Effect of Storage of Colostrum in Various Containers

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Objective: To compare the effect of storage on expressed colostrum kept at room and refrigeration temperature in 2 different types of containers (steel and plastic) at different time intervals (0 hours to 7 hours). Design: Prospective immunological study. Setting: Maternity ward. Methods: Colostrum was collected from 60 healthy lactating mothers and tested for total and differential cell counts and cell viability in plastic (polypropylene) and steel containers at 0 hours and 7 hours after storage at 28°C and 4°C. Results: Colostrum stored for 7 hours in plastic containers had a significantly (p <0.001) higher cell count and viability compared to that stored in steel containers both at 4°C and 28°C. The differential cell count did not vary with time, temperature or storage vessel. Conclusion: If required, colostrum should be stored at 4°C in plastic (polypropylene) containers to maintain its protective quality.

Key words: *Breastmilk, Colostrum, Containers, Storage, Temperature.*

BREASTMILK contains significant amounts of cells and humoral factors that protect the infant from a variety of infections^) including diarrheal diseases, otitis media(2) and respiratory illnesses(3). Long polyunsaturated fatty acids in breastmilk play a vital role for brain development^). Breastfeeding not only protects young infants from hospitalization(5) but also from unnecessary exposure to potential allergens from supplements like cow's milk(6). The need for promoting exclusive breastfeeding, particularly in the developing countries, is obvious. As more women in these countries join the work force, the need to store breastmilk is becoming an increasing necessity. The present study was, therefore, conducted to observe the effect of storage and type of container on the cell count and viability of human colostrum.

Subjects and Methods

Colostrum samples were collected from 60 lactating mothers who had delivered normally at full term. The age group of these mothers was **from** 18-25 years. All donors were in good health and had no evidence of mastitis.

Collection and transport: The samples were collected by manual expression (using the stripping method)(6) into sterile glass test tubes. The samples were transported in an ice-box immediately to the laboratory **for** processing.

Processing: Samples were diluted with phosphate buffered saline (PBS, pH 7.4) after marking the original volume of milk and were centrifuged at 1500 rpm for 20 minutes to defat them. The pelleted cells were washed in PBS and the cell volume

was readjusted to the original milk volume. After the zero hour estimation of cell count and viability, the cellular deposit was then distributed in four containers-two of steel and two of plastic. One container each of steel and plastic, were placed at refrigeration and room temperature, respectively. The cell count and viability were again estimated at 7 hours in all 4 samples. The plastic containers used in the study were made of polypropylene.

Estimation of cell viability and count(7): Equal amount of Erythrocin B dye (0.4%) and cell suspension was taken. The mixture was loaded into the White Blood Cell (WBC) chamber of a hemocytometer. The viability was done within 3 minutes otherwise the live cells take up the stain and look pink. The viable cells do not take up the dye and appear colorless whereas the dead cells take up the stain and look pink.

The total cell count was done as per the routine method in hemocytometer without diluting the sample. Smears which were prepared from defatted milk were fixed and stained with Wright's stain. These slides were then observed under oil-immersion lens, for differential white cell count.

Statistical Analysis

Data were analyzed by paired and unpaired 't' tests. The change from 0 hours to 7 hours was evaluated by paired 't' test and the comparison between plastic and steel containers was assessed by unpaired Y test (two tailed). Based on an earlier pilot study, a sample size of 60 was considered sufficient to detect a difference in cell count of 600 cells/cu mm and a 10% difference in cell viability with a power of 90% and a probability of 5%.

Results

The cell count and viability showed a significant decline at 7 hours of storage both at 4°C and 28°C (Table I), both in plastic and steel containers. Comparing the influence of containers on cell count and viabilty, it was observed that at 4°C, there was a significantly greater decrease both in cell count and viability with steel containers compared to plastic containers. At 28° C, while the cell count showed a significantly greater decrease in steel containers com-

TABLE I- *Comparison of Cell Count and Viability of Colostrum Stored at Room Temperature (RT) and 4°C in Plastic and Steel Containers (Mean ± SD)*

	Oh	7 h			
		Plastic		Steel	
		RT	4°C	RT	4°C
Total Count (Cells/cu mm)	3861 ±814	2403 ±505 * +	2845 ±556 *@	1857 ±360 *+	2247 ±393 *@
Cell viability (%)	84.7 ±7.1	41.6 ±13.2 *+	63.8 ±12.1 *@	41.2 ±7.9 *+	50.3 ±8.6 *@

* p <.001, comparison of change from 0-7 hours.
@ Comparison of containers at 4°C; p <.001
\+ Comparison of containers at RT; p < .001 for cell count, p > .05 for cell viability

pared to plastic, no such difference was observed with regard to cell viability. The mean differential count of colostrum revealed macrophages and Donnes corpuscles (lipid-laden monocytic phagocytes with a diameter of about 40 nm) of 62%, neutrophils 26%, lymphoctyes 11.5% and other cells like epithelial cells and Smudge cells (nonspecific anucleated cells) 0.5%.

Discussion

The results of this study suggest that plastic container (polypropylene) is superior to steel with respect to viability and total count of milk cells. The probable reason for greater reduction in cell viability in steel could be due to the oligodynamic action of nickel which is present in such containers^) resulting in cytolysis of milk cells when stored in steel containers over a prolonged time. The differential cellular count of colostrum was not significantly influenced by duration, temperature or type of container used for storage. These observations are comparable to earlier published observations(l,8). It was observed in the present study that cell viability was better retained at 4°C for 7 hours in plastic. In a similar study(9), it was suggested that milk should be collected in sterile plastic containers and maintained in refrigerator for a short period of time until it is fed to the infant. It is concluded that if required, expressed human milk should be preferably stored in plastic container at 4°C (in a refrigerator) until it can be fed to the newborn.

REFERENCES

1. Lawton JWM, Shortridge KF. Protective factors in human milk and colostrum. Lancet 1977; 1:253.
2. Dewey KG, Heinig J, Nommsen-Rivers LA. Differences in morbidity between breast-fed and formula-fed infants. J Pediatr 1995; 126: 696-702.
3. Howie PW, Forsyth JS, Ogston SA, Clark A, Florey C du V. Protective effect of brastfeeding against infection. BMJ 1990; 300:11-16.
4. Lanting CI, Fidler V, Huisman M, Touwen BCL, Boersma ER. Neurological differences between 9-year old children fed breastmilk or formula-milk as babies. Lancet 1994; 344:1319-1322.
5. Leventhal JM, Shapira ED, Aten CB, Berg AT, Egerter SA. Does breastfeeding protect against infections in infants less than 3 months of age? Pediatrics 1986; 78: 896-903.
6. Asquith MT, Harrod JR. Reduction of bacterial contamination in banked human milk. J Pediatr 1979; 95: 993-994.
7. Williamson MT, Murti PK. Effects of storage, time, temperature and composition of containers on biologic components of human milk. J Hum Lact 1996; 12: 21-25.
8. Goldblum RM, Goldman AS, Garza C, Johnson CA, Nichols BL. Human milk banking. II Relative stability of immunologic factors in stored colostrum. Acta Pediatr Scand 1982; 71:143-144.
9. Paxson CL, Cress CC. Survival of human milk leukocytes. J Pediatr 1979; 94: 61-63.