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Original Research

Effect of Ischemia Durations, Transport, Cryopreservation and Storage Conditions on Tissue Integrity, DNA, RNA, Protein Quality and Primary Cell Culture Initiation Capacity in Liver Tissues

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Abstract

Objectives: Standardization of pre-analytical factors is essential for ensuring the quality and reproducibility of tissue-based biomedical research. Factors such as ischemia time and freezing methods significantly impact sample integrity and the quality of downstream analyses. However, systematic evaluations of how ischemia time, transfer conditions, freezing methods, and storage parameters affect liver tissue quality remain limited. This study aimed to evaluate the effects of ischemia duration, freezing techniques, transfer conditions, and storage conditions and durations on liver tissue integrity, nucleic acid quality, protein yield, and primary cell culture initiation capacity.

Methods: Liver tissues from healthy male Wistar albino rats were subjected to varying cold ischemia durations (2h, 8h, 24h) and transfer conditions (+4°C, vacuum-sealed, culture medium) to assess tissue integrity, nucleic acid and protein quality, and cell culture initiation potential. Optimal conditions were selected for subsequent freezing techniques (controlled-rate, rapid freezing in liquid nitrogen, and freezing with isopentane) over different storage periods (3, 6, 9 months) at –80°C and –196°C. Histological assessment (HE and Masson trichrome staining) was performed to evaluate sample integrity. Nucleic acid, and protein yield and quality as well as primary cell culture initiation capacity, were tested.

Results: Tissue integrity remained stable for 0–2 hours under cold ischemia, with moderate degeneration observed after 24 hours. RNA and DNA yields were consistent across transfer conditions, with no significant differences detected. However, RNA integrity was more sensitive to ischemic conditions compared to total nucleic acid quantities. Freezing methods did not significantly differ in preserving tissue quality, and both –80 °C and –196 °C storage effectively maintained nucleic acid integrity up to 9 months. Primary cell cultures were successfully established from tissues subjected to a maximum of 8 hours of ischemia, but not from those exposed to 24 hours.

Conclusion: Optimized transfer conditions and appropriate freezing and storage methods are key to preserving liver tissue quality for biobanking and downstream analyses. This study provides valuable insights for developing standardized protocols for liver tissue biobanking, which could enhance reproducibility and reliability in translational research.

Keywords: Biobanking, Cold ischemia, cryopreservation, liver tissue, pre-analytical factors, translational research

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Proper preservation of tissue samples is vital for translational research and clinical studies. Since tissue samples, especially liver tissues, are sensitive to environmental changes, correct storage and handling are essential to maintain sample quality and usefulness for future genome, transcriptome, and proteome analysis. Improper storage or mishandling can cause degradation and contamination, which can be influenced by factors independent of the collection methodology, such as tissue type and the duration of ischemia during/after surgery. Especially, the cellular ischemia tolerance, sensitivity to freeze-thaw events, and cryopreservation conditions need to be tailored to specific tissue types.

Even short delays before freezing liver samples can induce biochemical and metabolic alterations greater than inter-individual differences.[1] Prolonged cold ischemia leads to autolysis, transcriptomic and proteomic changes, and histomorphological artifacts, with hepatocytes, endothelial cells, and bile duct epithelium especially susceptible to damage.[2–4] Such changes compromise RNA and protein integrity, immuno-histochemistry results, and ultimately the reliability of translational findings. Therefore, minimizing ischemia time, controlling freezing conditions, and applying tissue-specific protocols are crucial to preserving structural, metabolic, and molecular integrity.[5,6]

Biobanks are structured collections of biological samples and associated data, maintained for current or future scientific research, where each processing step critically impacts sample quality.[7] Standardization and optimisation are therefore essential to ensure high-quality samples, reproducibility of research, and inter-center comparability, ultimately transforming biobanks from mere storage facilities into reliable infrastructures for translational research.[8–10] Without such practices, samples risk becoming scientifically unusable, ethically questionable, or legally noncompliant.[11]

The liver is a particularly challenging tissue for biobanking due to its high enzymatic activity, metabolic turnover, and vulnerability to ischemia-induced degradation. RNA, proteins, and metabolites degrade rapidly in liver tissue, and standard cold ischemia thresholds established for other tissues may not be applicable.[12,13] While several studies have demonstrated ischemia-related alterations in gene expression, phosphorylation, and metabolic pathways,[14,15] systematic analyses comparing the effects of freezing techniques and storage conditions on multiple molecular classes in liver tissue remain limited. Furthermore, classic quality metrics, including RNA integrity number (RIN), do not fully capture ischemia-induced biological shifts, particularly in metabolomics and single-cell analyses.[16]

The lack of standardized recording of ischemia time, freezing methods, and storage duration in many biobank databases further reduces inter-center comparability and the translational value of research. Although optimal ischemia limits vary by tissue type, the specific impact of different freezing and storage methods on the molecular integrity of liver tissues has not yet been fully optimized. Controlled, tissue-specific studies are urgently needed to define analyte-specific thresholds and generate practical guidelines for hepatic biobanking.[12,17]

To address this, we evaluated the effects of transfer conditions, freezing methods, and storage durations on liver tissue morphology, DNA, RNA, and protein quality/quantity, as well as the success rate of establishing primary cell cultures. This study provides direct evidence on how liver tissues should be transferred, frozen, and stored by assessing multiple molecular parameters from the same samples under defined pre-analytical conditions. Our findings may contribute to the development of standardized operating procedures (SOPs) and SPREC-based guidelines specific to liver tissue biobanking, thereby enhancing the reliability, reproducibility, and clinical applicability of downstream genomics and proteomics research, as well as innovative ideas for improving preservation methods of liver allografts for transplantation studies.

Methods

Study Design

The study was designed to answer three questions: in the first step, the effect of ischemia time (2 hours, 8 hours and 24 hours) and transfer conditions (+4°C, vacuumed at +4°C and in culture medium at +4°C) on tissue integrity, nucleic acid quality (DNA and RNA), protein quality, primary cell culture initiation potential were analyzed; the second step were planned to examine the effects of freezing techniques (freezing in a controlled freezer at -1°C/min, rapid freezing with liquid nitrogen, and freezing in liquid nitrogen accompanied by isopentane) over storage durations of 3, 6, and 9 months. In the third step, storage conditions (–80°C freezer and –196°C liquid nitrogen tank) and storage times under these conditions were analyzed using the four parameters defined in previous steps. When analyzing tissue quality using HE staining, the A260/A230 and A260/A280 absorbance ratios were used to determine DNA quality, and the RIN (RNA Integrity Number) value was used to measure RNA quality. Protein quality was analyzed using the BCA (Bicinchoninic Acid) method. To determine cell culture initiation success, primary cell cultures were established and the viability and morphological status of the cells were examined using both trypan blue staining and microscopy (Fig. 1).

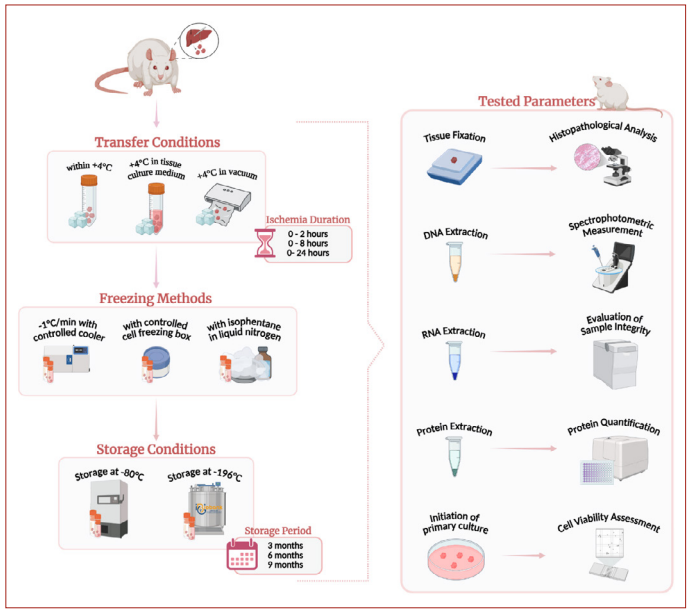

Figure 1. Schematic workflow of the experimental design. Liver tissues obtained from Wistar Albino rats were subjected to different transfer conditions, and the optimal protocol was selected based on histological analysis, DNA, RNA, protein, and cell viability. Using these same parameters, subsequent experiments investigated the effects of freezing methods, freezing conditions, and storage durations. The figure summarizes the workflow from tissue transfer to biomolecular and cellular analyses, where quality assessments served as a guiding criterion at each step.

Establishment of the Sample Collection

For this study, seven healthy male Wistar albino rats, aged 6–8 weeks and weighing 230–330 g, were obtained from the Vivarium unit of the Izmir Biomedicine and Genome Centre (IBG). Before the experiment, the animals were housed under standard laboratory conditions (12-hours light/dark cycle, 22±2°C temperature, 50–60% humidity) with free access to standard feed and water. Animal euthanasia was carried out by the institution's veterinary surgeon at the IBG-Vivarium unit in compliance with guidelines authorized by the IBG-Local Ethics Committee for Animal Experiments (IBG-HADYEK) (dated 29/11/2021, numbered 2021-022). Following euthanasia under deep anesthesia by cervical dislocation, macroscopic examination of the liver was performed. Subsequently, tissue samples were transferred to the IBG-Biobank under predefined conditions that reflect real clinical conditions. Ischemia times were planned according to scenarios routinely encountered in IBG-Biobank operations. Since the biobank is located within a health campus, some samples could be delivered within one hour, whereas in collaborations with distant centers, transfer could be delayed up to 24 hours. Accordingly, experimental conditions were established to evaluate how proximity of collection site, ischemia duration, and temperature influenced tissue integrity, nucleic acid quality/quantity and protein quantity, as well as cell viability. Samples were exposed to cold ischemia for 2 h, 8 h, and 24 h under three transfer conditions: (i) directly on ice at +4 °C in collection tubes, (ii) in tubes containing transfer medium/cell culture medium on ice at +4 °C, and (iii) in vacuum-sealed bags at +4 °C. Following ischemia, tissues were dissected into standardized pieces (5 mm^3/50 µg).

All procedures were documented using the BB_Vivarium_ Fresh Tissue Collection, Processing and Storage Tracking Form (Supplementary File 1). Sample information was registered in the biobank information management system (Openspecimen) and assigned a unique biobank code (PPID) (Table 1).

The entire sample collection, processing and storage processes were carried out in accordance with the SOP of the İBG-Biobank.

Evaluation of Freezing Methods and Storage Conditions on Liver Tissue Quality

Three freezing methods and two freezing conditions were compared. Each tissue sample was subjected (i) controlled freezer (PLANER; #Kryo560-16) (-1°C/min), (ii) rapid freezing in liquid nitrogen (LN2), and (iii) freezing in LN2 accompanied by isopentane, as well as all samples were treated with two different freezing conditions:(i) a freezing medium (70% complete RPMI1640, 20% FBS, and 10% DMSO) and (ii) fresh frozen tissue samples

Table 1. Sample record registered in Openspecimen with the project code

PPID	Registration Date	Gender	Animal Info ID	Genus	Weight (g)
V150	23-03-2023	Male	40082	Wister Albino	327.12
V151	12-06-2023	Male	40450	Wistar Albino	315
V152	11-07-2023	Male	40583	Wistar Albino	231.43
V184	05-10-2023	Male	41982	Wistar Albino	263.25
V183	05-10-2023	Male	41983	Wistar Albino	261.03
V185	12-10-2023	Male	42147	Wistar Albino	262.33
V291	06-02-2024	Male	47107	Wistar Albino	327.8

without medium. Then all samples with different freezing conditions were preserved under two different storage conditions: a -80°C deep freezer and a -196°C LN2 tank. Storage periods were set at times for 0, 3, 6, and 9 months. Thus, the effects of different freezing methods on each combination of storage temperature and storage duration were analysed (Fig. 1).

a. Histopathological Analyses

Tissue samples processed according to different ischemia periods and transfer and storage conditions were fixed in 10% buffered formalin solution for 48-72 hours for routine histopathological examinations. During the tissue processing procedure, samples were subjected to a stepwise alcohol series (70°, 80°, 90°, 96°, and 100°), xylene, and paraffin series in a tissue processor (Sakura Tissue-Tek Vip6-E2) before being embedded in paraffin blocks. Sections 4-6 µm thick were cut from these blocks using a microtome (Leica/Rm2245) and placed on slides. The sections were stained with Hematoxylin and Eosin (HE) in an automatic staining device (Leica/Stainer XI) and made ready for examination. Masson's Trichrome staining was used to analyze the integrity of the ECM[18] by using a commercial kit (BIO-OPTICA BO 04-010802). Sections of tissue samples obtained from each test condition were compared with sections prepared from fresh samples collected during necropsy. These analyses were performed under a light microscope (Olympus; BX53F), and microphotographic images were recorded using a camera (Olympus; DP27). Tissue integrity was evaluated by two veterinary pathologists using a scoring system: no damage = 0, minimal damage. = 1, moderate damage = 2, and severe damage = 3 according to preservation of cellular and ECM structural integrity, tissue architecture, and levels of autolysis.

b. DNA isolation and Quality Controls

Genomic DNA isolation was performed using the DNeasy Blood and Tissue Kit (QIAgen; #69504). The quantity and purity of the genomic DNA were determined using a NanoDrop (Thermo Scientific; ND-2000). DNA concentration was measured in ng/µL, and purity values were determined using the A260/A230 and A260/A280 absorbance ratios.

c. RNA isolation and Quality Control

Total RNA isolation was carried out using NucleoZOL (Macherey-Nagel; #69504). The homogenisation step was performed by a TissueLyser LT (Qiagen; #85600) device with 5 mm stainless steel beads (Qiagen; #69989) at a frequency of 50 Hz for 3 minutes, followed by the isolation process. RNA concentration was measured in ng/µL, and purity values were determined using the A260/A230 and A260/A280 absorbance ratios. RNA integrity and quality were analysed using the Agilent RNA 6000 Nano Kit (Agilent Technologies; #5067-1511) with the 2100 Bioanalyzer device (Agilent Technologies; #G2939BA). In this analysis, RIN (RNA Integrity Number) values were determined.

d. Protein Isolation and Quantitation

The isolation process was carried out using RIPA buffer (Radioimmunoprecipitation Buffer). The homogenization step was performed as described in RNA isolation section using a Tissue Lyser LT (Qiagen; #85600) device The protein concentration was determined using the BCA (Bicinchoninic Acid) method.[19] For this, a standard curve was created using bovine serum albumin (BSA) at known concentrations. The absorbance values of the samples were measured at 562 nm using a Varioskan Flash (Thermo Scientific; #5250030), and the protein quantities were calculated using the BSA standard curve with Microsoft Excel.

e. Assessment of Primary Cell Culture Initiation Capacity

A portion of the multiple samples were divided into five pieces of approximately 0.5 mm^3 each using a sterile scalpel in a cell culture dish.[20] The first tissue sample was directly cultured to analyze its potential for starting a cell culture. Four of these pieces were placed in cryotubes containing 1.5 mL of freezing medium and frozen using two different methods for further testing their primary cell culture initiation capacity: (i) two tissue pieces were placed in a CRF), (ii) two tissue pieces were placed in a CFE. These tissue samples were cut into as small pieces as possible using a sterile scalpel and washed with PBS after passing through a 100 µm filter. The tissue pieces remaining on the filter were washed repeatedly with PBS using a pipette until they were completely filtered out, resulting in a cell suspension.[20,21] The cells were seeded into 10 cm cell culture plates and incubated at 5% CO_2 and 37°C. The viability and morphological status of the cultured cells were analyzed both by trypan blue staining and under a microscope.

Statistical Analysis

For each condition, at least three biological replicates were generated, and when necessary, three technical replicates were included to ensure statistical robustness. Statistical analyses were performed using GraphPad Prism9. Comparisons across multiple groups were conducted with two-way ANOVA and mixed-effects models. Data are presented as mean and error (SEM), and p-values < 0.05 were considered statistically significant.

Results

To systematically evaluate the impact of different preanalytical variables on liver tissue quality, we first examined the influence of ischemia duration and transfer conditions on nucleic acid and protein quality, and the initiation capacity of primary cell culture.

1- The influence of transfer conditions and ischemia duration on tissue integrity, nucleic acid and protein quality:

No morphological changes were observed within 0–2 hours under cold ischemia (score:1±1). Under vacuum transfer conditions, signs of degeneration became evident at 8 hours score:2±1). After 24 hours of ischemia, moderate degeneration (score:3±1), was observed in the liver across all transfer conditions.

When we evaluated the effects of transfer duration for samples transferred on ice at +4 °C, independent of transfer condition, RNA, DNA and protein yields were between 100-300 ng/µl, 50-100 ng/µl, 20-35ug/µl, respectively (Fig. 2A, 2B, 2C). Although RNA yield was 2-3 times higher in samples transferred in tissue culture medium and in vacuum bags than in those directly transferred at +40C for 0-2 hours ischemia duration, no statistically significant differences were detected in any freezing and transfer conditions tested ($p<0.05$).

Despite this quantitative stability, RNA integrity showed

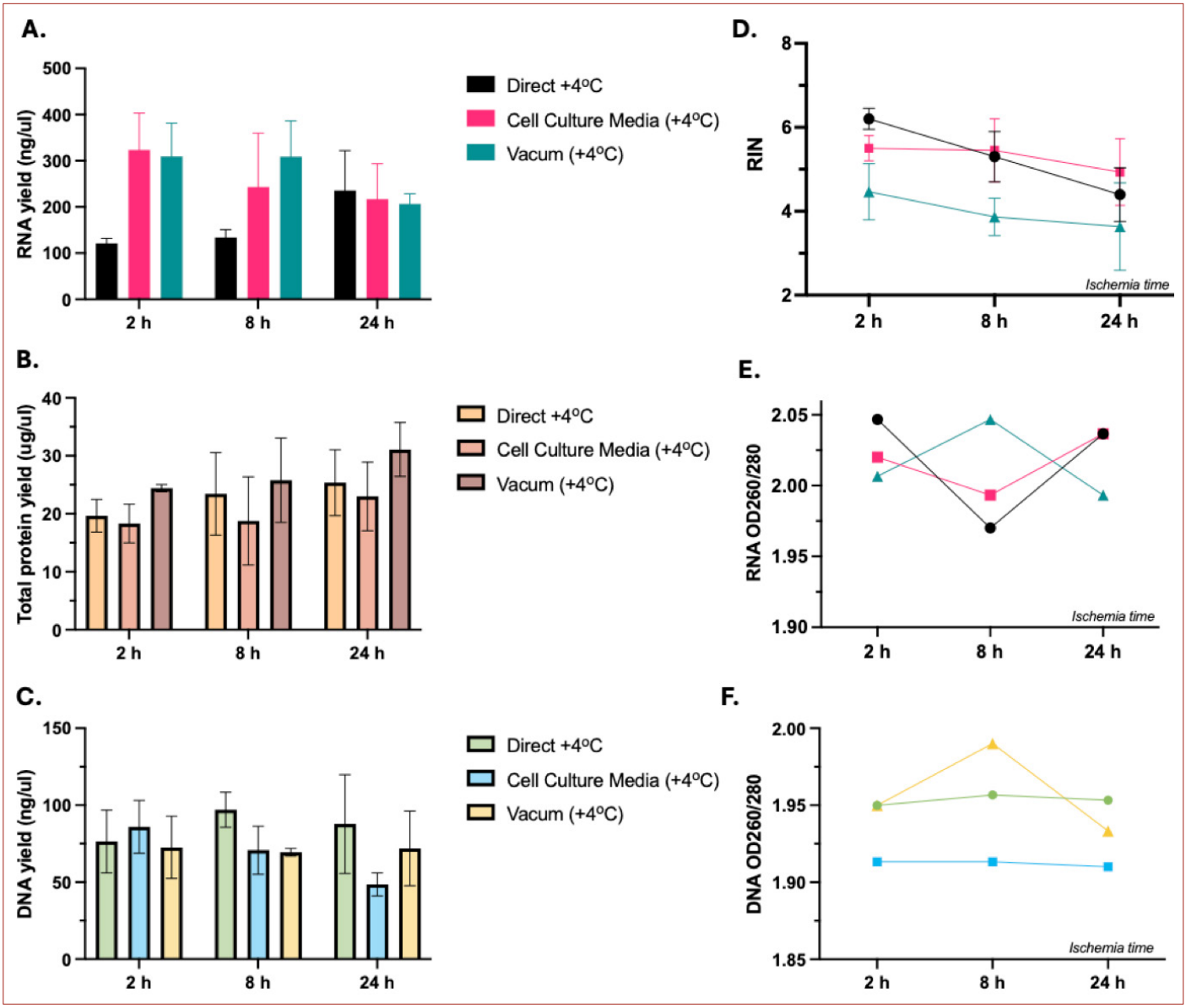

Figure 2. Effect of cold ischemia duration and transfer conditions on the quality and quantity of DNA, RNA, and proteins extracted from liver tissue samples.

condition-dependent variation. Samples transferred directly on ice consistently displayed preserved integrity, with RNA Integrity Numbers (RIN) ≥6 (Fig. 2D). RIN values remained around 6 when tissues were transferred in culture medium at +4 °C, but were substantially lower under vacuum transfer conditions (Fig. 2D) . However, there were no statistically significant differences ($p< 0.05$) in any freezing and transfer conditions compared to controls.

Furthermore, purity assessments confirmed optimal quality across all groups, with OD260/280 ratios of 1.8–2.1 for RNA and 1.7–2.0 for DNA. Taken together, these results demonstrate that nucleic acid amount and quality for all transfer conditions are suitable for downstream applications (Fig. 2D–F).

Across all ischemia durations tested (2, 8 and 24 h, +4 °C), the total yields of RNA, DNA, and proteins remained largely unchanged in liver tissues, with no statistically significant differences between transfer conditions.

The effects of transfer conditions and ischemia duration on primary cell culture initiation capacity

Primary cultures were successfully established from tissues transferred directly at +4°C or in culture medium/vacuum sealed conditions, and from tissues subjected to both 2 and 8 h ischemia, and they showed characteristic morphological features, whereas primary cultures could not be established from tissues subjected to 24 h ischemia (Fig. 3A and 3B).

These results demonstrated that only tissues transferred under 2 h ischemia, either in culture medium or vacuum-sealed at +4 °C, supported the successful establishment and maintenance of primary cell cultures. Under these conditions, cultures retained characteristic morphology, survived passaging, and enabled cryopreservation. Based on these optimized transfer conditions, subsequent analyses focused on evaluating the effects of different freezing techniques and storage conditions on tissue integrity and molecular quality.

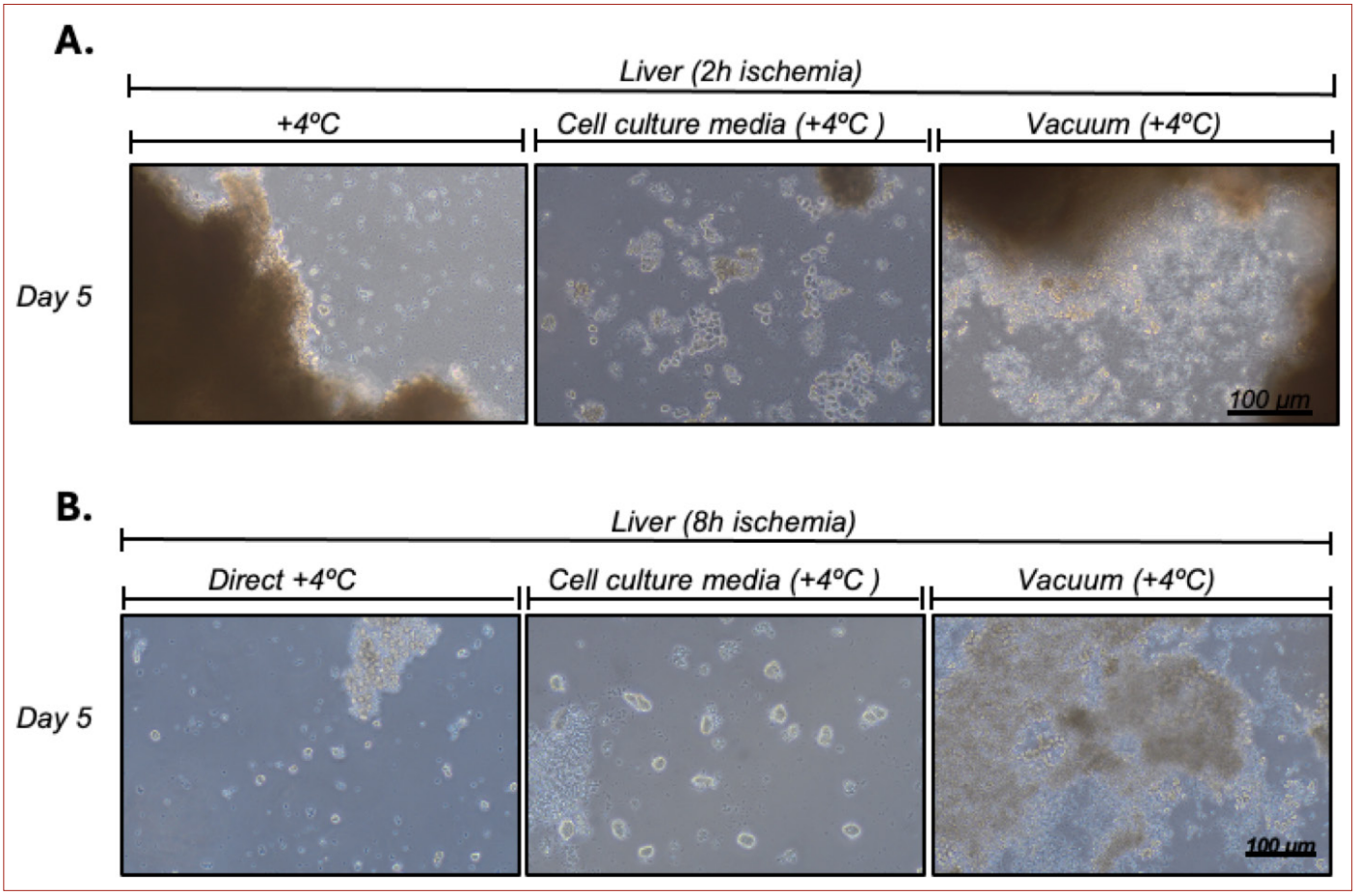

Figure 3. Primary liver cell culture outcomes following different cold ischemia durations and transfer conditions: Explants were prepared from transferred liver tissues and cultured under sterile conditions; cultures were monitored daily by light microscopy. **(A)** Representative phase-contrast image of a culture derived from tissue transferred in culture medium at 0–2 h cold ischemia, showing typical hepatocyte-like morphology, successful survival after passaging and attainment of confluence. **(B)** Representative image of a culture from the 2–8 h group showing initial attachment and morphological features, but failure to survive after passaging.

2- The influence of storage conditions on sample quality for samples frozen by different methods

To assess the impact of different freezing methods , liver tissue samples transferred under the previously identified optimal condition (direct transfer on ice at +4 °C) were frozen using three approaches as shown in Figure 1.

a. Effects on tissue integrity

No morphological changes or tissue damage were observed within 2 hours of cold ischemia compared to control conditions in FrFz and CFC and tissues (score:1±1), whereas low or moderate damage was observed for the IPA condition (score:2±1). In contrast, the damage score was determined as 2±1 for all storage conditions tested (3,6, and 9 months), supporting that FrFz of liver tissues transported at +40C is the best condition to prevent tissue integrity. Damage score for storing all frozen liver tissues at either -80 °C freezers or LN2 decreases tissue integrity slightly. No remarkable decrease was observed between storage at -80 °C freezers or LN2 up to 9 months.

b. Nucleic acid quality

Genomic DNA and RNA concentrations, RIN and A260/280 values from liver samples were measured as described in the Materials and Methods. The RIN value was determined as 6 at T0, and no differences were observed between

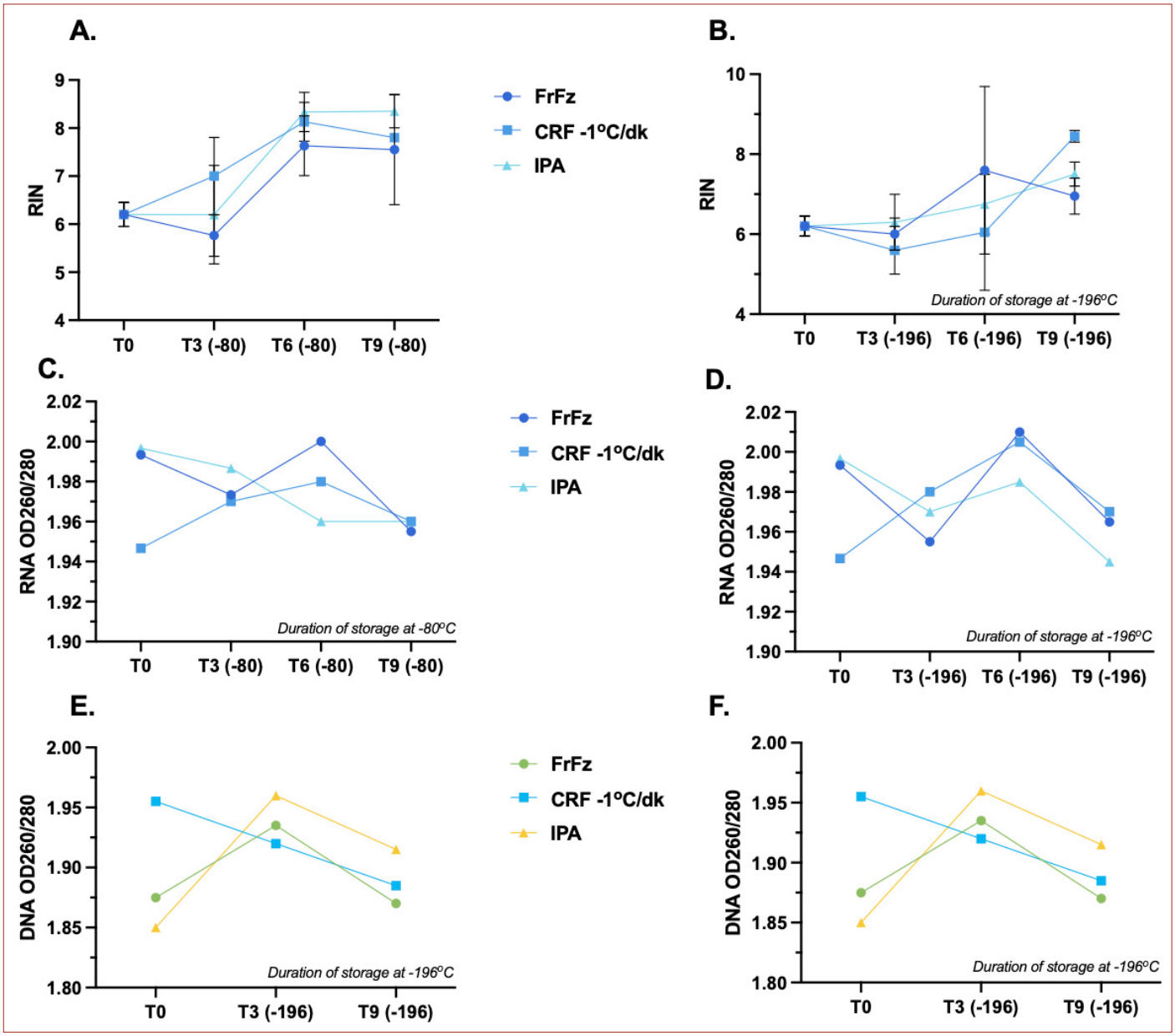

Figure 4. Evaluation of the impact of storage conditions (–80 °C and –196 °C) and storage durations (3, 6, and 9 months) on quality parameters for liver tissues.

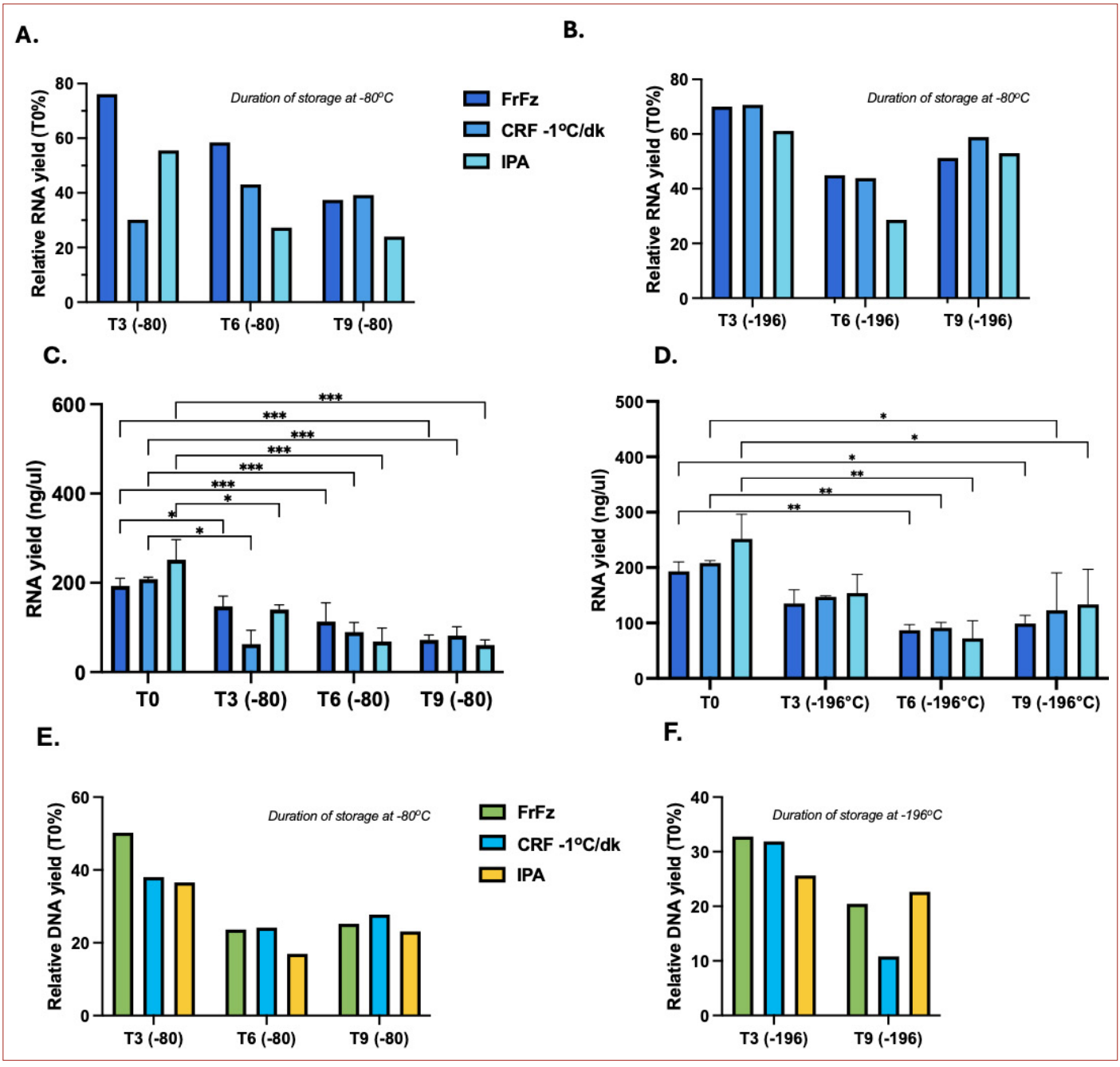

Figure 5. Evaluation of the impact of storage conditions (−80 °C and −196 °C) and durations (3, 6, and 9 months) on RNA and DNA quantity during the preanalytical phase. Comparisons across multiple groups were conducted with two-way ANOVA and mixed-effects models. Data are presented as mean and error (SEM), and p-values < 0.05 were considered statistically significant. Values presented as mean extraction yields from three biological replicates.

the freezing methods (Fig. 4 A-B). Similarly, no significant changes were detected across storage durations of 3, 6, and 9 months, representing short- to medium-term storage. RNA exhibited OD260/280 ratios of 1.89–2.11 (Fig. 4 C-D) while, DNA showed OD260/280 ratios ranging from 1.77 to 2.04 (Fig. 4 E-F). Overall, no statistically significant differences in nucleic acid quality were observed among the three freezing methods, storage durations, or temperatures (Fig. 4).

Extraction yields, expressed as micrograms of DNA or RNA per milliliter, were calculated for all samples. For each subject, a baseline yield (T0) was defined as the mean extraction yield obtained from three biological replicates processed immediately after collection. Comparisons across multiple groups were conducted with two-way ANOVA and mixed-effects models. Data are presented as mean and error (SEM), and p-values <0.05 were considered statistically significant.

The T0 value was used as a reference to calculate relative DNA or RNA yields (% of T0) for samples stored under different conditions. Relative RNA extraction yields under the different storage conditions relative to T0 are summarized in Figure 5A and 5B. The mean coefficient of variation (CV) at T0 RNA was 15%. Compared with T0, significant differences in nucleic acid yields were detected under −80 °C storage conditions at all durations, whereas samples stored at −196 °C showed changes in DNA and RNA amounts at T6 and T9. Similar analyses were performed for DNA; relative DNA extraction yields under the different storage conditions relative to T0 are summarized in Fig. 5E and 5F. The mean coefficient of variation (CV) at T0 DNA was 14%. Because no statistically significant differences were observed among the baseline samples yield, raw data were re-analyzed to compare relative changes over time and no significant differences were observed in either analysis (no data shown). Comparisons across multiple groups were conducted with two-way ANOVA and mixed-effects models. Data are presented as mean and error (SEM), and p-values < 0.05 were considered statistically significant.

c. Primary cell culture initiation capacity

The primary cell culture initiation, primary cell cultures were successfully established from liver tissues stored for 3 and 6 months under both freezing methods and storage temperatures (Fig. 6); however, the cells did not survive after passaging.

Discussion

In translational research, collaborative approaches combined with high-quality clinical data are essential to collect sufficient samples, while maintaining sample quality and homogeneity is crucial.[22] Low reproducibility remains a significant challenge for the development of clinical biomarkers for diagnosis, treatment, and monitoring.[23] Therefore, analyses and records used to assess tissue quality are critical to ensure reproducibility, yet standardized and comprehensive methods to evaluate tissue quality and usability have not been fully established.

However, it is unachievable to perform these analyses in clinical biobanking settings with human tissues due to the limitations, including sample size, genetic variability, a wide disease/lifestyle spectrum, and ethical issues. In this study, we used rat liver tissue which allow us to test a wide variety of conditions in a sequential experimental setting. By evaluating ideal and suboptimal pre-analytical scenarios using liver tissues obtained from one line of Wistar Albino rats, potential bias introduced by biobanked patient samples was minimized.

To find optimized transfer time and conditions for liver samples, we first tested three different cold ischemia durations and three different sample transfer conditions on sample quality. Since transfer at +4 °C has been reported to be critical for maintaining liver tissue stability[24,25] we tested all scenarios at +4 °C, and compared direct transfer, transferring tissues in culture medium, or vacuum sealed bags, to determine whether these conditions further improve sample quality for biomolecular analyses. Although keeping liver tissue samples in vacuum-sealed bags at 4 °C during transfer has been reported to inhibit enzymatic activity and prevent autolysis and mechanical stress,[26] in our experimental setting, vacuum-sealed bags did not produce a statistically significant result in any

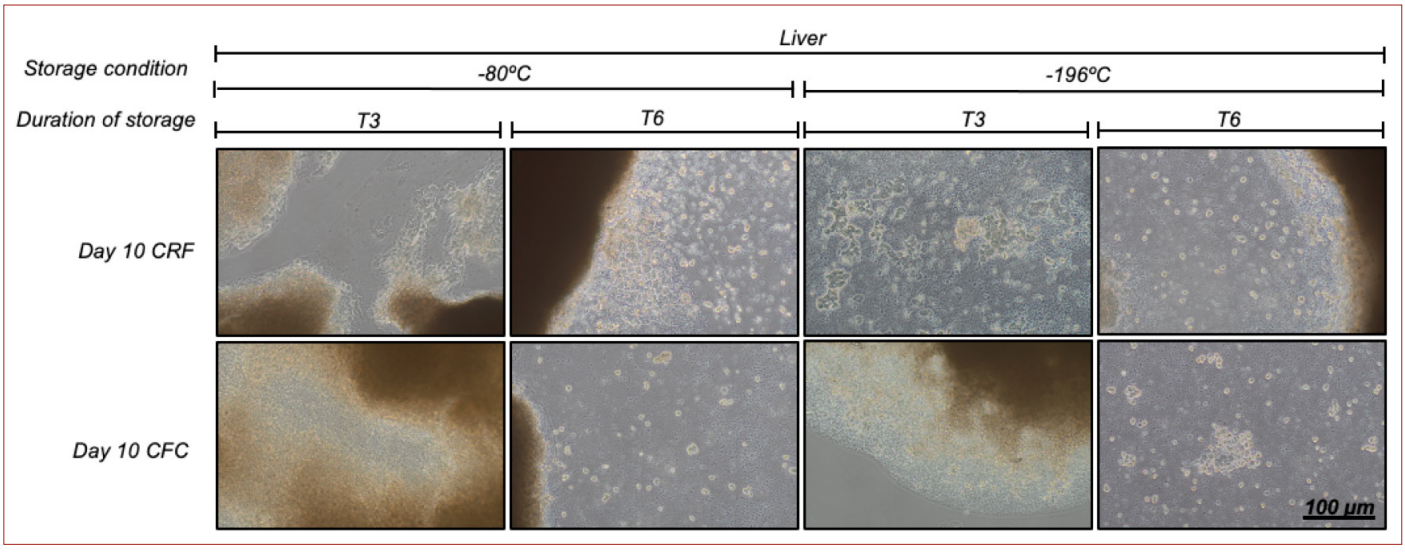

Figure 6. Primary liver cell culture outcomes following different storage durations and conditions: Primary cell cultures were successfully established from liver tissues stored for 3 and 6 months under both freezing methods and storage temperatures.

ischemia duration. Vacuum bags are commonly used to extend the shelf life of food or pharmaceutical products. However, in live tissue samples, particularly in biobanking, methods such as rapid cooling and cryopreservation are more effective. Therefore, the use of vacuum bags cannot compensate for the adverse effects of ischemia time.

Across all ischemia durations tested (2, 8 and 24 h, +4 °C), the total yields of RNA, DNA, and proteins remained stable in liver tissues, with no statistically significant differences between transfer conditions. These findings indicate that, although tissue morphology and RNA integrity showed gradual alterations over time, the overall molecular quantities were preserved regardless of ischemia duration. Data showing primary cell culture initiation capacity up to 8 hours of cold ischemia in all three transfer conditions support keeping cell viability in this time frame. Losing primary culture initiation capacity at longer cold ischemia (24 hours), parallel with the histopathological data showing cell damage.[27,28] Moreover, alterations in protein expression induced by cold ischemia have been found to correlate with concomitant changes at the gene expression level.[24]

The stability of RNA, DNA, and protein yields across different ischemia durations suggests that liver tissue possesses a relatively high resistance to short- and mid-term cold ischemia in terms of molecular quantity. This may be attributed to the dense cellular architecture and high tissue mass, which buffer against rapid degradation, and to the protective effect of storage at +4 °C, which slows enzymatic activity. However, despite similar quantities, RNA integrity was more sensitive to ischemia, highlighting that qualitative parameters rather than total amounts are more critical when evaluating the suitability of liver samples for downstream molecular and cellular applications.[29,30]

While freezing using a controlled-rate freezer (CRF) is commonly recommended in the literature,[31,32] in our study, freezing in isopentane or in a cell freezing container was as effective as using a controlled-rate freezer for preserving liver tissue. Notably, no significant difference was observed between –80 °C and –196 °C storage up to 9 months of storage period, supporting environmentally conscious "green biobanking" approaches.[33] It has been reported that maintaining tissues at –80 °C offers stable long-term preservation with minimal heat output and resource usage.[34,35] Sharing data on alternative preservation conditions, as presented here, can promote the development of more sustainable biobanking practices.

For nucleic acids, the significant decrease in RNA yield observed over time in liver tissues stored at –80 °C reflects the inherent vulnerability of RNA to enzymatic and chemical degradation, even under cold storage conditions. RNA is single-stranded and structurally less stable, making it more sensitive to residual RNase activity and ice crystal-induced damage.[29,36,37] In contrast, DNA is double-stranded and chemically more stable, explaining the lack of significant changes in DNA yields under storage at –80 °C or –196 °C. While ultralow temperatures effectively preserved RNA integrity during the first 3 months, prolonged storage may lead a gradual loss, highlighting the need to consider both storage temperature and duration for RNA-based analyses. The RIN value was 6 at T0, and no significant differences were observed between freezing methods or across storage durations of 3, 6, and 9 months, likely reflecting sample heterogeneity and differential preservation of RNA fragments.

Taken together, our study provides a comprehensive examination of how preanalytical factors, including cold ischemia time, freezing, and storage methods and affect quality parameters of liver tissue and the likelihood of preanalytical errors in a controlled experimental setup. Further studies to assess the impact of long-term storage on the liver tissue quality are needed.

Disclosures

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Ethics Committee Approval: (IBG-HADYEK) (Dated 29/11/2021, numbered 2021-022).

Authorship Contributions: Concept – NA, STA; Design – NA, STA; Supervision – NA, SB; Materials – STA, NA; Data collection &/or processing – STA, CC, BO, CU; Analysis and/or interpretation – STA, CC, BO, NA; Literature search – STA, CC, BO, NA; Writing – STA, CC, BO, NA; Critical review – STA, NA.

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