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ORIGINAL PAPER

Pancreatic Temperature During Transport for Transplantation: A Preclinical Study

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ABSTRACT

Objectives: To compare the viability of porcine pancreases transported under static cold conditions using two transport models. **Methods:** A prospective preclinical case-control study with an adapted design, conducted on Landrace pig organs. Data were collected from three variables: temperature, macroscopic assessment, and histology. The variables were compared using two packaging models: the experimental model, Autonomous and Intelligent Packaging for Cold Chain in Healthcare Systems (Embalagem Autônoma e Inteligente para Cadeia Fria de Sistemas de Saúde [EMAIS-SR]), and the control, a conventional thermal container widely used and filled with ice. Three thermometers were used for temperature measurements: two for internal temperature and one for surface temperature. The macroscopic evaluation analyzed color, tissue integrity, consistency, and the presence of lesions, edema, or hematomas in the organ. For histology, the samples were fixed and stained with hematoxylin-eosin, which allows visualization of tissue morphology under light microscopy, followed by immunohistochemical analysis. **Results:** Ten experimental surgeries were performed: six pancreases were stored under static cold conditions and transported in the active-cooling container, while four were transported in the conventional ice-filled container. Among all cases, only one pancreas had an exit temperature higher (12.1 °C) than the recommended maximum of 8 °C. However, in the macroscopic evaluation, the organ with the higher exit temperature received a better evaluation, while the others maintained a qualitative macroscopic evaluation. In histological analysis, on average, pancreases transported in the active-cooling container demonstrated better tissue quality by presenting more preserved cellular structures. **Conclusion:** The preclinical experimental model demonstrated that, although the organ was kept within the recommended temperature range, organs maintained in the technological solution EMAIS-SR, which does not use ice, were better preserved. Furthermore, the only case in which temperature exceeded the recommended limit, that is, in which the organ warmed after packaging, occurred in the traditional ice-filled container. The research encourages the development of new and more robust studies on clinical decisions regarding safe thermal limits and best practices for transporting pancreases intended for transplantation.

Descriptors: Pancreas; Pancreas Transplant; Organ Transplant; Logistics; Cold Ischemia.

Temperatura de Pâncreas Durante o Transporte para Transplante: Estudo Pré-Clínico

RESUMO

Objetivos: Comparar a viabilidade de pâncreas suínos transportados a frio estático em dois modelos de transporte. **Métodos:** Estudo pré-clínico prospectivo com delineamento caso-controle adaptado, realizado em órgãos de suínos Landrace. A coleta de dados foi realizada a partir de três variáveis: temperatura, avaliação macroscópica e histologia. As variáveis foram comparadas utilizando dois modelos de acondicionamento: o caso, Embalagem Autônoma e Inteligente para Cadeia Fria de Sistemas de Saúde (EMAIS-SR), e o controle, embalagem térmica convencional amplamente utilizada e preenchida com gelo. Para temperatura, foram utilizados três termômetros: dois para temperatura interna e um para temperatura de superfície. A avaliação macroscópica analisou coloração, integridade tecidual, consistência e presença de lesões, edema ou hematomas no órgão. Para histologia, as amostras foram fixadas e coradas por hematoxilina-eosina, que possibilita a visualização da morfologia tecidual em microscopia óptica seguida de

análise imuno-histoquímica. **Resultados:** Foram realizadas 10 cirurgias experimentais; seis pâncreas foram acondicionados a frio estático e transportados em embalagem com refrigeração ativa, enquanto quatro foram transportados em embalagem convencional com gelo. Dentre os casos, somente um pâncreas apresentou temperatura de saída mais alta (12,1 ˚C) que a preconizada de até 8 ˚C. Contudo, frente à avaliação macroscópica, o órgão com temperatura mais alta após o transporte apresentou melhor avaliação, enquanto os demais mantiveram avaliação macroscópica qualitativa. Na análise histológica, em média, os pâncreas transportados na embalagem de refrigeração ativa demonstraram melhor qualidade tecidual por apresentarem estruturas celulares mais preservadas. **Conclusão:** O estudo demonstrou, por meio de modelo experimental pré-clínico, que, apesar de terem sido mantidos em padrão de temperatura recomendado, os órgãos preservados na solução tecnológica EMAIS-SR, que não utiliza gelo, demonstraram-se mais bem preservados. Ademais, o único caso em que houve temperatura superior, ou seja, em que o órgão aqueceu após acondicionamento, ocorreu na embalagem tradicional com gelo. A pesquisa instiga a realização de novos e mais robustos estudos acerca de decisões clínicas sobre limites térmicos seguros e boas práticas para o transporte de pâncreas destinados ao transplante.

Descritores: Pâncreas; Transplante de Pâncreas; Transplante de Órgãos; Logística; Isquemia Fria.

INTRODUCTION

The pancreas plays vital endocrine and exocrine roles; its failure, especially in difficult-to-control type 1 diabetes mellitus, the main clinical indication, and more rarely in conditions such as chronic pancreatitis, may require transplantation as definitive therapy[1,2]. However, the organ is particularly sensitive to cold ischemia: temperature deviations or prolonged storage time cause endothelial injury, edema, microvascular thrombosis, and graft pancreatitis, reducing patient and organ survival. Furthermore, increased cold ischemia time is associated with a higher incidence of graft pancreatitis, vascular thrombosis, delayed graft function, and the need for surgical interventions[3], making thermal control during transport one of the main clinical and surgical challenges in pancreatic transplantation. Current guidelines recommend maintaining the temperature between 2°C and 8°C and limiting cold ischemia time to 12 hours; exceeding these limits exponentially increases the risk of graft loss[3].

In the clinical setting, the pancreas is still transported in conventional thermal boxes filled with ice, as with other organs. These containers provide passive insulation but generate unpredictable thermal gradients: regions near the ice can reach temperatures below 0 °C, leading to freezing. In comparison, others can exceed 8 °C due to melting ice or frequent lid openings. Furthermore, the technique lacks continuous temperature recording, preventing complete traceability of the process. The logistics stage, therefore, remains a critical and often underreported point in the transplant chain.

To address this gap, an academic-industrial partnership developed the Autonomous and Intelligent Packaging for the Cold Chain of Healthcare Systems (*Embalagem Autônoma e Inteligente para Cadeia Fria de Sistemas de Saúde* / EMAIS-SR) over five years, integrating studies in thermodynamics, mechanics, electronics, and ergonomics. The device combines electronic temperature control, air cushions for impact absorption, high-efficiency insulating materials, and digital technology that records and transmits environmental data in real time[4].

To validate the efficiency of the EMAIS-SR for the safe transport of organs for transplantation, preclinical research was conducted using porcine organs and, in a novel approach, the core temperature of the pancreas was measured with probes simultaneously with the surface temperature, allowing the identification of possible internal gradients undetectable by external methods. These readings were correlated with macroscopic viability parameters and standardized histological changes, providing a detailed view of the impact of transport in both a thermal box with ice and the EMAIS-SR.

Therefore, this study aims to compare the viability of porcine pancreases transported under static cold conditions in two transport models.

METHODS

Study design

A prospective preclinical study with an adapted case-control design was conducted using Landrace pig pancreases. The intervention (case) consisted of using the EMAIS-SR active thermal packaging, compared to a conventional cooler with ice (control). The primary outcome was organ viability, assessed by (i) core and surface temperature, (ii) macroscopic appearance, and (iii) histology. This design was chosen because thermal boxes with ice, which are widely used, already show satisfactory results and serve as a reference. A previous pilot study adjusted the procedures[5]. The article focuses specifically on the temperature and viability outcomes of the pancreas.

Study location

The study was developed at the Federal University of São Paulo (UNIFESP), in the Laboratory of Operative Technique and Experimental Surgery.

Animals, anesthesia and intraoperative monitoring

Ten Landrace porcines (~25 kg) from a farm affiliated with UNIFESP were included in the study. The animals received appropriate care before transport and were housed in ventilated wooden crates under continuous veterinary supervision. Anesthesia consisted of ketamine 15 mg/kg and midazolam 0.2 mg/kg intramuscularly, followed by intravenous induction with propofol 7 mg/kg. After intubation, they were maintained under isoflurane 1.5-2% for 4 hours. Analgesia was provided with fentanyl 2.5 mg/kg, and additional doses of propofol were administered as needed.

Pancreas uptake

Following a thoracoabdominal incision and aortic cannulation, the descending aorta was clamped, and the abdominal cavity was filled with crushed ice (frozen saline solution) to induce rapid cooling. Simultaneously, 2 liters of Custodiol® were infused through the cannulation. The organs were removed in this order: heart, liver, pancreas, and kidneys. Each organ was then transferred to the auxiliary table, and the pancreas was not perfused; it was only prepared for storage[5].

Transportation and packaging

Each pancreas was packaged using a sterile three-layer method: (i) a sterile inner bag containing the organ immersed in Custodiol® solution; (ii) a second sterile bag filled with cold saline solution; and (iii) a sterile outer bag. The assemblies were allocated to two transport modes in each experiment: a conventional plastic thermal box with ice (control) or the EMAIS-SR device (case). The EMAIS-SR was tested at two different technology readiness levels (TRLs).

In the experiments, the active packaging prototype, called EMAIS-SR (TRL6 and TRL7), was used. Both share the same operating principle (active refrigeration with electronic temperature control within the range indicated for solid organs). The TRL7 model represents a more advanced version, with engineering improvements (temperature set points and calibration) that result in greater core temperature stability. These parameters were adjusted after the first tests. Conceptually, both comprise the same EMAIS-SR Experimental Packaging group, since they were adjustments and improvements to the prototype and not a modification or replacement of the device.

After being packaged, they were transported by car for 30 minutes on a simulated urban route and then rested for 90 minutes in the laboratory, totaling at least 120 minutes of continuous exposure within the packaging system[5].

Data collection

The data collection method was described in a previous study[5]. Briefly, the data were collected in two phases: first, as soon as the organ was removed from the cavity and during preparation for packaging (H0), and then, after the time each organ remained in the packaging had elapsed (H2). Therefore, the time the organ remained in the packaging corresponds to the difference between H2 and H0.

Regarding temperature, this was measured using two different methods. First, two probe thermometers (culinary thermometers) were used, inserted at a 45° angle and held until the readings stabilized, according to the manufacturer's instructions. Then, the surface temperature was measured with an infrared thermometer, positioned 2 cm from the organ and held for 2 to 3 seconds, as per the manufacturer's instructions. The insertion and temperature measurement sites were standardized: the core thermometers were inserted into the head and body of the pancreas, and the surface temperature was measured in the center of the organ, regions where thermal variation could be greater.

The organ's viability was measured through macroscopic analysis, that is, the appearance of the pancreas at the two data collection points, and histological analysis, which aimed to visualize the organ's tissue architecture.

For macroscopic analysis, a data collection instrument was developed based on a previous morphological evaluation study[6]. The instrument considers: color (milky white, yellow, light brown, pink, or grayish-pink); organ quality, composed of tissue injury (absent = 0; contusion = 1; laceration = 2; other = 3); calcification (absent = 0; partial = 1; total = 2); consistency (rigidity absent = 0; partial = 1; total = 2); and presence of hematoma or edema (yes = 1; no = 0). The closer the score obtained in the evaluation is to 0, the better the organ's condition; for color, maintaining the original tone was considered ideal.

For histology, standardized samples were collected from the pancreatic tail before packaging (H0) and again after transport (H2). The fragments were fixed in 4% paraformaldehyde (phosphate buffer) for 24 hours, dehydrated in an increasing series of ethanol, cleared in xylene, and embedded in paraffin at 60 °C. 4 µm sections were obtained using a Minot microtome (50 µm spacing) and stained with hematoxylin-eosin (H&E) for histomorphometric analysis.

H&E-stained sections were examined using an optical microscope. Image acquisition was performed with a high-resolution AxioCam ICc 5 camera coupled to an AxioLab A1 light microscope (ZEISS®), equipped with an A-Plan 40× objective and a

capture magnification of 0.63×. Images were obtained using AxioVision 40x64 software, version 4.9.1.0 (ZEISS®), ensuring adequate documentation of the evaluated histological characteristics. In total, 20 representative images were analyzed, distributed between the control and EMAIS-SR groups.

The initial morphological evaluation of H&E-stained histological sections served as a criterion for selecting representative slides for immunohistochemical analysis. For immunohistochemical analysis of BCL-2 and p53 protein expression in the pancreas, 4 µm-thick histological sections were used, obtained from pancreatic fragments that had been previously fixed in 10% buffered formaldehyde and subsequently embedded in paraffin. The histological sections were mounted on silanized slides, dried at room temperature, and then incubated at 60 °C for 1 hour to promote adequate tissue adhesion.

The sections were deparaffinized in three consecutive xylene baths, each lasting 10 minutes, followed by rehydration in ethanol at decreasing concentrations of 100%, 95%, and 70%, remaining in each solution for 5 minutes, with a final wash in distilled water. Antigen retrieval was conducted in a 10 mM citrate buffer, pH 6.0, by heating in a steamer for 20 minutes. After cooling to room temperature, endogenous peroxidase was blocked by incubation with a 3% hydrogen peroxide solution in methanol (1:9) for 10 minutes, followed by washing in 0.1 M phosphate-buffered saline, pH 7.4.

Nonspecific binding was blocked with 3% bovine serum albumin for 30 minutes at room temperature. Subsequently, incubation was performed with the primary antibodies BCL-2 (clone sc-7846, Santa Cruz Biotechnology®, polyclonal, 1:100) and p53 (clone sc-126, Santa Cruz Biotechnology®, polyclonal, 1:100).

Incubation with primary antibodies was performed for 16 hours at 4 °C. After this period, the slides were washed in PBS and incubated with biotinylated secondary antibody for 30 minutes, followed by application of the streptavidin-peroxidase complex for another 30 minutes, both diluted in TRIS buffer, pH 7.6. The reaction was revealed with DAB solution, prepared immediately before use, until the brown color indicative of immunopositivity was observed.

The sections were counterstained with Mayer's hematoxylin for 20 seconds, washed in running water, dehydrated in an increasing series of ethanol, cleared in xylene, and mounted in Entellan® synthetic medium. Spleen tissue slides were used as a positive control, while negative controls were obtained by replacing the primary antibody with PBS.

Microscopic analyses were performed using an AxioLab A1 light microscope (Carl Zeiss®) with a 40× objective. Representative fields were selected per slide to assess staining intensity. Quantification of the brown coloration indicative of immunopositivity was performed using ImageJ® software, with values expressed in arbitrary optical density units (0-255).

Data analysis

The temperature data were analyzed using measures of central tendency, considering the mean and standard deviation.

In the macroscopic analysis, the quantitative scores comprised the average related to the variable "organ quality". Stable average values indicated preservation of visual quality, while increases or decreases reflected, respectively, improvement or worsening of the appearance. For the pancreas, lower scores corresponded to better quality. The comparison between the values obtained before (H0) and after transport (H2) indicated whether the organ's appearance had improved or deteriorated, including the pattern of the referred shade.

Histological analysis was based on H&E-stained sections, which allow visualization of tissue morphology under light microscopy. Basophilic structures (e.g., nuclei containing DNA) appeared in shades of blue to purple. At the same time, acidophilic components (cytoplasm) showed pink to red coloration, allowing the identification of structural alterations in pancreatic tissue. Immunohistochemical results were expressed as mean ± standard deviation, considering cytoplasmic staining as the standard for BCL-2 positivity and nuclear staining as the standard for p53 positivity. After quantification, photomicrographs corresponding to the observed immunostaining pattern were selected.

Ethical aspects

The Ethics Committee approved the experimental protocol on the Use of Animals at UNIFESP (no. 4197081221). All procedures were planned to minimize animal suffering.

RESULTS

Ten experimental surgeries were performed on porcines. Pancreases were packaged and transported in six experiments using EMAIS-SR packaging and in four using the control packaging (a 16-liter thermal box filled with ice). The average cold ischemia time for organs transported in the control packaging was 3 hours and 34 minutes (± 14 minutes). In comparison, the average residence time of organs in the control packaging was 2 hours and 41 minutes (± 12 minutes). For EMAIS-SR, the average cold ischemia time was 3 hours and 28 minutes (± less than 1 minute), and the average residence time of pancreases in the packaging was 2 hours and 35 minutes (± less than 1 minute).

Although small variations in organ residence time were observed in the packaging (difference between H2 and H0) in both the control and EMAIS-SR groups, the average times were similar, with a maximum difference of 12 minutes. These differences reflect operational adjustments inherent to the experimental model and do not constitute systematic discrepancies between the groups.

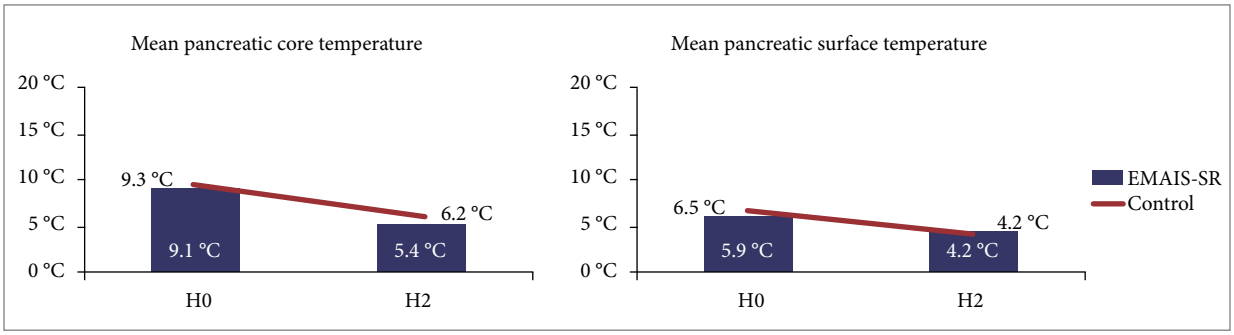
Regarding temperature, Table 1 presents the average values and standard deviation of the core and surface temperatures of the organs evaluated in the experiment. It is noteworthy that, among the cases, only one pancreas, transported in the control packaging, showed a higher exit temperature (12.1 °C) measured by the core thermometer. In contrast, the surface thermometer showed 6.1 °C.

Table 1. Temperature values in °C (H0 and H2) of organs transported in the control packaging and in the EMAIS-SR (mean ± SD).

Packaging	Core temperature		Surface temperature	
	H0	H2	H0	H2
Control (n = 4)	9.3 ± 2.1	6.2 ± 4.1	6.5 ± 2.9	4.2 ± 1.3
EMAIS-SR (n = 6)	9.1 ± 2.4	5.4 ± 1.4	5.9 ± 2.6	4.2 ± 1.3

Source: Elaborated by the authors.

Figure 1 shows the average temperature behavior pattern. Note that the core temperature value obtained is the average of the values measured by the two thermometers.



Source: Elaborated by the authors.

Figure 1. Graphical representation of the average temperatures (°C) obtained in the experiment.

Regarding the macroscopic evaluation, the organs transported in the control packaging had an average length of 9.9 cm (± 2.8 cm) and a width of 5.4 cm (± 1.9 cm). The organs transported in the EMAIS-SR packaging had an average length of 18.2 cm (± 1.7 cm) and a width of 7.4 cm (± 1.8 cm).

Concerning organ viability, Table 2 presents the results from macroscopic analysis. It is noted that the only organ evaluated to show improvement in overall appearance was the one with an exit temperature of 12.1 °C, which was higher than the recommended value.

Table 2. Macroscopic evaluation of the pancreas using a developed form.

Packing	Organ quality*		Coloration		Assessment
	Pre-transport	Post-transport	Pre-transport	Post-transport	
Control (n = 4)	0.4	0.0	Milky white	Milky white	Enhancement
	0.0	0.0	Milky white	Milky white	No change
	0.0	0.0	Milky white	Milky white	No change
	0.0	0.0	Milky white	Milky white	No change
EMAIS-SR (n = 6)	0.0	0.0	Yellow	Yellow	No change
	0.0	0.0	Milky white	Milky white	No change
	0.0	0.0	Milky white	Milky white	No change
	0.0	0.0	Milky white	Milky white	No change
	0.0	0.0	Milky white	Milky white	No change
	0.0	0.0	Milky white	Milky white	No change

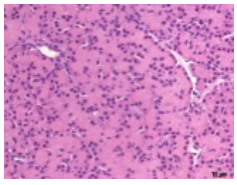
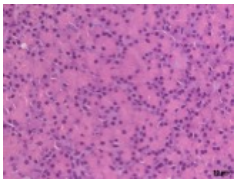
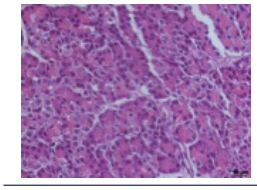
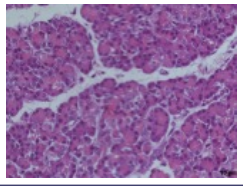
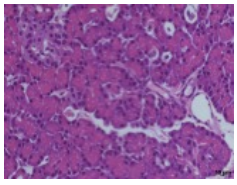
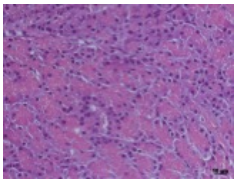
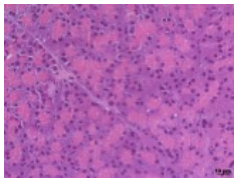
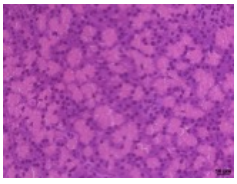
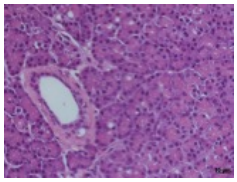
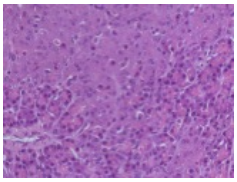
Source: Elaborated by the authors. *Average of the values obtained in the assessments of the variables tissue injury, calcification, consistency, and presence of hematoma or edema.

Related to histology, in the control group before transport, exocrine structures (responsible for the production of digestive enzymes) and a small endocrine portion (pancreatic islets) were observed. The exocrine pancreas forms tubuloacinar structures organized into lobules. The acini are composed of polyhedral cells with rounded, clear nuclei located in the basal portion; the apical portion presents eosinophilic granules. Loose connective tissue was noted around the acini and pancreatic ducts. Between the lobules, ducts lined by simple squamous or low cuboidal epithelium were observed. The exocrine portion consists of the pancreatic islets, which are dispersed throughout the exocrine parenchyma. The islets contain endocrine cells with clear cytoplasm and well-defined nuclei.

The basic histological architecture of pancreas sections from animals pancreases in the EMAIS-SR group also shows the same structure as in the control group. However, in pancreas sections from the control packaging group, precise acinar delimitation was not observed, with some poorly preserved areas, as also observed in the pancreas that reached an exit temperature of 12.1 °C. Although this organ showed improvement in macroscopic appearance, this improvement was not maintained when cellular structures were observed. In the Experimental Packaging group (EMAIS-SR), most nuclei are well preserved.

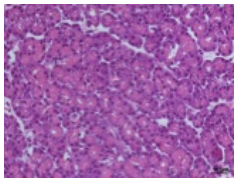
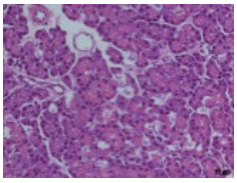
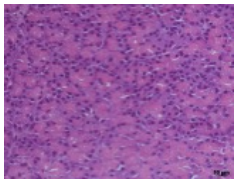
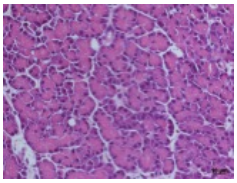
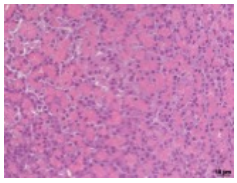
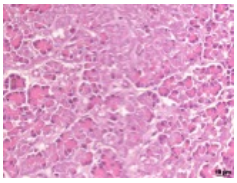
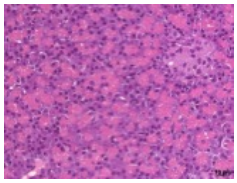
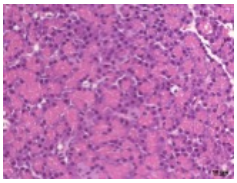
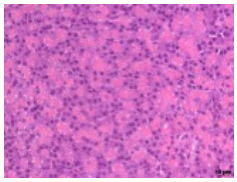
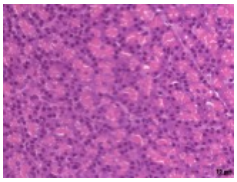
Regarding the endocrine portion, the pancreatic islets showed good morphological preservation in all groups evaluated. Well-defined contours and typical cellular organization were observed, with endocrine cells exhibiting clear cytoplasm and well-defined nuclei. No structural alterations, cellular disorganization, or evident signs of degeneration of the pancreatic islets were identified, indicating preservation of the endocrine architecture of the pancreas under the experimental conditions evaluated. Table 3 presents representative photomicrographs of pancreas histological sections that illustrate this description.

Table 3. Representative photomicrographs of pancreatic histological sections stained with Hematoxylin and Eosin (H&E), depicting morphological integrity at baseline (pre-transport) and following transport within the Control and EMAIS-SR groups.

Control packaging		
Before transport	**After transport**	**Assessment**
		Altered coalescence of pancreatic acini and pyknotic (dark) nuclei.
		Changes were present even before transport. After transport, the condition remained the same.
		Altered coalescence of pancreatic acini and pyknotic (dark) nuclei.
		Altered coalescence of pancreatic acini and pyknotic (dark) nuclei.
EMAIS-SR		
Before transport	**After transport**	**Assessment**
		Alterations were observed in only one region; however, the nuclei remained intact.

Continue...

Table 3. Continuation.

EMAIS-SR		
Before transport	**After transport**	**Assessment**
		No changes were observed.
		No changes were observed.
		Presence of alterations both before and after transport, preservation of nuclei (possible problem with fixation).
		No changes were observed.
		No changes were observed.

Source: Elaborated by the authors. 40× magnification. Scale bar = 10 μm.

Immunohistochemical analysis of pancreatic fragments stained for BCL-2 revealed positive immunoreactivity across all evaluated groups, characterized by a predominance of cytoplasmic expression within the parenchymal cells. This immunopositivity exhibited a homogeneous distribution, consistently observed in both baseline samples and those subjected to transport protocols, regardless of the container utilized (Control or EMAIS-SR)

Although no statistically significant difference was observed between the groups, a slight trend toward greater labeling intensity was observed in the EMAIS-SR group compared with the control packaging group, suggesting adequate preservation of cellular integrity and anti-apoptotic mechanisms in pancreatic tissue after transport.

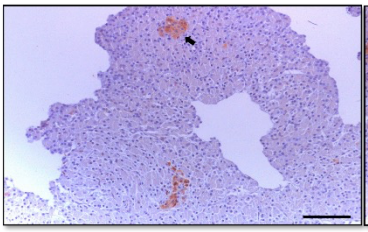
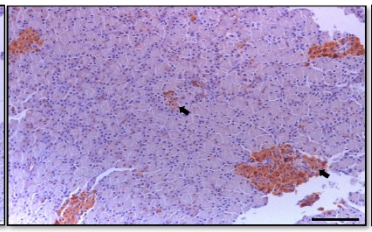
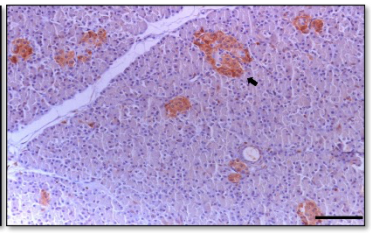
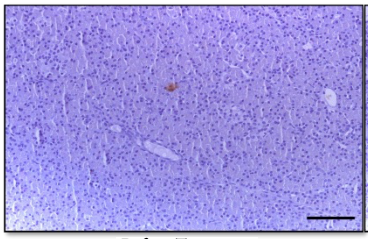
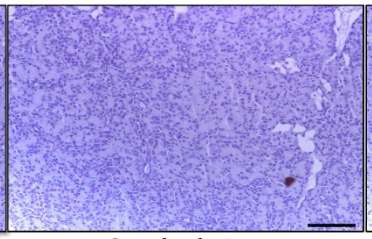
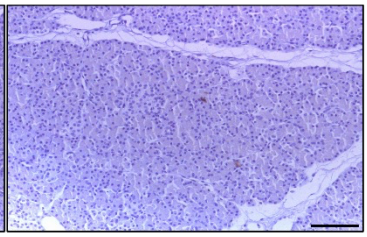
Densitometric analysis of BCL-2 immunostaining in the pancreas showed similar values between the evaluated groups. The control group, before transport, presented a mean of 188.6 ± 2.2 arbitrary units. Among the analyses of organs subjected to transport, the control packaging group had a mean of 168.1 ± 6.6 arbitrary units, while the EMAIS-SR group had a mean of 175.4 ± 5.1 arbitrary units. A slight tendency towards greater staining intensity was observed in the experimental group, suggesting better preservation of cellular integrity during transport.

In immunohistochemical sections for p53, no specific nuclear immunoreactivity was observed, indicating the absence of detectable p53 expression in the pancreatic samples analyzed. This finding is biologically compatible with the structural and functional integrity of the pancreatic tissue studied.

Although morphological preservation of pancreatic islets was demonstrated, specific identification of insulin granules by immunostaining was not performed in this study, which constitutes an objective for future investigations.

Table 4 presents photomicrographs of pancreas histological sections immunostained for BCL-2 and p53 in the different groups analyzed.

Table 4. Photomicrographs of histological sections of pancreas subjected to immunohistochemistry for BCL-2 and p53 in the different groups analyzed.

BCL-2		
		
Before Transport	Control packaging	Experimental packaging
p53		
		
Before Transport	Control packaging	Experimental packaging

Source: Elaborated by the authors. 20× magnification. Scale bar = 50 µm.

DISCUSSION

Discussing the thermal standards for pancreas preservation for transplantation is important, as the organ is particularly sensitive to temperature variations during storage and transport. Current guidelines suggest temperatures between 2 °C and 8 °C, with the ideal being close to 4 °C[9]. However, a significant gap exists regarding the definitive clinical validation of these thermal limits, especially with the introduction of new technologies and preservation solutions. This study highlighted the superiority of the EMAIS-SR technological solution, which eliminates the need for ice and provides better cell preservation than conventional ice-based packaging. This indicates that the current standard may require future revisions in light of emerging technologies[7-9].

Although the results are presented separately for the EMAIS-SR TRL6 and TRL7 prototypes, both represent distinct stages of maturity for the same active packaging technology, not independent systems but evolutionary versions with the same thermal control principle.

Considering that all storage times remained well below the clinical limit for cold ischemia described in the literature, the variation in storage time was not, in isolation, considered sufficient to justify relevant differences in histological viability between the groups[6-8].

Additionally, it is important to note that temperature measurements using core and surface thermometers have yielded divergent results in some cases, suggesting that surface measurements may underestimate or overestimate the core temperature of organs. This implies a need for more precise standardized methods in clinical practice. The use of surface thermometers is more indicated because they do not come into contact with the organs; however, standardization and margins of error need to be established. This is evident in other studies that evaluated kidney temperature in real-world settings, using different measuring instruments and varying degrees of reliability, which limit the comparability of findings[10,11].

Recent research with other organs, such as the lung[12,13], has also revisited traditional thermal standards, suggesting adjustments to temperature ranges to maximize post-transplant viability and optimize the time between procurement and transplantation. These studies reinforce the importance of continued investigation into specific, individualized thermal limits for each organ type, accounting for its metabolic characteristics and susceptibility to ischemic injury.

Regarding the macroscopic assessment used in the study, visual analysis provides important initial information on the organ's physical integrity[6]. However, this method has significant limitations: it relies on the observer's subjectivity and fails to detect big structural and functional changes, particularly at the cellular level. Therefore, it is recommended that visual qualitative assessment be complemented by objective methods, such as histology, to ensure a more comprehensive evaluation of viability.

Morphological analysis of the pancreas stained with H&E revealed adequate structural preservation of the pancreatic parenchyma in the samples analyzed before transport, involving both the exocrine and endocrine compartments. In these

samples, preserved organization of the pancreatic acini was observed, with intact cytoplasm and well-defined cell boundaries, as well as pancreatic islets with regular contours, preserved cellular architecture, and clear distinction from the adjacent exocrine tissue, characterizing a morphological pattern compatible with basal tissue integrity[14].

After transport, subtle differences were observed between the experimental groups. In the control packaging group, H&E-stained sections revealed alterations consistent with mild tissue stress, including focal rarefaction of the exocrine parenchyma, heterogeneity in acinar arrangement, and punctate cytoplasmic vacuolization. The pancreatic islets, although preserved and identifiable, presented, in some fields, less defined boundaries and slight cellular heterogeneity, suggesting greater sensitivity of the endocrine compartment to the packaging conditions. In contrast, in the EMAIS-SR group, the pancreatic parenchyma showed a more homogeneous organization, with well-preserved acini and pancreatic islets maintaining regular contours and intact cellular architecture, approaching the pattern observed in the samples before transport.

Immunohistochemical analysis supplemented the morphological findings. The p53 protein functions as a pivotal regulator of genomic stability, undergoing activation primarily in response to DNA damage, hypoxia, or oxidative stress; conversely, under physiological conditions, its expression levels remain constitutively low, governed by continuous MDM2-mediated degradation[15,16]. In the study, no specific nuclear immunoreactivity for p53 was observed in the analyzed samples, both before and after transport, in the control packaging and EMAIS-SR groups, encompassing the exocrine parenchyma and pancreatic islets. This pattern is consistent with the maintenance of genomic homeostasis and the absence of significant activation of p53-dependent apoptotic pathways[17,18].

The absence of detectable p53 expression in the samples before transport reinforces the interpretation that this tissue represents the basal state of pancreatic integrity. In the post-transport groups, the maintenance of this profile indicates that, despite the subtle morphological changes observed in the control packaging group, no sufficiently damaging stimulus was present to trigger a classic p53-mediated apoptotic response. In the EMAIS-SR group, the association between preserved morphology and the absence of p53 further supports the effectiveness of the packaging method in maintaining the physiological conditions of pancreatic tissue[19].

The cytoplasmic expression of BCL-2 observed in pancreatic tissue, including exocrine cells and pancreatic islets, supports this scenario. The BCL-2 protein plays a fundamental role in inhibiting the intrinsic apoptosis pathway by blocking the mitochondrial release of cytochrome c[20,21]. The presence of BCL-2 immunopositivity in samples before transport indicates basal expression of cellular survival mechanisms. After transport, the maintenance of BCL-2 expression in the control packaging and EMAIS-SR groups suggests preservation of these mechanisms, with a slight tendency towards greater staining intensity in the experimental group, consistent with the densitometric results[22].

The integration between morphological and immunohistochemical findings demonstrates coherence between structure and cellular function in the evaluated pancreas. Samples analyzed before transport represent the baseline pattern of tissue integrity, while the EMAIS-SR group maintained morphological and molecular characteristics closer to this pattern after transport. The control packaging group showed discrete structural alterations, without evidence of p53-dependent apoptotic pathway activation, and maintained BCL-2 expression, suggesting a possible adaptive mechanism of cellular protection.

Immunohistochemical analysis was performed on selected slides after prior morphological evaluation by H&E, in accordance with established methodological literature[23,24]. Thus, by associating the morphological findings obtained by H&E with the molecular pathways evaluated, it is concluded that the conditioning method employed in the EMAIS-SR group was effective in preserving the pancreatic parenchyma and pancreatic islets, maintaining conditions close to those observed before transport and contributing to the maintenance of cellular homeostasis.

This study may also provide important insights into the methods used in current clinical practice to assess organ viability. The combination of macroscopic and histological analyses used here suggests that future protocols could benefit significantly from the inclusion of more objective and technological measures, not only in the final pre-transplant stage but throughout the entire transport and storage process.

Finally, the study's limitations must be considered. As this is a preclinical study conducted in an animal model, the results cannot be directly extrapolated to human clinical practice without further validation in robust clinical studies. Furthermore, the small number of organs studied limits the generalizability of the results. Future studies with larger and more diverse samples are needed to corroborate these initial findings.

CONCLUSION

The study demonstrated, using a preclinical experimental model, that although the pancreas was kept within the recommended temperature range in 9 of 10 experiments, organs packaged in EMAIS-SR were better preserved. The only case in which temperature increased after packaging, with a final temperature of 12.1 °C, occurred with traditional packaging

using ice. Although this pancreas showed improvement in visual appearance (macroscopic evaluation), histological analysis revealed changes indicative of impaired organ preservation.

Given the innovations in preservation solutions and available technologies, further studies are needed to confirm the established thermal limits. Therefore, this research reinforces the importance and need for more robust future studies to support clinical decisions on safe thermal limits and to improve best practices for pancreas transport for transplantation.

CONFLICT OF INTEREST

Nothing to declare.

AUTHOR'S CONTRIBUTION

Substantive scientific and intellectual contributions to the study: Paim SMS, Cruz VA, Gomes KSC, Araujo JKS, Coutinho GMM, Teraoka EC, Leite RF, Gonçalves VA, Carbonel AA, Simões MJ, Soares JH, Taha MO, David AI, Schirmer J, Roza BA; **Conception and design:** Paim SMS, Cruz VA, Coutinho GMM, Teraoka EC, Leite RF, Gonçalves VA, Carbonel AA, Simões MJ, Taha MO, David AI, Schirmer J, Roza BA; **Data analysis and interpretation:** Paim SMS, Cruz VA, Gomes KSC, Araujo JKS, Coutinho GMM, Teraoka EC, Leite RF, Gonçalves VA, Carbonel AA, Simões MJ, Soares JH, Taha MO, David AI, Schirmer J, Roza BA; **Article writing:** Paim SMS, Cruz VA, Gomes KSC, Araujo JKS, Coutinho GMM, Teraoka EC, Leite RF, Gonçalves VA, Carbonel AA, Simões MJ, Soares JH, Taha MO, David AI, Schirmer J, Roza BA; **Critical revision:** Paim SMS, Cruz VA, Gomes KSC, Araujo JKS, Coutinho GMM, Teraoka EC, Leite RF, Gonçalves VA, Carbonel AA, Simões MJ, Soares JH, Taha MO, David AI, Schirmer J, Roza BA; **Final approval:** Paim SMS.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

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DECLARATION OF USE OF ARTIFICIAL INTELIGENCE TOOLS

The authors declare that no artificial intelligence tools were used in the preparation, writing, data analysis, or review of this manuscript.

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