



Evaluation of the Effect of Breast Milk Storage Conditions on the Viability of Cells in Breast Milk: A Pilot Study

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To the Editor: The fresh expressed breast milk (FBM) can be safely stored at room temperature for 3–4 h, in the refrigerator for up to 72 h, and in deep freezers for six months [1]. This study aimed to investigate the impact of storage conditions on the viability of various cell types, including potential stem cells, in FBM.

The study, designed as a single-center, prospective, experimental pilot study, obtained mature FBM samples (>15th d) from healthy mothers. FBM samples were immediately analyzed (Control Group) and compared to those stored in a refrigerator at +4 °C for three days (Group I) and frozen at -20 °C for three months (Group II). Viability analyses using propidium iodide (PI), trypan blue staining and examination of CD90 and CD73 profiles were conducted through flow cytometry.

The cell viability with total PI was 92% in the Control group and 83% and 78% in Groups I and II, respectively ($p < 0.05$). With trypan blue, total cell viability was 81.6% in the Control group and 73% and 72.9% in Groups I and II, respectively ($p > 0.05$) (Supplementary Fig. S1). In FBM, 12% of total cells with 92% viability expressed CD90, and 20% expressed CD73. In Group I, CD90 and CD73 were expressed by 18% and 20%, respectively, of the 83% viable cells.

Freezing was found to impact the quantity and activity of bioactive cells [2, 3]. While previous studies observed a 19% survival rate of viable cells after freezing [4], this study noted a higher viability ratio (78%) after three months of storage at -20 °C.

This study, utilizing a flow cytometric PI viability test, is the first to evaluate how storage conditions affect breast milk cell viability. The decrease in FBM cell viability over time, albeit better than anticipated, highlights the importance of consuming fresh milk to maximize its benefits.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12098-024-05143-9>.

Declarations

Conflict of Interest None.

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