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Exploring donor demographics effects on hepatocyte yield and viability: Results of whole human liver isolation from one center

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Abstract

Due to the growth of cell-based therapeutic alternatives addressing the shortage of livers for transplant, there is necessity for a reliable source of human hepatocytes. In addition, pharmaceutical research often requires human hepatocytes to assess new drug therapies during development or to achieve FDA approval. Whole human livers producing large quantities of cells from the same donor are ideal, enhancing reproducibility for all purposes, while also allowing for capturing variances in drug-metabolism across different demographics for pharmaceutical testing and development but are limited in availability and quality for research purposes. The present study investigates the effect of donor and liver procurement factors of 16 human livers on cell viability and yield, showing that typical exclusion criteria for transplant still produce viable hepatocytes with significant yields. Although limited in number of data points, which should be taken into consideration, the conclusions of this study could be utilized as indications, allowing for expansion of liver selection criteria for hepatocyte isolation and provide the necessary quality hepatocytes in large quantities for the growing pharmaceutical, biomedical, and therapeutic research fields.

Keywords

Hepatocytes; Hepatocyte Isolation; Whole Human Livers; Hepatocyte Cell Yield; Hepatocyte Cell Viability; Liver Procurement

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COMPETING FINANCIAL INTERESTS

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INNOVATION

In order to sustain research focusing on cell therapies for transplantation, bioengineering techniques, or development of drug therapies, the need for whole human livers for hepatocyte isolation is at an all-time high. This study provides the evidence that livers deemed unsuitable for transplantation are still capable of producing high quality cells, high yields and viabilities. Using these findings may support expansion of criteria for accepting livers for hepatocyte isolation thereby increasing the number of whole livers used for these purposes and decreasing the amount of livers that would otherwise be discarded, allowing a greater amount of human livers to be used for innovations that address the organ shortage and promote the future in drug therapies and bioengineering.

INTRODUCTION

Primary human hepatocytes continue to be a valuable tool for pharmaceutical, biomedical, and therapeutic purposes. Primary human hepatocytes provide a solid foundation of important in vivo cell function whereas alternate sources such as cell lines lack the ability to function like a true primary cell¹. The utilization of primary hepatocytes has a wide range and are used to study the interaction of drugs, metabolism, and drug toxicity as well as performance in hepatocytes transplantation and recellularized transplanted liver grafts. The usage of primary hepatocytes that are isolated from donor livers continues to be the most valuable resource as these show the clearest picture of therapeutic effects as they would be in vivo.

These fields of research are becoming increasingly important to address the liver organ shortage as the primary therapy for end stage liver failure remains orthotopic liver transplantation. According to the United Network of Organ Sharing, the waitlist for a liver transplant exceeds 14,000 people and many people will expire prior to receiving a liver transplant². This shows how much alternate therapies are needed. Many new and exciting therapies are being developed such as recellularized bioengineered livers³⁻⁵ and hepatic cell transplantation⁶ but remain limited on a therapeutic level⁷. On a pharmaceutical level, primary hepatocytes are used to determine the toxicity, metabolism, and drug-drug interactions necessary for FDA approval^{8,9}. A large source of human hepatocytes is thereby beneficial for continuing this research as well as potentially developing new lines of therapy both clinically and pharmaceutically.

A limitation persists in the availability of donor tissue as most healthy tissue is used for transplantation and thus isolation of hepatocytes from donor tissue has extended to attaining liver tissue that is typically not suitable for transplantation. Examples of such livers include: steatotic livers, death by cardiac death (DCD) donors, and prolonged warm and cold ischemia time livers. The question remains at large however if these factors of unsuitability and donor demographics affect the cell yield and viability of freshly isolated hepatocytes.

This study looked at a multitude of demographics and other variables of numerous donor livers and if they had a significant impact on cell yield and viability. All liver tissue studied were whole livers that were deemed unsuitable for transplant and were received at the Cell

Resource Core at Massachusetts General Hospital in Boston, MA through credited procurement agencies. The purpose of reviewing how these donor demographics and other variables influence cell yield and viability is to give detailed data that less than suitable organs still produce viable cells upon isolation, and could provide an expansion in selection criteria of livers for hepatocyte isolation, as well as improve operational efficiencies in isolation by tuning the selection criteria.

METHODS

A previously established 2-step digestion protocol was followed with slight modifications¹⁰. Solutions used for perfusion containing Krebs Ringer Buffer (KRB), EDTA (Thermo-Fisher Scientific) and Collagenase Type IV (Sigma-Aldrich) were made prior to the arrival of the liver. The perfusion system comprised of two water baths to maintain adequate temperature of the liver and the solutions, Masterflex peristaltic pumps, oxygenators (Radnoti), and bubble traps, were flushed with an isopropanol solution prior to use.

Once the liver arrived, it was sliced into 2–3 segments keeping in mind the presence of vessels for adequate perfusion. Luer Locks (Harvard Apparatus) were used in order to attach each liver segment to the perfusion system. After the liver segment was weighed, it was placed in the water bath set to 37°C and connected to the perfusion system. Compressed oxygen (AirGas) was turned on to allow oxygenation of the solutions. Two different perfusion solutions were perfused through the liver resection. Both perfusion steps were long enough to ensure adequate cellular detachment from the basilar membrane.

The purification portion of the protocol consisted of washing the cells with DMEM and filtering cell-containing solution through 250 µm and 90 µm mesh filter (Amazon) and multiple centrifugation steps at 50 g and 100 g speeds. A Percoll (Amazon) purification step was added to remove any dead cells. Once cell counting and viability determination were completed using Trypan blue, a cryopreservation solution was made containing University of Wisconsin solution (Fisher Scientific), Glucose, BSA and DMSO (Sigma-Aldrich). Using a Fill-it Machine (TAPBiosystems), the cells were dispensed in Cryovials (Fisher). A controlled rate freezer (CryoMed®, Thermo-Fisher) was used to allow the cells to be gently cooled down to –180°C. After freezing, the cells were placed in a liquid tank with a temperature of –180°C.

LIVER DONOR AND PROCUREMENT DATA

Livers from donors with a history of cirrhosis, fibrosis, and steatosis were not accepted for the purposes of this study. Social history was considered but were ultimately not deciding factors on whether to accept or decline livers for hepatocyte isolation. A total of 16 livers were isolated using the methods described above. Donor demographics and liver status are summarized in Table 1; please note that in some cases not all demographic and liver procurement data was available, and the yields and viabilities were subsequently not used in that particular data analysis for which data was missing. All the livers came from a credited organ procurement agency and were screened for serologies prior to acceptance. The livers accepted for isolation purposes were declined for transplant mostly due to age and DCD

status. Liver demographics and other data that were noted were age, BMI, gender, race, cause of death, donor status, warm ischemia time, and cold ischemia time. Donor status was noted to be donation after cardiac death (DCD) or donation after brain death (DBD). Of the hemodynamic data, time spent under inadequate levels of saturation of oxygen (SpO₂) and Mean Arterial Pressure (MAP) was studied. Warm ischemia time (WIT) was noted to be the length of time of extubation to the time of the flush¹¹ and cold ischemia time (CIT) was noted to be the length of time of cross clamp of the aorta to the time the isolation was started.

STATISTICS

All analyses were completed using GraphPad Prism®. For each variable, we studied the effects on both the cell yield and the viabilities of the cells. Cell yield was calculated as million per gram of tissue (M/g) and viabilities were calculated as percentages of viable cells compared to total cell yield. Data values that were incorporated in the analyses were both of successful and non-successful hepatocyte isolations. Non-successful hepatocyte isolations were determined as isolations where no cells were extracted from the tissue and no viability could be determined.

Variables with wide ranges were grouped by using previously established groupings (i.e. BMI). If no previously established groups existed, a median split was performed to create groups for comparison (i.e. WIT, SpO₂, MAP). Since the range of WIT in our study ranged from 23 minutes to 51 minutes, a median split was performed to divide the data into groups for comparison. The median split gave a median time of 30 minutes thereby viabilities and yields of WIT below and above 30 minutes were compared; notably 30 minutes is also the traditional limit of WI for transplantation of DCD livers. The general value for inadequate oxygen saturation is typically below 90%¹², however this study determined to calculate the time spent under an oxygen saturation of 80% as the majority of the livers immediately plunged below 90% oxygen saturation during the extubation period. A median split was performed to address the wide ranges of time spent below 80% SpO₂ and to determine groups for comparison with the result being 90% of time spent below and SpO₂ of 80%. The Mean Arterial

Pressure (MAP) was calculated by using the general formula:

$$MAP = \frac{2(\text{Diastolic blood pressure}) + \text{systolic blood pressure}}{3}.$$

The general cut off for an inadequate MAP is 60–70 mmHg¹¹ thereby the time spent of WIT under this value (%tMAP) was calculated using the hemodynamic data provided by the procurement agency. A median split was performed to determine the groups for comparison with the result being 75% of time spent below 70 mmHg. Cold ischemia time (CIT) was calculated by referring to the cross-clamp time documented by the procurement agency and taking note of what time the isolation was started for 14 livers. Data was grouped in CIT less than 12 hours versus more than 12 hours as the generally accepted CIT is 6–10 hours¹³. Of the binary data sets (donor status, gender, CIT, hemodynamic data, and WIT) an unpaired *t*-

test was performed to determine if there was a significant difference between the demographic and its effect on cell yield or viability. If data was deemed to be non-parametric, a Mann-Whitney test was performed instead. The other statistical test done on the data sets was an ordinary one-way ANOVA to determine if any significance was present between the variables and cell counts and viability. Correlation analyses were also performed to determine the directionality. Significance and confidence level were set at $p < 0.10$ and 90% respectively. All the graphs, aside the correlation analyses, note the mean and 95% confidence interval.

RESULTS

A summary of significance analysis can be found in Table 2. Results for each factor considered are detailed below.

Age

Age in years were grouped as less than 18 years, 18 years to 40 years, and greater than 40 years of age. Ordinary one-way ANOVA determined that the effect of age on cell yields were not statistically significant ($p = 0.9451$). Along with the cell yields, age did not have a significant effect on the viability of the hepatocytes isolated ($p = 0.6954$). Both data sets can be seen in Fig. 1a and 1b respectively.

Donor status

An unpaired t -test was performed to determine if there is a significant difference between DBD ($n = 3$) or DCD ($n = 12$) having an influence on cell yield or viability. Statistical analysis showed no significant effect on either cell yield ($p = 0.2464$) or viability ($p = 0.5634$). Donor status graphical representation is shown in Fig. 2a and 2b.

Gender

More livers were from male donors ($n = 9$) than women ($n = 5$). There was no significant difference between male or female donor livers with respect to cell yield (Fig. 3a, $p = 0.2952$). There was however a statistically significant difference on male or female donor livers and viabilities (Fig. 3b, $p = 0.0450$).

Body mass index (BMI)

BMI was grouped as underweight and normal grouped together, over-weight, and obese per national standards¹⁴. Most of the livers fell in the obese group, however no statistical significance was seen on BMI influencing either cell yield (Fig. 4a, $p = 0.1219$) or viability (Fig. 4b, $p = 0.6716$).

Race

There was not a proportional representation amongst the different races in our data set, with only a single donor being African American and the majority being Caucasian. A significant difference was found between Caucasian and Hispanic donor livers (Fig. 5a) in terms of cell yield ($p = 0.0499$). No difference was found between Caucasian and African American donor livers ($p = 0.8765$) or Hispanic and African American ($p = 0.1512$) donor livers in

terms of cell yield. A significant difference was found between Hispanic donor livers and African American donor liver in terms of viability (Fig. 5b, $p = 0.0902$). There was no difference found in viability between Caucasian and Hispanic ($p = 0.7064$) and between Caucasian and African American ($p = 0.1842$).

Cause of death

In the donor demographics of this study, there were three categories for causes of death: anoxia, stroke, and head trauma. Out of the 15 livers with documented cause of death, anoxia was the majority cause of death with stroke and head trauma having equal representation in the data set. In either cell yield or viability, there was no effect seen by the varying causes of death (Fig. 6a, $p = 0.7888$ and 6b, $p = 0.7830$ respectively).

Warm ischemia time (WIT)

The yield was impacted by the duration of WIT (Fig. 7a, $p = 0.0628$). A correlation matrix shows a significant relationship between yield and WIT ($p = 0.009$, $r = 0.712$) suggesting a positive correlation between WIT and yield (Fig. 7b). The impact of WIT on viability was not significant (Fig. 7c, $p = 0.1492$) and no significant relationship was found between viability and WIT ($p = 0.249$) but a positive correlation trend was suggested (Fig. 7d, $r = 0.361$).

Hemodynamic data

Neither yield or viability seemed to be significantly impacted by the percentage of time spent under 80% oxygen saturation during WIT (Fig. 8a, $p = 0.3095$; Fig. 8b, $p = 0.8413$ respectively). Findings for Mean Arterial Pressure were not significant either for yield or viability (Fig. 8c, $p = 0.22210$; Fig. 8d, $p = 0.6905$ respectively).

Correlation between %tSpO₂ and yield was found to be significant with a negative direction (Fig. 9a, $p = 0.039$, $r = -0.657$). The correlation between %tSpO₂ and viability was found to be not significant yet in a negative direction (Fig. 9b, $p = 0.648$, $r = -0.165$). Correlations between %tMAP, yield, and viability showed no significant correlations (Fig. 9c, $p = 0.208$, $r = -0.436$; Fig. 9d, $p = 0.533$, $r = -0.224$ respectively).

Cold ischemia time (CIT)

The majority of livers have a CIT longer than 12 hours ($n = 10$) with the average CIT being 14 hours and 35 minutes. Mann-Whitney test showed no significant impact of cold ischemic time on yield ($p = 0.7365$) but a significant difference in viability ($p = 0.0989$). Correlation analysis between CIT and viability suggests a negative direction ($r = -0.1847$). Graphical representation can be seen in Fig. 10a, 10b, 10c respectively.

DISCUSSION

Overall cell yield and viability results from this study are in line with previously published data utilizing whole organ isolations^{1,4,15}. Based on previous experience we did not accept any livers with cirrhosis, fibrosis, or severe steatosis as hepatocyte isolations are extremely variable and difficult from these types of liver tissues. This experience is similar to Bhogal *et*

al. (2011) who studied hepatocyte isolations between normal and diseased livers and found that livers with advanced liver disease and advanced stage of cirrhosis were not as successful in hepatocyte isolation than normal livers. Lee *et al.* (2014) also found that conditions such as fibrosis and liver fat significantly decreased viability and yield of hepatocytes so we did not accept any livers with significant fat percentage or fibrosis.

The livers accepted for hepatocyte isolation in this study were rejected for transplantation mainly due to age and donor status (DCD vs. DBD). Since neither demographic had a significant impact on cell yield and viability, this is a good indicator that livers of both status and any age can be accepted for hepatocyte isolation. There is, however, a notable reduction of variability (i.e. standard deviation) in DCD liver viability, compared to DBD livers, which may suggest that standardizing operational protocols around DCD livers may prove advantageous to guarantee cell quality in each isolation.

In this study there were more livers from male donors than female donors but three of the livers of male donors were non-successful. Though gender did not have a significant effect on yield, a significant difference on viability was found between genders, with female donors having consistently higher viabilities than male donors, even when failed isolations were not considered. This is somewhat contrary to recent evaluation of hepatocytes isolated from resected livers, where it was found that hepatocytes from male donors were better suited by FACS analysis¹⁶. It is possible that the difference lies in the whole organ vs. resected livers as the tissue source for isolation; liver fragments are in general obtained during tumor resections and tend to be fresh and higher quality for isolation^{17–19}. However, the total amount of such liver resection tissue is much less compared to whole organs, and the amount of cells from a single lot can be vastly higher when the whole organ is used. From our own experience and what is reported in the literature, resection size has great influence on the total yield and using whole livers allows multiple resection segments to be used rather than a single fragment from a tumor resection^{18,20,21}. Our findings indicate when whole livers are used for hepatocyte isolation, female donor organs could give higher viabilities.

In this study, livers with any Body Mass Index (BMI) were accepted which allowed for the comparison of viabilities and yields from livers with a variable range of BMI. Most of the livers in this pool were from donors in the obese weight status (BMI = 30+). While high donor BMI is a negative factor for transplantation, in our case BMI was found to not significantly impact viabilities and yields. This is somewhat contrary to our expectations and indicates this is a donor pool that can be more utilized for primary human hepatocytes. For instance, Organ Procurement Organizations could recover livers from these donors more frequently, even if they are unlikely to be used for transplantation.

The effect of race and the impacts on viability and yield are constrained due to uneven distribution between groups. In particular, although a significant difference was found between Hispanic versus African American donor livers in terms of viability, this comes with a major disclaimer since there was only one liver from an African American donor, and it was deemed not successful. There was a significant difference in yield between livers from Caucasian donors and livers from Hispanic donors. However, this also appears to be the

result of a single isolation with very high yields, so comparisons should be further tested with additional isolations before definitive conclusions are drawn.

No significant difference in either yield or viability was found in the different causes of death. Two of the hepatocyte isolations of donors whose cause of death was anoxia were found to be non-successful yet this did not have a statistically significant impact. It would be interesting to determine if the duration of anoxia at the time of death has any significance on viability and yield of hepatocyte isolations. For this study, livers were accepted only with a cardiac arrest period of less than 20 minutes as prolonged periods of anoxia could lead to hypoxic liver injury and have significant impacts on organ function and viability²². The effect of anoxia in general on the liver leads to an increase in enzymes depicting acute liver damage, namely aspartate aminotransferase (AST) and alanine aminotransferase (ALT) causing increases of 203× the normal level in some cases^{23,24}. Although the liver has protection against ischemic insults due to the dual blood supply from the hepatic portal vein (80%) and hepatic artery (20%)²⁵, this mechanism plays no role during cardiac arrest²³. Livers for hepatocyte isolation in this study were declined if liver function test (LFT) showed high AST and ALT values as these enzymes are the most frequent indicators of liver injury^{24–26}. Other LFT values were also taken into consideration to determine the extent of liver injury in the donors such as bilirubin and alkaline phosphate levels. Further investigation determining if there is an exact cut off in these values with significant impacts on viability and yield would be of interest to researchers deciding which livers to accept for hepatocyte isolation purposes.

In this study, warm ischemia time (WIT) was defined as the time of extubation to the time of flush, although varying definitions exist²⁷. The period of time of extubation to aortic flush was chosen as it shows severely compromised blood flow and oxygen saturation. It has been previously determined that warm ischemia times longer than 20 minutes correlate strongly with graft failure and decreases in organ viability¹¹; however, in our case all DCD livers had a warm ischemia time greater than 20 minutes. Surprisingly, the warm ischemia time did not have a significant effect on viability, but the organs with less than 30 mins WIT led to significantly reduced yield. Correlation analysis also revealed a positive correlation between warm ischemia time and yield. One explanation is that for livers with higher WIT, there are secondary causes that lead to their rejection for transplantation purposes, and the combination of warm ischemia and these additional factors result in a liver that is worse for transplant purposes. This data also shows that despite long warm ischemia times, viable cells and good yields can still be achieved from the high WIT livers and they should be considered for isolation.

Given the surprising results we obtained with WIT, we decided to take a closer look at the hemodynamic data during procurement which could allow us to study the hypoxic conditions of the organ during this time. Along with Foley *et al.* (2014) and others, who consider that WIT is an inaccurate assessment of donor organ function as some donors may maintain normal blood pressure and oxygen saturation after withdrawal of care²⁸, it was of interest to see if the more specific hemodynamic data (oxygen saturation and mean arterial pressure specifically) had an impact on viability and yield of hepatocytes isolated from DCD donors.

Calculating the percentage time spent under inadequate conditions of both oxygen saturation and MAP, we found that neither oxygen saturation or mean arterial pressure had a significant impact on either yield or viability. A significant negative correlation was determined between oxygen saturation and yield, however, and other negative correlations, though non-significant, were found between oxygen saturation and viability, and MAP and viability and yield. This suggests a possible trend that the less time spent under inadequate oxygen saturation or MAP during the WIT period, regardless of actual expiration length, could result in higher yields of viable cells; however our data is not conclusive in this aspect.

Many studies looking at hepatocyte isolations work with a particular surgery center where a rigorous hepatectomy and resection protocol is followed. This allows for a much more controlled environment and a shorter transport time from surgery room to laboratory space. Typically, the duration of cold ischemia time for transplant purposes of the liver are noted to be 6–10 hours with longer cold ischemia times being unacceptable¹³. This study however used livers procured and brought to us via organ procurement organizations that work on a nationwide level. Therefore, procurement protocols may have varied and cold ischemia times were much longer, with the longest duration being 23 hours of cold ischemia time. Bhogal *et al.* (2011) stated that the time between hepatectomy and isolation procedures was the most important factor in producing a successful isolation and that 3 hours is the limit ideally. However this limit will pose difficulty for researchers who do not work directly with a single procurement center. Albert Li *et al.* (2007) stated that CIT can be up to 24 hours or even longer and viable cells can still be attained. Our results support this finding, and that livers with a CIT greater than 12 hours can still produce a large number of hepatocytes with no statistical difference in yield; however their average viability is reduced slightly but significantly. Notably this was due to failed isolations with long CIT livers in our hands, and if these were eliminated the results would be similar between the two groups. This indicates that a second marker of viability for the long CIT livers would be needed to eliminate these unviable livers prior to isolation.

Overall, the purpose of this study was to assess whether demographics of the donor, warm ischemia time, and cold ischemia time affect the yield and fresh isolation viability of hepatocyte isolations from whole livers. A limitation of most human tissue work, including this study, is the number of livers studied and future studies could be expanded to incorporate more livers and hepatocyte isolation outcomes to improve hepatocytes and if differences are seen based on donor demographics and liver procurement data.

In summary, donor demographics such as race and gender had significant impacts on viability and yield while demographics such as BMI, age, donor status, cause of death did not. In addition, liver procurement data such as warm ischemia time showed that organs with less than 30 mins WIT led to significantly reduced yield, but no impact was found on viability. Cold ischemia time had no impact on yield, but longer cold ischemia time did decrease the viability of hepatocytes. The conclusions from this study should take the limited number of livers into consideration, however these conclusions could be used as indications to expand liver selection criteria for hepatocyte isolations for many researchers and incorporate livers from donors that would have otherwise been discarded.

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Figure 1. Comparison of yield and viability with age.

(a) No statistical significance at $p < 0.1$ was found with cell yield and age (<18 $n = 2$, 18–40 $n = 9$, 40+ $n = 4$). (b) Comparison between age and viability showed no statistical significance at $p < 0.1$ (<18 $n = 2$, 18–40 $n = 10$, 40+ $n = 4$).

Figure 3. Comparison of yield and viability with gender.

(a) No statistical significance was found at $p < 0.1$ between male or female donors and cell yields (Male $n = 9$, Female $n = 5$). (b) Statistical significance was found between gender and viabilities ($p = 0.0450$) (Male $n = 9$, Female $n = 6$). *indicates statistical significance at $p < 0.1$.

Figure 4. Comparison of yield and viability with Body Mass Index (BMI).

Comparing yield (a, <18.5–25 $n = 2$, 25–30 $n = 3$, 30+ $n = 9$) and viability (b, <18.5–25 $n = 3$, 25–30 $n = 3$, 30+ $n = 9$) to body mass index found no statistical significance at $p < 0.1$.

Figure 5. Comparison between cell yield and viability with race.

(a) A significant difference was found between livers from Caucasian and Hispanic donors in terms of cell yield ($p = 0.0499$). No difference was found at $p < 0.1$ between Caucasian and African American donors or Hispanic and African American donors. (Caucasian $n = 9$, Hispanic $n = 4$, African American $n = 1$). (b) Cell viability differences was significant between Hispanic and African American donors ($p = 0.0902$) but no significant difference was found at $p < 0.1$ between Caucasian and Hispanic donors and between Caucasian and African American donors. (Caucasian $n = 10$, Hispanic $n = 4$, African American $n = 1$).

*Indicates statistical difference at $p < 0.1$.

Figure 6. Comparison of cell yield and viability between cause of death.

No statistical significance found at $p < 0.1$ between (a, Anoxia $n = 6$, Head Trauma $n = 4$, Stroke $n = 4$) cell yield and (b, Anoxia $n = 7$, Head Trauma $n = 4$, Stroke $n = 4$) cell viability and cause of death.

Figure 7. Warm ischemia time (WIT) comparison between yield and viability.

(a) Statistical significance was found between the WIT and yield ($p = 0.0628$, WIT <30 $n = 6$, WIT >30 $n = 6$). (b) Significant positive relationship between WIT and Yield ($p = 0.009$, $r = 0.712$). (c) Impact of WIT was not significant at $p < 0.1$ on viability (WIT <30 $n = 6$, WIT >30 $n = 7$). (d) WIT and viability indicating a positive correlation trend. *Indicates statistical difference at $p < 0.1$.

Figure 8. Effects of oxygen saturation (SpO₂) and mean arterial pressure (MAP) and cell yield and viability.

Neither (a) yield or (b) viability was significantly impacted by the oxygen saturation at $p < 0.1$ (<90% $n = 5$, >90% $n = 5$). No significant impact was found on (c) cell yield and (d) cell viability by mean arterial pressure at $p < 0.1$ (<90% $n = 5$, >90% $n = 5$).

Figure 9. Correlation of oxygen saturation (SpO₂) and mean arterial pressure (MAP) on cell yield and viability.

(a) Significant correlation found between %tSpO₂ and yield ($p = 0.039$, $r = -0.657$). (b)

Correlation between %tSpO₂ and viability was non-significant ($p = 0.648$, $r = 0.165$).

Correlations between %tMAP, yield (c), and viability (d) showed no significant correlations ($p = 0.208$, $r = -0.436$; $p = 0.533$, $r = -0.224$ respectively) ($n = 10$).

Figure 10. Effect of cold ischemia time (CIT) on cell viability and yield.

(a) No significant impact was found at $p < 0.1$ between cell yield and cold ischemia time (<12 hrs $n = 4$, >12 hrs $n = 10$). (b) A significant impact was determined ($p = 0.0989$) between CIT and cell viability (<12 hrs $n = 4$, >12 hrs $n = 10$). (c) CIT and viability indicating a negative correlation trend. *indicates statistical difference at $p < 0.1$.

Table 1

Variables considered in analysis.

Liver variables tested	N	Mean $\pm$ SD
Age	16	31.4 $\pm$ 11.07
BMI	15	33.34 $\pm$ 9.31
Gender		
Male	9	N/A
Female	6	
Race		
Caucasian	10	N/A
Hispanic	3	
African American	1	
Cause of Death		
Anoxia	7	N/A
Stroke	4	
Head Trauma	4	
Donor Status		
DCD	12	N/A
DBD	3	
Warm Ischemia Time		
<30 minutes	6	25.3 $\pm$ 2.34
>30 minutes	6	40.0 $\pm$ 10.94
Cold Ischemia Time		
<12 hours	4	8.56 $\pm$ 2.12
>12 hours	11	16.08 $\pm$ 3.85

Table 2

Effect on cell yield and viability.

Variable	Significance on yield	Significance on viability
Age	$p = 0.9451$	$p = 0.6954$
Donor Status	$p = 0.2464$	$p = 0.5634$
Gender	$p = 0.2952$	$^*p = 0.0450$
BMI	$p = 0.1219$	$p = 0.6716$
Race	$^*p = 0.0499$	$^*p = 0.0902$
Cause of death	$p = 0.7888$	$p = 0.7830$
Warm Ischemia Time	$^*p = 0.0628$	$p = 0.1492$
Saturation of Oxygen	$p = 0.3095$	$p = 0.8413$
Mean Arterial Pressure	$p = 0.2221$	$p = 0.6905$
Cold ischemia time	$p = 0.7365$	$^*p = 0.0989$

* indicates statistical different at $p < 0.1$