

Article type : Original

Enhancement of immune response mediated by oropharyngeal colostrum administration in preterm neonates

Jorge Moreno-Fernandez MSc^{1,2}, Belén Sánchez-Martínez BD³, Laura Serrano-López MD³, Estefanía Martín-Álvarez MD³, Javier Diaz-Castro PhD^{1,2}, Manuela Peña-Caballero MD³, Francisca Martín-Peregrina BD³, Mercedes Alonso-Moya BD³, José Maldonado-Lozano MD^{4,5}, Julio J. Ochoa PhD^{1,2*}, Jose A. Hurtado-Suazo MD³.

¹Department of Physiology, University of Granada, Granada, Spain

²Institute of Nutrition and Food Technology “José Mataix Verdú”, University of Granada, Granada, Spain. ³Unit of Neonatology, Pediatric Service, Hospital Universitario Materno-Infantil Virgen de las Nieves, Granada, Spain. ⁴Pediatrics Department. University of Granada. Virgen de las Nieves University Hospital. Granada, Spain. ⁵Institute of Biosanitary Research of Granada. Maternal and Child Health Network, Carlos III Institute.

Running title: Oropharyngeal colostrum and immune response

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.13008

This article is protected by copyright. All rights reserved.

* Corresponding author: Julio José Ochoa Herrera, Institute of Nutrition and Food Technology “José Mataix Verdú”, University of Granada, Biomedical Research Centre, Health Sciences Technological Park, Avenida del Conocimiento s/n, Armilla, 18071 Granada, Spain, Tel.: +34 958241000 ext. 20317.
E-mail address: jjoh@ugr.es

Disclosure of potential conflict of interest

The authors declare that they have no relevant conflicts of interest.

Funding

This research was funded by the Andalusian Service of Health (Public Foundation Progress and Health, grant number PI-0374-2014).

Abstract

Background: The immune system of preterm infants is immature, being a significant cause of morbidity and mortality, particularly in the preterm infant. Oropharyngeal colostrum administration could be an immunomodulatory aid. Our aim was to evaluate the effect of oropharyngeal colostrum on the serum levels of immunoglobulins, lactoferrin and resistin during the first month of life and to track the clinical outcome of the neonates.

Methods: 100 preterm neonates born at < 32 weeks of gestation and/or weighing less than 1,500g and assisted in the Neonatal Intensive Care Unit were enrolled and divided into two groups: colostrum (n=48) and control (n=52). The subjects assigned to the colostrum group received 0.2 ml of colostrum (oropharyngeal route) every 4 hours for the first 15 days of life and if mothers have inability to breastfeed, they were included in the control group (no oropharyngeal colostrum). Serum concentrations of IgA, IgM and IgG1, lactoferrin and resistin were assessed in both groups at 1, 3, 15 and 30 days of life. Clinical data during hospitalization were collected.

Results: IgA and IgM increased in preterm neonates who were administered colostrum for 15 and 30 days. Lactoferrin increased after 30 days and resistin increased after 15 days of supplying oropharyngeal colostrum. The colostrum group underwent full enteral nutrition before and no differences were observed in the common neonatal morbidities.

Conclusion: Oropharyngeal colostrum administration is safe in preterm neonates and improves their immunological profile, showing a potential role as an immunomodulatory agent.

Keywords: Oropharyngeal colostrum, preterm neonates, immune response, immunoglobulins, clinical outcomes.

Impact statement: The immune system of preterm infants is immature, being a significant cause of morbidity and mortality, particularly in the preterm infant and oropharyngeal colostrum administration provides additional protection against infections, enhancing the immune system and improving the clinical outcome of the preterm neonates.

1. INTRODUCTION

Preterm infants have an immature immune system, with deficiencies in both innate and adaptive immunity and in the interaction between these two systems. These deficiencies increase the risk of acquiring infections, which are the major causes for mortality. In this sense, late-onset sepsis and necrotizing enterocolitis are significant causes of morbidity and mortality, especially in very lowbirth weight infants.¹⁻⁶

Exclusive human milk feedings have been shown to be beneficial in reducing the rate of infections.⁷ In this sense, colostrum is very rich in cytokines and other immune agents such as secretory immunoglobulin (Ig) A and lactoferrin, which provide high protection against infection.^{8,9} However, many preterm infants are unable to take substantial enteral volumes of human milk in the first days of life and do not receive adequate amounts of these immunomodulatory compounds, possibly leading to increased susceptibility to various infections and inflammatory conditions.¹⁰

Taking into account these considerations, a possible way to provide the premature newborn the advantages of breast milk could be the administration of oropharyngeal colostrum, also known as oral immune therapy. Although preterm infant are unable to take enteral volumes in the first few days, oropharyngeal administration does not involve the infant swallowing. During this easy intervention, a small amount of colostrum is placed directly onto the oropharyngeal mucosa in the buccal cavity for absorption.¹¹ This intervention has been demonstrated to be safe and well-tolerated by even the smallest and sickest extremely low birth weight infants.⁹

The scarce scientific literature available supports several benefits of this practice.^{8,12} However, more studies with bigger populations and at different ages to confirm these results and its clinical benefits are needed.^{9,12} To shed more light on this issue, the current study has been designed to evaluate the immunologic effects of oropharyngeal colostrum administration in premature infants (< 32 weeks of gestation and/or weighing less than 1,500 g).

2. METHODS

2.1 Experimental design and subjects

This is a non-randomized interventional study with control group developed since April 2014 through June 2016. The study was approved by the Bioethical Committee on Research involving Human Subjects at the University Hospital “Virgen de las Nieves” and followed the ethical principles of Helsinki Declaration of the World Medical Association (64th General WMA Assembly, Fortaleza, Brazil, October 2013). Inclusion criteria were: Neonates born with < 32 weeks of gestation and/or weighing less than 1,500 g in the Neonatal Intensive Care Unit of the University Hospital “Virgen de las Nieves” (Granada) and appropriate for gestational age. Exclusion criteria: neonates with congenital anomalies, chromosomopathies, malformations, congenital aneurysms, gastrointestinal disorders, renal disorders, receipt of vasopressor medications > 10 mcg/kg/minute; positive maternal HIV status. In hours prior to delivery or in the immediate postpartum period an information visit was carried out to the parents by a member of the study team, explaining the procedure of oropharyngeal feeding, the benefits of the study and the need for early stimulation for collecting colostrum as soon as possible. Consent was obtained from the parents after the nature and purpose of the study had been explained. Subsequently, the subjects (n=100) were assigned to the group receiving maternal colostrum (colostrum group, n=48) if this was available within the first 24 hours of life. In case of maternal inability to breastfeed or not availability of colostrum in the first 24 hours of life, the neonates were included in the control group (no oropharyngeal administration of maternal colostrum, n=52). The flowchart for participant enrolment and dropouts is shown in Figure 1. Subjects in the colostrum group received every 4 hours for the first 15 days of life a total of 0.2 ml of colostrum per treatment session.

2.2 Colostrum sampling and administration

Investigators met the mothers and educated them about hand-expression and electric pumping of breast milk every 2 to 3 hours. Mothers were given instructions and pre-labeled sterile milk collection flasks to collect their colostrum by using a sanitary hand-expression method. A trained nurse administered this colostrum by means of sterile Tuberculin syringes via the oropharyngeal route. The syringes were prepared daily with 0.1 mL of mother's colostrum, labelled and stored at 4°C in a specified breast milk refrigerator in the Neonatology Unit. The syringe (warmed in the infant's incubator for 5 minutes) was placed in the baby's mouth, along the buccal mucosal tissue to administer 0.1 ml over a period of less than 2 minutes on one side and then another administration of 0.1 ml on the contralateral oral mucosa. In each treatment session, a total of 0.2 ml, were therefore administered. The procedure was carried out every 4 hours for 15 days. During the procedure, heart rate, respiratory frequency, cutaneous oxygen saturation and arterial pressure were recorded if invasive monitoring was available. In case of dermal oxygen desaturation maintained below 88% or bradycardia <100 bpm or tachycardia >200bpm, the procedure would be discontinued.

2.3 Sample collection and analysis

To assess the immunological effects of administration of the oropharyngeal colostrum, 4 blood samples were collected during the first month of life: M1 (at enrolment), M2 (3rd day of life), M3 (15th day of life) and M4 (30th day of life). Blood samples were collected in micro-method tubes (0.6 mL). Blood samples were allowed to coagulate for 30 minutes and then centrifuged for 10 min at 1,000 x g. The obtained serum was aliquoted and was stored at - 80°C until further analysis.

2.4 Clinical outcomes

Independent variables

Maternal variables (infectious risk factors, use of prenatal/intrapartum antibiotic for infectious risk, type of delivery (vaginal, caesarean birth) and prenatal steroids use); Neonate's variables (gestational age, gender, Apgar score 1/5, anthropometric parameters at birth (weight, length, cephalic perimeter) and Clinical Risk Index for Babies (CRIB)), days to reach full enteral feeding, volume of enteral nutrition, need and type of ventilatory support, alteration in cerebral ultrasound as scored according to the classification by de Vries et al.¹³ and retinopathy of prematurity.

Dependent variables

Necrotizing enterocolitis (NEC). NEC is defined according to modified Bell's criteria stage ≥ 2 with clinical signs and radiological evidence including any of the following: pneumatosis intestinalis, portal venous gas with or without pneumoperitoneum; proven sepsis (symptoms or clinical signs of infection, biological markers of systemic inflammatory response syndrome (SIRS) (altered leukocyte count, CRP > 12 mg/l) and positive blood culture; Analytics (IgA, IgM, IgG1, Lactoferrin, Resistin)

2.5 Immunological parameters measurement

Luminex xMAP technology based MILLIPLEX MAP kits were used. Human Isotyping Magnetic Bead Panel Multiplex assay (cat. No. HGAMMAG-301K) was used for IgA, IgM and IgG1 determination, and Human Sepsis Magnetic Bead Panel 3 Multiplex Assay (cat. No. HSP3MAG-63K) was used for Lactoferrin and Resistin determination. Plate was read on LABScan 100 analyzer (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. Average values for each set of

duplicate samples or standards were within 15% of the mean. Cytokines concentrations in serum samples were determined by comparing the mean of duplicate samples with the standard curve for each assay.

2.6 Statistical Analysis

All data are presented as the Mean $\pm$ SEM. Taking into account previous studies, in which average levels of IgA in the first month of life were 22.48 ± 8.80 ($\mu\text{g/ml}$) in the control group, and 30.34 ± 11.92 ($\mu\text{g/ml}$) in the colostrum group, and also the population of other studies of colostrum administration (16), to obtain an asymptotic relative efficacy on the 90% power in the contrast of the null hypothesis $H_0: \mu_1 = \mu_2$, taking into account a level of significance of 5% and assuming the averages and standard deviations previously mentioned, it would be necessary to include 37 subjects per group. If we consider a possible dropout of 20%, the number would be a minimum of 45 individuals per group. All variables were tested to check if they followed the criteria of normality and homogeneity of variance using the Kolmogorov-Smirnov's and Levene's tests, respectively. For the qualitative variables, the Pearson or Fisher test was used in those cases in which the conditions of applicability were not met. To compare the differences between groups in the quantitative variables, the unpaired Student t test and the Mann-Whitney U test for non-normally distributed cases. To analyze the differences in each group over time of each variable studied a general linear model of variance for repeated measures was used. A value of $p < 0.05$ was considered significant. For data analysis we used the SPSS version 21.0 (SPSS Statistics for Windows, SPSS INC. Chicago, IL, USA).

3. RESULTS

The characteristics of mothers and their infants are summarized in Tables 1 and 2. There were no statistically significant differences in the maternal and infant characteristics studied.

Table 3 summarizes the clinical outcomes (morbidity) for infants in both groups. Infants in the colostrum group reached full enteral feedings sooner at an average of 7.2 ± 0.6 days compared to 9.1 ± 0.7 days for the control group, and this was the only characteristic that was statistically significant ($p < 0.05$).

Immunological biomarkers are shown in Figure 2. IgA was higher in colostrum group compared to the control group in M3 and M4 ($p < 0.05$). With regard to the evolution, IgA increased following the same trend in both groups, showing an increase in M4 compared to M1, M2 and M3 ($p < 0.05$) and in M3 compared to M1 and M2 ($p < 0.05$) (Figure 2A).

No differences were observed in IgG1 due to the oral colostrum administration compared to the control groups. With regard to the evolution, in colostrum group, IgG1 decreased in M4 compared to M1, M2 and M3 ($p < 0.05$), while in control group an increase was observed in M2 compared to M3 and M4 ($p < 0.05$) and a decrease in M4 compared to M1 ($p < 0.05$) (Figure 2B).

IgM was higher in colostrum group compared to the control group in M3 and M4 ($p < 0.05$). Both groups followed the same trend in the evolution, showing the highest value in M4 compared to M1, M2 and M3 ($p < 0.05$) (Figure 2C).

Lactoferrin was higher in colostrum groups compared to control groups in M4 ($p < 0.05$). With regard to the evolution, lactoferrin increased in M2 compared to M1 and in M3 compared to M1 and M2, decreasing in M4 compared to M3 and M1 ($p < 0.05$) in colostrum group, while in control group an increase was observed in M2 and M3 compared to M1 and M4 ($p < 0.05$) (Figure 3A).

Resistin was higher after 15 days of supplying oropharyngeal colostrum in M3 with regard to their counterparts controls ($p<0.05$). Resistin increased in M3 compared to M1 and M2, while decreased in M4 compared to M2 and M3 ($p<0.05$) and in M2 compared to M1 and M4 ($p<0.05$) in colostrum group. In addition, resistin increased in M2 compared to M1 and M4 in control group ($p<0.05$) (Figure 3B).

4. DISCUSSION

In preterm infants the rate of infections is inversely related to birth weight and gestational age and it is associated with increased mortality rates, immediate and long term morbidity, prolonged hospital stay and increased cost of care.⁶

Colostrum contains many types of protective agents and its oropharyngeal administration appears safe and may be associated with several clinical benefits.^{3,9,12} Oropharyngeal colostrum administration can improve the immune system thanks to the high concentration of IgA and lactoferrin and the interaction with gut-associated lymphoid tissues.^{8,9} However, there are controversies and limitations in the scarce scientific literature available due to small sample sizes¹⁶ and number of immunoglobulins studied,^{8,12} use of animal models¹³ and use of secretions (urine and saliva) instead of blood.^{9,12,14} For these existing limitations, this study was designed including a higher population of preterm neonates and more complete characterization of the immune biomarkers, being the first study to characterize the immune profile of the preterm neonate in blood and not in secretions which strengthens the results and conclusions obtained.

Oropharyngeal colostrum administration is a safer therapy for preterm neonates, as previously described.^{9,14} In the current study, we have observed that in the preterm neonates who received oropharyngeal colostrum, they reached full enteral nutrition before those that did not receive it. This could indicate a metabolic advantage for the underdeveloped gastrointestinal tract and reducing the presence of prematurity associated morbidities that compromise gastrointestinal

perfusion and that typically preclude enteral feedings during this period. The prolonged inability to feed enterally can lead to intestinal atrophy which increases the risk of localized inflammation, feeding intolerance, NEC and nosocomial infections.¹⁴ Thus, administering preterm colostrum could be a potential “immune therapy” to extremely low weight preterm infants in the first days of life when they cannot be fed enterally.

Among many humoral immune factors present in colostrum, IgA plays an important role, being the major immunoglobulin of human lacteal secretions and present in high concentrations.¹⁵ Newborn antibody production is low and insufficient to produce an effective immune response¹⁵ and therefore, for several months after birth, the main defense against infections is the passive immunity provided by maternal antibodies through lactation.¹⁵ In the current study, preterm neonates receiving oropharyngeal colostrum featured higher levels of IgA, especially after 15 or 30 days of administration, result that is in agreement with the previously reported by Lee et al.,¹² although only during the first 15 days of life. This result could be interpreted mainly as the passage through the systemic circulation after being absorbed by the oral or gastrointestinal mucosa. The excretion of IgA in the urine of infants receiving oropharyngeal colostrum support the absorption of this molecule from the mucosal surface,¹⁶ protecting the neonates against infections, modulating their immune system¹⁷ and providing an auxiliary physiological defense mechanism.¹⁸ Human colostrum is particularly rich in IgA, which has the ability to block bacterial adherence to epithelial cells,¹⁵ acts as an opsonin and plays a protective role against a number of microorganisms.¹⁹ In addition, *in vitro* studies have shown transepithelial uptake of IgA and binding to specific dendritic cells in Peyer’s patches in gut-associated lymphoid tissue, upregulating IgA-producing B cells.¹⁶ These findings suggest that colostrum sIgA may serve to promote an anti-inflammatory or tolerant environment as well as upregulating an infant’s IgA production.¹⁶

IgM²¹ is the first antibody made in a primary response^{20,21} and the second most common immunoglobulin in breast milk. It appears spontaneously in the absence of antigen stimulation, and it is a strong complement activator.²¹ Therefore the increase of IgM in the neonates receiving colostrum at day 15 and 30 represents once more, a beneficial effect in the immunological defense of the preterm neonate.

Finally, with respect to the IgG, the lack of statistical differences and the decline in their levels reflect the maternal immune response change, because it is a strictly transferable biomarker.²²

Little information is available on lactoferrin levels in neonates and especially in preterm neonates. Lactoferrin is considered a cell-secreted mediator that links the innate and adaptive immune responses.²³ This glycoprotein has antibacterial, antiviral, antifungal, anticancer, anti-oxidant, anti-inflammatory, anti-parasitic, anti-allergic, iron absorption regulatory functions and immunomodulatory activities.^{24,25} The decrease in the concentration of lactoferrin in breast milk can be correlated with the decrease in blood at 30 days of life recorded in the current study. However, the decrease recorded in the control group is more apparent than in the colostrum group, with higher lactoferrin serum levels in those patients who received colostrum at 15 days of life. This data suggests, once again, that oropharyngeal colostrum would increase lactoferrin absorption and would modulate the immune system through local interaction with the lymphoid tissue associated with mucous membranes (where the absorption takes place).

The group receiving oropharyngeal colostrum displayed markedly elevated levels of serum resistin compared with the controls at day 15 of the study. In the same way as lactoferrin does, resistin decreases progressively in maternal milk.²⁶ Resistin is a metabolic hormone that plays important roles in regulating energy homeostasis and carbohydrate/lipid metabolism, is mainly secreted by adipocytes, impairs glucose homeostasis and opposes the action of insulin in peripheral tissues.²⁷ On the other hand, pro-inflammatory cytokines IL-1 β , TNF- α and IL-6 stimulate the production of resistin.²⁸ IL-1Ra, as an acute phase protein, has also been linked to serum resistin

levels. Forsblad d'Elia et al.²⁸ found that resistin was related to markers of inflammation, particularly with the increase of IL-1ra. The high level of resistin at day 15 of the study in the colostrum group may induce hyperglycemia and play a critical role in ensuring the survival of the newborn by providing adequate substrate glucose for central nervous system utilization,²⁹ although was normalized at the end of the study, reflecting maybe a punctual glucose requirement.

Our findings contribute to the understanding of the beneficial effects of colostrum administration on immune system through a characterization for the first time of the immunological profile of the neonate during the first month of life. In addition, preterm neonates who receive oropharyngeal colostrum, reached full enteral nutrition before than those who did not received it, indicating a metabolic advantage for the underdeveloped gastrointestinal tract and reducing the presence of prematurity associated morbidities that compromise gastrointestinal perfusion and typically preclude enteral feedings during this period. The study sustains that oropharyngeal colostrum administration during 15 days in preterm neonates improves their immunological profile, indicating a positive influence on the developing immune system.

Acknowledgments

The authors are grateful to the Initiative for the Humanization of Birth Assistance and Breastfeeding for the support during the study. Jorge Moreno-Fernandez was supported by fellowship from the Ministry of Education, Culture and Sport (Spain) and is grateful to the Excellence Ph.D. Program “Nutrición y Ciencias de los Alimentos” from the University of Granada. The authors are grateful to Ms. Susan Stevenson for her efficient support in the revision with the English language.

Author contributions

Jorge Moreno-Fernandez and Javier Diaz-Castro drafted the initial manuscript and carried out the analyses. Manuela Peña-Caballero, Laura Serrano-López and Estefanía Martín-Álvarez carried out the analyses, reviewed and revised the manuscript. Belen Sánchez-Martínez, Francisca Martín-Peregrina and Mercedes Alonso-Moya participated in data collection process. José Maldonado-Lozano supervised the analyses development and revised the manuscript. Julio J. Ochoa conceptualized and designed the study, coordinated and supervised data collection at Institute of Nutrition and Department of Physiology. Jose A. Hurtado-Suazo designed the data collection instruments, and coordinated and supervised data collection at Unit of Neonatology, Pediatric Service, Hospital Universitario Materno-Infantil Virgen de las Nieves and critically reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

REFERENCES

1. Howson CP, Kinney MV, McDougall L, Lawn JE. Born Too Soon: Preterm birth matters. *Reproductive Health*. 2013;10(Suppl 1):S1-S1.
2. Wynn JL, Neu J, Moldawer LL, Levy O. Potential of immunomodulatory agents for prevention and treatment of neonatal sepsis. *J Perinatol*. 2009;29(2):79-88.
3. Strunk T, Currie A, Richmond P, Simmer K, Burgner D. Innate immunity in human newborn infants: prematurity means more than immaturity. *J Matern Fetal Neonatal Med*. 2011;24(1):25-31.
4. Kallman J, Schollin J, Schalen C, Erlandsson A, Kihlstrom E. Impaired phagocytosis and opsonisation towards group B streptococci in preterm neonates. *Archives of Disease in Childhood Fetal and Neonatal Edition*. 1998;78(1):F46-F50.
5. Horbar JD, Carpenter JH, Badger GJ, et al. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. *Pediatrics*. 2012;129(6):1019-1026.
6. Boghossian NS, Page GP, Bell EF, et al. Late-Onset Sepsis in Very Low Birth Weight Infants from Singleton and Multiple Gestation Births. *The Journal of pediatrics*. 2013;162(6):1120-1124.e1121.
7. Schanler RJ. Outcomes of human milk-fed premature infants. *Semin Perinatol*. 2011;35(1):29-33.
8. Gephart SM, Weller M. Colostrum as oral immune therapy to promote neonatal health. *Adv Neonatal Care*. 2014;14(1):44-51.
9. Zhang Y, Ji F, Hu X, Cao Y, Latour JM. Oropharyngeal Colostrum Administration in Very Low Birth Weight Infants: A Randomized Controlled Trial. *Pediatr Crit Care Med*. 2017;18(9):869-875.

- Accepted Article
10. Cristofalo EA, Schanler RJ, Blanco CL, et al. Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. *J Pediatr*. 2013;163(6):1592-1595.e1591.
 11. Rodriguez NA, Meier PP, Mw G, Zeller JM. Oropharyngeal administration of colostrum to extremely low birth weight infants: theoretical perspectives. *Journal of perinatology : official journal of the California Perinatal Association*. 2009;29(1):1-7.
 12. Lee J, Kim HS, Jung YH, et al. Oropharyngeal colostrum administration in extremely premature infants: an RCT. *Pediatrics*. 2015;135(2):e357-366.
 13. Sty AC, Sangild PT, Skovgaard K, et al. Spray Dried, Pasteurised Bovine Colostrum Protects Against Gut Dysfunction and Inflammation in Preterm Pigs. *J Pediatr Gastroenterol Nutr*. 2016;63(2):280-287.
 14. Rodriguez NA, Meier PP, Groer MW, Zeller JM, Engstrom JL, Fogg L. A pilot study to determine the safety and feasibility of oropharyngeal administration of own mother's colostrum to extremely low-birth-weight infants. *Adv Neonatal Care*. 2010;10(4):206-212.
 15. Hanson LA. Session 1: Feeding and infant development breast-feeding and immune function. *Proc Nutr Soc*. 2007;66(3):384-396.
 16. Glass KM, Greecher CP, Doheny KK. Oropharyngeal Administration of Colostrum Increases Salivary Secretory IgA Levels in Very Low-Birth-Weight Infants. *Am J Perinatol*. 2017;34(14):1389-1395.
 17. Chirico G, Marzollo R, Cortinovis S, Fonte C, Gasparoni A. Antiinfective properties of human milk. *J Nutr*. 2008;138(9):1801s-1806s.

- Accepted Article
18. França EL, Nicomedes TdR, Calderon IdMP, França ACH. Time-dependent alterations of soluble and cellular components in human milk. *Biological Rhythm Research*. 2010;41(5):333-347.
 19. França EL, Bitencourt RV, Fujimori M, Cristina de Moraes T, de Mattos Paranhos Calderon I, Honorio-França AC. Human colostrum phagocytes eliminate enterotoxigenic *Escherichia coli* opsonized by colostrum supernatant. *Journal of Microbiology, Immunology and Infection*. 2011;44(1):1-7.
 20. Janeway CA, Travers P, Walport M, Shlomchik M. *Immunobiology: The Immune System in Health and Disease*. 6th ed. New York: Garland Science; 2005.
 21. Boes M. Role of natural and immune IgM antibodies in immune responses. *Mol Immunol*. 2000;37(18):1141-1149.
 22. Janeway CA, Travers P. *Immunobiology: The Immune System in Health and Disease*. 3rd ed. New York: Garland Publishing Inc; 1997.
 23. Siqueiros-Cendon T, Arevalo-Gallegos S, Iglesias-Figueroa BF, Garcia-Montoya IA, Salazar-Martinez J, Rascon-Cruz Q. Immunomodulatory effects of lactoferrin. *Acta Pharmacol Sin*. 2014;35(5):557-566.
 24. Trend S, Strunk T, Hibbert J, et al. Antimicrobial protein and Peptide concentrations and activity in human breast milk consumed by preterm infants at risk of late-onset neonatal sepsis. *PLoS One*. 2015;10(2):e0117038.
 25. Decembrino L, DeAmici M, De Silvestri A, Manzoni P, Paolillo P, Stronati M. Plasma lactoferrin levels in newborn preterm infants with sepsis. *J Matern Fetal Neonatal Med*. 2017;30(23):2890-2893.

- Accepted Article
26. Ilcol YO, Hizli ZB, Eroz E. Resistin is present in human breast milk and it correlates with maternal hormonal status and serum level of C-reactive protein. *Clin Chem Lab Med*. 2008;46(1):118-124.
 27. Yeung EH, McLain AC, Anderson N, et al. Newborn Adipokines and Birth Outcomes. *Paediatr Perinat Epidemiol*. 2015;29(4):317-325.
 28. Forsblad d'Elia H, Pullerits R, Carlsten H, Bokarewa M. Resistin in serum is associated with higher levels of IL-1Ra in post-menopausal women with rheumatoid arthritis. *Rheumatology (Oxford)*. 2008;47(7):1082-1087.
 29. Ng PC, Lee CH, Lam CW, Chan IH, Wong E, Fok TF. Resistin in preterm and term newborns: relation to anthropometry, leptin, and insulin. *Pediatr Res*. 2005;58(4):725-730.

Table 1. Characteristics of the mothers enrolled in the study

		Colostrum group	Control group	<i>p</i>
Antenatal characteristics				
Delivery method	Vaginal birth (%)	47.5 %	34.8 %	0.33
	Cesarean birth (%)	52.5 %	65.2 %	
Antenatal steroids	No (%)	12.5 %	10.9 %	0.47
	Yes (%)	89.1 %	87.5 %	
Antenatal antibiotics	No (%)	20.5 %	15.2 %	0.49
	Yes(%)	76.9 %	84.8 %	
Amniotic infection syndrome*	No (%)	32.5 %	39.1 %	0.41
	Yes (%)	67.5 %	60.9 %	

*Amniotic infection syndrome (maternal group B streptococci; maternal fever in labor; Chorioamnionitis; premature rupture of placental membranes).

Table 2. Characteristics of the neonates enrolled in the study

		Colostrum group	Control group	p-value
Gestational age (weeks)		29.9 ± 0.4	29.5 ± 0.3	0.34
Gender	boys (%)	60.0 %	56.5 %	0.91
	girls (%)	40.0 %	43.5 %	
Apgar 1 min.		6.4 ± 0.3	6.6 ± 0.3	0.65
Apgar 5 min.		8.1 ± 0.2	8.1 ± 0.2	0.83
Weight (g)		1230.1 ± 48.2	1267.6 ± 52.4	0.60
Height (cm)		38.6 ± 0.5	38.4 ± 0.7	0.69
Cephalic Perimeter (cm)		27.1 ± 0.4	27.0 ± 0.4	0.83
Weight gaining first year (g)		612.9 ± 31.7	527.4 ± 49.2	0.06
CRIB Score		2.2 ± 0.4	2.0 ± 0.4	0.17

Values are means ± SEM.

CRIB (Clinical Risk Index for Babies)

Table 3. Clinical outcomes at the end of the study

	Colostrum Group	Control Group	p-value
Days to reach full enteral feeding *	7.2 ± 0.6	9.1 ± 0.7	0.04
Volume of full enteral feeding (ml)*	118.9 ± 5.3	107.8 ± 5.7	0.38
NEC at 15 days (Bell stage ≥ 2)	1 (2.4 %)	0 (0 %)	0.46
NEC at 30 days (Bell stage ≥ 2)	2 (4.9 %)	2 (4.3 %)	1
Proven Sepsis at 15 days	7 (17.1 %)	5 (10.9 %)	0.37
Proven Sepsis at 30 days	3 (7.3 %)	2 (4.4 %)	0.66
NIV during 1st month of life	35 (85.4 %)	39 (84.8 %)	0.87
MV during 1st month of life	9 (21.9 %)	13 (28.2 %)	0.72
ROP at 1st month of life	2 (4.9 %)	4 (8.7 %)	0.42
Abnormalities in ultrasound brain scan at 1st month of life	14 (34.1 %)	10 (21.7 %)	0.11

* Data expressed as Mean ± EEM.

NEC necrotizing enterocolitis; NIV noninvasive ventilation; MV mechanical ventilation; ROP retinopathy of prematurity

Figure 1. Flowchart showing participant progress and dropouts in the study.

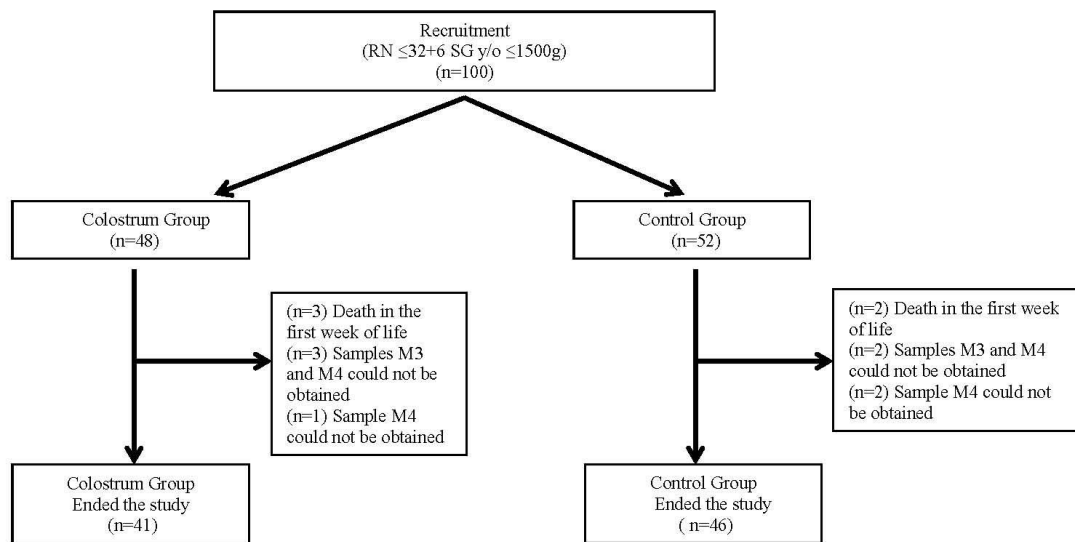


Figure 2. Effect of oropharyngeal colostrum administration on the levels of Immunoglobulin A (A), Immunoglobulin G1 (B) and immunoglobulin M (C) in serum. Results are expressed as mean $\pm$ standard error of the mean. A line between bars means statistically significant differences between groups ($P < 0.05$). Different letters in every group indicates significant differences due to the time (Colostrum (A, B, C, D, E); Control (a, b, c, d, e) ($P < 0.05$). M1: Birth (basal value); M2: 3rd day of life; M3: 15th day of life; M4: 30th day of life.

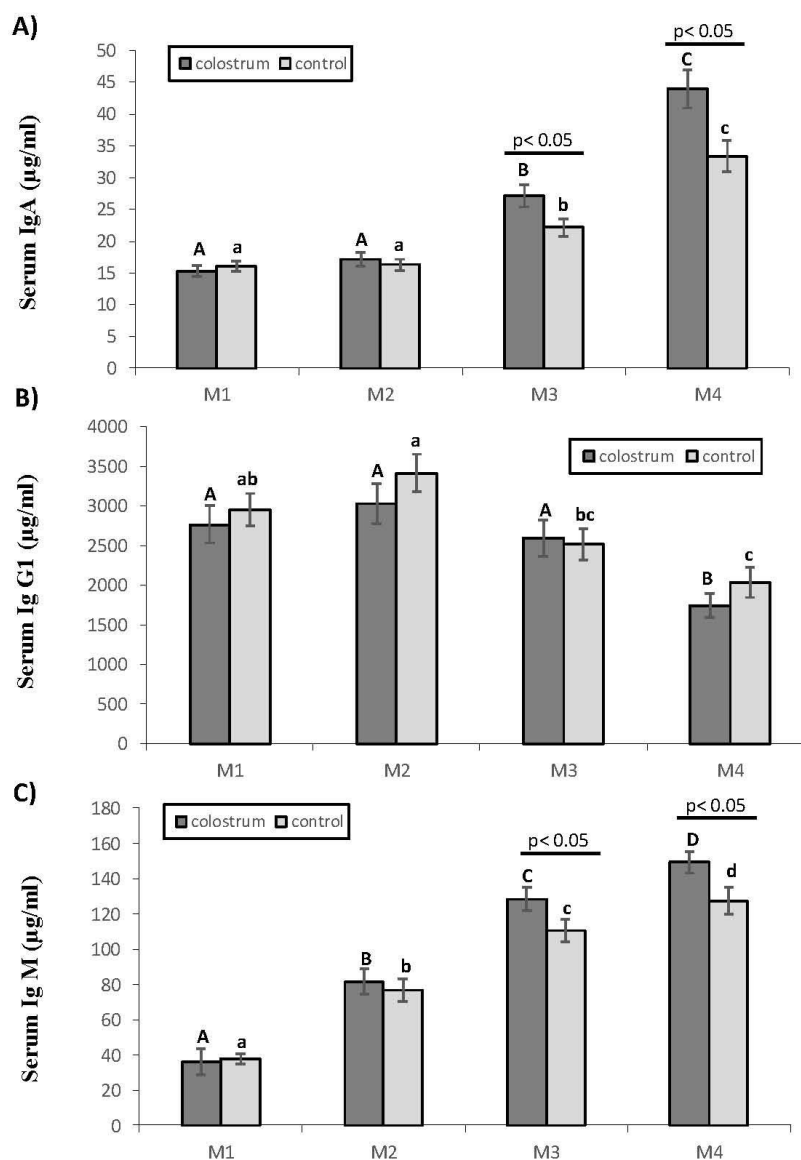


Figure 3. Effect of oropharyngeal colostrum administration on the levels of lactoferrin (A) and resistin (B) in serum. Results are expressed as mean $\pm$ standard error of the mean. A line between adjacent bars means statistically significant differences between groups ($P < 0.05$). Different letters in every group indicates significant differences due to the time (Colostrum (A, B, C, D, E); Control (a, b, c, d, e) ($P < 0.05$). M1: Birth (basal value); M2: 3rd day of life; M3: 15th day of life; M4: 30th day of life.

