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Original Research

Comparison of Different Organ Storage Techniques for Transplantation: Experimental Study

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Abstract

Objectives: The aim is to compare techniques of storage organs for transplantation. The ideal range of temperature is between 2 and 6 oC.

Methods: Six storage techniques were tested: 1- Standard: set of plastic bags filled with preservation solution and immersed in a thermal box (34 liters) filled with cubed ice; 2- Standard + plastic pot: plastic pot wrapping bags before immersion in ice; 3- Standard + metal box: metal box involving bags before immersion in ice; 4- Standard + crushed ice: Crushed ice instead of cubes; 5- Standard + ice bar: Ice bars instead of cubes; 6- High volume box: Standard in a 50 liter cooler. Variables: a) Temperature inside de plastic bags, the box and the room; b) time to reach the lower temperature; c) time of the temperature within the ideal range. One-way ANOVA was used to compare means, with Tukey's post hoc test and 5% of significance.

Results: Each assay was repeated ten times. All groups presented mean temperature inside the bags bellow the expected range, although the group 5 was higher than 2 (p=0.014) and 6 (p=0.006). The group 5 also presented higher temperature inside the box than group 1 (p=0.03), 2 (p=0.007), 4 (p=0.016) and 6 (p=0.001). There was no difference between the groups regarding to neither room temperatures (p=0.106) nor the time to reach the nadir of temperature (p=0.395). The time within the ideal range of temperature were higher in group 5 when compared to group 2 (p=0.027) and 6 (p=0.026).

Conclusions: Storage in thermal box, regardless of the technique, results in temperatures below the ideal range most of the time. Technical variations do not significantly impact on temperature inside the organ packaging, although using of bar ice leads to a closer approximation of the ideal range.

ABBREVIATIONS: SD: Standard Deviation

INTRODUCTION

Organ transplantation is a treatment widely used in cases of organ dysfunction, in order to provide longer survival and better quality of life for patients [1]. Over decades of evolution of surgical techniques, organs storage still requires special attention, as the ischemia time and the temperature of the organ are determinants of the outcomes after the transplant [2]. It is recommended that the organ should be kept at temperatures between 2 and 8 ºC during ischemia time [3-4]. It was observed that temperatures below 2 °C might cause the formation of water crystals, which can compromise cell metabolism and tissue physiology. On the other hand, temperatures above 6 °C, in the absence of oxygen, activate the anaerobic metabolism of the cells, causing ionic transmembrane imbalance, cell edema and cytolysis [5-6]. In addition, the decrease of intracellular pH activates proteases that lead to cell death as well as facilitates microbiological growth [7-8].

Other features are also crucial and thermodynamically important for thermal packaging, such as the ice shape, the composition of containers, the mass of ice used and the volume of the thermal box [4, 6]. Despite knowing the importance of these aspects, few countries have clear packaging and storage standards which contemplate and describe all these characteristics [9].

Recently, the use of the perfusion machine in organ preservation among transplant teams has become progressively more common and most of the aspects mentioned are already considered when using it [10, 11]. However, high costs still limit access to this technology in most countries[12]. Therefore, the use of ice and thermal container-based storage is still extremely necessary for the steady growth in the number of transplants. This study aims to evaluate the temperature variations in 6 different experimental groups, which represent some of the most used techniques of graft storage in order to verify whether they are effective in reaching and maintaining the temperature to which the organ is exposed within an appropriate range.

MATERIALS AND METHODS

Study design

Experimental *in vitro* study. Temperatures and times were measured and different organ storage techniques have been compared using the guidelines of the Brazilian Health Agency (figure 1) as the reference for storing organs in hypothermia for transplants [9].

Description of the study and groups

A first package (sterile and waterproof plastic bag – 2 liters in volume) was filled with 500ml of organ preservation solution (IGL®) at room temperature, received the sensor of the first thermometer (Ta) and was sealed. This first package was then coated with another similar package, but this time, filled with 500ml of sterile saline at room temperature. The second package was then covered with a third sterile and waterproof plastic bag without any solution, which was also sealed with cotton tape. The set of packages was then stored according to the group to which it belongs (Figure 1):

- Group 1 (standard renal storage): The set of packages was immersed in a polystyrene thermal box (medium size - 34 liters) fully filled with cube ice.
- Group 2 (standard + plastic pot): The set of packaging (plastic bags) was placed in a polystyrene plastic pot, similar to the one used to pack ice cream, which was then immersed in a polystyrene thermal box (medium size - 34 liters) fully filled with cube ice. The purpose of this plastic pot is to reduce direct contact between ice cubes and plastic bags.
- Group 3 (standard + metal box): The set of packaging (plastic bags) was packed in a steel box, similar to the one used to sterilize surgical instruments, which was then immersed in a polystyrene thermal box (medium size - 34 liters) fully filled with cube ice. The purpose of this metal box is to reduce the direct contact of ice cubes with plastic packaging using material with different caloric exchange properties compared to plastic.
- Group 4 (standard + crushed ice): The set of packages was immersed in a polystyrene thermal box (medium size - 34 liters) fully filled with crushed ice, in order to generate a greater total mass of conditioned ice.
- Group 5 (standard + bar ice): The set of packaging was immersed in a polystyrene thermal box (medium size - 34 liters) fully filled with bar ice, in order to generate a smaller contact surface of the ice and therefore less heat loss.
- Group 6 (high volume storage): The set of packages was immersed in a polystyrene thermal box (large size - 50 liters) fully filled with cube ice. This is the standard in liver hypothermic storage.

In all groups, a second thermometer (Tb) was immersed in the thermal box but outside the set of bags. In addition, the room temperature (Tc) was also monitored. The experimental room was climatized by an air conditioner and set up at 24 ºC.

The display of the thermometers (Ta, Tb and Tc) were recorded every 30 minutes for 48 hours, which produced 96 measurements from each thermometer in each assay. The assay in each of the six groups studied was repeated ten times. Then sixty assays have been performed in total.

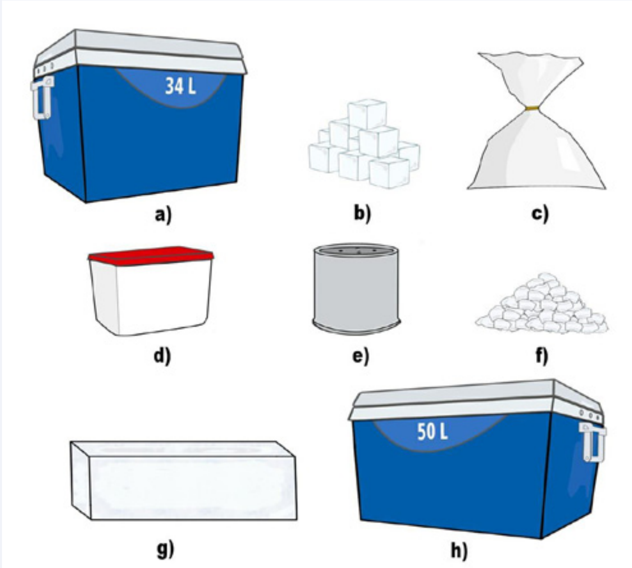

Figure 1 Technical variations of storage - Group 1: Standard renal storage – (a)34L thermal container + (b) cube ice + (c) plastic bags set; Group 2: standard + (d)plastic pot; Group 3: standard + (e)metal box; Group 4: standard + (f)crushed ice; Group 5 - Standard + (g) bar ice; Group 6 - standard + (h)high volume (50L) container.

Statistical Analysis

All variables are numeric. Each assay generated three set of 96 measured temperatures (Ta, Tb and Tc). We calculated the area under the curve for each set of temperatures obtained. Therefore, each assay produced three numbers representing the area under the curve of Ta, Tb and Tc. We then calculated the mean and standard deviation of each group studied (ten assays each). So, the numbers shown here as Ta, Tb and Tc are actually the mean of ten area under the curve for a group studied.

We yet measured the time (in hours) to reach the lowest temperature over time (Tnadir) as well as the time (in hours) the temperature was within the ideal range (between 2 °C and 8 °C) (Tideal).

The values obtained on the thermometers were exported to the IBM SPSS statistics® software. The one-way analysis of variance (ANOVA) test was used for comparison between groups, followed by Tukey's post hoc test to identify differences. The level of significance was 5%.

RESULT AND DISCUSION

A total of 17,280 temperatures were measured considering the ten experiments of each of the six groups and their three temperatures (primary packaging (Ta), thermal box (Tb) and environment (Tc)), measured 96 times in each test. The average room temperature (Tc) in all groups was 22.06ºC (SD = 1.2).

The expected mean area under the curve of Ta should be between 192 to 576 in order to represent a steady temperature within the ideal range of 2 – 6 ºC. However, we observed every group presented lower curve of temperatures (Table1).

The temperatures observed inside the primary packaging (Ta)

Table 1: Average temperatures (Ta, Tb and Tc) and times (for nadir and in the ideal range) of the six groups.							
Groups	**Group1** Mean (SD)	**Group2** Mean (SD)	**Group3** Mean (SD)	**Group4** Mean (SD)	**Group5** Mean (SD)	**Group6** Mean (SD)	***p* value** (ANOVA)
Temperature (Ta)	85,71 (89,1)	10,36 (40,38)	121,04 (111)	36,05 (80,07)	165,29 (175,2)	-3,35 (48,37)	0,002
Temperature (Tb)	91,32 (127,4)	63,40 (61,01)	166,87 (91,36)	79,34 (80,88)	265,78 (217,1)	30,08 (98,02)	0,001
Temperature (Tc)	2148,30 (77,12)	2081,83 (112,3)	2159,03 (86,23)	2097,88 (217,5)	2127,00 (91,5)	2012,52 (91,13)	0,106
Time to nadir (Tnadir)	4,70 (3,97)	5,65 (3,01)	10,25 (10,4)	5,80 (5,51)	4,85 (8,85)	4,65 (4,46)	0,395
Time within ideal (Tideal)	4,20 (9,82)	0,50 (0,47)	5,40 (4,82)	6,10 (12,35)	15,35 (19,38)	0,45 (0,43)	0,027

were significantly higher in groups 5 (bar ice) when compared either to group 2 (plastic pot) - p = 0.014 or group 6 (volume box 54L) - p = 0.006 (Figure 2). The group 5 also presented higher temperatures inside the thermal box (Tb) than group 1 (standard) - p = 0.030, group 2 (plastic pot) - p = 0.007, group 4 (crushed ice) - p = 0.016 and group 6 (volume box 54L) - p = 0.001 (Figure 3). There was no difference between groups with respect to ambient temperatures (Tc) over time. (p = 0.106).

The time in the ideal temperature range (Tideal) inside the primary packaging also showed a disparity between the groups (p = 0.027). Group 5 had a longer time in the expected range when compared to group 2 (p = 0.027) and group 6 (p= 0.026) – (Figure 4).

The time for nadir of temperature (Tnadir) also showed no significant difference between the groups inside the primary sac (p = 0.395). However, it is worth noting that this time was longer, in absolute numbers, in group 3 (metal box) in relation to the other groups.

The importance of packaging and storing organs in hypothermia for transplantation has been widely studied. The temperature range considered ideal by the Brazilian technical standard is between 2 and 6 ºC [9]. However, some studies have suggested a wider range of 2 to 8 ºC [4, 7]. The present study observed that, regardless of the technical variation used, during most of the time, the temperature inside the organ packaging is at levels below those considered desirable. This excessive cooling can be harmful to the cells of the graft, since it may result in molecular changes and protein denaturation that modify the expected biological response after reperfusion in the recipient organism [6].

It is desirable that the temperature decreases evenly and quickly in the package in which the organ is stored, reaching the desired temperature range as soon as possible. Thus, it will decrease the time of warm ischemia and present better conservation, avoiding metabolic demand, energy expenditure and release of free radicals until the ideal temperature is reached[4]. In the present study, we chose to fill the first package with a solution at room temperature, in order to simulate the situation in which the organ, which will be at body temperature, will be subjected. We observed that the mean time to reach the temperature nadir of the 60 experiments was 5.98h, being even longer in group 3 (10.25h), although there was no statistical difference between the groups. These data suggest that the

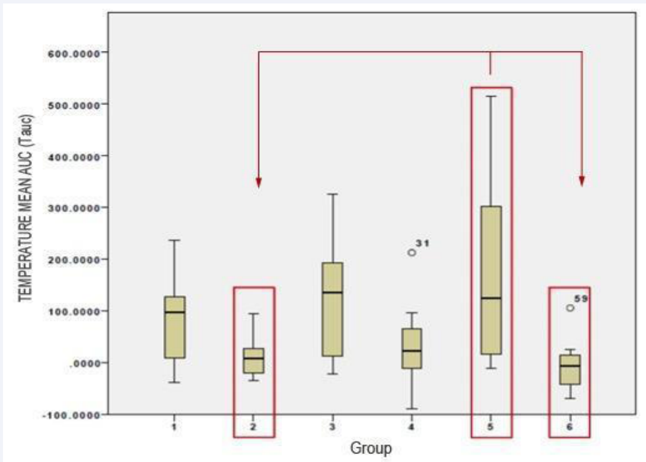

Figure 2 Temperature inside the 1st package (Ta). Groups 5 x 2 (p = 0.014) and Groups 5 x 6 (p = 0.006) - ANOVA.

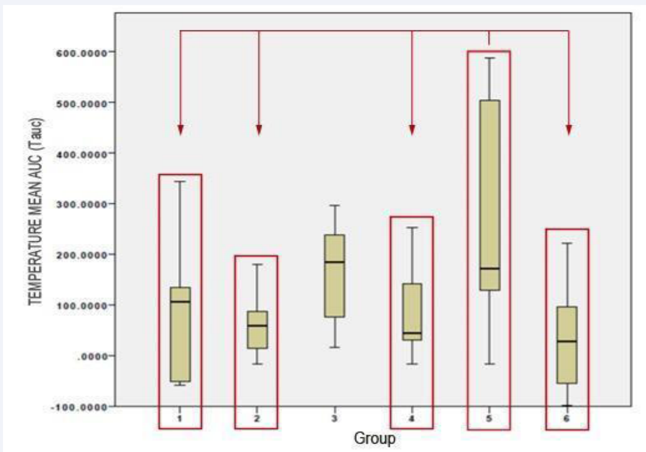

Figure 3 Temperature inside the thermal box (Tb). Groups 5 x 1 (p = 0.030), Groups 5 x 2 (p = 0.007), Groups 5 x 4 (p = 0.016) and Groups 5 x 6 (p = 0.001) - ANOVA.

models studied are ineffective in quickly providing the ideal temperature for organ maintenance. However, it should be considered that the cooling in practical conditions should be faster, since the organ will be perfused with a solution at 4 ºC and then immersed within the same solution.

The temperature inside the first package and inside the

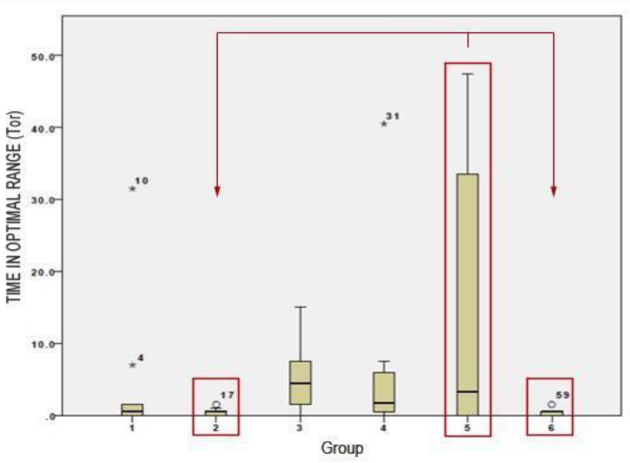

Figure 4 Time (h) of the temperature of the interior of the 1st package (Ta) in the ideal range (Tideal) - ($p = 0.027$). Groups 5 x 2 ($p = 0.027$) and Groups 5 x 6 (0.026) - ANOVA.

thermal box was higher in group 5, which represented the model with ice bars. The group 5 also presented a longer time within the ideal range of temperature. In addition, the time to reach the nadir of temperature was among the lowest in the same group, although without statistical significance. From these findings, it can be inferred that the storage with bar ice seems to present a thermodynamic behavior closer to that which would be desired, that is, a rapid reach of the ideal temperature and stability in the desired range. A possible explanation for this phenomenon would be that the shape of the bar ice decreases the area of contact with the medium, thus absorbing little energy from the system by convection, while, being in contact with the primary packaging, there is greater and steady transfer of thermal energy [13]. In group 1, 2, 4 and 6 we had the lowest mass / volume ratio, that is, the lowest ice density. In addition, the ice shape allows the energy to distribute throughout the thermal box and not only in contact with the primary packaging.

Some transplant teams advocate the use of a plastic pot in which the bags containing the organ should be stored, such as the one used in group 2, or a metal box, such as the one used in group 3. In this way, the organ would have an additional protection against mechanical trauma of transport and would also avoid direct contact of the ice with the plastic bags surrounding the graft [9]. The present study observed that there was no statistical difference in the temperatures of the first package (Ta), time to reach the nadir of temperature (Tnadir) or in the time within the ideal temperature range (Tideal) when these techniques were compared with the standard. Consequently, based on these results, there is no problem regarding temperatures whether a team decides to use a plastic or metal protection around the graft. However, it is noteworthy that the values in group 3 (metal box) were closer to those observed in group 5 (bar ice). Therefore, if the team wants additional protection for the bag with the organs, apparently, the metal box would be better than the plastic pot.

There was a coherence between the temperature relationships observed inside the first package (Ta) and those observed in the thermal box (Tb), when comparing the different groups. Thus, it can be said that gauging the temperature of the thermal box is an indirect but reliable assessment of the temperatures to which the graft is being subjected. This finding corroborates the literature's recommendation to use transport devices (thermal boxes) with temperature control, although this is not yet mandatory in Brazilian standards [7, 9].

The hypothermia perfusion machine has been shown to be efficient in the purpose of keeping the organ at an adequate temperature, avoiding cell injuries and consequent clinical repercussions [14, 15]. The comparison between static storage (thermal box) and the perfusion machine demonstrated advantages in the use of this technology to prevent delayed graft function and ensure greater survival in the first post-transplant year [15-17]. Another study demonstrated the possibility of using the perfusion machine even after storage in a thermal box, with success in decreasing the time of delayed graft function[14]. Despite the good results regarding the reduction of reperfusion injury and the delayed function of the graft, there is a need for studies that demonstrate long-term advantages with the use of the machine and that investigate possible vascular disorders and its adaptation to low-flow organs blood, such as the pancreas [5, 10]. In addition, there are studies skeptics about the supposed advantages of the perfusion machine, raising the hypothesis that the perfusion machine could cause deleterious edema in the organ with consequent atherosclerosis or glomerulosclerosis [5]. However, the main question behind the routine use of perfusion machines is whether they present themselves as a cost-effective method, since, for most countries, the costs of the machine and its inputs do not override their supposed advantages, so that its use is restricted to expanded criteria organs and with high ischemia time, where the lower rate of delayed graft function and even the best evaluation of the vascular resistance of the graft may represent advantages that justify the costs of the treatment [12].

The present work has some positive points, such as the comparison of technical variations commonly made in the practice of organ procurement, without necessarily prior proof their benefits or risks. In addition, assays repeated 10 times, with temperature control of the room and parametric statistical evaluation, also attribute a higher degree of reliability and reproducibility to the findings. On the other hand, it is an experimental study and, therefore, does not reproduce exactly what we are going to observe in vivo. In this sense, it will be interesting, in a next stage of the study, to use animal organs and histological studies. Moreover, at the real scenario, we observe changes in surrounding temperatures. Consequently, there could be worthy to try to simulate some of those modifications and observe the response of the model.

CONCLUSION

Storage in a thermal box, regardless of technique, results in temperatures slightly below the ideal range most of the time. The technical variations shown do not significantly change the temperature inside the organ package nor the time to reach the nadir of temperature. Among the reproduced groups, the use of bar ice causes more stable temperatures and closer to the desired range, which represent a greater approximation of the ideal when compared with other groups studied.

REFERENCES

1. Aceto P, Perilli V, et al. Perioperative-, Recipient-, and Donor-Related Factors Affecting Delayed Graft Function in Kidney Transplantation. Exp Clin Transplant. 2019; 17: 575-579.
2. Peng P, Ding Z, He Y, Zhang J, Wang X, et al. Hypothermic Machine Perfusion Versus Static Cold Storage in Deceased Donor Kidney Transplantation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Artif Organs. 2019; 43: 478-489.
3. Michel SG, LaMuraglia Ii GM, Madariaga MLL, Anderson LM. Innovative cold storage of donor organs using the Paragonix Sherpa Pak ™ devices. Heart Lung Vessel. 2015; 7: 246-255.
4. Horch DF, Mehlitz T, Laurich O. Organ transport temperature box: multicenter study on transport temperature of organs. Transplant Proc. 2002; 34: 2320.
5. Patel SK, Pankewycz OG, Weber-Shrikant E. Graft arteriosclerosis and glomerulosclerosis correlate with flow and resistance to machine perfusion in kidney transplantation. Transplant Proc. 2012; 44: 2197-2201.
6. Rowland R, Ponticorvo A, Jarrin Lopez A. Monitoring kidney optical properties during cold storage preservation with spatial frequency domain imaging. J Biomed Opt. 2019; 24:116003-116007.
7. Net M, Trias E, Navarro A. Cold chain monitoring during cold transportation of human corneas for transplantation. Transplant Proc. 2003; 35: 2036-2038.
8. Jing L, Yao L, Zhao M, Peng L-P, Liu M. Organ preservation: from the past to the future. Acta Pharmacol Sin. 2018; 39: 845-857.
9. Oliveira GYL, Santos ACP, Cunha LS. Evaluation of Current Organ Packaging and Storage Patterns Between Brazilian Renal Transplant Teams. Transplantation Journal. 2017;101: S35.
10. Balfoussia D, Yerrakalva D, Hamaoui K, Papalois V. Advances in machine perfusion graft viability assessment in kidney, liver, pancreas, lung, and heart transplant. Exp Clin Transplant. 2012; 10: 87-100.
11. Lam VWT, Laurence JM, Richardson AJ, Pleass HCC, Allen RDM. Hypothermic machine perfusion in deceased donor kidney transplantation: a systematic review. Journal of Surgical Research. 2013; 180: 176-182.
12. Gómez V, Galeano C, Diez V, Bueno C, Díaz F, et al. Economic impact of the introduction of machine perfusion preservation in a kidney transplantation program in the expanded donor era: cost-effectiveness assessment. Transplant Proc. 2012; 44: 2521-2524.
13. DeHoff RT. Thermodynamics in Materials Science. New York: McGraw-Hill; 1993.
14. Matos ACC, Requiao Moura LR, Borrelli M. Impact of machine perfusion after long static cold storage on delayed graft function incidence and duration and time to hospital discharge. Clin Transplant. 2017;32.
15. Montenovo MI, Perkins JD, Kling CE, Sibulesky L, Dick AA, et al. Machine Perfusion Decreases Delayed Graft Function in Donor Grafts With High Kidney Donor Profile Index. Exp Clin Transplant. 2021;19:8-13.
16. Cannon RM, Brock GN, Garrison RN, Smith JW, Marvin MR, et al. To pump or not to pump: a comparison of machine perfusion vs cold storage for deceased donor kidney transplantation. J Am Coll Surg. 2013; 216:625–633.
17. Deng R, Gu G, Wang D. Machine perfusion versus cold storage of kidneys derived from donation after cardiac death: a meta-analysis. PLoS One. 2013; 8: e56368-e56368.

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