Colostrum immunoglobulins and oxidative capacity may be affected by infant sex and maternal age and parity

DAVUT SİNAN KAPLAN
CAHİT BAĞCI
MUSTAFA ÖRKMEZ
ÖZGE KÖMÜRÇÜ KARUSERCİ
SEYHUN SUCU

See next page for additional authors

Follow this and additional works at: https://journals.tubitak.gov.tr/medical

Part of the Medical Sciences Commons

Recommended Citation
KAPLAN, DAVUT SİNAN; BAĞCI, CAHİT; ÖRKMEZ, MUSTAFA; KARUSERCİ, ÖZGE KÖMÜRÇÜ; SUCU, SEYHUN; ÇELİK, HAKİM; and TAYSİ, SEYİTHAN (2019) "Colostrum immunoglobulins and oxidative capacity may be affected by infant sex and maternal age and parity," Turkish Journal of Medical Sciences: Vol. 49: No. 1, Article 14. https://doi.org/10.3906/sag-1810-66
Available at: https://journals.tubitak.gov.tr/medical/vol49/iss1/14

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Medical Sciences by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.
Colostrum immunoglobulins and oxidative capacity may be affected by infant sex and maternal age and parity

Authors
DAVUT SİNAN KAPLAN, CAHİT BAĞCI, MUSTAFA ÖRKMEZ, ÖZGE KÖMÜRCÜ KARUSERCI, SEYHUN SUCU, HAKİM ÇELİK, and SEYİTHAN TAYSİ

This article is available in Turkish Journal of Medical Sciences: https://journals.tubitak.gov.tr/medical/vol49/iss1/14
Colostrum immunoglobulins and oxidative capacity may be affected by infant sex and maternal age and parity

Davut Sinan KAPLAN1,*, Cahit BAĞCİ1, Mustafa ÖRKMEZ2, Özge KÖMÜRÇU KARUSERCİ3, Seyhun SUCU4, Hakim ÇELİK5, Seyithan TAYSI6

1Department of Physiology, Sakarya University, Sakarya, Turkey
2Department of Biochemistry, Şehitkamil Public Hospital, Gaziantep, Turkey
3Department of Gynecology and Obstetrics, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey
4Department of Gynecology and Obstetrics, Cengiz Gökçek Gynecology and Obstetrics Hospital, Gaziantep, Turkey
5Department of Physiology, Faculty of Medicine, Harran University, Şanlıurfa, Turkey
6Department of Biochemistry, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Background/aim: The aims of this study were to determine the levels of the total antioxidant status (TAS), the total oxidant status (TOS), and the concentration of immunoglobulin A (IgA) and M (IgM) in colostrum, and evaluate relationships between these parameters and maternal age, maternal parity, and infant sex.

Materials and methods: The analysis was performed in serum samples of colostrum which were collected from 90 mothers on the first day of lactation between 10:00 and 12:00 AM.

Results: The measurements established that no significant association existed between the TAS level of colostrum and parity, maternal age, or infant sex. However, mothers 18 to 30 years of age had significantly lower colostrum TOS and OSI levels compared with mothers older than 30 years of age. IgA and IgM values of the colostrum of primiparous mothers were significantly higher than those of multiparous mothers, whereas no correlations existed with the age of the mother. Additionally, significantly higher colostrum IgA and IgM values were observed in female infants fed colostrum compared with male infants.

Conclusion: In conclusion, sex-based hormonal changes in mothers during pregnancy may be associated with the different colostral immunoglobulin levels for male and female infants.

Key words: Total antioxidant status, total oxidant status, breast milk

Received: 10.10.2018 ● Accepted/Published Online: 24.11.2018 ● Final Version: 11.02.2019

1. Introduction
Because optimal growth and development of infants is possible only with breast-feeding, breast milk is considered to be the gold standard for infant nutrition. Breast milk transmits nutrients to the infant, affects biochemical systems, enhances immunity, and eliminates many pathogens.

Increased concentrations of the oxidation products of lipids, proteins, and nucleic acids in bodily fluids indicate oxidative stress. The balance between the oxidation power of reactive oxygen species (ROS) and antioxidant defense mechanisms determines the degree of oxidative stress. The aim of the antioxidant defense system is to protect the organism against the adverse effects of ROSs (1). Infants are exposed to increased oxidative stress due to increased ROS production during delivery and a decrease in antioxidative components, such as vitamin E or glutathione peroxidase (2). Immunoglobulin A (IgA) is the principal immunoglobulin in colostrum and milk. IgA concentrations decline by the fourth week postpartum. However, significant levels of IgA are maintained in milk during the first year of lactation and in the second year of life, milk contains considerable amounts of IgA even if a baby is partially breast-fed (3). IgA levels may vary according to the personal characteristics of the mother and the gestational age of the baby. More IgA and immune factors are found in the colostrum and the milk of mothers who deliver preterm ( <37 weeks) compared to a longer birth term (37–42 weeks) (4). The IgM concentration in colostrum is low and decreases progressively during lactation because IgM is produced by the neonate soon after birth in response to infection (5). Compared to IgA

* Correspondence: dskaplan@gantep.edu.tr

This work is licensed under a Creative Commons Attribution 4.0 International License.
concentrations, IgM concentrations exhibit less variability associated with the personal characteristics of the mother and the gestational age of the baby. No changes in IgM concentrations were observed in the colostrum and milk of mothers who gave birth at preterm compared to those of mothers who gave birth at full term (5,6). Similar to IgA, IgM levels were higher in primiparous mothers than in multiparous mothers (7).

The purpose of the current study was to (1) investigate the levels of the total antioxidant status (TAS), the total oxidant status (TOS), the oxidative stress index (OSI), and the concentration of IgA and IgM in the colostrum, and (2) evaluate relationships between these parameters and maternal age, maternal parity, and infant sex.

2. Materials and methods

2.1. Subjects

This study was performed according to the rules of the ethics committee of Gaziantep University (Gaziantep, Turkey) and with informed consent from the mothers. Inclusion criteria for mothers were spontaneous vaginal delivery (SVD) at term, absence of premature or postmature birth, absence of any complications of pregnancy, delivery, and puerperium (particularly hypertension, eclampsia, infection, dystocia, or agalactia), and absence of plastic surgery in mammary. Inclusion criteria for the neonates comprised a birth weight of 2500–4000 g, absence of fetal distress, birth asphyxia, sepsis, major congenital abnormalities, or other significant diseases.

2.2. Sample collection and preparation

Colostrum samples were obtained from mothers on the first day of lactation between 10:00 and 12:00 AM from January to March 2013 using a commercial breast pump. Samples were frozen immediately and stored at −80 °C until analysis. Collection dates and times were recorded as were the demographic and anthropometrical characteristics of the infants and the mothers (Table 1). The previous suckling occurred approximately 2 h prior to sample collection.

Colostrum samples were prepared using a double centrifugal process. Samples were allowed to reach ambient temperature until dissolved. After thawing, colostrum samples were centrifuged for 10 min at 680 × g at 4 °C, after which the lipid layer and cellular elements were removed. The aqueous fraction was centrifuged again for 30 min at 10,000 × g at 4 °C, and the lipid layer and cellular elements were removed. Clear, ready-to-use serum fractions were separated into 1.5 mL sterile tubes for measurements (8).

2.3. Measurement of colostrum TAS, TOS, and OSI levels

The TAS, TOS, and OSI levels of the serum were measured using an automated analyzer (Tokyo Boeki, Prestige 24, Tokyo, Japan) with a commercially available measurement kit for TAS (Rel Assay Diagnostics, Gaziantep, Turkey) (9) and TOS (Rel Assay Diagnostics, Gaziantep, Turkey) developed by Erel (10). The OSI index was expressed as a percentage ratio of the TOS level to the TAS level. For calculation, the units of the TAS concentration were changed to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H2O2 equiv/L) / TAS (mmol Trolox equiv/L) (11).

2.4. Determination of colostrum IgA and IgM concentrations

Serum IgA and IgM levels were detected by nephelometry (BN ProSpec, Siemens Healthcare Diagnostics, Marburg, Germany) using commercially available kits (Siemens N Antiserum to Human IgA, Siemens N Antiserum to Human IgM) according to the manufacturer’s instructions.

2.4.1. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) Version 22.0. Multiple linear regression models were built for comparison of adjusted means with the colostrum samples and also for statistical comparisons between all group measurements. Descriptive statistics for numerical variables were expressed as the mean ± standard deviation (SD); P-values less than 0.05 were considered significant.

3. Results

Maternal and infant characteristics are listed in Table 1. The levels of TAS, TOS, and OSI values determined in
the colostrum of mothers are reported in three primary categories, i.e. maternal age, maternal parity, and infant sex, on the first day of lactation (Table 2).

As anticipated, no significant differences were found in TAS levels of colostrum across all three groups. The mean TAS levels were significantly reduced in the colostrum of mothers aged 18–30 years (2.57 ± 1.3) compared with mothers aged >30 years (3.28 ± 1.4) (P = 0.038). No significant differences were determined between primiparous and multiparous mothers. No differences were determined for the colostrum OSI between male and female infants. The OSI provided an integrative perspective for TAS and TOS levels in the colostrum. In the current study, the OSI ratios in the colostrum of mothers were observed to be higher after age 30, when compared with the colostrum of mothers aged 18–30 (P = 0.042). No significant differences were found between primiparous and multiparous mothers. No differences were determined for the colostrum OSI between male and female infants.

IgA and IgM concentrations (Table 3) were significantly higher in the colostrum of mothers of female infants compared to mothers of male infants and higher in the colostrum of primiparous compared with multiparous

### Table 2. Levels of TAS, TOS, and OSI.

| Groups      | TAS (mmol Trolox equiv/L) | P     | Regression coefficients | TOS (μmol H_2O_2 Eqiv/L) | P     | Regression coefficients | OSI       | P      | Regression coefficients |
|-------------|---------------------------|-------|-------------------------|---------------------------|-------|-------------------------|-----------|--------|-------------------------|
| Sex         |                           | B     | B                       |                           | B     |                         |           |        |                         |
| Female      | 1.85 ± 0.2                | 0.90  | 0.006                   | 2.81 ± 1.6                | 0.51  | −0.21                   | 0.15 ± 0.08| 0.46   | −0.01                   |
| Male        | 1.84 ± 0.2                |       |                         | 2.79 ± 1.3                |       |                         | 0.15 ± 0.06|        |                         |
| Maternal age |                            |       |                         |                           |       |                         |           |        |                         |
| 18–30       | 1.84 ± 0.2                | 0.51  | 0.033                   | 2.57 ± 1.3                | 0.04* | 0.73                    | 0.14 ± 0.06| 0.04*  | 0.03                    |
| >30         | 1.86 ± 0.3                |       |                         | 3.28 ± 1.4                |       |                         | 0.17 ± 0.07|        |                         |
| Parity      |                           |       |                         |                           |       |                         |           |        |                         |
| Primiparous | 1.90 ± 0.2                | 0.17  | −0.08                   | 2.76 ± 1.3                | 0.48  | −0.28                   | 0.14 ± 0.05| 0.73   | −0.07                   |
| Multiparous | 1.83 ± 0.2                |       |                         | 2.81 ± 1.4                |       |                         | 0.15 ± 0.07|        |                         |
| General mean| 1.84 ± 0.2                |       |                         | 2.80 ± 1.4                |       |                         | 0.15 ± 0.07|        |                         |

Data are presented as mean ± SD, and P-value from multiple linear regression analysis.

* P < 0.05.

### Table 3. Concentrations of IgA and IgM.

| Groups      | IgA (g/L) | P     | Regression coefficients | IgM (g/L) | P     | Regression coefficients |
|-------------|-----------|-------|-------------------------|-----------|-------|-------------------------|
| Sex         |           | B     | B                       |           | B     |                         |
| Female      | 33.75 ± 15.0 | 0.01* | 8.80                    | 2.12 ± 1.4| 0.01* | 0.83                    |
| Male        | 25.82 ± 14.2|       |                         | 1.45 ± 0.9|       |                         |
| Maternal age |           |       |                         |           |       |                         |
| 18–30       | 29.16 ± 15.2| 0.62  | 1.76                    | 1.80 ±1.2 | 0.86  | 0.06                    |
| >30         | 29.75 ± 14.9|       |                         | 1.57 ±1.1 |       |                         |
| Parity      |           |       |                         |           |       |                         |
| Primiparous | 35.95 ± 15.7| 0.01* | −10.5                   | 2.73 ± 1.3| 0.001*| −1.53                   |
| Multiparous | 27.33 ± 14.3|       |                         | 1.54 ± 1.0|       |                         |
| General mean| 29.37 ± 15.0|       |                         | 1.74 ± 1.2|       |                         |

Data are presented as mean ± SD, and P-value from multiple linear regression analysis.

* P < 0.05.
mothers. No significant differences existed between maternal age and immunoglobulin concentrations.

4. Discussion
Breast milk and colostrum contain many antioxidant constituents, including albumin, bilirubin, cysteine, uric acid, glutathione, coenzyme Q10, lactoferrin, and vitamins A, C, and E (12–14). In addition, breast milk contains several times greater concentrations of antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase than serum (15,16). Prior studies have noted that breast-fed children may have higher albumin, uric acid, vitamin C, and TAS levels than formula-fed children, even when formula milk contained much higher concentrations of antioxidants and vitamins in vitro than fresh breast milk did (12,17). Reports have shown that TAS was higher in the colostrum than in transitional and mature milk (18) and that the TAS levels of colostrum was not affected by storage at −80 °C (19). Each of these studies demonstrated the importance of colostrum and mature milk consumption as an antioxidant source to decrease the risk of disease due to the occurrence of an imbalance between antioxidant status and oxidative stress.

In this study, the importance of TAS in colostrum samples on the first day of lactation was assessed. No significant effects of maternal age, maternal parity, or infant sex on colostrum TAS levels were observed. This study demonstrated that TAS levels were constant and did not change according to maternal or infant characteristics. Consequently, colostrum is a constant source of antioxidants that protect newborns postnatally from higher oxygen levels than encountered prenatally.

Another finding was that TOS levels in the colostrum were approximately 5 times less than in the blood serum of healthy people (20,21). Both the high persistence of TAS levels in the colostrum relative to blood serum (20) and the up to 5 times less levels of TOS in the colostrum may indicate that antioxidants and oxidants are selectively passed to the colostrum, thereby providing increased evidence of colostrum as important to life support. Older mothers reported significantly higher TOS levels than younger mothers (Table 3). In addition, this study reported no significant difference between maternal parity and infant sex with TOS levels of colostrum compared to the TAS levels. Although the colostrum TOS value exhibited concentrations approximately five times less than in blood serum, an increased maternal age caused increased transport of oxidants to the colostrum. Therefore, it is possible to hypothesize that maternal age is associated with colostrum quality.

The OSI is an important parameter that reveals the general oxidant and antioxidant balance of colostrum on the first day of lactation because it is obtained from proportioning the TAS and TOS levels. The OSI values obtained in this study were significantly associated with maternal age.

Colostrum contains more immunoglobulin than mature milk and its concentration changes throughout lactation (22). In contrast to our research, studies in which milk samples were collected over longer lactation periods, i.e. 1–3 or 1–5 days, report IgA concentrations in the colostrum that differ from those reported in this study and among each other, such as 10 g/L (23), 12 g/L (24), 5.61 g/L (6), 6.5 g/L (4), 2.25 g/L (5), and 17.4 g/L (25). However, Hennart et al. reported dramatic differences in the concentration of IgA between the first day and second day of lactation. On the second day of lactation, colostrum IgA concentrations were decreased by nearly two-fold (26). Comparable studies reported that total protein concentrations were also decreased in colostrum by approximately two-fold between the first and third days of lactation (27). A number of authors demonstrated that the colostrum of primiparous mothers contained higher IgA concentrations compared to multiparous mothers (29,30), while others reported no difference in the colostrum and mature milk (6,26). In the present study, colostrum IgA concentrations were determined to be significantly different between primiparous mothers and multiparous mothers, 35.95 g/L and 27.33 g/L, respectively. This unique spectrum of antibody specificity is achieved by the "homing" of B lymphocytes, synthesized in the mother's gastrointestinal tract and transferred to her mammary glands. The antibody composition of breast milk compensates partly for the deficiency of antibodies directed against enteric antigens in placentally transferred IgG (31). In prolonged births in primiparous mothers, fetuses can be exposed to enteric pathogens for a long time, so they need more immunoglobulin. This study demonstrated that no significant difference existed between IgA concentrations and maternal age, and was similar to that published previously for colostrum (6,32). A sufficient amount of IgA is vital for a newborn regardless of the age of the mother.

In conclusion, sex-based hormonal changes in mothers during pregnancy may be associated with the differentcolostal immunoglobulin levels for male and female infants. The results of this study indicate that IgA levels were significantly higher in colostrum consumed by female infants. This study provides an exciting opportunity to advance our knowledge of sex-based changes in breast milk. Important in the first days of life, the presence of higher amounts of immunoglobulins, which are found in female infants compared to male infants, could suggest better protection against various diseases. In general, studies comparing infant sex and
disease, such as middle ear infections (33), wheezing (34), necrotizing enterocolitis (35), sudden infant death syndrome (36), growth retardation (37), sepsis (38), and deaths due to asphyxia (39), remarkably report the occurrence of disease significantly more often in male infants. Further research should be undertaken to investigate the relationship between colostral immunoglobulin levels and newborn’s disease.

Newborns have IgM production capacity to protect against infection. For this reason, the rate of IgM tends to fall gradually throughout breast-feeding (5). Similar to IgA concentrations, the colostrum of primiparous mothers contained higher IgM concentrations compared to multiparous mothers (7). Although it is not yet clear exactly how the number of births to an individual mother influences the composition of the colostrum, previous studies reported an effect on certain hormones, such as prolactin (40). This study appears to be consistent with other research that found no difference between maternal age and the amount of IgM in the colostrum (6). The present study determined that, similar to IgA, IgM levels were significantly higher in the colostrum consumed by female infants. Male sex was considered immunologically disadvantageous compared to female infants.

References

1. Szlagatys-Sidorkiewicz A, Zagierski M, Jankowska A, Luczak G, Macur K, Baczek T, Korzon M, Krzykowski G, Martyssiak-Zurowska D, Kaminska B. Longitudinal study of vitamins A, E and lipid oxidative damage in human milk throughout lactation. Early Hum Dev 2012; 88: 421-424.
2. Friel JK, Friesen RW, Harding SV, Roberts LJ. Evidence of oxidative stress in full-term healthy infants. Pediatr Res 2004; 56: 878-882.
3. Riordan J, Wambach K. Breastfeeding and Human Lactation. 4th ed. Sudbury, MA, USA: Jones and Bartlett Publishers; 2010.
4. Castellote C, Casillas R, Ramirez-Santana C, Perez-Cano FJ, Castell M, Moretones MG, Lopez-Sabater MC, Franch A. Premature delivery influences the immunological composition of colostrum and transitional and mature human milk. J Nutr 2011; 141: 1181-1187.
5. Koenig A, de Albuquerque Diniz EM, Barbosa SF, Vaz FA. Immunologic factors in human milk: the effects of gestational age and pasteurization. J Hum Lact 2005; 21: 439-443.
6. Islam SK, Ahmed L, Khan MN, Huque S, Begum A, Yunus AB. Immune components (IgA, IgM, IgG, immune cells) of colostrum of Bangladeshi mothers. Pediatr Int 2006; 48: 543-548.
7. Striker GA, Casanova LD, Nagao AT. Influence of type of delivery on A, G and M immunoglobulin concentration in maternal colostrum. J Pediatr (Rio J) 2004; 80: 123-128.
8. Ombrá MN, Musumeci M, Simpore J, Palano GM, Musumeci S. beta-Endorphin concentration in colostrums of Burkinabe women. Nutrition 2006; 22: 31-36.
9. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37: 112-119.
10. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-1111.
11. Selek S, Aslan M, Horoz M, Gur M, Erel O. Oxidative status and serum PON1 activity in beta-thalassemia minor. Clin Biochem 2007; 40: 287-291.
12. Aycicek A, Erel O, Kocyiigit A, Selek S, Demirkol MR. Breast milk provides better antioxidant power than does formula. Nutrition 2006; 22: 616-619.
13. Buescher ES, MclHeran SM. Colostral antioxidants: separation and characterization of two activities in human colostrum. J Pediatr Gastroenterol Nutr 1992; 14: 47-56.
14. Goldman AS, Goldblum RM, Hanson LA. Anti-inflammatory systems in human milk. Adv Exp Med Biol 1990; 269: 69-76.
15. Friel JK, Martin SM, Langdon M, Herzberg GR, Buettner GR. Milk from mothers of both premature and full-term infants provides better antioxidant protection than does infant formula. Pediatr Res 2002; 51: 612-618.
16. I’Abbe MR, Friel JK. Superoxide dismutase and glutathione peroxidase content of human milk from mothers of premature and full-term infants during the first 3 months of lactation. J Pediatr Gastroenterol Nutr 2000; 31: 270-274.
17. Alberti-Fidanza A, Burini G, Perielli G. Total antioxidant capacity of colostrum, and transitional and mature human milk. J Matern Fetal Neonatal Med 2002; 11: 275-279.
18. Zarban A, Taheri F, Chakhkandi T, Sharifzadeh G, Khorashadizadeh M. Antioxidant and radical scavenging activity of human colostrum, transitional and mature milk. J Clin Biochem Nutr 2009; 45: 150-154.
19. Sari FN, Akdag A, Dizdar EA, Uras N, Erdeve O, Erel O, Dilmén, U. Antioxidant capacity of fresh and stored breast milk: is −80°C optimal temperature for freeze storage? J Matern Fetal Neonatal Med. 2012; 25: 777-782.
20. Atlı M, Aslan M, Emin Kucukoglu M, Temur HB, Taskin A, Celik H. Peripheral lymphocyte DNA damage and oxidative status in football players after a three-day football tournament. Intern Med 2013; 52: 213-217.
21. Baysal E, Taysi S, Aksoy N, Uyar M, Celenk F, Karatas ZA, Tarakcioglu M, Bilinc H, Mumucu S, Kandlikama M. Serum paraoxonase, arylesterase activity and oxidative status in patients with obstructive sleep apnea syndrome (OSAS). Eur Rev Med Pharmacol Sci 2012; 16: 770-774.
22. Michael JG, Ringenback R, Hottenstein S. The antimicrobial activity of human colostral antibody in the newborn. J Infect Dis 1971; 124: 445-448.

23. Chirico G, Marzollo R, Cortinovis S, Fonte C, Gasparoni A. Antiinfective properties of human milk. J Nutr 2008; 138: 1801S-6S.

24. Newburg DS, Walker WA. Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatr Res 2007; 61: 2-8.

25. Lawrence RA, Lawrence RM. Breastfeeding: A Guide for the Medical Profession. 7th ed. Maryland Heights, MO, USA: Mosby/Elsevier; 2011.

26. Hennart PF, Brasseur DJ, Delogne-Desnoeck JB, Dramaix MM, Robyn CE. Lysozyme, lactoferrin, and secretory immunoglobulin A content in breast milk: influence of duration of lactation, nutrition status, prolactin status, and parity of mother. Am J Clin Nutr 1991; 53: 32-39.

27. Saint L, Smith M, Hartmann PE. The yield and nutrient content of colostrum and milk of women from giving birth to 1 month post-partum. Br J Nutr 1984; 52: 87-95.

28. Hibberd CM, Brooke OG, Carter ND, Haug M, Harzer G. Variation in the composition of breast milk during the first 5 weeks of lactation: implications for the feeding of preterm infants. Arch Dis Child 1982;57:658-662.

29. Prentice A, Prentice AM, Cole TJ, Whitehead RG. Determinants of variations in breast milk protective factor concentrations of rural Gambian mothers. Arch Dis Child 1983; 58: 518-522.

30. Kawano A, Emori Y. Changes in maternal secretory immunoglobulin A levels in human milk during 12 weeks after parturition. Am J Hum Biol 2013; 25: 399-403.

31. Martin RJ, Fanaroff AA, Walsh MC. Fanaroff and Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant. 9th ed. PA, USA: Saunders/Elsevier; 2015.

32. Bachour P, Yafawi R, Jaber F, Choueiri E, Abdel-Razzak Z. Effects of smoking, mother's age, body mass index, and parity number on lipid, protein, and secretory immunoglobulin A concentrations of human milk. Breastfeed Med 2012; 7: 179-188.

33. Lok W, Anteunis LJ, Meesters C, Chenaault MN, Haggard MP. Risk factors for failing the hearing screen due to otitis media in Dutch infants. Eur Arch Otorhinolaryngol 2012; 269: 2485-2496.

34. Venero-Fernandez SJ, Suarez-Medina R, Mora-Faife EC, Garcia-Garcia G, Valle-Infante I, Gomez-Marrero L, Abreu-Suarez G, Gonzalez-Valdez J, Fabro-Ortiz DD, Fundora-Hernandez H et al. Risk factors for wheezing in infants born in Cuba. QJM 2013; 106: 1023-1029.

35. Ahle M, Drott P, Andersson RE. Epidemiology and trends of necrotizing enterocolitis in Sweden: 1987-2009. Pediatrics 2013; 132: e443-51.

36. Moscovis SM, Hall ST, Burns CJ, Scott RJ, Blackwell CC. The male excess in sudden infant deaths. Innate Immun 2013.

37. Kerstjens JM, de Winter AF, Sollie KM, Bocca-Tjeertes IF, Potijk MR, Reijneveld SA, Bos AF. Maternal and pregnancy-related factors associated with developmental delay in moderately preterm-born children. Obstet Gynecol 2013; 121: 727-733.

38. Herz K, Wohlmuth P, Liedtke B, Schmidt S, Hackeloer BJ, Hellmeyer L. Late preterms: the influence of foetal gender on neonatal outcome. Z Geburtshilfe Neonatol 2012; 216: 141-146.

39. Baez-Baez GL, Orozco-Valero Mde J, Davalos-Guzman JC, Mendez-Magana AC, Celis A. Drowning mortality trends in children younger than 5 years old in Mexico, 1979-2008. Rev Invest Clin 2012; 64: 529-534.

40. Zuppa AA, Tornesello A, Papacci P, Tortorolo G, Segni G, Lafuente G, Moneta E, Diodato A, Sorcini M, Cart S. Relationship between maternal parity, basal prolactin levels and neonatal breast-milk intake. Biology of the Neonate 1988; 53: 144-147.