



Influence of type of delivery on A, G and M immunoglobulin concentration in maternal colostrum

Gabriel A. J. Striker¹, Lucy D. Casanova², Aparecida Tiemi Nagao³

Abstract

Objective: To investigate the association between type of delivery and immunoglobulin concentration in maternal colostrum.

Methods: We studied 82 women who were giving birth. Age was between 21 and 41 years, gestational age was 37 or more weeks and parity up to IV pregnancies. The women were in good nutritional condition and did not have any pregnancy or puerperium-associated diseases. The following aspects were also considered as inclusion criteria for the newborn: weight $\geq 2,500$ g, Apgar score > 7 in the first minute and exclusive breastfeeding until discharge from the nursery. The women were divided into three groups: A - vaginal delivery, B - caesarean section with labor and C - elective caesarean section. Colostrum was collected manually between 48 and 72h after delivery. Immunoglobulins were dosed using the ELISA technique.

Results: There were no differences between the three groups in terms of time of colostrum collection. The shorter the time of colostrum collection, the greater the concentration of immunoglobulin A. Primiparous women had higher concentrations of IgA and IgM in maternal colostrum than did multiparous women. The group submitted to caesarean section with labor had higher concentrations of IgA in maternal colostrum than did the normal delivery group. IgM and IgG concentrations in colostrum were not influenced by type of delivery.

Conclusion: The occurrence of labor together with surgical stress induce higher IgA concentrations in the colostrum of women submitted to caesarean section with labor.

J Pediatr (Rio J). 2004;80(2):123-8: Delivery, immunoglobulin A, immunoglobulin G, immunoglobulin M, colostrum.

Introduction

According to the World Health Organization (WHO) a reasonable rate of caesarian deliveries would be below 20%.¹ In Brazil, this rate had already passed 15% of all births by 1970 and reached 30% by 1980.² Authors first began to call attention to the inexorable tendency towards caesarians in western societies during the eighties. Today the country holds the world record in this practice.³ Private

maternity units or those affiliated to healthcare plans, in the large urban centers of the Southeast, have reached rates bordering on 90%. Some public teaching hospitals in the same region record rates between 30 and 60%. These levels are explained by the fact that these hospitals are centers of excellence for high-risk expectant mothers.⁴ The rates have evolved geometrically, with an acceleration over the last 10 years. According to the 1996 Annual Statistics Bulletin, released by the Foundation for the São Paulo State Data Analysis System (SEADE *Fundação Sistema Estadual de Análise de Dados do Estado de São Paulo*) caesarian deliveries performed by the Brazilian National Health System (SUS - *Sistema Único de Saúde*) make up 41.2% of all births for the state as a whole and 38% of births in the state capital.⁵

1. Master's Degree, Hospital do Servidor Público Estadual de São Paulo, São Paulo, SP, Brazil.

2. PhD, School of Medicine, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil.

3. PhD. Professor, Department of Clinical Analysis, School of Pharmacy, Universidade Federal do Ceará (UFC), Fortaleza, CE, Brazil.

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As a result of the striking increase in caesarian delivery rates, much research has been performed to analyze the effect of the procedure on newborns (NB). Respiratory complaints have received special attention due to their influence on high morbidity and mortality rates during the first few extrauterine minutes. According to Casanova & Badaró-Marques,⁶ references have been being made to an association between caesarian deliveries and increased frequency of neonatal respiratory distress syndrome since the start of the twentieth century.

A neonate depends on many different mechanisms that aid its adaptation to extrauterine life. These include: chemical mediators, hormones liberated by stress and the immuno-hematological system, which may undergo changes depending on type of delivery. A number of studies have been performed to investigate these changes.

Neonates born vaginally have been found to have higher levels of epinephrine, norepinephrine and dopamine in umbilical plasma when compared to those delivered by caesarian.⁶⁻¹⁰ Cortisol and TSH were also found to be at higher concentrations in the umbilical cords of those delivered vaginally.¹¹⁻¹⁵ It is, therefore, possible that these findings are linked to labor and/or vaginal delivery.

Certain defense cells can also undergo changes as a result of the type of delivery. Studies have shown a significant increase in total leukocyte counts and in polymorphonuclear neutrophil (PMN) numbers in the umbilical plasma of NB born vaginally; leukocyte function, however, as defined by the phagocytic activity of neutrophils, monocytes and eosinophils was shown to be best among those born by caesarian.¹⁶⁻¹⁷

Studies of the NB immune system found higher concentrations of immunoglobulin G (IgG) and interleukin 6 (IL-6) in the umbilical cord of those born by vaginal delivery. In addition it was found that vaginal delivery results in higher plasma concentrations of interleukin 1 (IL-1), IL-6 and tumor necrosis factor (TNF) in the recently delivered mother. In view of these results it has been suggested that labor and/or vaginal delivery may have an important role to play in the modulation of the defenses of both NB and recently delivered mother.¹⁸⁻²¹

Even though colostrum and breast milk are the best food for an infant, the possible changes which may occur to their composition as a result of the type of delivery have been little studied. Kulski *et al.* studied the concentration of the immunoglobulins A, G and M in the colostrum of recently delivered mothers who had given birth normally or by elective caesarian. Samples were collected during the first seven days postpartum. The authors did not find any alterations to immunoglobulin concentrations related to the type of delivery, the sample was, however, just 19 recently delivered mothers.²²

The observed differences between mothers and NB cellular and humoral constituents after normal and caesarian deliveries justify curiosity about possible alterations to the composition of maternal colostrum that

may occur depending on the type of delivery. The important role played by colostrum immunoglobulins in the NB's earliest defenses together with the limited number of studies that have been performed of variations in their concentrations related to the type of delivery was the central motivation for this investigation.

Methods

This study was carried out at the Maternity unit of the Hospital Israelita Albert Einstein (HIAE) in the city of São Paulo, Brazil. A survey was made of all medical records pertaining to recently delivered mothers who had given birth at the hospital's Maternity Unit during the period from May to June 1999 systematically and continuously. One hundred and two were selected as fulfilling the previously defined criteria (inclusion and exclusion) for the study. The criteria for recently delivered mothers were: age (21 to 41 years), gestational age (37 weeks or more), birth order (one to four pregnancies), good nutritional status, no plastic surgery to breasts and no pathologies during gestation and puerperium. The criteria for NB were: gestational age as calculated by Capurro of 37 weeks or more, birth weight $\geq 2,500$ g, first minute Apgar score above 7, remained in the communal ward or the nursery for normal NB and exclusively breastfed throughout the hospital stay.

The final sample size ($n = 82$) used for the 2x2 contingency tables proved sufficient for the majority of the calculations performed for an α of 0.05, and a test power of $(1-\beta) = 0.80$.

Once approval for the study protocol had been granted by the HIAE Commission for Ethics in Medical Research, the recently delivered mothers selected were interviewed in person by the responsible researcher and only participated after signing an Informed Consent Form

The study was designed as cross-sectional, involving three groups of recently delivered mothers: group A – recently delivered mothers who had given birth by vaginal delivery; group B – recently delivered mothers who had given birth by caesarian preceded by labor and group C – recently delivered mothers who had had elective caesarians.

Colostrum was collected between 48 and 72 hours postpartum, by two workers from the HIAE Human Milk Bank. Samples were 3 ml and obtained by manual expression from one breast during the morning at the bedside. The samples were sent to the HIAE Human Milk Bank where they were stored at -20 °C. Once all the samples had been collected the material was transported to the Mucus Immunology Laboratory at the Biomedical Sciences Institute at the Universidade de São Paulo (ICB-USP), where the laboratory-based phases were carried out.

Test tubes containing the colostrum samples were first subjected to high speed centrifugation (approximately 3,500 rpm) for delipidization. The lipid fraction was

discarded and the liquid fractions used for immunoglobulin A, G and M assay, using the Enzyme Linked Immunosorbent Assay (ELISA) technique.

One hundred microliters (100 µl) of purified Anti-IgA, anti-IgM or anti-IgG capture antibodies (Sigma, USA) were pipetted at a concentration of 5 µg/ml, (Costar, USA), onto 96-well plates, which were then covered with plastic film, put into an incubator for 16 hours at 4 °C and, promptly washed in PBS, pH 7.4 with Tween at 0.05% three times. Samples were diluted in buffered diluting solution (PBS-NaCl 0.5 M and Tween at 0.2%) in four serial twofold dilutions. Reference standards were purified, secretory IgA, IgM or IgG (Sigma, USA) at concentrations of 3.9 to 250 µg/ml. For positive reaction control we used a pooled colostrum sample at 4 serial twofold dilutions, of 1/8,000 to 1/64,000 for IgA and from 1/200 to 1/1,600 for IgM and IgG. The colostrum samples were diluted at from 1/5,000 to 1/40,000 for IgA assay, at from 1/100 to 1/800 for IgM assay and at dilutions from 1/50 to 1/400 for IgG assay. The reference, the positive control and the samples were incubated at 37 °C for two hours. After three washes in PBS Tween at 0.05%, the conjugates were added (anti-IgA, anti-IgM or anti-IgG) marked with peroxidase (Sigma, USA), at a dilution of 1/5,000 in buffered diluting solution and the plates were incubated for 1 hour and 30 minutes at 37 °C. The plates were washed again in PBS Tween 0.05% and, 100 µl of the substrate (citrate-phosphate buffer 0.1M, pH 5.0; H₂O₂ at 0.01% and orthophenyldiamine at 0.4 mg/ml) were added to each well to reveal the reaction. Plates were left in the dark for 30 minutes. In order to fix the reaction, 50 µl of H₂SO₄ 2.5 N were added to each well. The spectrophotometer was set to the chosen wavelength of 492 nm for viewing.

Data was processed and analyzed using the version 6.04 of the program Epi-Info. Quantitative variables were described in terms of central tendencies (mean and median), dispersal (standard deviation) and maximum and minimum values, while categorical variables were expressed in percentages. Categorical variables were compared using the Chi-square test with and without Yates' correction. Contingency tables have been used to present this data. Averages for continuous variables were

compared using the Fisher exact test. Pearson's parametric correlation method was adopted for comparing the correlation between two variables. Values for p of less than 0.05 were accepted as indicating statistical significance.

The median for each immunoglobulin isotype was calculated using concentration values for the whole population (n = 82). Based on these medians, the contingency tables were split into percentages and numbers of mothers above the median in terms of their individual colostrum immunoglobulin concentrations and percentages and numbers below or equal to the median.

An analysis based on the study of medians was chosen because of the wide spread of no colostrum immunoglobulin concentration results.

Results

Five of the total of 102 recently delivered mothers who had previously been selected did not agree to collection and in 15 cases it was not possible to collect 3 ml of colostrum. Table 1 shows the distribution of the variables studied across the group of mothers.

Table 2 demonstrates that there was no significant difference between the time taken before collection between the three groups. This is highly relevant since, as Figure 1 shows, when the entire group of mothers is analyzed by time, the earlier that colostrum is taken the higher the concentration of IgA. The same is not observed in relation to immunoglobulins G and M.

Analyzing the relationship between birth order and immunoglobulin A, G and M concentrations, it was found that first-time mothers have higher colostrum IgA and IgM concentrations than do multiparous mothers. No influence was seen to be exerted by birth order on the concentration of IgG in maternal colostrum. There was also no observed statistically significant difference in terms of time to colostrum collection based on birth order for the sample as a whole ($\chi^2_c = 1,24$ p = 0,26587).

Table 1 - Description of the variables studied and the values of measures taken

Variable measures	Median	Minimum value	Maximum value	Mean	Standard deviation
Mother's age	30.00	21.00	41.00	30.61	4.50
Gestational age	39.28	37.00	41.14	39.25	0.93
NB's weight	3,272.50	2,665.00	4,310.00	3,279.76	404.55
Collection time	59.72	48.00	72.00	58.81	7.14
ELISA IgA	750.00	92.00	5,500.00	1,600.00	1,800.00
ELISA IgG	2.80	0.90	53.00	6.26	9.95
ELISA IgM	112.50	11.00	3,184.00	2,500.10	475.10

Mother's age in years; gestational age in weeks; weight in grams; time to collection in hours; immunoglobulins in mg/dl.

Table 2 - Correlation between type of delivery and time taken before collection of maternal colostrum

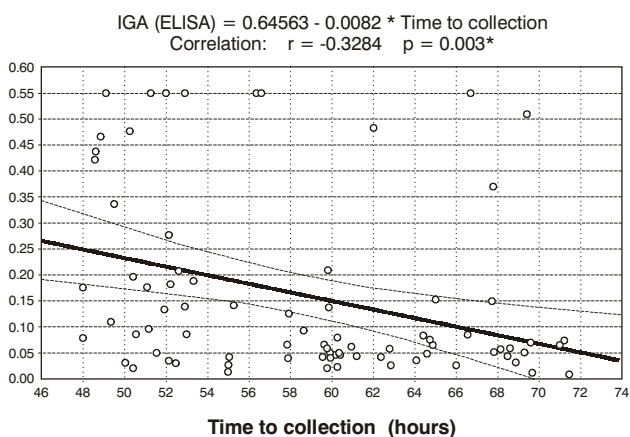
Type of delivery	n. of mothers	Collection period (hours) (mean±standard deviation)
Normal	33	60.00±7.45
Caesarian with labor	17	57.51±7.04
Elective caesarian	32	58.27±6.89
Total	82	58.81±7.14

F = 0.83; p = 0.440090 (non-significant).

In order to study the influence of labor on immunoglobulin A, G and M concentrations in maternal colostrum, the natural delivery group were joined with the group who had had caesarians only after starting labor (group A + B) and compared them with the elective caesarian group (group C). This procedure revealed that there were no statistically significant differences in immunoglobulins A, G and M concentrations between the colostrum of recently delivered mothers who had and who had not undergone labor (Table 3). The group that had had caesarians after labor exhibited higher IgA concentrations than the natural delivery group. The levels of immunoglobulins G and M did not differ significantly between the three groups.

Discussion

Within the primary focus of this study it was found that the type of delivery appears to influence IgA concentrations in maternal colostrum, in that mothers that had undergone labor followed by caesarian exhibited higher concentrations than did mothers who had given birth normally. Concentrations of IgM and IgG, however, were not influenced by the type of delivery. The only published work on humans that studied the correlation between type of delivery and the concentrations of different elements

**Figure 1** - Correlation between concentrations of IgA in the colostrum, using the ELISA technique, and the time to collection of maternal colostrum**Table 3** - Number of mothers who presented immunoglobulins concentrations (mg/dl) in the colostrum below or above the median, measured by ELISA technique, regarding the variables studied

Variables	IgA concentration		IgM concentration		IgG concentration	
	≤ median n (%)	> median n (%)	≤ median n (%)	> median n (%)	≤ median n (%)	> median n (%)
Type of delivery						
Normal	21 (63.64)	12 (36.36)	21 (63.64)	12 (36.36)	21 (63.64)	12 (36.36)
Caesarian with labor	4 (23.53)	13 (76.47)*	7 (41.18)	10 (58.82)	7 (41.18)	10 (58.82)
Elective caesarian	16 (50.00)	16 (50.00)	13 (40.63)	19 (59.37)	13 (40.63)	19 (59.37)
Labor						
Yes	25 (50.00)	25 (50.00)	28 (56.00)	22 (44.00)	28 (56.00)	22 (44.00)
No	16 (50.00)	16 (50.00)†	13 (40.63)	19 (59.37) ‡	13 (40.63)	19 (59.37)
Number of pregnancies						
One = 36	13 (36.11)	23 (63.89)	12 (33.33)	24 (66.67)	14 (38.89)	22 (61.11)
More than one = 46	28 (60.87)	18 (39.13)	30 (65.22)	16 (34.78)	27 (58.69)	19 (41.30)

Medians (mg/dl): IgA, 750.00; IgM, 112.50; IgG, 2.80.

* p = 0.016925 caesarian with labor x vaginal delivery.

† p = 0.04522 IgA one pregnancy x more than one pregnancy.

‡ p = 0.00820 IgM one pregnancy x more than one pregnancy.

within maternal colostrum and milk was performed by Kulski *et al.*,²² in 1981. Among other elements, the authors analyzed the immunoglobulin A, G and M levels in a sample of 11 recently delivered mothers who had given birth vaginally and 8 who had had caesarians, finding no alterations in the concentrations of these immunoglobulins in the colostrum related to type of delivery.

The finding that colostrum IgA levels were higher among recently delivered mothers who had had caesarians after labor than among the vaginal delivery group cannot be explained merely by surgical stress since the difference did not exist in relation to the elective caesarian group. Labor, in isolation, also fails to explain this finding since when both groups who had undergone labor are combined there is no significant difference in colostrum IgA concentration when compared with the elective caesarian group. It is possible that the combination of the stress of labor with surgical stress caused the recently delivered mothers who had undergone both to exhibit higher colostrum IgA concentrations between 48 and 72 hours postpartum.

It is known that the concentrations of breast milk components vary as lactation progresses: lactoferrin, total IgA and leukocyte concentrations fall during the first weeks of lactation and then stabilize. Lysozyme concentrations are low immediately postpartum and increase with time.²³⁻²⁵

The average time taken to collection of colostrum did not show any statistically significant difference between the three groups. When the group is taken as a whole, however, it was found that the shorter the time to collection, the higher the IgA concentration. This did not apply to IgG or IgM. The fact that the average time to collection did not vary between the groups reveals that this variable did not exert influence over immunoglobulin concentration differences between groups. The finding that IgA concentrations are higher the earlier they are sampled reinforces the importance of early suckling – in the delivery room if possible.

There was no difference in terms of birth order between the three groups. However, when the sample was studied as a whole, it was found that primiparous mothers had higher concentrations of IgA and IgM in their colostrum than did multiparous mothers. In the face of this result, the relationship between time to collection and birth order was studied, but no statistically significant relationship was found between the two factors. It is possible that primiparous mothers presented higher concentrations of IgA and IgM in their colostrum because their breasts were slower to fill than were the breasts of mothers who had given birth before²⁶ and that this resulted in more concentrated colostrum. There was no observed influence of birth order on colostrum IgG concentrations. It is appropriate to point out here that Coelho *et al.* did not find that birth order exerted any influence over concentrations of immunoglobulins A, M or G in maternal colostrum.²⁷

The combination of the stress of labor with surgical stress is possibly involved in the observation of elevated IgA concentrations in the maternal colostrum of the group of recently delivered mothers who underwent both caesarian and labor; but the observation of colostrum immunoglobulin concentrations of such variation begs the question of what other factors may explain some recently delivered mothers having IgA concentrations tens of times higher than others. Further studies should be performed in order to better understand the factors that influence the levels of these elements that are so important to the newborn's defenses.

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Corresponding author:

Gabriel A. J. Striker

Rua Dr. Alceu de Campos Rodrigues, 229/210

CEP 04544-000 - São Paulo, SP, Brazil

Tel.: +55 (11) 3845.6834 – Fax: +55 (11) 3849.6573

E-mail: striker@terra.com.br