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CLINICAL ARTICLE

# Viability and functional capacity after thawing of hematopoietic progenitor cells cryopreserved at a cord blood stem cell bank in Colombia

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ABSTRACT

*Objective:* To evaluate the viability and functional capacity of hematopoietic progenitor cells from cord blood samples cryopreserved at the Banco de Células Stem de Colombia. *Methods:* After thawing and centrifugation of 20 samples, viable white blood cells were numbered by the trypan blue method and $CD34^+CD45^{+\,dim}$ hematopoietic progenitor cells were numbered by flow cytometry. Clonogenic assays also tested the functional capacity of viable $CD34^+CD45^{+\,dim}$ cells. *Results:* The median rates of viable $CD34^+CD45^{+\,dim}$ cells were 99.6% before freezing and 73.0% after thawing ($P<0.001$). The 20 cultures yielded a median of 12 cells with a lineage of red cells, 17.5 cells with a lineage of white cells, and 10 cells with a mixed lineage. *Conclusion:* Although the rate of viable $CD34^+CD45^{+\,dim}$ cells was decreased by 26.6% after thawing by the method we used, the numbers of $CD34^+CD45^{+\,dim}$ cells that formed colonies were similar to those obtained by other published methods.


## 1. Introduction

Not only are progenitor hematopoietic cells (PHCs) from umbilical cord blood immunologically immature, they are also more proliferative and less likely to be rejected than marrow or blood stem cells from adult donors [1]. Moreover, PHCs are tested for contamination before they are frozen, and when frozen immediately after delivery they escape the aging processes undergone by stem cells collected from the blood of adults. Since the first cord blood transplantation was performed, in 1988, many families have requested to have cord blood from their newborns preserved in the event that siblings may need it to treat genetic diseases [1–3].

The transplantation of PHCs can be of great utility in the treatment of diseases such as acute lymphoid leukemia, acute myeloid leukemia, myelodisplastic syndromes, chronic myeloid leukemia, paroxysmal nocturnal hemoglobinuria, advanced lymphomas, acute aplastic anemia, and Fanconi anemia [4]. Other innate metabolic errors can be treated with these cells, such as leukodystrophies of the white matter of the brain, metachromatic leukodystrophy, adrenoleukodystrophy, mannosidosis, and fucosidosis, as well as primary immunodeficiency disorders such as Wiskott-Aldrich syndrome, Chediak-Higashi syndrome, leukocytic adhesion deficiency, and Blackfan-Diamond syndrome [5].

Hematopoietic progenitor cells are not abundant in cord blood, where they can only be collected once. Moreover, these cells can be harmed by the osmotic stress induced by the cryopreservatives used and by the formation of ice crystals [6,7]. It is therefore of great importance to assess both the quantity of cryopreserved $CD34^+CD45^{+\,dim}$ cells still viable after thawing, and the growth of the cells' different lineages. The objective of this investigation was to assess the viability and functional capacity of cord blood stem cells stored for a period of 10 months at the Banco de Celulas Stem de Colombia and thawed according to the method described by Rubenstein et al. [8].

## 2. Materials and methods

Twenty cord blood samples were randomly selected from 788 samples voluntary stored by families at the Banco de Celulas Stem de Colombia. These samples (1.0 mL) were cryopreserved at −120 °C in liquid nitrogen for a period of 10 months. They were thawed in a

**Table 1**
Median number and percentage (interquartile range) of viable progenitor hematopoietic cells before freezing and after thawing.[a]

| Viable $CD34^+CD45^{+\,dim}$ cells | Before freezing No. (IQR) | After thawing No. (IQR) | *P* value |
|---|---|---|---|
| Number | 48.53 (29.40) | 41.49 (23.17) | <0.001 |
| Percentage | 99.60 (2.01) | 73.00 (12.75) | <0.001 |

[a] Twenty 1.0-mL samples of umbilical cord blood were tested.

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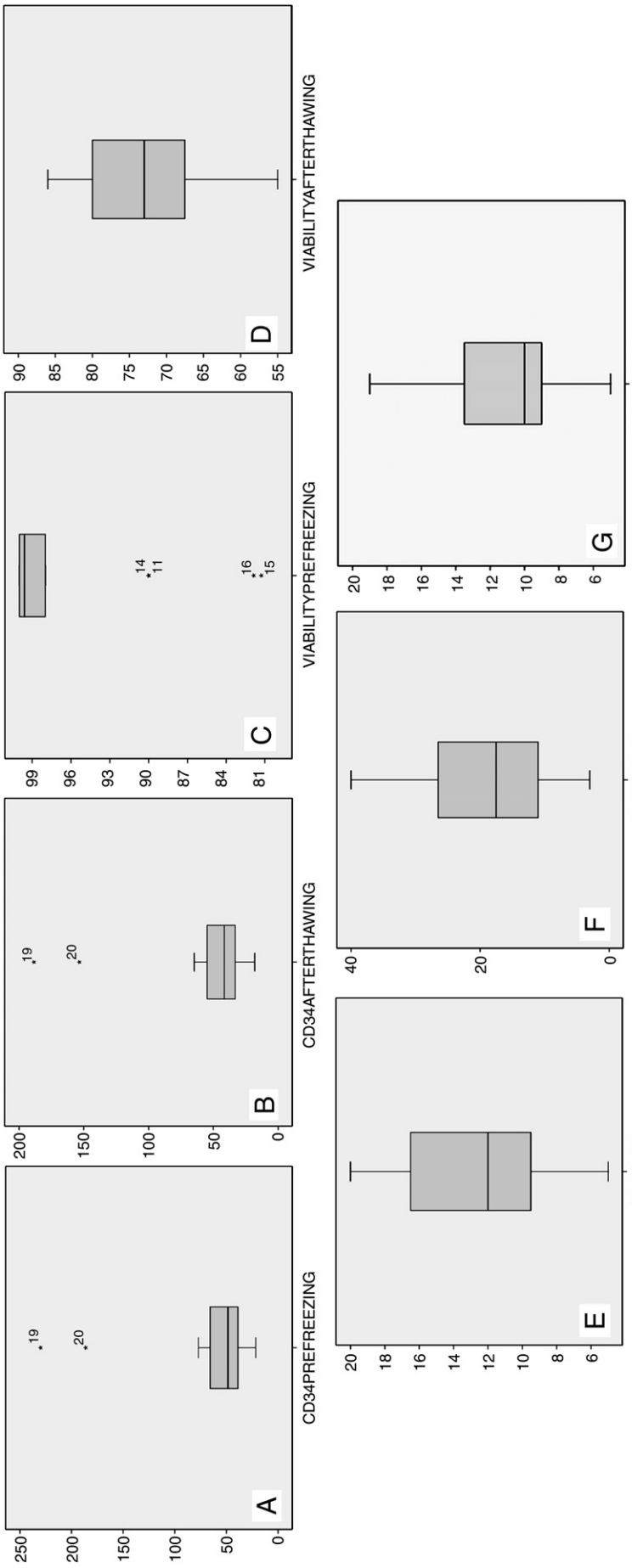


**Fig. 1.** Box plots describing and showing the distribution of the findings. A, median number of CD34 + $CD34^{dim}$ cells before freezing; B, median number of CD34 + $CD34^{dim}$ cells after thawing; C, viability rate of the CD34 + $CD34^{dim}$ cells before freezing; D, viability rate of the CD34 + $CD34^{dim}$ cells after thawing; E, median number of CD34 + $CD34^{dim}$ cells progenitors of red blood cells; F, median number of CD34 + $CD34^{dim}$ cells progenitors of white blood cells; and G, median number of CD34 + $CD34^{dim}$ cells progenitors of mixed colonies.

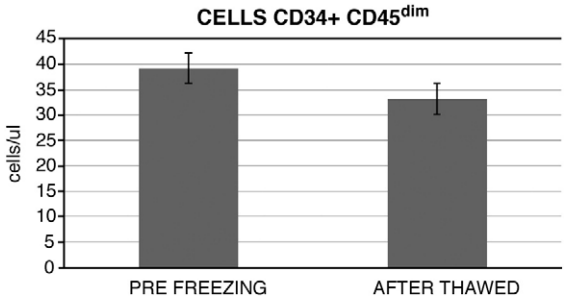


**Fig. 2.** Numbers of $CD34^{+}CD45^{+\,dim}$ cells per milliliter of cord blood before freezing and after thawing ($P<0.05$).

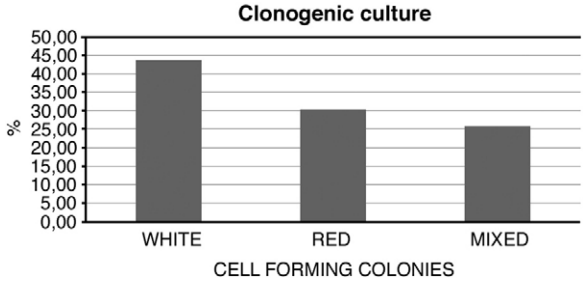


**Fig. 3.** Percentages of $CD34^{+}CD45^{+\,dim}$ cells progenitors of white blood cells, red blood cells, and both white and red blood cells.

water bath at 37 °C using human albumin solution (Baxter Health Care, Deerfield, IL, USA) and Plasma-Lyte (Baxter Health Care) in a proportion of 10% for each. The thawing solution was mixed in a 2:1 ratio with cord blood samples, adding a small amount every 15 seconds until the osmotic equilibrium was re-established. After the thawing process, 500 µL of cord blood cells from each sample were collected and centrifuged at 200*g* with isotonic saline solution for 5 minutes to remove the red blood cells.

To evaluate the viability of white blood cells, 10 µL of the remaining cell suspension was mixed with 20 µL of trypan blue and the live cells were counted in a Neubauer chamber. The number of live $CD34^{+}CD45^{+\,dim}$ cells found in the white blood cells was detected using antihuman phycoerythrin (PE)-conjugated antibodies CD34 and fluoresceine isothianate (FICT)-conjugated CD45 antibody in a FACScan flow cytometer, with CellQuest software for analysis (all from Becton Dickinson, San Diego, CA, USA). We followed the protocol proposed by the International Society of Hematotherapy and Graft Engineering [9].

To assess the functional capacity of the $CD34^{+}CD45^{+\,dim}$ cells, cells from the reserved 500 µL of cord blood were cultured according to the manufacturer's instructions in a semisolid growth medium (Methocult; Stem cells Technologies, Vancouver, Canada) and the colony-forming cells were counted.

The institutional ethics committee approved the study and all donors signed a consent form. Data are presented as median and interquartile range (IQR). The Shapiro-Wilk test for normality was used to check the distribution of the variables and the Wilcoxon signed-rank test was used to compare the findings between the state before freezing and after thawing. $P<0.05$ was considered significant. We checked for correlations by calculating Kendall $\tau$ rank correlation coefficients. SPSS software for Windows, version 14 (SPSS, Chicago, IL, USA) was used for statistical analysis.

## 3. Results

The characteristics of cell viability at baseline and after thawing are shown in Table 1. The median (IQR) of $CD34^{+}CD45^{+\,dim}$ cells/µL were 48.53 (29.4) before freezing and 41.49 (23.17) after thawing ($P<0.001$), and the median (IQR) viability rates were 99.6% (2.01) before freezing and 73.0% (12.75) after thawing, for a drop of 26% ($P<0.001$) (Table 1; Figs. 1 and 2). The median of colony-forming cells were 12 (7.5) for progenitors of red blood cells, 17.5 (16.25) for progenitors of white blood cells, and 10 (4.75) for progenitors of either red or white cells (Table 2 and Fig. 3).

The correlation indices between $CD34^{+}CD45^{+\,dim}$ cells and colony-forming units were 0.45, 0.21, and 0.18 for progenitors of white, red, and white or red cells, respectively.

**Table 2**
Median number (interquartile range) of colony-forming progenitor hematopoietic cells after thawing according to lineage. [a]

| Lineage of $CD34^{+}CD45^{+\,dim}$ cells | No. (IQR) |
|---|---|
| Red | 12.00 (7.57) |
| White | 17.50 (16.25) |
| Mixed | 10.00 (4.75) |

[a] Twenty 500-mL samples of umbilical cord blood were tested.

## 4. Discussion

The success of treatments with PHCs depends on the viability and functional capacity of the cells. The quality control of thawing of cord blood is fundamental, and necessary to be able to anticipate the cell loss that will occur during both cryopreservation and the restoration of the blood's physiological conditions.

Many in vitro studies have reported that the freezing phase can lower by as much as 30% the viability and functional capacity of the cryopreserved product [8–11]. In the present study, whose aim was to evaluate the manual method of thawing described by Rubinstein et al. [8], we obtained a cell viability of approximately 73%. Multiple studies based on this method, but with modifications, have reported cell recovery rates as high as 95% after thawing. The differences may be due to changes made in the basic protocol, e.g., the use of a larger sample volume, which would contain a larger number of progenitor cells, or of automated equipment.

In this study we used cord blood samples of about 1 mL, which, of course, had a smaller number of $CD34^{+}CD45^{+\,dim}$ cells than a full unit of umbilical cord blood, which contains about 25 mL. Moreover, the significant difference between the numbers of $CD34^{+}CD45^{+\,dim}$ cells obtained before freezing and after thawing in our study show the importance of preserving and monitoring cell viability. Thawing larger samples and using automated thawing equipment allow for a greater control over factors such as temperature, reagent concentration, number of agitation, and others factors involved in the thawing process [10]. Although cell viability is better preserved by automated thawing methods, which control the quality of the processes, than by manual thawing, the latter is still used because of its low cost.

The cells must not only to be viable, but also retain their differentiation capacity. Our clonogenic cultures yielded colony-forming progenitors of leukocytes, of erythrocytes, and of both leukocytes and erythrocytes—the latter colony-forming progenitor cells tending to be larger than those of leukocytes. Progenitor cells with the $CD34^{+}CD45^{+\,dim}$ phenotype seem to favor the leukocyte lineage [12,13].

The thawing protocol based on osmotic cell stabilization proposed by Rubinstein et al. [8] has been used and modified over the past 20 years. The Banco de Células Stem de Colombia adopted this method for cell cryopreservation and thawing, and we evaluated the procedures and modified them when necessary. In this study, as a measure to ascertain the good preservation of blood cord units at our institution, we tested the viability of progenitor cells with the $CD34^{+}CD45^{+\,dim}$ phenotype after thawing.

Even though we noted a significant loss in the total number of viable $CD34^{+}CD45^{+\,dim}$ cells, from 99% to 73%, our results were within

the ranges reported worldwide for cell viability and differentiation capacity—thereby guaranteeing the quality of the graft at the time of transplantation. In order to increase the quality of the laboratory processes, we believe that we needed to use a larger sample size and standardize such variables as sample volume, temperature, and thawing speed.

## 5. Conflict of interest

The authors declare that they have no conflict of interest.

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