Immunologic Factors in Human Milk: The Effects of Gestational Age and Pasteurization

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Immunologic Factors in Human Milk: The Effects of Gestational Age and Pasteurization

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Abstract

This study compared concentrations of total protein, lysozyme, and immunoglobulins (IgA, IgG, IgM) in samples of colostrum (n = 101) obtained from mothers of infants < 32 weeks, 32 to 36⁄7 weeks, and ≥ 37 weeks gestational age, both before and after pasteurization. Total protein was measured by refraction index, lysozyme by the lysoplate method, and immunoglobulins through the radial immunodiffusion technique. The total protein concentration was greater in colostrum of the < 32 weeks and 32 to 36⁄7 weeks categories compared to full-term ($P < .001$), while concentrations of lysozyme and IgM were similar. IgA concentrations were higher in the < 32 weeks group compared to the full-term and similar to the 32 to 36⁄7 weeks group ($P < .05$). The IgG was higher in the < 32 weeks category compared to 32 to 36⁄7 weeks, and both were similar to the full-term ($P < .05$). Pasteurization significantly decreased all of the factors analyzed. *J Hum Lact*. 21(4):439-443.

Keywords: human milk, colostrum, gestational age, IgA, IgG, IgM, lysozyme, pasteurization

Growing evidence favors the use of human milk for the feeding of preterm newborns based on its many benefits; these benefits include improved defense against infections, better absorption and digestion of nutrients, and superior neurologic development of the infant as well as improved maternal well-being.[1,2] However, offering low birth weight infants hospitalized for long periods their own mother's milk can sometimes become difficult. For example, mothers may experience low milk supply due to a lack of direct suckling and a lack of suitable conditions for mechanical milk expression for prolonged periods.[3] Thus, the use of pasteurized human milk has been identified as a good alternative to feeding the preterm newborn when fresh milk from his or her own mother is lacking because many of the human milk's immunologic properties remain present after the pasteurization,[4] as supported by clinical studies demonstrating that pasteurized human milk also protects newborns against infections.[5,6]

Several studies have revealed differences in the concentrations of immunologic factors between the milk of mothers with term versus preterm infants.[7-9] Nevertheless, few data address how concentrations of these immunologic factors vary with gestational ages of < 37 weeks, especially < 32 weeks,[10,11] or how pasteurization affects these concentrations. Therefore, we investigated potential differences in concentrations of total protein, lysozyme, immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) in raw and pasteurized milk of mothers at 3 different gestational age ranges.

Methods

All mothers with babies born in the Darcy Vargas Maternity Hospital were eligible to participate in the

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No reported competing interests.

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study, except those who met the following exclusion criteria: refusal to participate, acquired immunodeficiency, peripartum or congenital infection, and insufficient milk production by day 4 postpartum.

Between the second and fourth day after delivery, we collected 10 to 15 mL of colostrum by hand expression from mothers of newborns representing 3 different gestational age ranges: < 32 weeks, 32 to 36$\frac{6}{7}$ weeks, and ≥ 37 weeks. One aliquot was pasteurized at 62.5°C for 30 minutes, and the other aliquot was kept raw. Then both were frozen and stored at –20°C.

Before the laboratory analysis, the colostrum samples were thawed and centrifuged at 25 000 rpm (4°C) for 30 minutes and the cream layer discarded. The remaining fractions were assayed for total protein concentration by the refraction index method; for lysozyme by the lysoplate method,[12] using lyophilized *Micrococcus lysodeikticus* cells (M –3770; Sigma Chemical Co, St Louis, Mo); and for IgA, IgG, and IgM by simple radial immunodiffusion,[13] using Nor Partigen IgA and LC-Partigen IgG and IgM plaques (Dade Behring Marburg GmbH, Liederbach, Germany). All samples were assayed twice for all factors, and the reported results represent the mean of these 2 measurements.

Statistical Analysis

Repeated-measures analysis of variance with gestational age group modeled as a between-group factor and raw versus pasteurized modeled as a within-group factor was used to compare the mean concentrations of each outcome measure (total protein, lysozyme, IgA, IgG, and IgM). The presence of a possible interaction effect among gestational age and pasteurization was also evaluated (P^1). In cases in which the analysis indicated a significant effect of the gestational age ($P^3 < .05$), post hoc Bonferroni analysis was used to identify significant differences between the pairs (coefficient of global confidence of 95%). In a complementary manner, confidence intervals of 95% were calculated for differences in variables before and after pasteurization (P^2).

Mothers provided informed consent to participate.

This study was approved by the Ethics Committee for Analysis of Research Projects of the School of Medicine, University of São Paulo, Brazil.

Results

A total of 101 colostrum samples were analyzed: 36 from mothers of newborns < 32 weeks, 32 from mothers of newborns 32 to 36$\frac{6}{7}$ weeks, and 33 from mothers of newborns ≥ 37 weeks. Table 1 summarizes characteristics of the newborns whose mothers donated the milk. Table 2 presents the mean concentrations (± SDs) of total protein, lysozyme, and IgA, IgG, and IgM in these colostrum samples.

Table 1. Demographic Data for Newborns*

	Gestational Age Group		
	< 32 wk (n = 36)	*32-36 $\frac{6}{7}$ wk (n = 32)*	*≥ 37 wk (n = 33)*
Gestational age, wk ($\bar{x}$ ± SD)	28.9 ± 2.1	33.8 ± 1.2	39.5 ± 0.7
Birth weight, g ($\bar{x}$ ± SD)	1164 ± 329	1963 ± 414	3301 ± 325
Small for gestational age, %	13.0	21.0	0.0

*Data are from the newborns of 101 women who donated colostrum samples.

The mean total protein concentrations between the 3 gestational age ranges differed significantly, with a significant and similar reduction after pasteurization in all 3 groups. Total protein concentrations were highest in colostrum from the mothers of newborns with a gestational age of 32 to 36$\frac{6}{7}$ weeks and lowest in the colostrum from mothers of term infants.

The mean lysozyme concentrations in the colostrum samples were similar between the 3 gestational age groups, and a significant and comparable reduction in these concentrations occurred after pasteurization in all 3 groups.

The IgA concentration in raw colostrum from the mothers of < 32 weeks was significantly higher than that in raw colostrum from mothers of term infants, and both were similar to the 32 to 36$\frac{6}{7}$ weeks group. Pasteurization significantly lowered IgA concentrations in colostrum within all 3 gestational ages. However, compared to the other gestation ages, the effect of pasteurization on IgA concentrations was less in the < 32 weeks group, indicating an interaction effect.

The IgG concentration in the raw colostrum from mothers of newborns < 32 weeks was significantly higher than that in raw colostrum from mothers of newborns 32 to 36$\frac{6}{7}$ weeks ($P < .05$), and both were similar to term colostrum. Pasteurization significantly reduced IgG concentrations in colostrum associated with all analyzed gestational ages; however, this effect was still higher in the < 32 weeks colostrum, indicating an interaction effect.

IgM was detected in only a small percentage of the raw colostrum samples and presented similar concentrations in the different gestational age ranges. After

Table 2. Total Protein, Lysozyme, and Immunoglobulin Concentrations in Colostrum*

	Gestational Age Group					
	< 32 wk (n = 36)		*32-36 6/7 wk (n = 32)*		*≥ 37 wk (n = 33)*	
Colostrum	*Raw*	*Pasteurized*	*Raw*	*Pasteurized*	*Raw*	*Pasteurized*
Total proteins, g/dL ($\bar{x} \pm$ SD)**	8.9 ± 2.4	7.7 ± 2.1	10.2 ± 1.9	8.8 ± 1.9	7.7 ± 1.5	6.7 ± 1.1
Lysozyme, µg/L ($\bar{x} \pm$ SD)†	705.6 ± 847.1	242.7 ± 343.3	788.3 ± 937.9	110.5 ± 139.0	545.5 ± 429.8	140.1 ± 246.6
Immunoglobulin A, g/L ($\bar{x} \pm$ SD)‡	3.102 ± 1.360	2.032 ± 1.115	3.004 ± 1.303	1.331 ± 0.878	2.250 ± 1.267	0.858 ± 0.521
Immunoglobulin G, g/L ($\bar{x} \pm$ SD)§	0.076 ± 0.038	0.018 ± 0.026	0.047 ± 0.042	0.010 ± 0.020	0.054 ± 0.037	0.015 ± 0.023
Immunoglobulin M, g/L ($\bar{x} \pm$ SD)‖	0.017 ± 0.038	0.000 ± 0.000	0.005 ± 0.015	0.000 ± 0.000	0.014 ± 0.034	0.000 ± 0.000

*Values reflect $\bar{x} \pm$ SD concentrations of proteins in raw and pasteurized colostrum samples associated with different infant gestational ages. P^1 = interaction effect P value; P^2 = pasteurization effect P value; P^3 = gestational age effect P value.
**$P^1 = .433$, $P^2 < .001$, $P^3 < .001$
†$P^1 = .253$, $P^2 < .001$, $P^3 < .386$**
‡$P^1 = .048$
§$P^1 = .022$
‖$P^1 = .237$, $P^2 < .001$, $P^3 = .089$

pasteurization, it was not possible to quantify IgM in any of the analyzed samples.

Discussion

Several factors have awakened interest in the properties and characteristics of human milk from the mothers of preterm infants, especially those of very low birth weight; these include the growing survival rates of very low birth weight infants and the recommended use of human milk, which is considered most beneficial for the preterm infant.[1,2] Human milk has already been the object of various studies, particularly in relation to its immunoprotein characteristics. However, most studies have simply compared the immunoglobulin profiles of the milk from preterm and full-term mothers without assessing how results in the preterm group may vary with different gestational age ranges.[7,8] In addition, only the raw (unpasteurized) form of human milk has generally been analyzed. In contrast, we characterized the immunoprotein content of colostrum associated with 3 different gestational age ranges, in addition to the effects of pasteurization on this content.

The preterm infant requires a greater quantity of proteins for growth than the full-term infant does. In our study, the mean total protein concentration in the raw colostrum from the mothers of the 2 groups of preterm infants was greater than that in the colostrum from the mothers of the full-term infants. Between the 2 preterm groups, the total protein concentration was greater in the raw colostrum from mothers of 32- to 36 6/7-week gestation infants. Montagne et al[11] previously compared total protein concentrations in colostrum samples from mothers of preterm infants < 33 weeks, infants 33 to 36 weeks, and full-term infants. They also found evidence of greater protein concentrations in the colostrum from mothers of preterm versus full-term infants, indicating that the milk of a preterm infant's mother can adequately meet the infant's need for greater protein ingestion. After pasteurization, mean total protein concentrations are still greater in both preterm colostrums compared to term colostrums.

Lysozyme is an important factor in the defense of mucus membranes against infections, presenting at low concentrations in colostrum and progressively increasing during the course of breastfeeding.[14] Previous studies that compared milk from mothers of preterm and full-term infants have shown similar concentrations of lysozyme in colostrum.[11,14] In this study, the mean lysozyme concentrations were similar in raw colostrum associated with the 3 gestational age ranges. Similarly, Velona et al[15] reported similar lysozyme concentrations in raw colostrum from the mothers of preterm infants < 30 weeks and full-term infants. After pasteurization, lysozyme concentrations in the raw colostrum associated with the 3 groups decreased by 65% to 85%. This decrease was greater than that observed in a study of mature milk from mothers of full-term infants, in which only a 23% to 33% reduction in the lysozyme concentration was observed.[16] Even with this reduction, the lysozyme concentrations are still much higher than those found in nonhuman milk.

IgA is much more abundant in colostrum than in mature milk. IgA levels in human milk decrease progressively during lactation yet they decrease more

slowly in milk from mothers of preterm infants. Studies comparing milk from a single group of mothers of preterm infants with that from mothers of full-term infants revealed higher IgA concentrations in the preterm milk.[7-9] Similarly, the mean IgA concentration was significantly higher in the colostrum of mothers of preterm infants < 32 weeks versus full-term infants in our study. Montagne et al[11] also found higher concentrations of IgA in the crude colostrum of mothers of preterm infants (< 33 weeks and 33 to 36 weeks) compared with full-term infants.

Compared to the other 2 groups, we also observed a lower percentage reduction in IgA after pasteurization in the colostrum from mothers of infants < 32 weeks gestational age. This finding indicates a possible interaction effect between gestational age and pasteurization. Although it was not possible to conduct a qualitative analysis of IgA in this study, this finding may be of particular importance to the preterm infant fed this milk. That is, the higher levels of IgA may provide greater protection against infections to the more immature infant, mainly necrotizing enterocolitis.

IgG is found in small concentrations in human colostrum and milk. Its importance lies in its ability to counteract the infant's deficiencies in opsonization and antibody-mediated cytotoxicity. In the present study, IgG was detected in 82% of the raw colostrum samples. This percentage is higher than that determined by Liebhaber et al[17] who, using the same radial immunodiffusion technique, detected IgG in 47% of their samples.

Previous studies have demonstrated similar concentrations of IgG in preterm and full-term milk.[7-9] Our results also show similar IgG concentrations between preterm and full-term colostrum; however, IgG concentrations in colostrum obtained from mothers of infants < 32 weeks were higher than those obtained from mothers of infants 32 to 36⁄7 weeks. This could be seen as a mechanism to compensate for the low transference of IgG through the placenta up to that gestational age. As observed in other studies,[16,17] pasteurization significantly reduced the IgG concentrations in colostrum associated with all gestational ages. The fact that this reduction was more acute in the colostrum from mothers of infants < 32 weeks gestational age suggests the need for further study.

The newborn is fully capable of producing IgM in response to infection. Thus, the IgM concentration in colostrum is low, and there is a progressive reduction in this concentration during lactation. In the present study, IgM was only detected in 17% of the raw colostrum samples. This percentage is similar to that reported by Liebhaber et al,[17] who managed to quantify IgM in 21% of their samples. In addition, the IgM concentrations in the colostrum samples associated with all 3 gestational age ranges were similar. Thus, our results are similar to those of other studies that showed no differences in IgM concentrations in colostrum samples from the mothers of preterm and full-term infants.[7-9] As in other studies,[17,18] we were not able to quantify IgM in any of the colostrum samples after pasteurization.

Based on these results, we conclude that although all factors analyzed had a significant reduction after pasteurization, total protein and IgA retained appreciable concentrations, especially in colostrum from mothers of both preterm groups, indicating that pasteurized preterm milk can be a beneficial alternative for feeding the preterm infant. Higher concentrations of proteins, IgA, and IgG in raw milk of the mothers of more preterm infants may be a compensatory protective mechanism for these babies, and a great effort should be made to feed the preterm with his or her own mother's milk.

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Resumen

Este estudio compara concentraciones de proteína total, lisosima, e inmunoglobulinas (IgA, IgG, IgM) en muestras de calostro (n = 101) de madres de bebés < 32 semanas, 32 a $36^{6/7}$ semanas, y ≥ 37 semanas de gestación, ambos antes y después de pasteurización. Las proteínas totales se midieron con índice de refracción, las lisozimas, por medio del método de lisoplato; y las inmunoglobulinas, por medio de la técnica de inmunodifusion radial. La concentración de proteínas totales era mayor en la categoría de el calostro de < 32 semanas y 32 a $36^{6/7}$ semanas comparado con el de termino ($P < .001$), mientras que las concentraciones de lisozima e IgM fueron similares. Las concentraciones de IgA fueron mayores en el grupo de < 32 semanas comparado con el de termino y similar a las 32 a $36^{6/7}$ semanas y ambos similares en el de termino ($P < .05$). La pasteurización disminuye significativamente todos los factores analizados en este estudio. Altas concentraciones de proteína total e IgA en el calostro pretermino, crudo y pasteurizado, indica que la leche de prematuros tiene una cantidad importante de factores inmunológicos, aun después de pasteurizar.