**Additional Review Methods**

*Search methods and study selection*

We searched PubMed to identify relevant studies with keywords related to fluid preservation of brain tissue. We searched the PubMed database from its inception to Aug 4, 2023, using the following search terms: (brain OR "Brain"[Mesh] OR "nerve" OR "Nervous System"[Mesh]) AND (“fluid preservation” OR “liquid storage” OR “brain banking” OR formaldehyde OR formalin OR paraformaldehyde OR glutaraldehyde OR glyoxal OR acrolein) AND (storage OR stored OR conservation OR decades OR century OR museum OR indefinite OR repository OR prolonged OR archive OR archival OR archives OR ancient). Using SysRev, one reviewer screened the titles and abstracts and identified studies for full-text review based on pre-determined eligibility criteria (Bozada et al., 2021). Next, the same reviewer evaluated the full texts and selected studies to include. To find to find additional eligible studies, we hand-searched the reference lists and referencing studies of the most relevant papers. Any studies meeting the inclusion criteria that were identified outside the formal search process, such as in the initial literature scoping process, were also included.

*Eligibility criteria*

To be formally included in this review, studies needed to report original empirical data relevant to the effects of different methods of brain tissue fluid preservation on histological and morphological integrity. More specifically, we included studies that met the following criteria:

* Examined preservation of brain or nervous system tissue in a liquid state.
* Assessed histological and/or morphological preservation through microscopy or other structural analysis methods.
* Had a minimum preservation duration of 4 weeks.

We focused on structural and morphological integrity rather than biomolecular preservation alone. Therefore, studies utilizing only neuroimaging without cellular histological analysis were excluded, as were studies focused solely on biomolecular preservation without histological or morphological assessment. Both human and animal studies were eligible for inclusion. If multiple publications reported on the same underlying data, we included the study that drew the most relevant conclusions about preservation effects.

*Data extraction*

For each included study, we extracted relevant data on the method of fluid preservation, such as the preservative fluid used, the storage conditions, the storage duration, the visualization method, and the histological/morphological outcome(s) described. We treated each distinct preservation condition, duration, or visualization method reported in a study as a separate "observation." For example, if a study reported outcomes for tissue stored in two different fluids, these were recorded as two observations. However, minor variations in otherwise highly similar preservation conditions or visualization methods from the same study were grouped into a single observation. The determination of whether two similar preservation conditions or visualization methods should be grouped into one observation or separated was based on an assessment of the level of detail and independence reported for each in the text.

*Qualitative data analysis*

We summarized the key mechanisms of action described for different fluid preservation methods based on the extracted data. For the fluid preserved tissues stored in aldehyde, to perform visualization and summarization of the data, two raters independently graded each histologic outcome using the following scale:

1. Absent/minimal storage artifact: No or minimal differences of the feature compared to baseline state. Example adjectives might include “no significant change”.
2. Partial storage artifact: Observable differences of the feature due to changes in the postmortem interval, but not so severe as to render the histologic feature useless. For example, loss of intensity of a label without a significant reported change in localization of the label. Example adjectives might include “unreliable”.
3. Near-total/total storage artifact: Near-complete or complete inability to appreciate the original state of the feature, making inference of the original state likely impossible with the available data. Example adjectives might include “unable to detect” or “absent”.

Grading was performed by multiple raters in an independent fashion, similar to our previous review (Krassner et al., 2023). For each observation, one rater reviewed the full text of the study, while other raters reviewed the extracted decomposition outcome, referring to the full text where needed for context. A pilot round of grading was performed with all the raters using multiple representative studies to ensure that the raters were using the same general framework. The interrater reliability of the grades was calculated with the intraclass correlation (ICC) statistic, specifically using a single-rating, absolute-agreement, two-way random-effects model. Grades discussed in the pilot round of grading were not included in the interrater reliability calculation. The ICC value and its 95% confidence interval was interpreted based on the guidelines of Koo and Li (Koo and Li, 2016). For determining the final grade, if there was a discrepancy between the first two grades, then the final grade was decided by coming to a consensus between them.

*Gross examination of brain tissue stored in fluid preservative*

The brain tissue used for gross examination was obtained and de-identified at the Icahn School of Medicine at Mount Sinai in accordance with its policies, regulations, and institutional review board recommendations. Photos were taken with a digital camera and photo brightness was adjusted to ensure visual consistency across samples.

*Differences between the protocol and the review*

We categorized the included studies into five types of chemicals used for fluid preservation, providing a more granular analysis of the preservation methods. We only performed qualitative data analysis for the studies reporting aldehyde preservation, because this was the only category that had a sufficient sample size to allow for useful summarization and visualization. We also included a gross examination of brain tissue stored for several years in fluid.

**References**

Bozada, T., Borden, J., Workman, J., Del Cid, M., Malinowski, J., Luechtefeld, T., 2021. Sysrev: A FAIR Platform for Data Curation and Systematic Evidence Review. Front. Artif. Intell. 4, 685298. https://doi.org/10.3389/frai.2021.685298

Koo, T.K., Li, M.Y., 2016. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J. Chiropr. Med. 15, 155–163. https://doi.org/10.1016/j.jcm.2016.02.012

Krassner, M.M., Kauffman, J., Sowa, A., Cialowicz, K., Walsh, S., Farrell, K., Crary, J.F., McKenzie, A.T., 2023. Postmortem changes in brain cell structure: a review. Free Neuropathol. 4, 4–10. https://doi.org/10.17879/freeneuropathology-2023-4790