ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2521-0807 (Print)
ISSN: 2521-0424 (Online)
CODEN: MSMAGK

Matrix Science Medica (MSM)

DOI: *http://doi.org/10.26480/msm.01.2026.01.04*

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RESEARCH ARTICLE

IMPROVED PRESERVATION OUTCOMES OF CLUSTER OF DIFFERENTIATION 34+ STEM CELLS IN CORD BLOOD FOLLOWING LONG-TERM STORAGE AT ULTRA-LOW TEMPERATURE

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ARTICLE DETAILS

Article History:

Received 27 January 2026
Revised 20 February 2026
Accepted 25 February 2026
Available online 16 March 2026

ABSTRACT

Background: Umbilical cord blood is a rich source of Cluster of Differentiation (CD) 34+ stem cells with therapeutic value, but its use in Nigeria is limited due to inadequate cryopreservation infrastructure and underdeveloped cord blood banking systems. Objective: This study aimed to assess the viability of CD34+ stem cells in cord blood after long-term storage at ultra-low temperature. Methods: In this longitudinal study, cord blood was collected and cryopreserved at −190°C in a liquid nitrogen tank. CD34+ stem cell viability was assessed biweekly over 48 weeks using flow cytometry. Data were analyzed with SPSS to evaluate viability trends and predict future cell preservation. Results: Fifty postpartum women (mean age: 32.4 ± 5.91 years) participated. Baseline CD34+ counts averaged 330 cells/mL, declining steadily to about 220 cells/mL over 48 weeks. Forecasting predicts a continued gradual decline, with viability potentially lost after approximately 200 weeks if current trends persist. Conclusion: CD34+ stem cells remain viable after 48 weeks at −190°C, with viability expected beyond 3 years, confirming the effectiveness of ultra-low-temperature storage. This demonstrates the potential for sustainable cord blood banking in Nigeria, where funding is limited for its establishment. To fully realize this opportunity, increased public awareness, healthcare training, supportive policies, and further research on the optimization and clinical application are necessary.

KEYWORDS

Cluster of differentiation 34+, cord blood banking, cryopreservation, hematopoietic stem cells

1. INTRODUCTION

Umbilical cord blood (UCB), the biological material remaining in the umbilical cord and placenta after childbirth, is a rich and noninvasive source of hematopoietic stem cells (HSCs), particularly cluster of differentiation (CD) 34+ cells with high proliferative potential (Bień et al., 2024). Once considered medical waste in many countries, UCB is now widely collected, processed, and cryopreserved for clinical use due to its proven therapeutic value in treating hematological, immunological, and genetic disorders such as leukemia, lymphoma, sickle cell disease, and Fanconi anemia (Gudauskaitė et al., 2023).

Cord blood offers several advantages over traditional sources of HSCs like bone marrow, including faster availability, reduced risk of graft-versus-host disease, and potential for a stronger graft-versus-leukemia effect (Sanchez-Petitto et al., 2023). These attributes have supported its growing application in both allogeneic and autologous stem cell transplantations globally. Countries leading in cord blood stem cell transplantation include Japan, China, India, South Korea, Australia, and the United States (Iida et al., 2022). Europe also maintains robust cord blood banking systems, while countries in the Arab world, such as Saudi Arabia, Jordan, Egypt, and the United Arab Emirates, have developed both public and private banking infrastructures (Matsumoto et al., 2015). Despite this global progress, low- and middle-income countries, particularly in Africa, face significant limitations in implementing HSC transplantation. In Nigeria and other sub-Saharan African nations, the absence of structured cord blood banking systems, high operational costs, and limited infrastructure hinder access to potentially curative therapies for diseases such as sickle cell anemia and hematological malignancies (Muhibi & Obeta, 2022; Grobbelaar et al., 2019).

The successful clinical use of cord blood stem cells relies heavily on the quality and viability of cells post-storage (Kim et al., 2015). Cryopreservation methods, including controlled-rate freezing and the use of cryoprotective agents, have been developed to minimize cellular damage. In our previous study, cord blood stem cells cryopreserved at −20°C remained viable after 6 months of storage (Muhibi et al., 2019). However, ultra-low temperature preservation at approximately −190°C, using liquid nitrogen vapor phase, is considered more effective for maintaining long-term stem cell integrity and viability (Hunt, 2011). This study was designed to evaluate the viability of CD34+ stem cells isolated from UCB following 48 weeks of storage at −190°C. The findings are intended to support improved cryopreservation strategies and underscore the potential of ultra-low temperature storage in establishing sustainable and effective cord blood banks, especially in resource-limited settings like Nigeria.

2. METHOD

2.1 Study Area

The study was conducted at the Uniosun Teaching Hospital and Asubiaro Specialist Hospital, both located in Osogbo, Osun State, Nigeria. Osogbo is the state capital of Osun and is primarily populated by the Yoruba people. Uniosun Teaching Hospital is located in Olorunda Local Government,

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	Website: www.matrixscmedica.com	**DOI:** 10.26480/msm.01.2026.01.04

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Osogbo, at 7° 45 ' North latitude and 4° 33′ East longitude. Uniosun Teaching Hospital, established in 2006, serves as a major referral center with 13 medical departments, providing comprehensive healthcare services. Asubiaro Specialist Hospital is known for its specialized maternal care, making it an ideal location for this study.

2.2 Study Protocol

This study employed a longitudinal research design, which allowed for the observation of the viability and quantitative assessment of CD34+ positive stem cells in cord blood samples over 48 weeks. Cord blood was collected from the placenta and umbilical cord of each participating mother immediately after delivery. Approximately 50 mL of cord blood was aseptically withdrawn using a sterile syringe. The collected blood was dispensed into sterile bottles containing 5 mL glycerol as cryoprotectant and 7 mL of citrate phosphate dextrose adenine anticoagulant and mixed to preserve the viability of the stem cells. Two milliliters were dispensed into 25 microvials; a vial was analyzed within 2 h of sample collection; the other 24 vials were frozen at −190°C in a liquid nitrogen tank. Before quantitative analysis of the CD34+ marker, cells from the frozen samples were washed multiple times with sterile phosphate buffer saline solution, stained, and erythrolyzed. A 50% cell suspension was prepared, and the CD34+ markers were quantified using an immunophenotyping technique. Viable cells were measured using the Sysmex Partec CyFlow Cube 6 flow cytometer, which identified anti-CD34/PE monoclonal antibodies. All reagents were procured from Sysmex Partec, Germany. This procedure was repeated at 2-week intervals for 48 weeks. All results were appropriately documented.

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 22.0 (Sysmex Partec GmbH, Görlitz, Saxony, Germany). The CD34+ count was expressed in mean and standard deviation and presented as a line graph to show the trend in count over time. A predictive model was used to forecast future trends of CD34+ count over the next 300 weeks.

3. Results

A cohort of 50 postpartum women participated in the study. The age distribution reveals that the majority of the participants were between 31 and 40 years (50%), followed by those aged 21–30 years (42%), and a smaller proportion above 40 years (8%). The mean age was 32.42 ± 5.91 years [Table 1].

Table 1: Age distribution of study participants

Age group (years)	Frequency (%)
21–30	21 (42.0)
31–40	25 (50.0)
Above 40	4 (8.0)
Mean±SD	32.42±5.91
SD: Standard deviation	

The mean CD34+ stem cell counts of all cord blood samples analyzed were 274.68 ± 33.24. The least count was 224.22, and the maximum count was 333.92 [Table 2].

Table 2: Descriptive statistics of cluster of differentiation 34+ stem cell counts

	Minimum	Maximum	Median	Mean±SD
CD34+	224.22	333.92	272.40	274.68±33.24
SD: Standard deviation, CD: Cluster of differentiation				

The line graph illustrates the trend in CD34+ stem cell counts in cord blood units stored over a 48-week period. At baseline, the CD34+ cell count starts at approximately 330 cells/μL. The cell count decreased at a steady progression up to about 225 cells/μL after 48 weeks of storage [Figure 1].

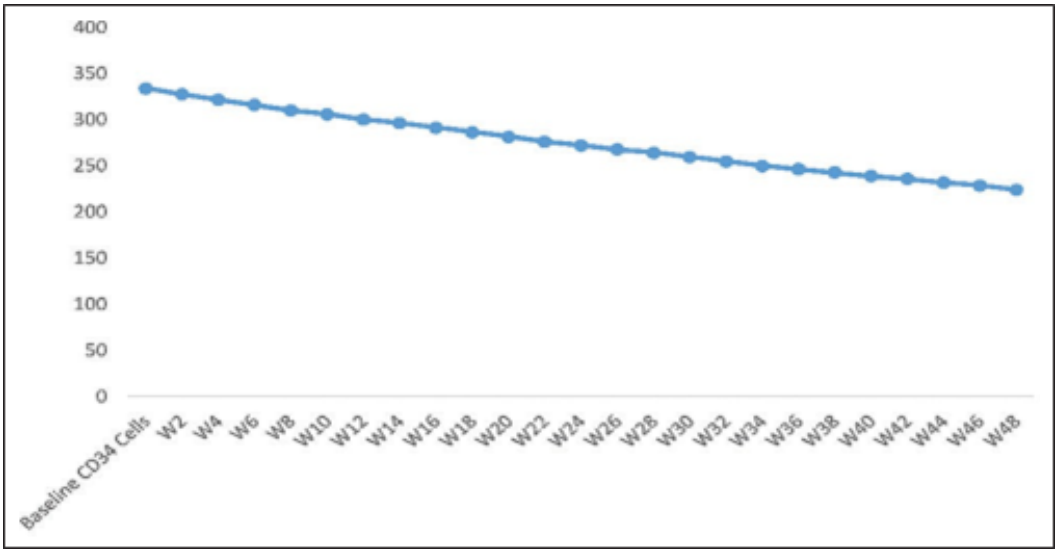

Figure 1: Trend of stored Cluster of Differentiation 34+ stem cells over 48 weeks. CD: Cluster of Differentiation

Figure 2 presents both observed and forecasted trends in CD34+ stem cell counts over time. The observed data (represented by solid blue markers) covers the initial 48-week period, during which CD34+ counts exhibit a gradual, consistent decline from approximately 330 cells/μL to around 220 cells/μL, with each point showing the mean ± SEM (standard error of the mean). Beyond week 48, the graph extends into a forecasted period (indicated by a red dashed line), projecting the likely trajectory of CD34+ cell viability if current trends persist. The forecast suggests a continued linear decline, with CD34+ counts predicted to drop below zero after approximately 150 weeks.

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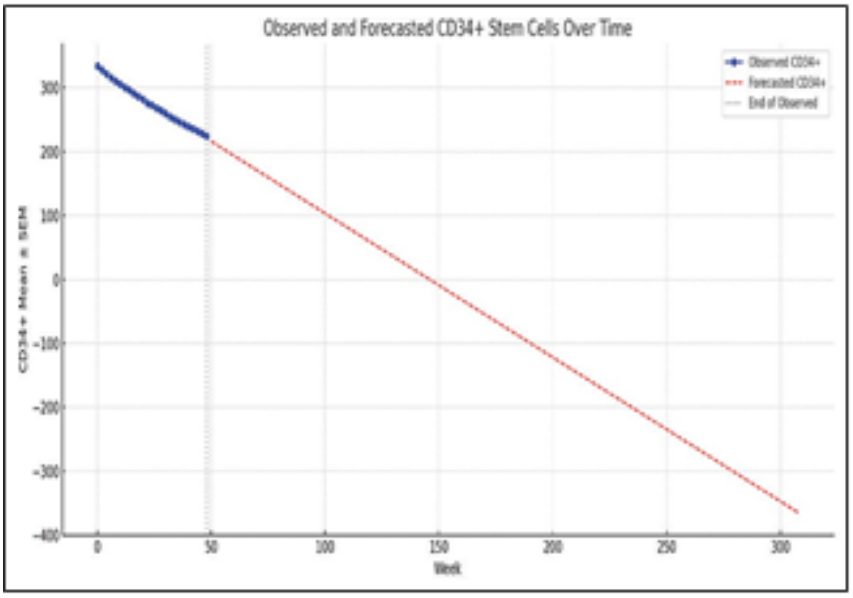

Figure 2: Forecasting Cluster of Differentiation 34+ stem cells count over 300 weeks. CD: Cluster of Differentiation

4. Discussion

Cord blood-derived HSCs have emerged as a viable alternative for stem cell transplantation, particularly in developed countries where standardized guidelines support their collection, storage, and clinical application. Several countries in the Asia–Pacific region, North America, and Europe have advanced cord blood banking systems (Iida et al., 2022), whereas some African nations, such as South Africa, currently run private cord blood banking, storing samples from South Africa, Kenya, Namibia, Zimbabwe, and Mauritius (Eze et al., 2023). Nigeria, despite being the most populous country in Africa, has yet to fully embrace the practice of cord blood banking. The slow uptake of this practice in Nigeria can be attributed to multiple factors. These include low public and professional awareness, inadequate infrastructure, and the absence of specialized cryopreservation facilities (Muhibi & Obeta, 2022). Despite these challenges, studies have shown a high willingness among pregnant women to donate cord blood (John-Olabode et al., 2021), suggesting a favorable attitude that could be harnessed if awareness and access are improved.

This study aimed to evaluate the long-term viability of CD34+ stem cells in UCB stored at an ultra-low temperature of approximately −190°C using liquid nitrogen vapor phase, a significant advancement over our previous research, which demonstrated successful preservation at −20°C for up to 6 months (Muhibi et al., 2019). The current findings indicate that even after 48 weeks of storage at −190°C, CD34+ cells remained viable, with only a gradual decline observed. It was also predicted that the stem cells would remain viable after storage for over 3 years. This suggests that ultra-low temperature cryopreservation can effectively preserve the cellular integrity and functional potential of stem cells over extended periods.

The implication of this result is particularly relevant for resource-limited settings like Nigeria, where the development of a sustainable and reliable cord blood banking system could have significant healthcare benefits. In many communities, including among the Yoruba population, the placenta and umbilical cord are often discarded due to cultural beliefs. However, with the right sensitization and infrastructural support, these biological materials could become valuable resources for therapeutic applications, especially in treating hematological and genetic disorders, which are prevalent in the region.

5. Conclusion

The long-term preservation of CD34+ stem cells from UCB holds significant promise for advancing regenerative medicine and expanding access to stem cell therapies. In the context of Nigeria, where the practice of cord blood banking is still in its infancy, the findings of this study show the untapped potential of a resource that is often discarded after childbirth. Establishing reliable cryopreservation protocols and investing in sustainable biobanking infrastructure could help bridge the gap in treatment options for various hematological and genetic disorders prevalent in the country.

To fully harness this potential, there is a pressing need for increased public awareness, targeted education of healthcare professionals, and the development of national policies that support ethical collection, storage, and utilization of cord blood. Further research is also recommended to optimize preservation techniques, evaluate clinical outcomes, and assess the feasibility of large-scale implementation in resource-limited settings. Building a robust framework for cord blood banking in Nigeria could mark a pivotal step toward improving healthcare equity and expanding the frontiers of therapeutic possibilities.

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