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ORIGINAL ARTICLE – OPEN ACCESS

The Effect of Protein Supplementation on Body Growth Indices and Immune System Development in Premature Neonates with Very Low Birth Weight

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ABSTRACT

Objective: Complications in very low birth weight (VLBW) infants include an increase in mortality, sepsis, failure to thrive, and immune system development disorder. However, breast milk is the preferred choice for premature infants. However, no useful nutrition support exists for well-being growth in preterm infants, especially in protein quantity. Thus, this study aims to add protein supplements to breast milk for increased growth criteria and the development of the immune system.

Materials and Methods: This double-blind clinical trial included 30 VLBW infants whose weight was <1,500 g and with gestational age <32 weeks. The infants were divided into three equal groups (n=10). Groups A and B received 4.8 and 3.5 gm/kg/day, respectively. However, group C was without supplement. Growth criteria (e.g., daily weight and head circumference) and weekly length were measured in all groups. Moreover, the blood sample was given pre (day 1) and post (day 21) study to analyze white blood cells, neutrophils, lymphocyte, immunoglobulin G, immunoglobulin M, and immunoglobulin A (IgA).

Results: Based on the results of the current study, a dose of 4.8 g/kg/day of protein supplement caused a significant increase in weight and head circumference but did not affect length development. Furthermore, receiving high-dose protein supplements caused an increase in neutrophil and lymphocyte count and serum IgA concentration.

Conclusion: Receiving high-dose protein supplement caused the development of growth criteria and the development and evolution of immune system criteria especially the innate immune system that caused decrease infectious diseases such as sepsis.

Keywords: Protein supplementation, very low birth weight, powder, body growth indices, premature neonates

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INTRODUCTION

Newborns born before week 37 of gestation from the first day of the last menstruation are considered preterm by the World Health Organization (WHO) (1, 2). The growing percentage of mortalities in children <5 years old occur during infancy. About 57% of mortalities in this age group occur within the first month of life (1, 2). The very low birth weight (VLBW) level is an accurate indicator for predicting neonatal mortality. In addition, VLBW infants account for more than 50% of neonatal mortalities and 50% of neurological complications and disabilities. Infants weighing between 1,501 and 2,500 g have a 95% chance of survival (2). Physical growth of premature infants at the end of 2 years reaches that of a term newborn. However, VLBW babies will not be able to have the same level of growth and development as term neonates in the event of severe chronic complications, inadequate nutrition, or lack of adequate care (2). Birth weight and gestational age are the strongest indicators of neonatal mortality. Thus, nutrition in preterm infants is a major and important challenge for physicians (2). Premature infants have slow growth and do not show the desired growth. The higher the premature and low birth weight, the greater the mental, neurological, and maybe immune defects will be. Poor postbirth weight gaining and low weight of newborns during discharge result in some complications. The growth and development of neonatal periods form the vital foundation for the entire childhood and life (1). Premature infants receive lower nutrients intake, especially protein levels. Premature babies need special dietary supplements because they have not stayed enough in the womb to store the food they need (Fig. 1) (3). However, supplementary proteins are effective in improving weight gain and head circumference growth, height, and probably the immune system (4). The American Academy of Pediatrics and the WHO strongly recommend breastfeeding for preterm and premature infants, but it does not meet newborns' protein requirements (3). Thus, enriching breast milk to meet the needs of VLBW infants is necessary. A common strategy for enriching breast milk is the addition of a fortified milk supplement (FMS) (5–7). FMS that contains protein, calcium, and phosphorus can be added to breast milk when a premature baby can drink 120 cm^3/kg/day from his/her mother's milk (1). Studies and evidence suggest that immune systems in preterm newborns are evolved less and are less advanced than full-term neonates, and therefore the body may not show the necessary immune responses to environmental stimuli (8–15). The immature underdeveloped immune system in premature infants increases the risk of various infections, especially early and late sepsis (16–26).

The incidence of sepsis in the USA is 7.9 per 1,000 live births (12). Moreover, animal studies have shown that the addition of protein to an immature animal at birth strengthens the immune system and the maturation of cells in this system compared with the control groups. Unfortunately, no clinical studies exist in this regard. Therefore, this study was conducted to evaluate the effects of protein supplements.

MATERIALS and METHODS

Population studied

The present study was a double-blind clinical trial performed on healthy preterm infants of <32 weeks gestational age and <1,500 g in the neonatal intensive care unit (NICU) Ward of Hajar Hospital in Shahrekord City, southwest of Iran. The inclusion criteria included preterm infants of <32 weeks gestational age with a birth weight of <1,500 g who were born and fed with fortified milk supplements. Exclusion criteria included the presence of congenital malformations, necrotizing enterocolitis, the need for major surgery, evidence of intrauterine infection, chromosomal abnormalities, sepsis, and neonates weighing <1,000 g.

Sample Size and Sampling Method

Ten subjects were selected in each group using a simple random sampling method considering the limited number of eligible infants. The babies were randomly placed in one of the three groups (groups A, B, and C) and underwent the intended intervention after each baby was selected according to the inclusion criteria.

Data Collection Method and Instruments

The data were collected by measuring and observing the weight, height, and circumference of the head. The instruments used included a digital balance, nonelastic rubber tape measure, bun urea nitrogen (BUN), and pH tests. Moreover, the total number of neutrophils, lymphocytes. and immunoglobulin was measured and calculated using CBC.diff and enzyme-linked immunosorbent assay (ELISA) tests to study the evolution of the immune system.

Method

Healthy preterm infants with birth weights <1,500 g and gestational age of <32 weeks who were admitted to the NICU Ward of Hajar Hospital in Shahrekord city (Iran) based on ultrasonography or the first day of the last menstrual period LMP were enrolled in the current study according to the inclusion and exclusion criteria. The necessary intervention was performed later after receiving breast milk, 80–90 cm^3/kg fortified with a specified dose of FMS (one cup in 100 cm^3 of breast milk; Danone, Aptamil Company, Ashton Common, UK), and parental consent. They were later divided into three groups in a double-blind, randomized manner, after being homogenized in terms of gestational age, birth weight, and Apgar score at 1 and 5 min.

Group A: Received supplementary protein with a maximum dose of 4.8 g/kg/day with FMS-fortified breast milk.

Group B: Received protein with a moderate dose of 3.5 g/kg/day with FMS-fortified breast milk.

Group C: Received FMS-fortified breast milk only without receiving the supplementary protein.

This study recorded premature infant data using a digital scale at the beginning of the study and daily thereafter. The height and head circumference of the samples were later measured using a nonelastic rubber tape measure at the beginning of the study and every week. The study lasted 3 weeks (21 days) and the protein powder (1.8 g/kg/day) was started in group A on the first day. Moreover, 1.5 g/kg/day was added to the initial dose on days 7 and 14 of the study so that it reached a maximum dose of 4.8 g/kg/day, which continued until the end of the study using a precise electronic scale. In group B, protein at 1.1 g/kg/day was started on the first day and 1.1 g/kg/day was added to the initial dose on days 7 and 14 of the study to reach a maximum dose of 3.5 g/kg/day. The amount of supplementary protein was calculated and measured accurately based on the intended weights and groups. It was then dissolved in 10 cm^3 of breast milk and given daily to the newborns in groups A and B. In addition, group C received only FMS-fortified breast milk (FMS Aptamil Company). The protein amount in the FMS-fortified breast milk is 2.9 g/100 cm^3. During the protein use phase, BUN and the blood PH of the infants were checked every 2 weeks to evaluate the possible complications associated with the use of protein supplements. Some information was obtained about the average gestational age of the infant, birth weight, infant gender, Apgar score at 1 and 5 min in each of the three groups using infants' hospitalization records.

Blood samples were taken from the newborns to measure the immune system factors in two stages (the beginning of the study, before receiving the protein supplement; 24 h after receiving the last dose of protein supplement on day 22). Blood samples were sent to the laboratory to undergo CBC.diff test so that the total number of neutrophils and lymphocytes as well as immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) using the ELISA method could be examined. The kits used were manufactured in Germany and made available through the Pishtazteb Research Company. The sensitivity and specificity for this kit are 100% and 97.56%, respectively. The height, weight, and head circumference were measured three times and their mean was reported to increase the validity of the study.

Data Analysis Method

Quantitative data (height, weight, and head circumference) were described using mean and standard deviation and analyzed using analysis of variance (ANOVA) and post hoc tests such as the Tukey's test. Paired t-test was used in cases that were compared before and after treatment. All groups had three repetitions to increase credibility. After being collected, laboratory findings on the development of the immune system were analyzed using the Statistical Package for the Social Sciences statistical method, and results were later announced.

RESULTS

The basic information for each of the newborns included in the current study is shown in Table 1. This information includes the infants' gender, delivery method, receiving or not receiving ventilation aids, and the development of some of the complications caused by premature birth.

Table 1. Specifications and clinical symptoms in groups

Group indicator	Group A (n=10)	Group B (n=10)	Group C (n=10)
Male	4	5	9
Female	6	5	1
Cesarean section	10	10	10
Natural childbirth	–	–	–
RDS	9	8	8
Mechanical ventilation	3	2	1
NCPAP	7	8	9
Sepsis	–	–	–
NEC	–	–	–
IVH	–	–	–

RDS: Respiratory distress syndrome; NCPAP: Nasal continuous positive airway pressure; NEC: Necrotizing enterocolitis; IVH: Intraventricular hemorrhage

The results of the growth indices (e.g., mean weight of newborns, height, and roundness of the head) are indicated in Table 2. The data obtained from this study were analyzed using the repeated-measurement ANOVA test. Table 2 shows the mean of neonatal weight in groups A, B, and C at the beginning of the study and on days 7, 14, and 21. Table 2 also shows the weight comparison between groups (weight difference before and after study) using Mauchly's sphericity test. Table 2 shows the average height of the infants in groups A, B, and C at the beginning and days 7, 14, and 21 of the study. Table 2 shows the average head circumference of the newborns in groups A, B, and C at the beginning, and days 7, 14, and 21 of the study. Also, immune system evolution indicators, i.e., neutrophil, lymphocyte, white blood cells, IgG, IgM, IgA, and secondary factors have been identified in Table 3.

Table 4 shows the average number of neutrophils in groups A, B, and C at the beginning (day 1) and end (day 21) of the study. The results of Mauchly's sphericity test showed no significant difference in terms of the absolute number of neutrophil counts ($p>0.05$), but sphericity-assumed test showed significant mean changes in neutrophils count over time ($p=<0.0001$). Moreover, groups A and C ($p=<0.0001$) and groups A and B ($p=0.006$) showed significant difference. Conversely, groups B and C ($p=0.236$) did not show a significant difference. Table 5 shows the mean number of lymphocytes in groups A, B, and C at the baseline (day 1) and end (day 21) of the study. No significant difference exists between the groups in terms of the number of lymphocyte cells according to Mauchly's test ($p>0.05$). However, the sphericity-assumed test showed a significant mean changes in lymphocytes over time ($p=0.26$). The results of the post hoc test showed no significant difference between groups A and B ($p=0.163$) and groups B and C ($p=0.702$). However, a significant difference exists between the groups A and C ($p=0.031$) as shown in Table 5. Moreover, Table 5 shows also the average number of white blood cells in groups A, B, and C at the baseline (day 1) and end (day 21) of the study. Furthermore, no significant difference was noted between the above groups in terms of the total number of white blood cells ($p<0.05$) according to Mauchly's test ($p<0.05$). However, the results of the sphericity-assumed test showed no significant difference between the above groups in terms of changes in mean white blood cells over time ($p=<0.0001$). According to the post hoc tests, a significant difference exists between groups A and B ($p=<0.0001$) and groups C and A ($p=<0.0001$) in terms of the mean total white blood cell count. In addition, no significant difference was noted between groups B and C ($p=0.078$) which is shown in Table 5.

Table 4 shows the mean IgG value in groups A, B, and C at baseline (day 1) and end (day 21) of the study. The results of Mauchly's test of sphericity test was not significant concerning IgG level ($p>0.50$), but the sphericity-assumed test showed a significant difference between the three groups in terms of mean IgG over time ($p=<0.0001$), but the intergroup comparison indicates nonsignificant changes. Table 4 shows the mean IgM level in groups A, B, and C at baseline (day 1) and end (day 21) of the study. No significant difference between the two groups in terms of IgM values according to Mauchly's test ($p>0.05$). However, the sphericity-assumed test showed significant changes between the groups in terms of the mean IgM values over time ($p=0.001$), but the intergroup comparison indicates nonsignificant changes

Table 2. Results of changes in the mean weight of newborns, height, and roundness of the head

The variable studied	Day	A (n=10)	B (n=10)	C (n=10)	Coefficient of variation (CV)
Weight (gram)	1	122.97±1320	110.5±1260	111.96±1245.5	9.02
	7	184.06±1665.5	140.37±1400	243.66±1428.5	12.64
	14	386.55±1997.5	119.02±1582	238.66±1548	14.51
	21	37±1014.37	130.16±1819	243.23±1689	9.07
Height (centimeter)	1	39.55±1.83	39.85±1.43	41.15±1.88	4.25
	7	41.8±2.04	40.75±1.51	41.8±1.97	4.43
	14	43.15±2.39	41.7±1.33	42.4±2.04	4.52
	21	45.2±2.47	42.75±1.29	43.25±2.18	4.52
Round the head (centimeter)	1	28.5±1.24	28.8±1.11	28.3±1.22	4.17
	7	30.4±1.71	29.7±1.27	28.6±1.04	4.53
	14	32.1±1.71	30.65±1.29	29.35±1.13	4.46
	21	33.85±1.87	32.35±1.29	30±1.20	4.52

Table 3. Results of changes in the mean IgG, IgM, and IgA values in the newborns in different days by groups

The variable studied	Day	A (n=10)	B (n=10)	C (n=10)	p
IgG	1 (Beginning of the study)	4.50±0.87	4.48±1.04	4.61±0.90	IgG level (p>0.50)
	21 (End of study)	7.01±1.65	5.85±1.3	5.35±1.78	Mean IgG over time (p=<0.0001)
IgM	1 (Beginning of the study)	0.338±0.057	0.336±0.403	0.335±0.411	Groups A, B (p=0.029)
	21 (End of study)	0.393±0.056	0.388±0.057	0.391±0.057	Groups A, C (p=<0.0001)
					Groups C, B (p=0.452)
IgA	1 (Beginning of the study)	0.477±0.096	0.477±0.096	0.516±0.097	Groups A, B (p=0.029)
	21 (End of study)	1±0.109	0.774±0.105	0.612±0.100	Groups A, C (p=<0.0001)
					Groups C, B (p=0.452)

Table 4. Results of changes in the mean number of neutrophils, lymphocytes, and white blood cells in newborns

The variable studied	Day	A (n=10)	B (n=10)	C (n=10)	p
Number of neutrophils	1 (Beginning of the study)	1447.9±352.5	1382.8±454	1416.7±506.8	Groups A, C (p=<0.0001)
	21 (End of study)	5328.5±697.4	3797±480.5	2975.3±887.1	Groups A, B (p=0.006)
					Groups B, C (p=0.236)
Number of lymphocytes	1 (Beginning of the study)	4279.9±1404.9	3767±490.23	3587. 792.43	Groups A, B (p=0.163)
	21 (End of study)	5702.5±1443.78	5148.5±601.43	4297.6±641.34	Groups B and C (p=0.702)
					Groups A and C (p=0.031)
Number of white blood cells	1 (Beginning of the study)	7150.6±563.8	4254±203.5	4313.5±361.65	Groups A, B (p=<0.0001)
	21 (End of study)	10545±1760.5	8320.20±650.6	4639±487.7	Groups C, A (p=<0.0001)
					Groups B and C (p=0.078)

as shown in Table 4. Moreover, Table 4 shows the mean IgA values in groups A, B, and C at baseline (day 1) and end (day 21) of the study. The results of the sphericity-assumed test showed a significant difference between the three groups in terms of the IgA values (p=<0.0001). Moreover, no significant differences were noted between groups B and A (p=0.029) and groups A and C (p=<0.0001). However, no significant difference was noted between groups C and B (p=0.452). Table 5 shows the mean values of BUN and pH of the different groups. These two variables were within the normal range considering the mean results (BUN <9, pH = 7.35–7.45).

Table 5. Mean changes in pH and BUN in groups A, B, and C

Parameter	Studied groups	Mean±SD
PH	A	7.38 0.07
	B	7.39 0.02
	C	7.36 0.05
BUN	A	9 0.09
	B	6 0.05
	C	9 0.05

SD: Standard deviation; BUN: Blood urea nitrogen

DISCUSSION

Thirty preterm infants were randomly divided into three groups of 10 in the present study. The results of weight gaining showed that significant differences were noted between groups A and B (p=0.004), A and C (p=0.002), and B and C (p=0.002) after consuming protein supplements.

This study was performed with the assumption that high-dose supplementary proteins improve weight gain in preterm infants compared with moderate doses or the absence of the supplementary protein. Miller et al. (3) showed that the group receiving protein (4.2 g/kg/day) showed a higher weight gain compared to the group receiving the protein (3.6 g/kg/day). Moreover, the results of the study of Rochow and Biasin (6, 7) showed similar effects of high-dose protein supplements in increasing weight gain. In the present study, 4.8 g/kg/day of supplementa-

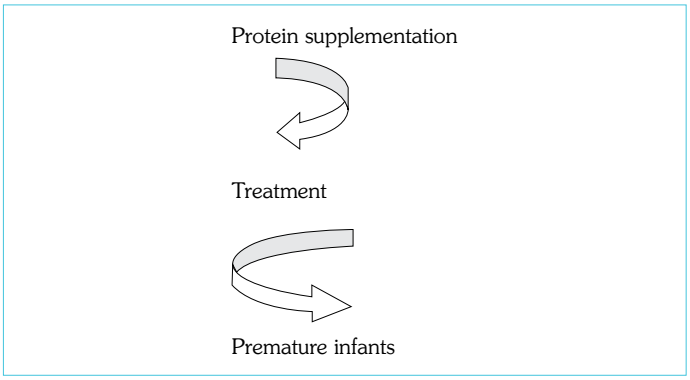

Figure 1. Protein supplementation on body growth indices and immune system development in premature neonates with VLBW

ry protein resulted in improved weight gain in group A compared with groups C and B. In addition, receiving a moderate dose of supplementary protein (3.5 g/kg/day) has a positive effect on weight gain compared with group C. The use of low-dose supplementary proteins is effective on weight gain. However, this weight gain is lower compared with high protein doses. Thus, the increased weight gain in group A is approximately twice as much as groups B and C. Moreover, the mean weight gain at the baseline and end of the study in group A was 1,478±573 g compared with groups B (556±120 g) and C (444±56 g). The study of Miller et al. (3) showed that supplementary proteins (mean dose, 4.2 g/kg/day) did not affect height increase, but Rochow et al. (10) showed that the supplementary proteins (dose, 4.5 g/kg/day) had an effect on height increase compared with the group receiving the same protein at a dose of 3 g/kg/day. Moreover, Orvay et al. (4) showed the positive effects of supplementary proteins at a dose of 4.7 g/kg/day compared with a dose of 3.7 g/kg/day on height increase. Considering the foregoing, it seems that increasing the height in preterm infants requires the use of high doses of supplementary proteins. The final mean height increase in groups A, B, and C in this study was 3.65, 2.9, and 2.1 cm, respectively. The results of statistical studies on the head circumference changes of the newborns after consuming the powder of the supplementary proteins showed significant differences between groups A and C (p=0.004) and the absence of significant differences between groups A and B (p=0.375) and groups B and C (p=0.101) over time. According to the study of Miller et al., (3) the head circumference was significantly increased in the group receiving supplementary protein (4.2 g/kg/day) compared with the group receiving supplementary protein (3.6 g/kg/day. Moreover, Biasini et al. (7) referred in their study the higher efficacy of high supplementary protein dose (4.8 g/kg/day) in increasing the head circumference compared with supplementary protein dose of 3.5 g/kg/day. However, Orvay et al. (4) did not show that the head circumference was not significantly improved in the group receiving the supplementary protein (4.7 g/kg/day) compared with the group receiving the same protein at a dose of 4.8 and 3.7 g/kg/day.

The present study showed that the high protein dose has a positive effect on head circumference increase in the studied samples. However, no significant difference was noted between groups B and C in this respect. The expected head circumference is 6 cm in healthy newborns in the first trimester of birth (2 cm per month) (1). At the end of this study, 5, 3.5, and 1.72 cm increase in head circumference of infants was obtained in groups A, B, and C, respectively. Postbirth increased head circumference is one of the important clinical indications for brain development. Thus, a strong correlation exists between poor head circumference growth after birth and insufficient development of the nervous system and cerebral palsy in the future (1). Statistical results on the absolute number of neutrophils after administration of protein supplements showed the presence of a significant difference between groups A and B (p=0.006) and groups A and C (p=0.000) and the absence of a significant difference between groups B and C (p=0.236). In other words, high protein intake can increase the number of neutrophils. The normal IgG and IgM values in neonates are 2.93–10.81 and 0.04–2.28, respectively. In addition, Rusu et al. (27) investigated the effects of whey protein on neutrophils in newborn mice. The results confirm the increase in the number of neutrophils and enhanced function of these cells, including chemotaxis, phagocytosis, and adhesion in the groups receiving high-dose protein compared with those receiving low-dose proteins (27, 28). The results of the study of Smith et al. (29) showed a reduction in diarrhea frequencies caused by rotaviruses and the improvement of immune responses in HIV-infected mice following the use of whey proteins, which is probably due to an increase in the number of neutrophils. The increased number of neutrophils in animal specimens following the use of whey proteins is probably due to the reduction of early and late neutrophils apoptosis (27, 29). In the present study, the use of supplementary proteins has led to an increase in the number of neutrophils, which is probably due to the presence and effects of whey protein in this supplement. Whey protein is a rich source of essential amino acids such as tyrosine, cysteine, and histidine, which cannot be produced by the body itself (1). A significant difference was observed between groups A and C (p=0.031) in terms of the number of lymphocytes after using protein powder supplements. However, no significant difference was noted between groups A and B (p=0.702) and groups B and C (p=0.163). In other words, the use of high-dose supplementary proteins increases the number of lymphocytes (30). The results of the study of Boudry et al. (31) were consistent with the present study and showed an increase in the number of T and B lymphocytes. Moreover, whey protein is a factor affecting the number of lymphocytes and the result obtained in the present study is probably attributed to whey protein compounds in supplementary powders and their effect on the incidence of CD markers.

The results of the study by Kashaki et al. (2018) (32) showed that the effect of protein supplementation on the growth of infants weighing <1,000 g admitted to the NICU had an average daily weight gain of 55.92 and 36.90, respectively, in the case and control groups. 91/13 92 was 30.80 g (p=0.001). The mean weekly linear growth in the case and control groups was 0.77 and 77.77 and 0.76 and 0.76 cm (p=0.939), respectively. The mean weekly growth of head circumference in the case and control groups was 0.01 and 0.10 and 0.16 and 34.34 cm, respectively (p<0.001). Various studies have focused on the protein requirements of ELBW infants proposing contradictory results. The total amounts of 150 kcal/kg/day energy and 4.2 g/kg/day protein are adequate in increasing fat-free mass in premature infants (33). The findings of another study indicated that infants receiving more protein showed a higher rate of weight gain, which indicated the pivotal role of protein in the daily nutrition of premature ELBW infants, and head circumference growth was observed in both groups, while it was significantly higher in the case group (32). The results of those studies are consistent with the current study.

The use of high-dose protein supplements improves growth and development indices, especially weight and head circumference in preterm infants. Moreover, the use of these supplements improves and evolves immune indices, especially neutrophils, lymphocytes, and IgA values, and thus reduces the risk of threatening infectious diseases such as sepsis.

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Ethics Committee Approval: The Shahrekord University of Medical Sciences Ethics Committee granted approval for this study (date: 22.11.2016, number: IR.SKUMS.REC.91-7-15/16).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES

1. Azis NMN, Abidin, KZ. Knowledge, attitudes and practice behaviors of healthcare professionals on association between periodontal disease and preterm and/or low birth weight infants: a Malaysian study. Malaysian J Public Health Med 2016; 16(1): 45–52.
2. Khan A, Nasrullah FD, Jaleel R. Frequency and risk factors of low birth weight in term pregnancy. Pak J Med Sci 2016; 32(1): 138–42.
3. Miller J, Makrides M, Gibson RA, McPhee AJ, Standfor TE, Morris S, et al. Effect of increasing protein content of human milk fortifier on growth in preterm infants born at <31 wk gestation: a randomized controlled trial. Am J Clin Nutr 2012; 95(3): 648–55. [CrossRef]
4. Costa-Orvay JA, Figueras-Aloy J, Romera G, Closa-Monasterolo R, Carbonell-Estrany X. The effects of varying protein and energy intakes on the growth and body composition of very low birth weight infants. Nutr J 2011; 10: 140. [CrossRef]
5. O'Reilly D, Dorodnykh D, Avdeenko NV, Nekliudov NA, Garssen J, Elolimy AA, et al. Perspective: The role of human breast-milk extracellular vesicles in child health and disease. Adv Nutr 2021; 12(1): 59–70.
6. Syam A, Suhartatik S, Handayani L. Assessing Breastfeeding behavior in Indonesia: Does early skin-to-skin contact affect mothers' breastfeeding performance and confidence? Pakistan J Nutrition 2018; 18(1): 86–93. [CrossRef]
7. Biasini A, Marvulli L, Neri E, China M, Stella M, Monti F. Growth and neurological outcome in ELBW preterms fed with human milk and extra-protein supplementation as routine practice: do we need further evidence? J Matern Fetal Neonatal Med 2012; 25 (Suppl 4): 72–4.
8. Palmér L, Ericson J. A qualitative study on the breastfeeding experience of mothers of preterm infants in the first 12 months after birth. Int Breastfeed J 2019; 14: 35. [CrossRef]
9. Navarro-Tapia E, Sebastiani G, Sailer S, Toledano LA, Serra-Delgado M, García-Algar Ó, et al. Probiotic Supplementation During the Perinatal and Infant Period: Effects on Gut Dysbiosis and Disease. Nutrients 2020; 12(8): 2243. [CrossRef]
10. Rochow N, Landau-Crangle E, Fusch C. Challenges in breast milk fortification for preterm infants. Curr Opin Clin Nutr Metab Care 2015; 18(3): 276–84. [CrossRef]
11. Asadi G, Aslani A, Nayebinia AS, Fathnezhad-Kazemi A. Explaining breastfeeding experiences and assessing factors affecting breastfeeding self-efficacy in mothers of premature infants: a mixed method study protocol. Reprod Health 2020; 17(1): 42. [CrossRef]
12. Maddux AB, Douglas IS. Is the developmentally immature immune response in paediatric sepsis a recapitulation of immune tolerance?. Immunology 2015; 145(1): 1-10. [CrossRef]
13. Newman TB, Draper D, Puopolo KM, Wi S, Escobar GJ. Combining immature and total neutrophil counts to predict early onset sepsis in term and late preterm newborns: use of the I/T2. Pediatr Infect Dis J 2014; 33(8): 798–802. [CrossRef]
14. Einarson TR, Vicente C, Zilbershtein R, Piwko C, Bø CN, Pudas H, et al. Pharmacoeconomics of depot antipsychotics for treating chronic schizophrenia in Sweden. Nord J Psychiatry 2014; 68(6):: 416–27.
15. Koucký M, Malíčková K, Cindrová-Davies T, Germanová A, Pařízek A, Kalousová M, et al. Low levels of circulating T-regulatory lymphocytes and short cervical length are associated with preterm labor. J Reprod Immunol 2014; 106: 110–7. [CrossRef]
16. Quinello C, Silveira-Lessa AL, Ceccon ME, Cianciarullo MA, Carneiro-Sampaio M, Palmeira P. Phenotypic differences in leucocyte populations among healthy preterm and full-term newborns. Scand J Immunol 2014; 80(1): 57–70. [CrossRef]
17. Rueda CM, Wells CB, Gisslen T, Jobe AH, Kallapur SG, Chougnet CA. Effect of chorioamnionitis on regulatory T cells in moderate/late preterm neonates. Hum Immunol 2015; 76(1): 65–73. [CrossRef]
18. Borges MC, Sesso ML, Roberti LR, de Menezes Oliveira MA, Nogueira RD, Geraldo-Martins VR, et al. Salivary antibody response to streptococci in preterm and fullterm children: a prospective study. Arch Oral Biol 2015; 60(1): 116–25. [CrossRef]
19. Luciano AA, Arbona-Ramirez IM, Ruiz R, Llorens-Bonilla BJ, Martinez-Lopez DG, Funderburg N, et al. Alterations in regulatory T cell subpopulations seen in preterm infants. PLoS One 2014; 9(5): e95867.
20. Di Filippo P, Scaparrotta A, Rapino D, Cingolani A, Attanasi M, Petrosino MI, et al. Vitamin D supplementation modulates the immune system and improves atopic dermatitis in children. Int Arch Allergy Immunol 2015; 166(2): 91–6. [CrossRef]
21. Clancy N, Onwuneme C, Carroll A, McCarthy R, McKenna MJ, Murphy N, et al. Vitamin D and neonatal immune function. J Matern Fetal Neonatal Med 2013; 26(7): 639–46. [CrossRef]
22. Ahmad SM, Raqib R, Qadri F, Stephensen CB. The effect of newborn vitamin A supplementation on infant immune functions: trial design, interventions, and baseline data. Contemp Clin Trials 2014; 39(2): 269–79. [CrossRef]
23. Sheikh A, Shamsuzzaman S, Ahmad SM, Nasrin D, Nahar S, Alam MM, et al. Zinc influences innate immune responses in children with enterotoxigenic Escherichia coli-induced diarrhea. J Nutr 2010; 140(5): 1049–56. [CrossRef]
24. Patel N, Dalrymple KV, Briley AL, Pasupathy D, Seed PT, Flynn AC, et al; UPBEAT Consortium. Mode of infant feeding, eating behaviour and anthropometry in infants at 6-months of age born to obese women - a secondary analysis of the UPBEAT trial. BMC Pregnancy Childbirth 2018; 18(1): 355. [CrossRef]
25. Rochow N, Fusch G, Mühlinghaus A, Niesytto C, Straube S, Utzig N, et al. A nutritional program to improve outcome of very low birth weight infants. Clin Nutr 2012; 31(1): 124–31. [CrossRef]
26. Fenton TR, Premji SS, Al-Wassia H, Sauve RS. Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database Syst Rev 2014; 2014(4): CD003959. [CrossRef]
27. Rusu D, Drouin R, Pouliot Y, Gauthier S, Poubelle PE. A bovine whey protein extract can enhance innate immunity by priming normal human blood neutrophils. J Nutr 2009; 139(2): 386–93. [CrossRef]
28. Sugiharto S, Poulsen AS, Canibe N, Lauridsen C. Effect of bovine colostrum feeding in comparison with milk replacer and natural feeding on the immune responses and colonisation of enterotoxigenic Escherichia coli in the intestinal tissue of piglets. Br J Nutr 2015; 113(6): 923–34. [CrossRef]

29. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils *in vitro*. J Clin Invest 1989; 83(6): 2008–17. [CrossRef]
30. Pérez-Cano FJ, Castellote C, González-Castro AM, Pelegrí C, Castell M, Franch A. Developmental changes in intraepithelial T lymphocytes and NK cells in the small intestine of neonatal rats. Pediatr Res 2005; 58(5): 885–91. [CrossRef]
31. Boudry C, Buldgen A, Portetelle D, Collard A, Théwis A, Dehoux JP. Effects of oral supplementation with bovine colostrum on the immune system of weaned piglets. Res Vet Sci 2007; 83(1): 91–101. [CrossRef]
32. Kashaki M, Mazouri A, Bordbar A, Saboute M, Behnamfar Z, Talebi A. Effect of protein supplementation on the growth of infants weighing less than 1,000 grams hospitalized on the neonatal intensive care unit of Akbar Abadi Hospital in Tehran, Iran (2015-2016). Iranian J Neonatology 2018; 9(3): 49–56.
33. Hellmuth C, Uhl O, Demmelmair H, Grunewald M, Auricchio R, Castillejo G, et al. The impact of human breast milk components on the infant metabolism. PLoS One 2018; 13(6):e0197713. [CrossRef]