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*Published in:*  
 Transplantation

*DOI:*  
[10.1097/TP.0000000000002530](https://doi.org/10.1097/TP.0000000000002530)

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*Document Version*  
 Final author's version (accepted by publisher, after peer review)

*Publication date:*  
 2019

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Martins, P. N., Berendsen, T. A., Yeh, H., Bruinsma, B. G., Izamis, M.-L., Op den Dries, S., Gillooly, A. R., Porte, R., Yarmush, M. L., Uygun, K., & Markmann, J. F. (2019). Oxygenated UW solution decreases ATP decay and improves survival after transplantation of DCD liver grafts. *Transplantation*, 103(2), 363-370. <https://doi.org/10.1097/TP.0000000000002530>

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## **Oxygenated UW solution decreases ATP decay and improves survival after transplantation of DCD liver grafts**

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## **Authorship**

1. Paulo N. Martins and James F. Markmann participated in research design, performance of the research and writing of the paper.
2. Timothy A. Berendsen, Heidi Yeh, Bote G. Bruinsma, Maria-Louisa Izamis, Sanna Op den Dries, Robert Porte, Martin L. Yarmush, and Korkut Uygun participated in performance of the research and writing of the paper.
3. Andrew R. Gillooly participated in writing of the paper.

## **Disclosure**

The authors declare no conflicts of interest.

## **Funding**

Departmental grant

## **Abbreviations:**

ATP, adenosine triphosphate

DCD, donation after cardiac death

ECD, extended criteria donation

MDA, malondialdehyde

PFC, perfluorocarbon

UW, University of Wisconsin preservation solution

## ABSTRACT

**Background:** DCD liver grafts are known to be predisposed to primary nonfunction and ischemic cholangiopathy. Many DCD grafts are discarded because of older donor age or long warm ischemia times. Thus, it is critical to improve the quality of DCD liver grafts. Here, we have tested whether an enriched oxygen carrier added to the preservation solution can prolong graft survival and reduce biliary damage. **Methods:** We assessed the ATP content decay of mouse liver grafts after cold ischemia, warm ischemia, and combined warm+cold ischemia. In addition, we used a rat model of liver transplantation to compare survival of DCD grafts preserved in high-oxygen solution (pre-oxygenated PFC+UW solution) vs. lower-oxygen solution (pre-oxygenated UW solution). **Results:** ATP levels under UW preservation fall to less than 10% after 30 min of warm ischemia. Pre-oxygenated UW solution with PFC reached a significantly higher PaO<sub>2</sub>. After 45min of warm ischemia in oxygenated UW+PFC solution, grafts showed 63% higher levels of ATP (p=0.011). In addition, this was associated with better preservation of morphology when compared to grafts stored in standard UW solution. Animals that received DCD grafts preserved in higher oxygenation solution showed improved survival: 4 out of 6 animals survived long-term whereas all control group animals died within 24 hours. **Conclusions:** The additional oxygen provided by PFC during static cold preservation of DCD livers can better sustain ATP levels, and thereby reduce the severity of ischemic tissue damage. PFC-based preservation solution extends the tolerance to warm ischemia, and may reduce the rate of ischemic cholangiopathy.

## INTRODUCTION

Historically, continuous oxygenation was considered a crucial component of organ preservation, by maintaining cellular homeostasis and preventing cell damage <sup>[1]</sup>. However, with the introduction of the more sophisticated preservation solutions (e.g. UW solution), organ preservation without additional oxygenation was possible and became the standard of organ preservation. While the evidence comparing oxygenated with non-oxygenated preservation is limited, it seems that oxygenation may be particularly beneficial in extended criteria donation (ECD) such as donation-after-cardiac-death (DCD). These organs have a higher likelihood to develop primary-non-function and are predisposed to ischemic cholangiopathy <sup>[1-4]</sup>. It has been proposed that supplementation of oxygen during organ preservation may support oxidative phosphorylation thus driving ATP production. Cells then use ATP to uphold metabolic processes that protect the cell from ischemic damage <sup>[17, 21]</sup>. Novel preservation strategies may allow utilization of a larger pool of potential donors.

Perfluorocarbons (PFC) are biologically inert substances with a high solubility for oxygen approximately a hundred times higher than plasma. The amount of oxygen dissolved in plasma is less than 1% of the total amount of oxygen in blood, and the addition of PFC increases the content of dissolved oxygen to 10-15% <sup>[5]</sup>. A negligible O<sub>2</sub>-binding constant of PFC also allows the release of oxygen into the surrounding tissue more effectively than hemoglobin <sup>[6]</sup>. PFC-dissolved oxygen is characterized by a high extraction ratio, making it immediately available to tissues. Importantly, O<sub>2</sub> dissociation and tissue release are maintained at low temperatures <sup>[7]</sup>. Another advantage of PFC is its small particle size. Varying from 1/10<sup>th</sup> to 1/100<sup>th</sup> the size of an erythrocyte, PFC has a larger relative surface-to-volume ratio, which contributes to better

oxygenation of the microvasculature <sup>[8]</sup>. This is of particular importance to DCD grafts because the microvasculature can be compromised by micro-thrombi in the setting of cardiac death <sup>[9]</sup>. As a result of this unique combination of properties, PFC-based solutions have been examined as an oxygen carrier for blood substitutes, myocardial protection, ventilatory support, cell culture, and organ preservation before transplantation <sup>[10]</sup>.

PFC-based preservation solutions have been used in many models of organ preservation, including ischemic livers <sup>[11-16]</sup>. In addition, it has been investigated in an animal liver transplant model <sup>[46]</sup>. The aim of this study was to optimize oxygen delivery during the cold storage period by adding PFC to standard UW (University of Wisconsin) preservation solution, and confirm this using a rat liver transplant model. It is known that the period of warm ischemia associated with DCD exhausts ATP stores. We hypothesize that the addition of PFC may allow increased oxidative phosphorylation during cold storage, resulting in a more effective ATP recovery upon transplantation. This can alleviate ischemia reperfusion injury to the liver parenchyma and biliary tree and result in better outcomes post-transplantation.

#### **MATERIALS and METHODS:**

PFC (perfluorodecalin, FluoroMed L.P., Round Rock, TX, USA) was used in the concentration of 20% v/v and was preloaded with oxygen (1L/min) through direct bubbling for 30 min before preservation. Because the PFC has high density and does not mix with UW solution the graft was placed at the interface between PFC and UW solution (double layer method) <sup>[7]</sup>.

Animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Massachusetts General Hospital. After circulatory arrest livers from C57/B6 mice were flushed with UW solution and stored in either pre-oxygenated UW solution or UW+PFC solution in three different temperature groups (4°C for 24 h, 37°C for 60 min, and 37°C for 45 min followed by 36 h at 4°C, Fig. 1). The pre-oxygenation time was 30 min with an O<sub>2</sub> rate of 1 L/min. We used PFC at 20% v/v (3 mL PFC+12 mL UW solution) vs. 15 mL of UW in the control group. Storage of grafts at 37 °C for 45 min followed by 36 h at 4°C was used to simulate the warm ischemia experienced by DCD donors.

*ATP assay.* Liver biopsies were taken for analysis of tissue ATP content at different time points as described before (every 15 minutes in the warm ischemia group and every 2 hours in the first 12 hours, then every 6 hours to 36 hours in the cold ischemia group) <sup>[17]</sup>. Immediately after biopsy, the samples were snap frozen in liquid nitrogen and stored at -80°C. Prior to homogenization of the tissue, biopsies were mechanically pulverized in liquid nitrogen. Pulverized tissue was analyzed for ATP content using a luminescence-based cell viability assay (BioVision Inc.). Luminescence was measured using a single-tube detector (Molecular Devices, Sunnyvale, California). ATP content was normalized to protein content by the Bradford method, and measured spectrophotometrically after reaction with Coomassie dye.

*Rat orthotopic liver transplantation.* All animals were transplanted by an experienced microsurgeon (T.A.B.) according to the established protocol by Kamada et al. and optimized by Delrivière et al <sup>[18, 19]</sup>, and were divided in two groups differing only by the presence of PFC in

the UW solution (n=6/group, Fig. 2). Male Lewis rats weighing 250 to 300 g (Charles River Laboratories, Boston, MA, USA) were used as donors and recipients. The animals were maintained in accordance with National Research Council guidelines, and the experimental protocols were approved by the Subcommittee on Research Animal Care (SRAC), Committee on Research, Massachusetts General Hospital. All surgical procedures were performed under aseptic conditions. The animal was anesthetized with 5% isoflurane induction, followed by 1-2% maintenance (Baxter, Deerfield, IL, USA). Donor animals received heparin (1,000 units i.v) before opening of the chest and exsanguination of the animal until cardiac arrest occurred. We left the liver in situ for 30min (period of warm ischemia) and then flushed it either with oxygenated UW or with oxygenated UW+PFC. After that, the liver was removed and placed in a container containing UW solution or UW+PFC at 4°C for an additional 4 hours (period of cold ischemia). Both solutions were pre-oxygenated for 30 minutes before storage of the graft. Grafts were flushed with normal saline to remove the preservation solutions before transplantation. Livers were transplanted using the cuff-technique and without arterial anastomosis because an arterialized model was not available in our lab <sup>[18]</sup>.

*Histology.* Liver parenchyma biopsies were fixed in 10% formalin. After paraffin embedding, samples were sectioned and stained with hematoxylin and eosin (H&E). We used cytokeratin 19 antibody (sc-33119, Santa Cruz Biotechnology). Samples were stained for caspase-3 as markers of apoptosis. Bile duct damage was analyzed by light (H&E, and cytokeratin-19 immunohistochemical staining), and by electron microscopy.

*Electron microscopy.* Common bile duct specimens were fixed in a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer. After dehydration in a graded series with ethanol, samples were embedded in Epon 812 resin. Excised transplants were



fixed in 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). Electron microscopy was performed with a transmission electron microscope (EM902, Carl Zeiss, Oberkochen, Germany) at 80 kV. Images were recorded with a digital camera.

*Statistical analysis.* Continuous data are presented as mean  $\pm$  standard deviation. We used either ANOVA or student T test to compare continuous variables in different groups. P values  $<0.05$  were considered statistically significant.

## RESULTS

### *Oxygenated PFC+UW solution improves ATP stores after warm and cold ischemia*

First, the oxygen content ( $\text{PaO}_2$ ) of the solutions was measured in the absence of a liver graft. Following 30 min of pre-oxygenation, the  $\text{PaO}_2$  of the UW+PFC solution, as expected, was increased when compared to pre-oxygenated UW solution (Fig. 3). In the first hours, the oxygen content of both groups dropped with a similar trend, while the PFC+UW group retained higher dissolved oxygen content after longer preservation.

The solutions were then tested in an extreme warm ischemia model of the mouse liver. After a period of 45 min of warm ischemia at  $37^\circ\text{C}$ , the livers preserved in PFC+UW solution showed over 30% higher levels of ATP in the liver parenchyma when compared to UW solution (Fig. 4A). In comparison, livers that underwent cold ischemia only manifested a markedly slower decrease of ATP over many hours (Fig. 4B). Livers preserved in PFC+UW solution had ATP levels multi-fold higher than UW preservation alone up to 24 hours ( $p<0.05$ ), after which ATP was depleted in both groups. To simulate the clinical situation with both warm and cold ischemia, we included a group that underwent both warm (45 min) and cold (36h) ischemia, and

followed ATP. In this setting, livers stored in PFC+UW solution also retained significantly higher ATP levels after 6 h ( $p=0.021$ ), 12h ( $p=0.007$ ), and 18h ( $p=0.02$ ) (Fig. 4C).

#### ***Liver grafts preserved in PFC+UW solution show better histological morphology***

As expected for a non-transplant model and with short-term preservation, the histological changes in livers after 45 minutes of warm ischemia observed with light microscopy were modest. However, storage in UW+PFC was associated with less ballooning of hepatocytes (Fig. 5A). Using light microscopy, we found marked changes in the liver parenchyma between the two groups only after 72hrs of cold ischemia. The PFC+UW solution grafts show less cellular edema and necrosis (Fig. 5E). On the other hand, the ischemic damage suffered by the biliary epithelium was more intense after warm ischemia than the damage found in the liver parenchyma. Oxygenated PFC+UW solution was associated with improved preservation of the biliary epithelium. The epithelium of the gallbladder and bile duct (non-transplanted) preserved in oxygenated UW solution after 45 minutes of warm ischemia was flat and started sloughing-off (Figs. 6). When examined using EM, biliary epithelium preserved in UW solution without PFC showed loss of apical microvilli and increased apoptosis (Fig. 7D-F) and increased expression of caspase 3 (Fig. 8). These characteristics were improved in oxygenated PFC+UW solution (Figs. 7C and 8B).

#### ***PFC+UW solution improves survival of DCD liver grafts***

In a rat model of orthotopic liver transplantation, four out of six animals (66%) that received DCD grafts (30 min of warm ischemia+4 hours of cold ischemia) preserved in UW+PFC solution survived long term (>100 days), while all animal of the control group died within 24 hours after liver transplant ( $p=0.0005$ ). Two animals of the PFC+UW group survived for more than 6 months (Fig. 9).

## **DISCUSSION**

### ***Importance of oxygenation in liver preservation***

It has been suggested that oxygenation during organ storage is beneficial to extended criteria organs <sup>[1]</sup>. During liver machine preservation, oxygenation of the perfusate reduces alanine aminotransferase compared to controls. This also resulted in reduced oxygen free radical-mediated lipid peroxidation upon reperfusion, and activated the AMP salvage pathway. Enzyme release during reperfusion was reduced by 70% with additional oxygenation compared with controls. Functional recovery (bile production) was enhanced by approximately twofold with high oxygenation of the perfusate <sup>[20]</sup>.

Our group and others have demonstrated that liver metabolism can be optimized before transplantation to improve postoperative outcome <sup>[2,17,21–26]</sup>. Energy repletion processes during preservation are correlated with resumption of normal metabolic function of the liver <sup>[17,18,22–25,27]</sup>. We show that ATP stores drop dramatically after warm ischemia, and that subsequent addition of PFC mitigates this drop in ATP levels.

### ***PFC use in graft preservation and clinical transplantation***

Oxygen carriers have been used in experimental models of preservation of various organs (e.g. kidney, liver, pancreas, heart, lung, and intestine) <sup>[11,13,15,16,29]</sup>, and in clinical transplantation studies of kidney and pancreas <sup>[6,30]</sup>. These studies show that PFC oxygenation increased graft tolerance to ischemia. When the pancreas is preserved in PFC-based preservation solution, the organ continuously generates ATP for up to 96 hours <sup>[16]</sup>, which it uses to maintain cell integrity. Thus, PFC-based solution prevents pancreas swelling more effectively than UW solution alone <sup>[32, 33]</sup>. Furthermore, it improves the viability of vascular endothelium and microcirculation <sup>[34]</sup>. In a rat small bowel transplantation model, PFC based solution successfully preserved grafts twice

as long as UW storage only <sup>[13]</sup>. Similar results were seen in a canine small bowel model and a rat heart transplant model. Seven out of eight recipients of small bowel preserved with UW solution died within 3 days, while grafts preserved in PFC+UW solution survived <sup>[14, 35]</sup>. Kurota et al. showed that after 90 minutes of warm ischemic injury, the canine pancreas grafts lost ATP and were no longer viable. However, when the damaged pancreas was resuscitated by PFC-based solution for 24 to 48 hours at 4°C, the grafts regained viability <sup>[34]</sup>.

PFC-based solutions are simpler to use and cheaper than oxygenated machine preservation. To our knowledge, there is no report of liver preservation with PFC-based solutions in the clinical setting. However, in a rat model, Bezinover reported that when livers were flushed with PFC added to the preservation solution and stored for 8 hours, they showed less damage based on AST levels, histology score, and caspase-3 expression by immunohistochemistry <sup>[11]</sup>. In another study, Bezinover et al. showed that preservation of ischemic rat liver grafts with oxygenated UW solution (with or without PFCs) produces superior preservation of the graft, based on the pattern of hepatic gene expression, intracellular fat score, degree of caspase-3 activation, as well as the ADP/ATP ratio when compared with standard storage in UW solution without oxygenation <sup>[12]</sup>. Another study using a rat DCD model showed that liver grafts subjected to 30 minutes of warm ischemia after cardiac arrest followed by 18 hours of cold ischemia could be reconditioned by gaseous oxygenation (persufflation) in the first 2hrs of cold storage <sup>[41]</sup>. However, the last three studies were not performed in a transplantation model. Our results are consistent with more recent rat liver transplant experiments performed by Okumura et al. In regards to diffusion of O<sub>2</sub> through our perfusion system, we hypothesize that O<sub>2</sub> preferentially flows from the area of highest affinity to lowest in the graft. In PFC alone after O<sub>2</sub> infusion, a PaO<sub>2</sub> of approximately 600mmHg was measured, compared to nearly 900mmHg in PFC+UW. Because PFC and UW do

not homogenize, the graft remains immersed in UW and is fed by O<sub>2</sub> moving from the PFC phase, through the UW and finally into graft tissue across the capsule. However, due to the passive nature of the O<sub>2</sub> diffusion, we recognize that deeper parenchymal tissue may be unequally exposed as those nearest the surface of the organ. DCD donor livers preserved in 20% preoxygenated UW solution for 3 hours before transplant showed a significant reduction in serum malondialdehyde (MDA) levels ( $p=0.03$ ) compared to control livers perfused in UW alone, indicating reduced oxidative stress <sup>[46]</sup>. Furthermore, histological examination of livers treated with preoxygenated UW demonstrated less focal necrosis and architecture loss plus significantly improved Suzuki IRI scores ( $p<0.001$ ) compared to controls <sup>[46]</sup>. The authors argue that reduced oxidative stress likely attenuated hepatocyte mitochondrial swelling, thereby mitigating histological damage. Biliary damage assessed with TEM showed preserved microvilli in the canaliculi of their UW+PFC group with significant loss of microvilli in controls. We also observe blunting of canaliculi in mouse bile ducts with UW only perfusion in contrast to UW+PFC. However, as neither this study nor ours investigated biliary complications after transplant – a major problem in using DCD grafts in humans – we are limited in drawing conclusions regarding biliary function after using PFC. In terms of overall survival after transplant, Okumura et al. show that DCD graft preservation in UW+PFC increased 14 day survival from 28.6% to 85.7% ( $p=0.02$ ) <sup>[46]</sup>. Interestingly, as in our study, mortality of control animals with plain UW preservation grafts was striking.

### ***Clinical Use***

Most of the experience with PFC-based solution was obtained with respect to the pancreas. Currently, PFC is used by a number of centers in pancreatic islet preservation <sup>[6]</sup>. For whole-

pancreas preservation, it was clinically used for the first time in 1999 at the University of Minnesota. In the first clinical trial of 10 pancreas transplants, no adverse effects on the recipients after transplantation were reported <sup>[6]</sup>. In a clinical study with 58 DCD kidney grafts flushed with 20% PFC added to supplemented UW solution, Reznik showed that the incidence of delayed graft function was reduced by 30% and serum creatinine was half that of the control group 21 days after transplant <sup>[30]</sup>.

### ***Limitations of PFC therapy and study limitations***

PFCs are biologically inert and cannot bind to any protein or enzyme <sup>[8]</sup>. Intravenous perfluorocarbon (PFC) forms emulsions with other agents and is cleared from the blood through phagocytosis by reticuloendothelial macrophages before ultimately being eliminated through the lungs approximately 4-12 hours after infusion. Although PFC use for organ or islet transplantation has not been associated with side-effects <sup>[7]</sup>, larger volumes of intravenous PFC can elicit flu-like symptoms and cutaneous flushing in rare cases. These effects are reversible <sup>[42]</sup>. In the 1980–1990s PFCs were pursued as “blood substitutes”. In 1989, the United States Food and Drug Administration approved the PFC emulsion Fluosol-DA-20% for IV use <sup>[43]</sup>. Fluosol was approved as an “oxygen therapeutic” for treatment of myocardial ischemia at the time of balloon angioplasty <sup>[43]</sup>. At least 15,000 patients received Fluosol-DA-20% <sup>[42]</sup>. For organ transplantation the risks of PFC therapy are even more negligible once the graft is flushed before transplantation to remove the potassium rich UW preservation solution.

One limitation of static PFC oxygenation is that oxygenation is done passively by diffusion from the oxygen rich (PFC) compartment in the bottom of the solution to the lower oxygen compartment (UW solution+graft) on the top. This creates a gradient of oxygen delivery that is not equally divided over the organ <sup>[44]</sup>. Another limitation of our study is that we could not assess

long-term complications of ischemic cholangiopathy because the transplant model we used is not arterialized (technique not available in our lab because of technical challenges). This creates a challenge in assessing the contribution of this organ preservation method on biliary complications <sup>[45]</sup>. In addition, no rat in the control group survived more than one day to allow meaningful comparison.

In conclusion, we show herein that PFC addition to UW preservation solution slows the decrease of ATP, thus reducing tissue damage/apoptosis and improving post-transplant survival in a rat liver transplant model. This suggests that a PFC based preservation solution may extend tolerance to ischemic damage and open a wider pool of ECD donors in human transplant.

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## FIGURE LEGENDS:

Fig. 1. Liver preservation model (no reperfusion). We first pre-loaded the preservation solutions (UW and UW+PFC) by bubbling O<sub>2</sub> 1L/min for 30 min. Next, we preserved liver grafts in either oxygenated UW or oxygenated UW+PFC at physiologic temperature (37°C), cold temperature (4°C) or warm followed (37°C) by cold temperature (4°C). We took liver biopsies at regular intervals to measure ATP content (every 15 min in the warm ischemia model and every 2 hours for the first 12 hours and every 6 hours until 36 hours in the cold ischemia model) to assess the kinetics of ATP decay. Legend. UW: University of Wisconsin solution, PFC: perfluorocarbon solution, O<sub>2</sub>: oxygen.

Fig 2. Transplantation model. We performed syngeneic transplantation after a period of 30 min of warm ischemia followed by 4 hours of cold ischemia in either oxygenated UW or oxygenated UW+PFC and followed survivors long-term. Legend: UW: University of Wisconsin solution, PFC: perfluorocarbon solution, O<sub>2</sub>: oxygen, LTx: liver transplant

Fig. 3. PaO<sub>2</sub> in different oxygenated preservation solutions; oxygenated UW solution, and UW solution plus oxygenated PFC, and baseline oxygen levels in UW solution (horizontal dashed line) over 20 hours at 4°C. The pre-oxygenation time was 30 minutes with 1L/min. Volume: PFC 20% (3ml PFC+12 ml UW) vs. UW 15ml. n=6. The PaO<sub>2</sub> in oxygenated PFC UW solution drops quickly and in the next 4 hours is very similar to the PaO<sub>2</sub> in oxygenated UW solution. However, after that point and up to 20 hours it is still higher.

Fig. 4. ATP decay in mouse liver grafts. (A) Decay in warm ischemia groups (37 °C, n=6). The level of ATP in liver tissue preserved in oxygenated UW solution alone (-■-) falls to less than 15% after 30 minutes of warm ischemia. Although grafts preserved in oxygenated UW+PFC solution (-●-) had significantly increased levels of ATP (at 60 min p=0.002), this increase was discrete. (B) Cold ischemia groups (4°C, n=6). Addition of oxygenated PFC to UW solution (-●-) is associated with a very significant increase of ATP stores during 24 hours of preservation. (C) ATP decay in mouse livers after warm ischemia (45 minutes at 37°C) followed by cold storage (4°C) for 36 hours in either oxygenated UW (-■-) or oxygenated PFC+UW solution (-●-); n= 6 mice/group, p<0.001.

Fig. 5. Representative sections of mouse livers. (A) Naïve liver, (B) Liver after 45 minutes of warm ischemia in oxygenated UW solution, and (C) Liver after 45 minutes of warm ischemia in oxygenated PFC+UW solution, respectively. Trichrome blue staining 400x. Representative H&E staining of mouse livers after 72 hours of cold ischemia in either oxygenated UW solution (D) or oxygenated PFC+UW (E). PFC supplemented solution led to less cellular edema and necrosis.

Fig. 6. Mouse gallbladder after 45 minutes of warm ischemia (37°C). Left panel (naïve gallbladder), middle panel (after 45 minutes of warm ischemia in oxygenated UW solution), right panel (after 45 minutes of warm ischemia in oxygenated PFC+UW solution). Cytokeratin-19 Staining (40x and 200x).

Fig. 7. Microscopic cross-section of mouse extra-hepatic bile ducts: Naïve (A), after 45 minutes of warm ischemia in either oxygenated UW preservation solution (B), or oxygenated PFC+UW

solution (C), Toluidine Blue staining 200x. The biliary epithelium preserved in UW solution without PFC duct shows loss of apical microvilli and apoptosis (7E) compared to native (7D) and UW+PFC (7F) treated livers, EM.

Fig. 8. Oxygenated PFC was associated with less caspase-3 expression in mouse livers after warm ischemia. Caspase staining (apoptosis) of the liver after 45 minutes of warm ischemia in oxygenated UW solution (A) vs oxygenated PFC+UW solution (B). Caspase-3 staining 100x.

Fig. 9. Oxygenated PFC+UW solution dramatically increased animal survival. Survival curves of rats that received DCD livers (30 minutes of warm ischemia + 4 hours of cold ischemia at 4°C) in oxygenated UW solution (—) vs. oxygenated UW+PFC (- - -).



Fig. 1

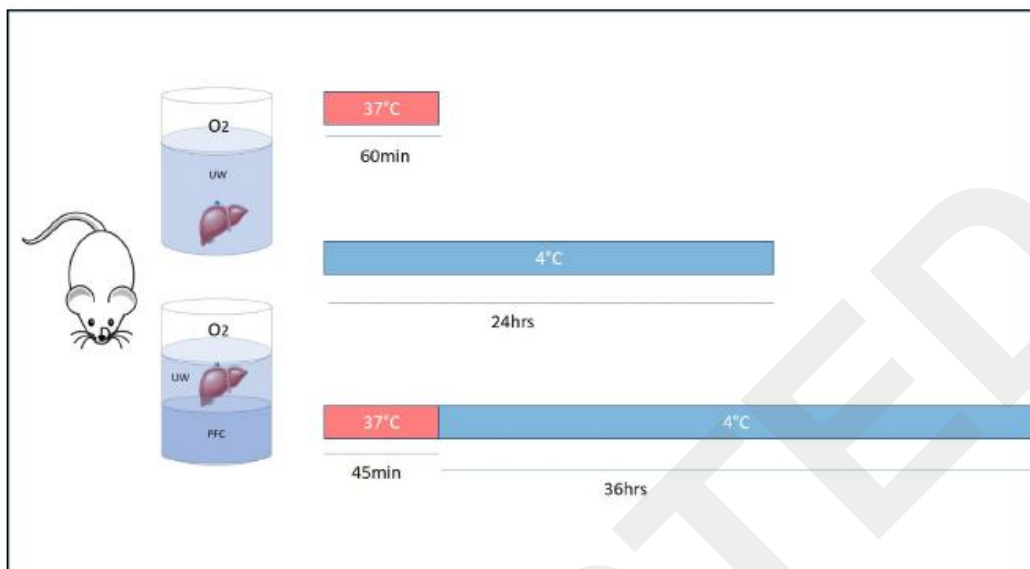


Fig. 2

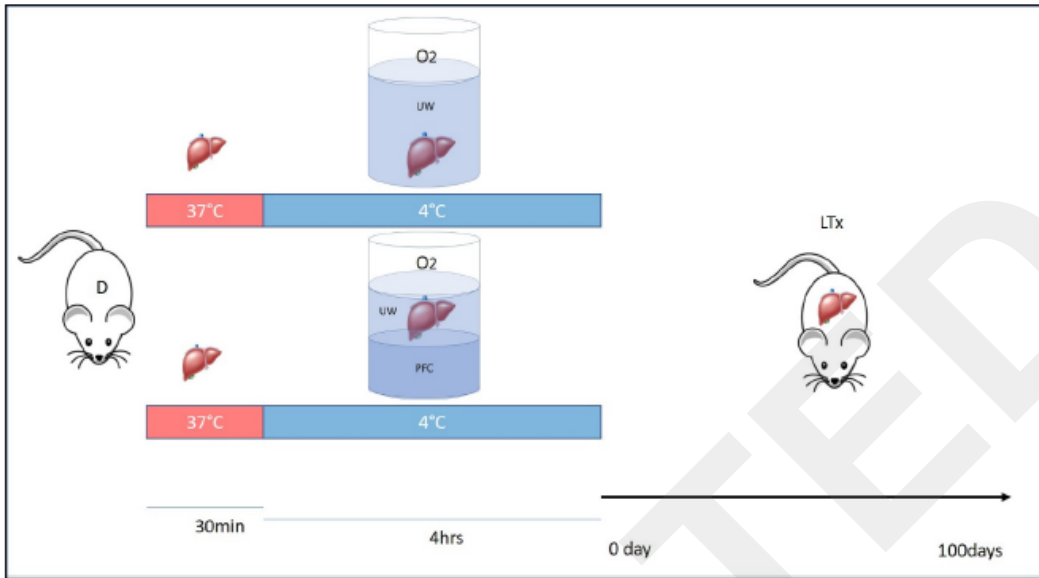


Fig. 3

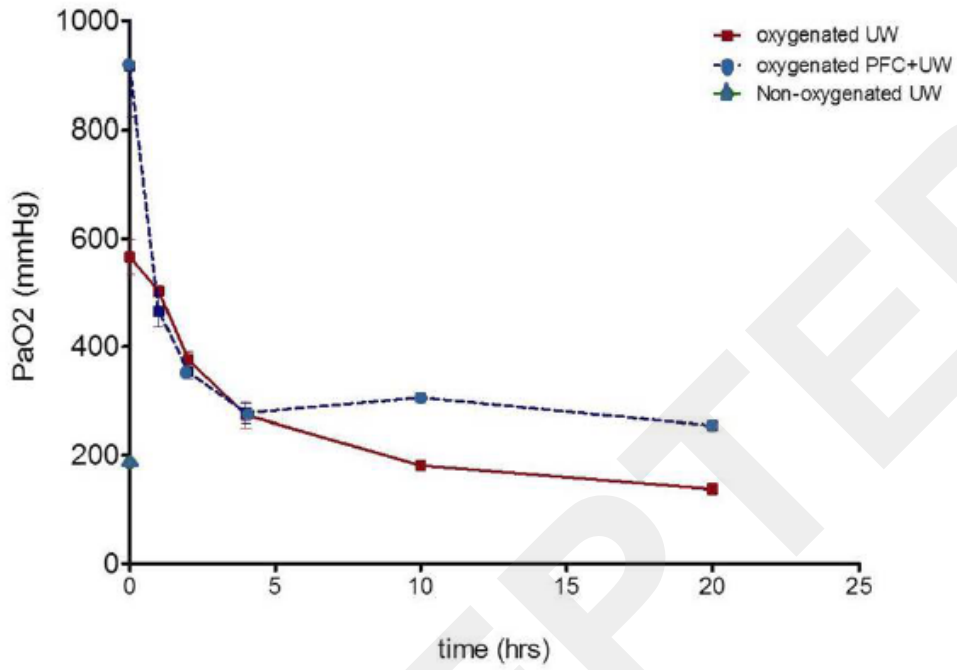


Fig. 4

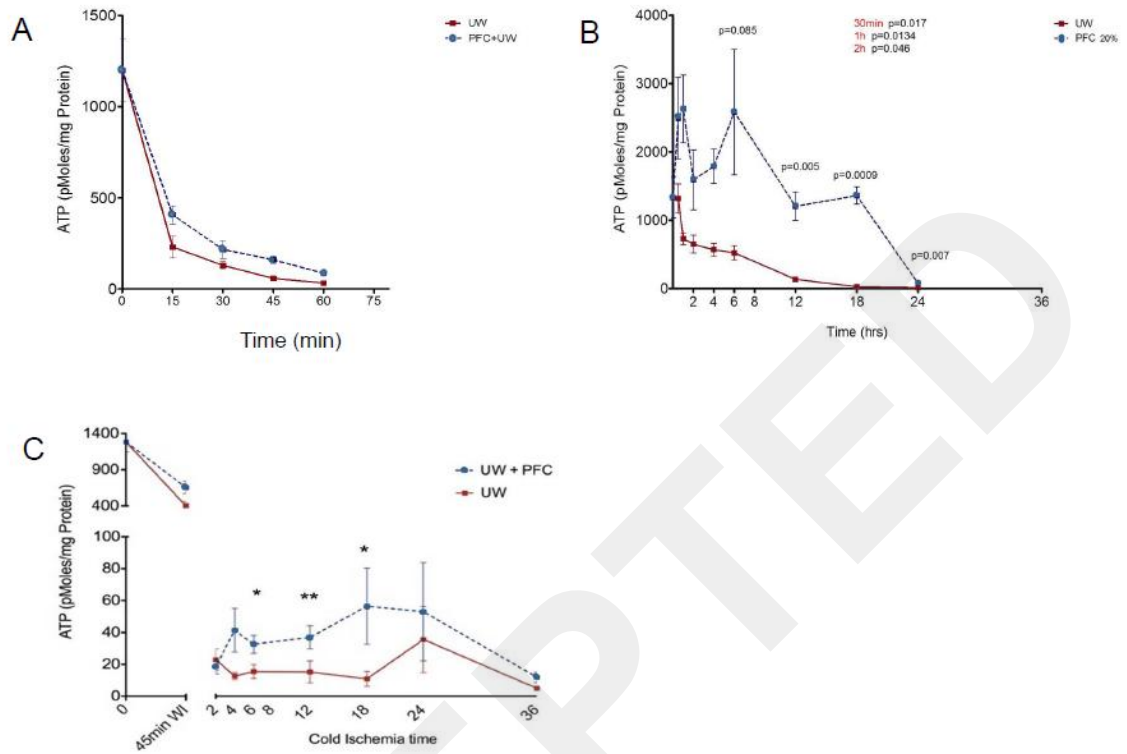


Fig. 5

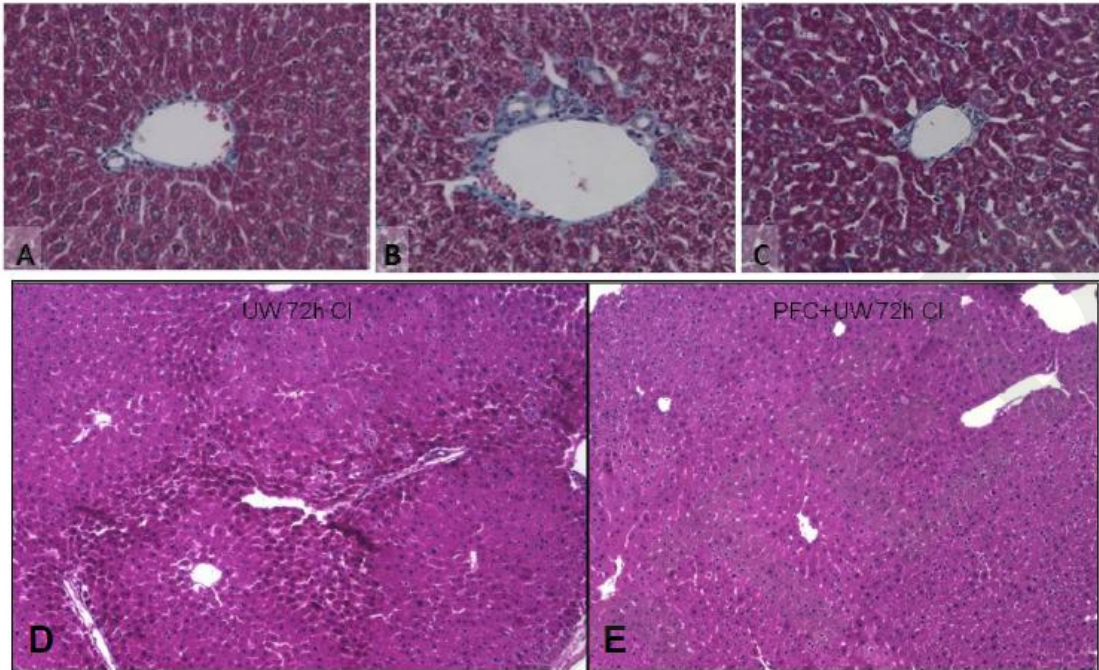


Fig. 6  
40X



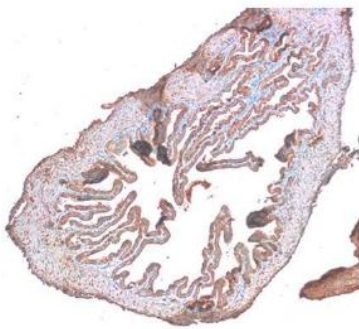
Naïve GB



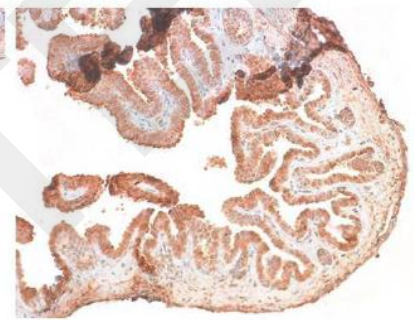
UW 45min WI



PFC+UW 45min WI



200x



ACCEPTED

Fig. 7

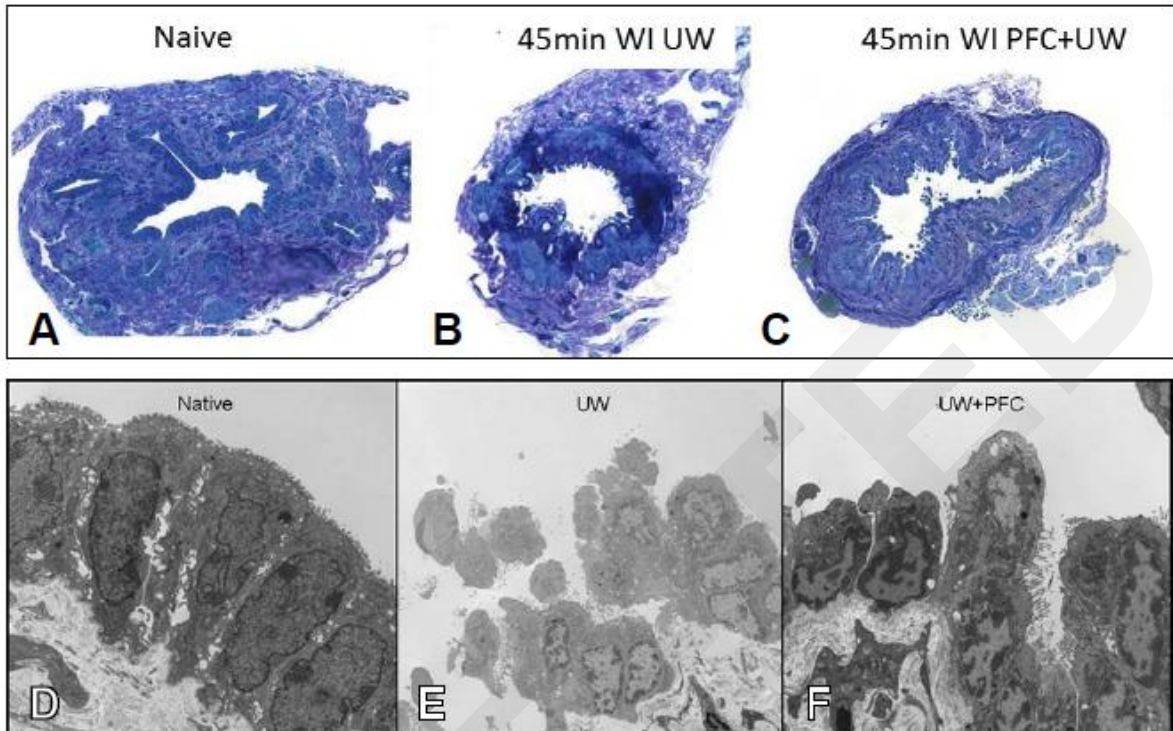
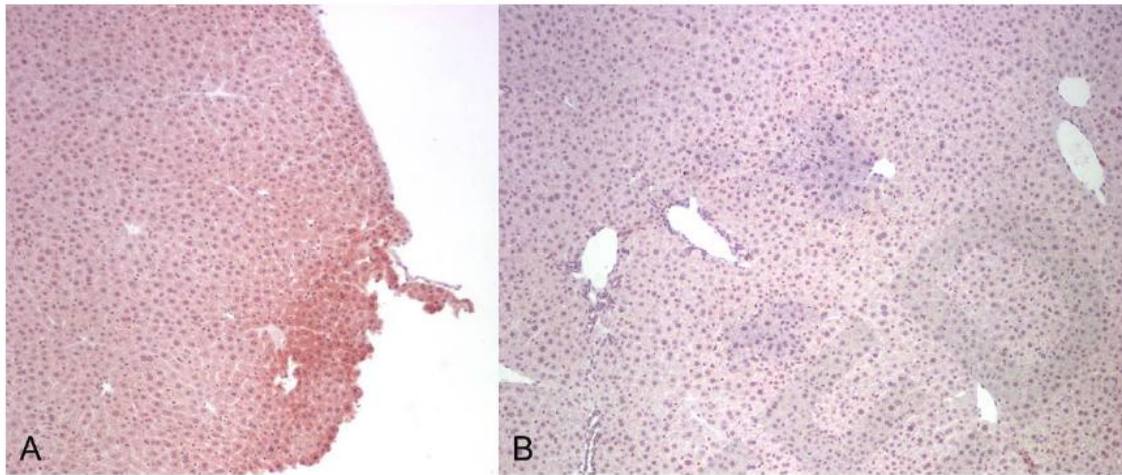




Fig. 8



ACCEPTED



Fig. 9

