there was no significant difference compared to UWMPS. There was no significant difference between Somah and UWMPSa.

Although the modifications in UWMPS did not improve heart function preservation, there was a trended increase in function preservation with UWMPSa. Additionally, the hearts preserved with UWMPSi had a trending decrease in heart function preservation compared to UWMPS.

Hearts preserved with Somah had the highest weight gain. As well as the hearts preserved with the two modified UWMPS had a trending edema formation increase compared to UWMPS. Somah had significantly higher edema formation compared to UWMPS hearts.

Although Somah presented the best results in preserving heart function, it also had the highest edema formation. This might be related to the flow rate applied. There is a recent study which published a considerably lower edema formation after a hypothermic ESHP for up to 8h using UWMPS[108]. In our study, Somah had an average weight gain around 100%. Future short-term outcomes investigations have to be performed (Figure 3.5). Therefore, analyzing whether the edema affects the early transplant results.

LIMITATIONS

We evaluated the effects of different modifications to UWMPS on heart function preservation over 12 hours of ESHP in a small sample size study. We have used a simulated transplantation model with the normothermic working mode perfusion device. Which can differ from the actual transplantation model, but also it significantly reduces the research cost in a porcine model[51,52].

The UWMPS was developed and first used for other organs preservation, such as kidney, liver and pancreas, not for heart preservation. This is a commercial product which

is available for an ample use in myocardial preservation[110,118,119]. Expanding their use to different temperatures may facilitate ESHP implementation.

Although Somah has demonstrated promising results in the cold storage and ESHP methods, in our prolonged subnormothermic ESHP study it has not shown a significantly higher CI compared to UWMPS. Nonetheless, it had a significant higher edema formation compared to UWMPS, which may impact heart transplant short-term outcomes.

Prolonged blood storage might affect the results in our normothermic working mode ESHP device, during the simulated transplant for heart function assessment. Although the storage time was the same for all the groups analyzed, the red blood cell quality may be affected during storage[112,120].

CONCLUSIONS

There was no improvement in heart function preservation after the modifications applied to UWMPS in this study. Additionally, studies are required to enhance heart function preservation with modified UWMPS in subnormothermic ESHP.

DISCLOSURE STATEMENT: Dr. Darren Freed and Dr. Jayan Nagendran are co-founders of Tevosol Inc., a Bridge-to-Life company. None of the remaining authors have any financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

FUNDING STATEMENT: This study was supported by grants from the Canadian Institutes for Health Research - Canadian National Transplant Research Program (CIHR-CNTRP), the University Hospital Foundation (UHF), and in part by the Natural Sciences and Engineering Research Council of Canada (NSERC-CRSNG).

TABLES & FIGURES

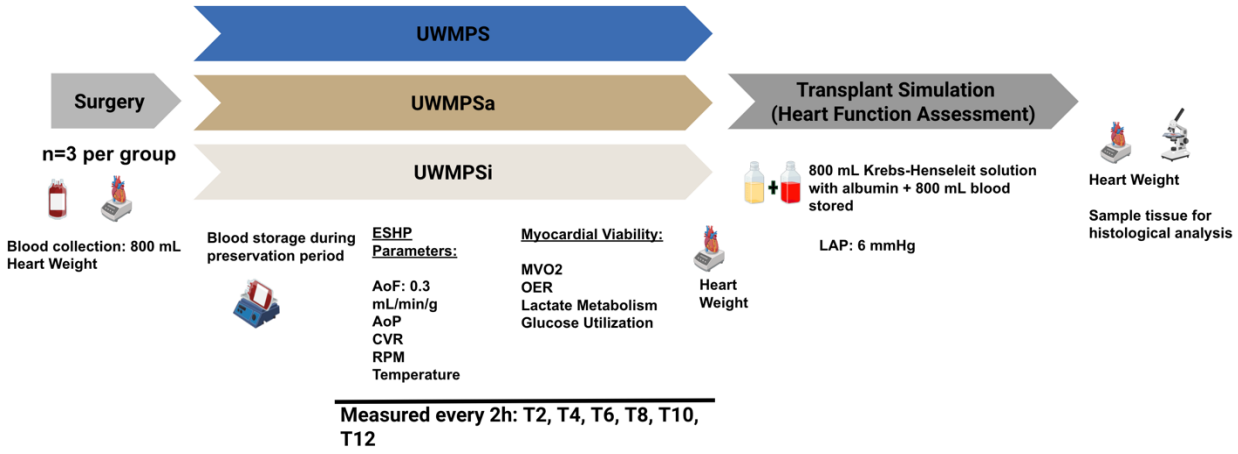


FIGURE 3.1: Summary of the procedures for the entire experiment

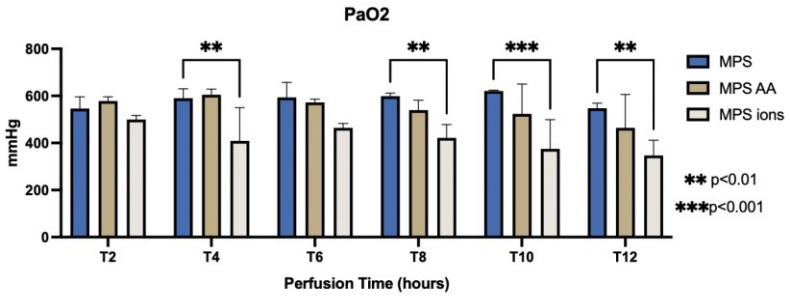


FIGURE 3.2: Mean PaO2 level for each perfusate: Significantly lower PaO2 in UWMPSi in different time points compared to other perfusates.

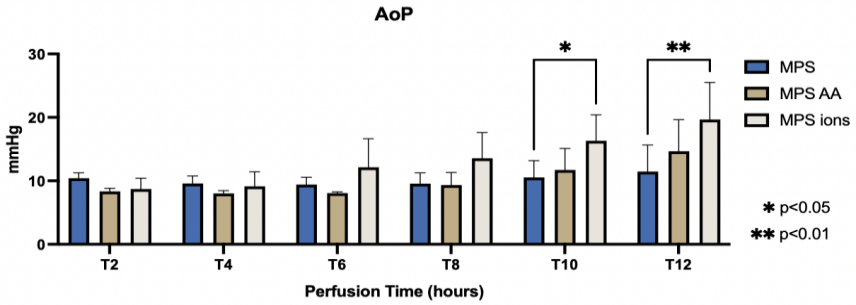


FIGURE 3.3: Mean aortic pressure (AoP) during ESHP for each group

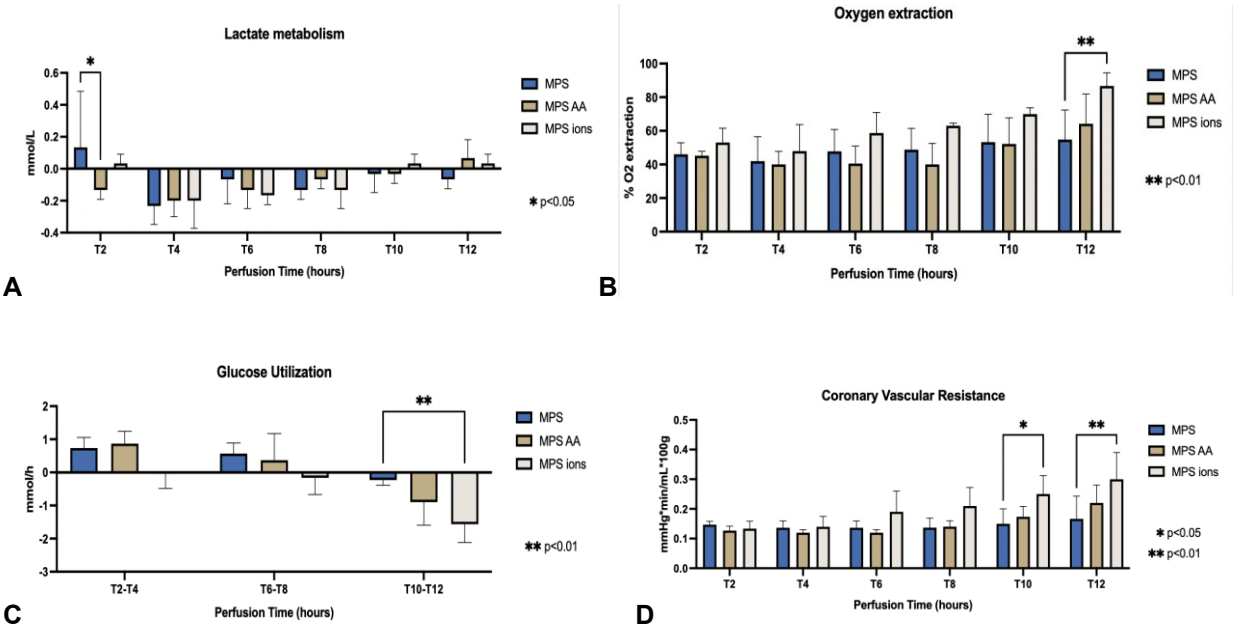


FIGURE 3.4: Metabolic parameters for each perfusate group

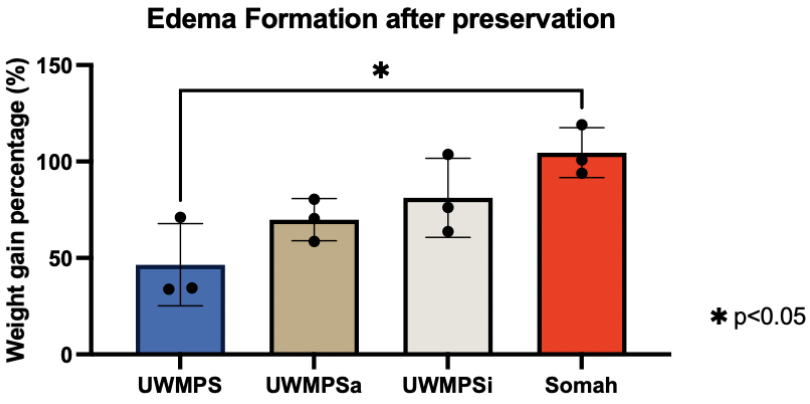


FIGURE 3.5: Edema formation for each group

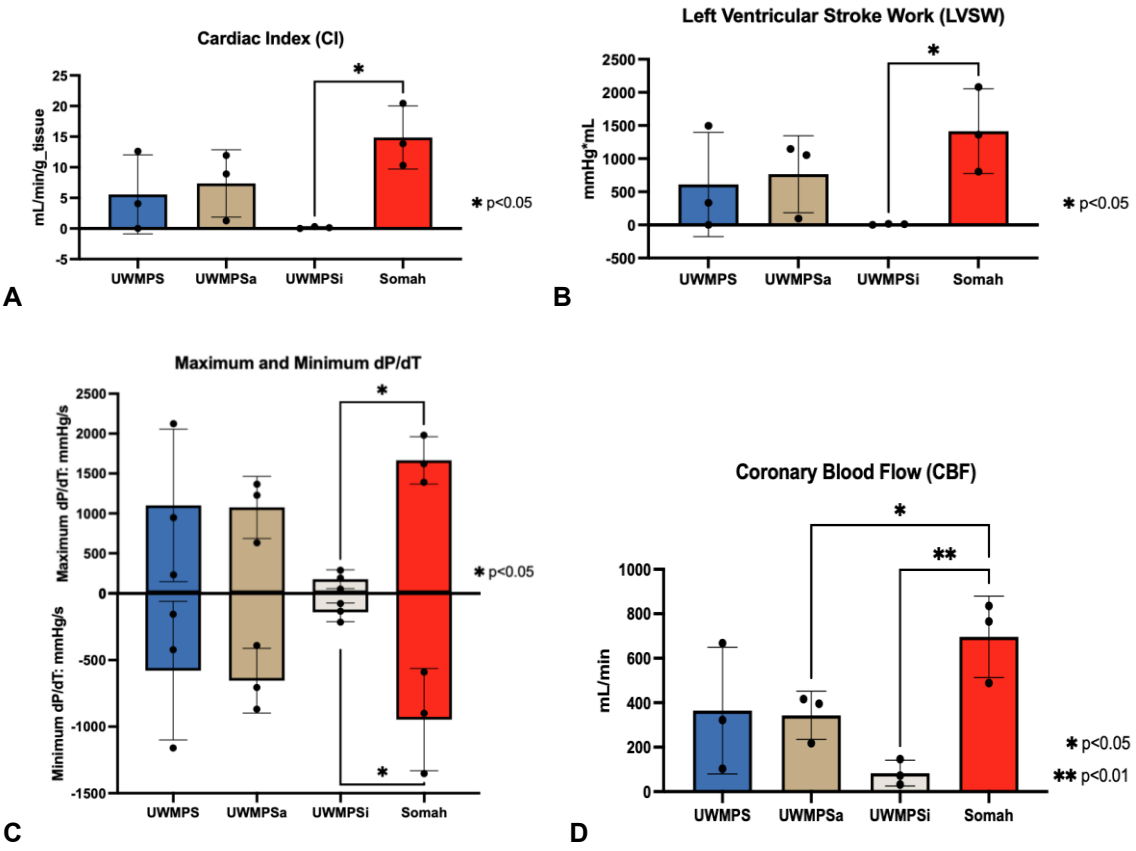


FIGURE 3.6: Heart function parameters measured for each group

TABLE 3.1: Components for each perfusate used

	UWMPS	UWMPSa	UWMPSi	SOMAH
Adenine (free base)	0.68 g/L	0.68 g/L	0.68 g/L	
Adenosine				0.534 g/L
Glutathione (reduced)	0.92 g/L	0.92 g/L	0.92 g/L	0.461 g/L
HEPES (free acid)	2.38 g/L	2.38 g/L	2.38 g/L	
Hydroxyethyl Starch	50 g	50 g	50 g	
Magnesium Gluconate	1.13 g/L	1.13 g/L	1.13 g/L	
Magnesium chloride (hexahydrate)				0.101 g/L
Magnesium sulfate (heptahydrate)				0.123 g/L
Mannitol	5.4 g/L	5.4 g/L	5.4 g/L	
Potassium Phosphate (monobasic)	3.4 g/L	3.4 g/L	3.4 g/L	0.06 g/L
Potassium chloride (monobasic)				0.522 g/L
Ribose, D(-)	0.75 g/L	0.75 g/L	0.75 g/L	
Sodium Gluconate	17.45 g/L	17.45 g/L	17.45 g/L	
Sodium Hydroxide	0.7 g/L	0.7 g/L	0.7 g/L	
Sterile Water for Injection	1000 mL	1000 mL	1000 mL	1000 mL
L-Arginine		1.073 g/L		1.073 g/L
L-Carnitine		2 g/L		2 g/L
L-Carnosine		2.26 g/L		2.26 g/L
L-Citrulline Malate		0.175 g/L		0.175 g/L
Ascorbic Acid		0.176 g/L		0.176 g/L
Dichloroacetate (DCA)		0.075		0.075
Creatine Orotate		0.274		0.274
Creatine Monohydrate		0.298		0.298
Calcium chloride	0.068 g/L	0.068 g/L	0.191 g/L	0.191 g/L
Glucose	1.8 g/L	1.8 g/L	1.92 g/L	1.92 g/L
Sodium Bicarbonate			0.420	0.420
Sodium Chloride			2.6316	2.6316
Sodium Phosphate			0.05	0.05
Insulin 10 mg/mL				1 g/L

TABLE 3.2: Mean heart function assessment parameters analyzed for each group

	Cardiac Index (mL/min/g_tissue) *Mean±SD	LVSW (mmHg*mL) *Mean±SD	dP/dT max (mmHg/s) *Mean±SD	dP/dT min (mmHg/s) *Mean±SD	Coronary Flow (mL/min) *Median±SD
UWMPS	5.56±6.44	610±786	1100.33±955.27	-579±520.57	364.33±284.87
UWMPSa	7.37±5.5	766±581.23	1075.33±389.33	-546.67±243.1	343±108.76
UWMPSi	0.16±0.15	8.67±7.09	176±117.46	-141±70.34	83.33±57.84
Somah	14.86±5.14	1414.33±640.23	1664.33±296.77	-947±384.26	696.33±182.94
SCS 6h	5.77±2.44	550.4±408.8	1630±404.8	-1115±599.2	405±48.15
Normal Function	16.10±2.44	2189±861.5	2026±612.3	-1269±93.13	455±25.51

CHAPTER 4- THESIS SUMMARY

The optimal treatment for patients with advanced heart failure is heart transplantation. However, the number of procedures performed per year is limited by several factors. The current standard preservation method, static cold storage (SCS), is one of the factors contributing negatively to this. SCS limits preservation time up to 4-6h, precluding long-distance organ transportation.

Ex-situ heart perfusion (ESHP) permits safe prolonged organ preservation. Consequently, it facilitates organ transportation, extending time for an appropriate match between donor and recipient, expanding donor pool for transplantation.

The hypothermic and subnormothermic methods simplify the ESHP preservation. While normothermic is a complex one and needs additional training compared to the other two methods. Being the hypothermic and subnormothermic ESHP a less complex and a safe method to be implemented to any surgical team with minimal training.

This thesis explored the different temperatures for hypothermic ESHP using UWMPS and investigated the effect of these temperatures in heart function preservation after a prolonged ESHP. The results encountered in this study demonstrate that hypothermic ESHP can safely extend the preservation time, from 6 to 12 hours, compared to SCS.

We have also demonstrated that hypothermic ESHP enhances heart function preservation compared to SCS. However, when we analyzed the three temperatures studied, we have not shown significant differences in heart function preservation. Despite that, we believe that in a device failure event the lowest temperature gives the transplantation team more time to resolve an issue or even switch from ESHP to SCS.

Additionally, to this observation, the hearts deprived from oxygen supplementation have demonstrated non-statistically significant differences in heart function preservation compared to SCS. We tend to believe that this result might be related to the constant temperature control applied in this method.

In our subnormothermic project, we aimed to optimize the UW machine perfusion solution (UWMPS). This perfusate has been safely used for hypothermic ESHP and SCS. However, it has not demonstrated the same results in higher temperatures.

Once Somah has been proposed as a promising solution for organ preservation, we decided to investigate its components. Therefore, we attempted to improve UWMPS with some of the Somah components.

In our thesis, we demonstrated in the UWMPSi (when added more calcium chloride, sodium bicarbonate, sodium phosphate and sodium chloride to UWMPS) a trended decrease in heart function preservation compared to UWMPS. We also had a significantly higher edema formation in hearts preserved with Somah than the hearts preserved with UWMPS. Despite that, Somah had the highest mean cardiac index that also was not significant compared to UWMPS.

The addition of amino acids and vitamin C to UWMPS did not reveal any significant differences in heart function preservation compared to UWMPS. Edema formation had a trended increase compared to UWMPS.

Further research investigating extended preservation time (up to 24 hours) and different perfusates for each ESHP method (hypothermic and subnormothermic) is important for heart function preservation improvement. Optimize perfusate and define the

best temperature, especially for hypothermic ESHP, is pivotal to guarantee a broader application of ESHP.

REFERENCES:

1. McKelvie RS, Moe GW, Ezekowitz JA, Heckman GA, Costigan J, Ducharme A, et al. The 2012 Canadian Cardiovascular Society heart failure management guidelines update: focus on acute and chronic heart failure. Can J Cardiol. 2013 Feb;29(2):168-81.
2. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation. 2022 May 3;145(18):e895-e1032.
3. Tsutsui H. Recent advances in the pharmacological therapy of chronic heart failure: Evidence and guidelines. Pharmacol Ther. 2022 Oct;238:108185.
4. Truby LK, Rogers JG. Advanced Heart Failure: Epidemiology, Diagnosis, and Therapeutic Approaches. JACC Heart Fail. 2020 Jul;8(7):523-536.
5. Crespo-Leiro MG, Metra M, Lund LH, Milicic D, Costanzo MR, Filippatos G, et al. Advanced heart failure: a position statement of the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail. 2018 Nov;20(11):1505-1535.
6. Severino P, Mather PJ, Pucci M, D'Amato A, Mariani MV, Infusino F, et al. Advanced Heart Failure and End-Stage Heart Failure: Does a Difference Exist. Diagnostics (Basel). 2019 Nov 1;9(4):170. Epidemiology, classification, Intermacs
7. Kirklin JK, Naftel DC, Pagani FD, Kormos RL, Stevenson LW, Blume ED, Myers SL, Miller MA, Baldwin JT, Young JB. Seventh INTERMACS annual report: 15,000 patients and counting. J Heart Lung Transplant. 2015 Dec;34(12):1495-504.

8. Molina EJ, Shah P, Kiernan MS, Cornwell WK 3rd, Copeland H, Takeda K, Fernandez FG, Badhwar V, Habib RH, Jacobs JP, Koehl D, Kirklin JK, Pagani FD, Cowger JA. The Society of Thoracic Surgeons Intermacs 2020 Annual Report. Ann Thorac Surg. 2021 Mar;111(3):778-792.
9. Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, et al. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. Circulation. 2022 Feb 22;145(8):e153-e639.
10. Lawrence S. Heart failure in Canada complex incurable and on the rise [Internet]. 2022[cited2024Mar18].Availablefrom:https://www.heartandstroke.ca/what-we-do/media-centre/news-releases/heart-failure-in-canada-complex-incurable-and-on-the-rise
11. Crespo-Leiro MG, Barge-Caballero E. Advanced Heart Failure: Definition, Epidemiology, and Clinical Course. Heart Fail Clin. 2021 Oct;17(4):533-545.
12. Bozkurt B, Coats AJ, Tsutsui H, Abdelhamid M, Adamopoulos S, Albert N, et al. Universal Definition and Classification of Heart Failure: A Report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure. J Card Fail. 2021 Mar 1:S1071-9164(21)00050-6.
13. Carrel A. The transplantation of organs: a preliminary communication. 1905 [classical article]. Yale J Biol Med. 2001 Jul-Aug;74(4):239-41.
14. Stolf NAG. History of Heart Transplantation: A Hard and Glorious Journey. Braz J Cardiovasc Surg. 2017 Sep-Oct;32(5):423-427.

15. Konstantinov IE. At the cutting edge of the impossible: a tribute to Vladimir P. Demikhov. Tex Heart Inst J. 2009;36(5):453-8.

16. Fann JI, Baumgartner WA. Historical perspectives of the American Association for Thoracic Surgery: Norman E. Shumway, Jr (1923-2006). J Thorac Cardiovasc Surg. 2011 Dec;142(6):1299-302.

17. Buchanan E. The operation: A human cardiac transplant: An interim report of a successful operation performed at Groote Schuur Hospital, Cape Town. Author: C N Barnard. S Afr Med J. 2017 Nov 28;107(12):1041-1044. TINHAM DOIS 34

18. Qin G, Jernryd V, Sjöberg T, Steen S, Nilsson J. Machine Perfusion for Human Heart Preservation: A Systematic Review. Transpl Int. 2022 Mar 21;35:10258.

19. Wang L, MacGowan GA, Ali S, Dark JH. Ex situ heart perfusion: The past, the present, and the future. J Heart Lung Transplant. 2021 Jan;40(1):69-86.

20. Khush KK, Potena L, Cherikh WS, Chambers DC, Harhay MO, Hayes D Jr, et al; International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: 37th adult heart transplantation report-2020; focus on deceased donor characteristics. J Heart Lung Transplant. 2020 Oct;39(10):1003-1015.

21. Goila AK, Pawar M. The diagnosis of brain death. Indian J Crit Care Med. 2009 Jan-Mar;13(1):7-11.

22. Dhanwate AD. Brainstem death: A comprehensive review in Indian perspective. Indian J Crit Care Med. 2014 Sep;18(9):596-605.

23. Greer DM, Shemie SD, Lewis A, Torrance S, Varelas P, Goldenberg FD, et al. Determination of Brain Death/Death by Neurologic Criteria: The World Brain Death Project. JAMA. 2020 Sep 15;324(11):1078-1097.

24. Chew HC, Iyer A, Connellan M, Scheuer S, Villanueva J, Gao L, et al. Outcomes of Donation After Circulatory Death Heart Transplantation in Australia. J Am Coll Cardiol. 2019 Apr 2;73(12):1447-1459.

25. Messer S, Page A, Axell R, Berman M, Hernández-Sánchez J, Colah S, et al. Outcome after heart transplantation from donation after circulatory-determined death donors. J Heart Lung Transplant. 2017 Dec;36(12):1311-1318.

26. García Sáez D, Bowles CT, Mohite PN, Zych B, Maunz O, Popov AF, et al. Heart transplantation after donor circulatory death in patients bridged to transplant with implantable left ventricular assist devices. J Heart Lung Transplant. 2016 Oct;35(10):1255-1260.

27. Canadian Institute for Health Information. Canadian Organ Replacement Register, e-Statistics on Organ Transplants, Waiting Lists and Donors - 2022. Available from URL: https://www.cihi.ca/en/organ-transplants-in-canada-donations-and-need

28. Longnus SL, Mathys V, Dornbierer M, Dick F, Carrel TP, Tevaearai HT. Heart transplantation with donation after circulatory determination of death. Nat Rev Cardiol. 2014 Jun;11(6):354-63.

29. Steinbrook R. Organ donation after cardiac death. N Engl J Med. 2007 Jul 19;357(3):209-13.

30. Manara AR, Murphy PG, O'Callaghan G. Donation after circulatory death. Br J Anaesth. 2012 Jan;108 Suppl 1:i108-21.

31. Detry O, Le Dinh H, Noterdaeme T, De Roover A, Honoré P, Squifflet JP, et al. Categories of donation after cardiocirculatory death. Transplant Proc. 2012 Jun;44(5):1189-95.

32. Shah KS, Kittleson MM, Kobashigawa JA. Updates on Heart Transplantation. Curr Heart Fail Rep. 2019 Oct;16(5):150-156.

33. de Jonge N, Kirkels JH, Klöpping C, Lahpor JR, Caliskan K, Maat AP, et al. Guidelines for heart transplantation. Neth Heart J. 2008;16(3):79-87.

34. Bifulco O, Bottio T, Caraffa R, Carrozzini M, Guariento A, Bejko J, et al. Marginal versus Standard Donors in Heart Transplantation: Proper Selection Means Heart Transplant Benefit. J Clin Med. 2022 May 9;11(9):2665.

35. Piperata A, Caraffa R, Bifulco O, Avesani M, Apostolo A, Gerosa G, et al. Marginal donors and organ shortness: concomitant surgical procedures during heart transplantation: a literature review. J Cardiovasc Med (Hagerstown). 2022 Mar 1;23(3):167-175.

36. Huckaby LV, Hickey G, Sultan I, Kilic A. Trends in the utilization of marginal donors for orthotopic heart transplantation. J Card Surg. 2021 Apr;36(4):1270-1276.

37. Cooper DK. A brief history of cross-species organ transplantation. Proc (Bayl Univ Med Cent). 2012 Jan;25(1):49-57.

38. Farr M, Stehlik J. Heart Xenotransplant: A Door That Is Finally Opening. Circulation. 2022 Mar 22;145(12):871-873.

39. Reardon S. First pig-to-human heart transplant: what can scientists learn? Nature. 2022 Jan;601(7893):305-306.

40. Arabi TZ, Sabbah BN, Lerman A, Zhu XY, Lerman LO. Xenotransplantation: Current Challenges and Emerging Solutions. Cell Transplant. 2023 Jan-Dec;32:9636897221148771

41. Osaki S, Anderson JE, Johnson MR, Edwards NM, Kohmoto T. The potential of cardiac allografts from donors after cardiac death at the University of Wisconsin Organ Procurement Organization. Eur J Cardiothorac Surg. 2010 Jan;37(1):74-9.

42. Hess NR, Ziegler LA, Kaczorowski DJ. Heart donation and preservation: Historical perspectives, current technologies, and Future Directions. Journal of Clinical Medicine. 2022 Sept 28;11(19):5762.

43. Kounatidis D, Brozou V, Anagnostopoulos D, Pantos C, Lourbopoulos A, Mourouzis I. Donor heart preservation: Current knowledge and the new era of machine perfusion. International Journal of Molecular Sciences. 2023 Nov 24;24(23):16693.

44. Wagner MJ, Hatami S, Freed DH. Thoracic Organ Machine Perfusion: A review of concepts with a focus on reconditioning therapies. Frontiers in Transplantation. 2023 Mar 22;2.

45. Hatami S, Conway J, Freed DH, Urschel S. Thoracic organ donation after circulatory determination of death. Transplantation Reports. 2023 Feb;8(1):100125.

46. Spencer BL, Wilhelm SK, Stephan C, Urrea KA, Palacio DP, Bartlett RH, et al. Extending heart preservation to 24 h with normothermic perfusion. Front Cardiovasc Med. 2024 Apr 4;11:1325169.

47. See Hoe LE, Li Bassi G, Wildi K, Passmore MR, Bouquet M, Sato K, et al. Donor heart ischemic time can be extended beyond 9 hours using hypothermic machine perfusion in sheep. J Heart Lung Transplant. 2023 Aug;42(8):1015-1029.

48. Funamoto M, Ong CS, Kilmarx SE, Li SS, Spencer PJ, Moonsamy P, et al. Utilization of new heart preservation technology in the era of new organ allocation and extended ischemic times. The Journal of Heart and Lung Transplantation. 2020 Apr;39(4).

49. Tong CKW, Khush KK. New Approaches to Donor Selection and Preparation in Heart Transplantation. Curr Treat Options Cardiovasc Med. 2021;23(5):28.

50. Kawabori M, Posawatz DA, Chen FY, Couper GS. A Simple Technique for Reliable Donor Organ Temperature Management With the SherpaPak System. ASAIO J. 2022 Aug 1;68(8):e134-e135.

51. Schmiady MO, Graf T, Ouda A, Aser R, Flammer AJ, Vogt PR, Wilhelm MJ. An innovative cold storage system for donor heart transportation-lessons learned from the first experience in Switzerland. J Thorac Dis. 2021 Dec;13(12):6790-6799.

52. Moayedifar R, Shudo Y, Kawabori M, Silvestry S, Schroder J, Meyer DM, et al. Recipient Outcomes With Extended Criteria Donors Using Advanced Heart Preservation: An Analysis of the GUARDIAN-Heart Registry. J Heart Lung Transplant. 2024 Apr;43(4):673-680.

53. Bixby CE, Balsam LB. Better Than Ice: Advancing the Technology of Donor Heart Storage With the Paragonix SherpaPak. ASAIO J. 2023 Apr 1;69(4):350-351.

54. Singh SS, Das De S, Spadaccio C, Berry C, Al-Attar N. An overview of different methods of myocardial protection currently employed peri-transplantation. Vessel Plus. 2017 Oct 26.

55. Minasian SM, Galagudza MM, Dmitriev YV, Karpov AA, Vlasov TD. Preservation of the donor heart: From basic science to clinical studies. Interactive CardioVascular and Thoracic Surgery. 2014 Dec 23;20(4):510–9.

56. Karduz G, Yaman MO, Altan M, Sahin G, Toraman F, Aksu U. St. Thomas and Del Nido Cardioplegia are superior to custodiol cardioplegia in a rat model of Donor Heart. Scandinavian Cardiovascular Journal. 2020 Nov 13;55(2):122–8.

57. Janssen H, Janssen PH, Broelsch CE. Celsior solution compared with University of Wisconsin solution (UW) and histidine-tryptophan-ketoglutarate solution (HTK) in the protection of human hepatocytes against ischemia-reperfusion injury. Transpl Int. 2003 Jul;16(7):515-22.

58. Weissenbacher A, Vrakas G, Nasralla D, Ceresa CDL. The future of organ perfusion and re-conditioning. Transpl Int. 2019 Jun;32(6):586-597.

59. White CW, Messer SJ, Large SR, Conway J, Kim DH, Kutsogiannis DJ, et al. Transplantation of Hearts Donated after Circulatory Death. Front Cardiovasc Med. 2018 Feb 13;5:8.

60. Skrzypiec-Spring M, Grotthus B, Szelag A, Schulz R. Isolated heart perfusion according to Langendorff---still viable in the new millennium. J Pharmacol Toxicol Methods. 2007 Mar-Apr;55(2):113-26.

61. Bell RM, Mocanu MM, Yellon DM. Retrograde heart perfusion: the Langendorff technique of isolated heart perfusion. J Mol Cell Cardiol. 2011 Jun;50(6):940-50.

62. Olejnickova V, Novakova M, Provaznik I. Isolated heart models: cardiovascular system studies and technological advances. Med Biol Eng Comput. 2015 Jul;53(7):669-78.

63. Jing L, Yao L, Zhao M, Peng LP, Liu M. Organ preservation: from the past to the future. Acta Pharmacol Sin. 2018 May;39(5):845-857.

64. Hatami, S., Freed, D.H. Machine Perfusion of Donor Heart: State of the Art. Curr Transpl Rep 6, 242–250 (2019).

65. White CW, Hasanally D, Mundt P, Li Y, Xiang B, Klein J, et al. A whole blood-based perfusate provides superior preservation of myocardial function during ex vivo heart perfusion. J Heart Lung Transplant. 2015 Jan;34(1):113-121.

66. Hatami S, White CW, Shan S, Haromy A, Qi X, Ondrus M, et al. Myocardial Functional Decline During Prolonged Ex Situ Heart Perfusion. Ann Thorac Surg. 2019 Aug;108(2):499-507.

67. Hatami S, Qi X, White CW, Bozso SJ, Himmat S, Sergi C, et al. The Position of the Heart During Normothermic Ex Situ Heart Perfusion is an Important Factor in Preservation and Recovery of Myocardial Function. ASAIO J. 2021 Nov 1;67(11):1222-1231.

68. Morito N, Obara H, Matsuno N, Enosawa S, Furukawa H. Oxygen consumption during hypothermic and subnormothermic machine perfusions of porcine liver grafts after cardiac death. J Artif Organs. 2018 Dec;21(4):450-457.

69. Bruinsma BG, Yeh H, Ozer S, Martins PN, Farmer A, Wu W, et al. Subnormothermic machine perfusion for ex vivo preservation and recovery of the human liver for transplantation. Am J Transplant. 2014 Jun;14(6):1400-9.

70. Quader M, Toldo S, Torrado J, Mishra S, Mauro AG, Mezzaroma E, et al. Determination of Optimal Coronary Flow for the Preservation of "Donation after Circulatory Death" in Murine Heart Model. ASAIO J. 2018 Mar/Apr;64(2):225-231.

71. Ta HQ, Teman NR, Kron IL, Roeser ME, Laubach VE. Steen solution protects pulmonary microvascular endothelial cells and preserves endothelial barrier after lipopolysaccharide-induced injury. The Journal of Thoracic and Cardiovascular Surgery. 2023 Jan;165(1).

72. Thatte HS, Rousou L, Hussaini BE, Lu XG, Treanor PR, Khuri SF. Development and evaluation of a novel solution, Somah, for the procurement and preservation of beating and nonbeating donor hearts for transplantation. Circulation. 2009 Oct 27;120(17):1704-13.

73. Lowalekar SK, Cao H, Lu XG, Treanor PR, Thatte HS. Subnormothermic preservation in somah: a novel approach for enhanced functional resuscitation of donor hearts for transplant. Am J Transplant. 2014 Oct;14(10):2253-62.

74. Lowalekar SK, Lu XG, Thatte HS. Further evaluation of Somah: long-term preservation, temperature effect, and prevention of ischemia-reperfusion injury in rat hearts harvested after cardiocirculatory death. Transplant Proc. 2013 Nov;45(9):3192-7.

75. Ribeiro RVP, Alvarez JS, Yu F, Paradiso E, Adamson MB, Maria Ruggeri G, et al. Hearts Donated After Circulatory Death and Reconditioned Using Normothermic Regional Perfusion Can Be Successfully Transplanted Following an Extended Period of Static Storage. Circ Heart Fail. 2019 Apr;12(4):e005364.

76. Amrani M, Ledingham S, Jayakumar J, Allen NJ, Rothery S, Severs N, et al. Detrimental effects of temperature on the efficacy of the University of Wisconsin solution when used for cardioplegia at moderate hypothermia. Comparison with the St. Thomas Hospital solution at 4 degrees C and 20 degrees C. Circulation. 1992 Nov;86(5 Suppl):II280-8.

77. Lowalekar SK, Cao H, Lu XG, Treanor PR, Thatte HS. Sub-normothermic preservation of donor hearts for transplantation using a novel solution, Somah: a comparative pre-clinical study. J Heart Lung Transplant. 2014 Sep;33(9):963-70.

78. Quader M, Torrado JF, Mangino MJ, Toldo S. Temperature and flow rate limit the optimal ex-vivo perfusion of the heart - an experimental study. J Cardiothorac Surg. 2020 Jul 22;15(1):180.

79. Duignan T, Guariento A, Doulamis IP, Kido T, Regan WL, Saeed M, et al. A Multi-Mode System for Myocardial Functional and Physiological Assessment during Ex Situ Heart Perfusion. J Extra Corpor Technol. 2020 Dec;52(4):303-313.

80. White CW, Ambrose E, Müller A, Li Y, Le H, Hiebert B, et al. Assessment of donor heart viability during ex vivo heart perfusion. Can J Physiol Pharmacol. 2015 Oct;93(10):893–901.

81. Bounader K, Flécher E. End-stage heart failure: The future of heart transplant and artificial heart. Presse Med. 2023 Oct 26;53(1):104191.

82. Pinnelas R, Kobashigawa JA. *Ex vivo* normothermic perfusion in heart transplantation: a review of the TransMedics® Organ Care System. Future Cardiol. 2022 Jan;18(1):5-15.

83. Lepoittevin M, Giraud S, Kerforne T, Barrou B, Badet L, Bucur P, et al. Preservation of Organs to Be Transplanted: An Essential Step in the Transplant Process. Int J Mol Sci. 2022 Apr 30;23(9):4989.

84. Rosenfeldt F, Ou R, Salamonsen R, Marasco S, Zimmet A, Byrne J, et al. A novel combination technique of cold crystalloid perfusion but not cold storage facilitates transplantation of canine hearts donated after circulatory death. J Heart Lung Transplant. 2016 Nov;35(11):1358-1364.

85. Foguenne M, MacMillan S, Kron P, Nath J, Devresse A, De Meyer M, et al. Current Evidence and Future Perspectives to Implement Continuous and End-Ischemic Use of Normothermic and Oxygenated Hypothermic Machine Perfusion in Clinical Practice. J Clin Med. 2023 Apr 29;12(9):3207.

86. Iske J, Schroeter A, Knoedler S, Nazari-Shafti TZ, Wert L, Roesel MJ, et al. Pushing the boundaries of innovation: the potential of *ex vivo* organ perfusion from an interdisciplinary point of view. Front Cardiovasc Med. 2023 Oct 12;10:1272945.

87. Wyss RK, Méndez Carmona N, Arnold M, Segiser A, Mueller M, Dutkowski P, et al. Hypothermic, oxygenated perfusion (HOPE) provides cardioprotection via succinate oxidation prior to normothermic perfusion in a rat model of donation after circulatory death (DCD). Am J Transplant. 2021 Mar;21(3):1003-1011.

88. Tingle SJ, Figueiredo RS, Moir JA, Goodfellow M, Thompson ER, Ibrahim IK, et al. Hypothermic machine perfusion is superior to static cold storage in deceased donor kidney transplantation: A meta-analysis. Clin Transplant. 2020 Apr;34(4):e13814.

89. Schlegel A, Muller X, Mueller M, Stepanova A, Kron P, de Rougemont O, et al. Hypothermic oxygenated perfusion protects from mitochondrial injury before liver transplantation. EBioMedicine. 2020 Oct;60:103014.

90. Tingle SJ, Figueiredo RS, Moir JA, Goodfellow M, Talbot D, Wilson CH. Machine perfusion preservation versus static cold storage for deceased donor kidney transplantation. Cochrane Database Syst Rev. 2019 Mar 15;3(3):CD011671.

91. Steen S, Paskevicius A, Liao Q, Sjöberg T. Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. Scand Cardiovasc J. 2016 Jun;50(3):193-200.

92. Ou R, Lim YW, Choong JW, Esmore DS, Salamonsen RF, McLean C, Forbes J, et al. Low-flow hypothermic crystalloid perfusion is superior to cold storage during prolonged heart preservation. Transplant Proc. 2014 Dec;46(10):3309-13.

93. Wicomb WN, Novitzky D, Cooper DK, Rose AG. Forty-eight hours hypothermic perfusion storage of pig and baboon hearts. J Surg Res. 1986 Mar;40(3):276-84.

94. Michel SG, La Muraglia GM 2nd, Madariaga ML, Titus JS, Selig MK, Farkash EA, et al. Twelve-Hour Hypothermic Machine Perfusion for Donor Heart Preservation Leads to Improved Ultrastructural Characteristics Compared to Conventional Cold Storage. Ann Transplant. 2015 Aug 11;20:461-8.

95. Hatami S, White CW, Ondrus M, Qi X, Buchko M, Himmat S, et al. Normothermic Ex Situ Heart Perfusion in Working Mode: Assessment of Cardiac Function and Metabolism. J Vis Exp. 2019 Jan 12;(143).

96. Sandha JK, White CW, Müller A, Avery E, Thliveris J, Dixon IMC, et al. Steroids Limit Myocardial Edema During Ex Vivo Perfusion of Hearts Donated After Circulatory Death. Ann Thorac Surg. 2018 Jun;105(6):1763-1770.

97. Subramani S, Kanthakumar P, Maneksh D, Sidharthan A, Rao SV, Parasuraman V, et al. O2-CO2 diagram as a tool for comprehension of blood gas abnormalities. Adv Physiol Educ. 2011 Sep;35(3):314-20.

98. Peltz M, Cobert ML, Rosenbaum DH, West LM, Jessen ME. Myocardial perfusion characteristics during machine perfusion for heart transplantation. Surgery. 2008 Aug;144(2):225-32.

99. Xia S, Chen G, Wang B, Yin Y, Sun Z, Zhao J, et al. Addition of Sodium Pyruvate to Stored Red Blood Cells Attenuates Liver Injury in a Murine Transfusion Model. Mediators Inflamm. 2016;2016:3549207. 005364.

100. Olkowicz M, Ribeiro RVP, Yu F, Alvarez JS, Xin L, Yu M, et al. Dynamic Metabolic Changes During Prolonged *Ex Situ* Heart Perfusion Are Associated With Myocardial Functional Decline. Front Immunol. 2022 Jun 24;13:859506.

101. Wipper S, Russ D, Iffland A, Burdorf L, Thein E, et al. Fluorescent cardiac imaging: a novel intraoperative method for quantitative assessment of myocardial perfusion during graded coronary artery stenosis. Circulation. 2007Aug28;116(9):1007-14.

102. Schmidt BM, Rezende-Neto JB, Andrade MV, Winter PC, Carvalho MG Jr, Lisboa TA, et al. Permissive hypotension does not reduce regional organ perfusion compared to normotensive resuscitation: animal study with fluorescent microspheres. World J Emerg Surg. 2012 Aug 22;7 Suppl 1(Suppl 1):S9.

103. Michaud K, Basso C, d'Amati G, Giordano C, Kholová I, Preston SD, et al; Association for European Cardiovascular Pathology (AECVP). Diagnosis of myocardial infarction at autopsy: AECVP reappraisal in the light of the current clinical classification. Virchows Arch. 2020 Feb;476(2):179-194.

104. Wilson HM, Cheyne L, Brown PAJ, Kerr K, Hannah A, Srinivasan J, et al. Characterization of the Myocardial Inflammatory Response in Acute Stress-Induced (Takotsubo) Cardiomyopathy. JACC Basic Transl Sci. 2018 Dec 31;3(6):766-778.

105. Spencer BL, Wilhelm SK, Stephan C, Urrea KA, Palacio DP, Bartlett RH, et al. Extending heart preservation to 24 h with normothermic perfusion. Front Cardiovasc Med. 2024 Apr 4;11:1325169.

106. Wicomb W, Boyd ST, Cooper DK, Rose AG, Barnard CN. Ex vivo functional evaluation of pig hearts subjected to 24 hours' preservation by hypothermic perfusion. S Afr Med J. 1981 Aug 8;60(6):245-8.

107. van Suylen V, Vandendriessche K, Neyrinck A, Nijhuis F, van der Plaats A, Verbeken EK, et al. Oxygenated machine perfusion at room temperature as an alternative for static cold storage in porcine donor hearts. Artif Organs. 2022 Feb;46(2):246-258.

108. Andrijauskaite K, Veraza RJ, Lopez RP, Maxwell Z, Cano I, Cisneros EE, et al. Novel portable hypothermic machine perfusion preservation device enhances cardiac viability of donated human hearts. Frontiers in Cardiovascular Medicine. 2024 Apr 2;11.

109. Copeland H, Hayanga JWA, Neyrinck A, MacDonald P, Dellgren G, Bertolotti A, et al. Donor heart and lung procurement: A consensus statement. J Heart Lung Transplant. 2020 Jun;39(6):501-517.

110. Li J, Xue C, Ling X, Xie Y, Pavan D, Chen H,et al. A Novel Rat Model of Cardiac Donation After Circulatory Death Combined With Normothermic *ex situ* Heart Perfusion. Front Cardiovasc Med. 2021 Jul 23;8:639701.

111. Ou R, Gavin JB, Esmore DS, Rosenfeldt FL. Increased temperature reduces the protective effect of University of Wisconsin solution in the heart. Ann Thorac Surg. 1999 Nov;68(5):1628-34; discussion 1634-5

112. García-Roa M, Del Carmen Vicente-Ayuso M, Bobes AM, Pedraza AC, González-Fernández A, Martín MP, et al. Red blood cell storage time and transfusion: current practice, concerns and future perspectives. Blood Transfus. 2017 May;15(3):222-231.

113. Mainardi G, Hatami S, Wagner M, Khan M, Wang X, Pidborochynski T, et al. (866) subnormothermic machine perfusion of neonatal and small pediatric sized Hearts. The Journal of Heart and Lung Transplantation. 2023 Apr;42(4).

114. Taylor MJ, Baicu SC. Current state of hypothermic machine perfusion preservation of organs: The clinical perspective. Cryobiology. 2010 Jul;60(3 Suppl):S20-35.

115. Trahanas JM, Witer LJ, Alghanem F, Bryner BS, Iyengar A, Hirschl JR, et al. Achieving 12 Hour Normothermic Ex Situ Heart Perfusion: An Experience of 40 Porcine Hearts. ASAIO J. 2016 Jul-Aug;62(4):470-6.

116. D' Alessandro AM, Southard JH, Love RB, Belzer FO. Organ preservation. Surgical Clinics of North America. 1994 Oct;74(5):1083–95.

117. Huang MQ, Zhang M, Zhan SL, Chen L, Xue ZL. Saturated Dissolved Oxygen Concentration in in situ Fragmentation Bioleaching of Copper Sulfide Ores. Front Microbiol. 2022 Apr 6;13:821635.

118. Crick SJ, Sheppard MN, Ho SY, Gebstein L, Anderson RH. Anatomy of the pig heart: comparisons with normal human cardiac structure. J Anat. 1998 Jul;193 (Pt 1)(Pt 1):105-19.

119. Pannekoek A, Ali U. Does the solution used for cold static storage of hearts impact on heart transplant survival? Interact Cardiovasc Thorac Surg. 2021 Oct 29;33(5):814-818.

120. Shah A, Brunskill SJ, Desborough MJ, Doree C, Trivella M, Stanworth SJ. Transfusion of red blood cells stored for shorter versus longer duration for all conditions. Cochrane Database Syst Rev. 2018 Dec 22;12(12):CD010801.