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Determination of breast milk cell immune function and maternal health education

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ABSTRACT

It was to investigate the breast milk cell immune function and the effect of health education on pregnant and lying-in women. 100 primiparas were randomly divided into the control group (50 cases): routine health education; the test group (50 cases): prenatal breastfeeding health education based on the control group. Breastfeeding status, as well as breast milk immune cell composition at each stage, were compared between the two groups after intervention. After the intervention, the maternal feeding knowledge score of the test group (17.3 ± 2.4) points was significantly higher than that of the control group (14.1 ± 2.9) points ($P < 0.05$); the total feeding self-efficacy score of the test group was significantly higher than that of the control group at four weeks after delivery and eight weeks after delivery ($P < 0.05$); at eight weeks after delivery, 42 parturients in test group chose exclusive breastfeeding, significantly more than 22 parturients in the control group ($P < 0.05$); during colostrum, CD3+ accounted for (57.8 ± 4.2)%, CD4+ accounted for (31.5 ± 3.7)%, CD8+ accounted for (26.2 ± 2.4)%, CD4+/CD8+ was (1.2 ± 0.3), significantly higher than those of transitional milk and mature milk ($P < 0.05$); during colostrum, IFN-γ was (1.4 ± 0.4) μg/L, IL-8 was (1.4 ± 0.4) μg/L, significantly higher than those of mature milk ($P < 0.05$). Breast milk is beneficial to improve the immune function of newborns. It is necessary to perform health education for pregnant and lying-in women and improve the breastfeeding rate.

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Introduction

The best food for newborns is breast milk, so breastfeeding is the best feeding method for infants and young children, which can provide the most needed nutrients for infants and promote their growth and development (1). Moreover, compared with other foods, breast milk is both healthy and safe, especially the immune substances contained in breast milk as well as anti-infective factors, which can help infants improve immunity and reduce the possibility of other diseases such as infection (2). The results of related studies have shown that early initiation of breastfeeding after the birth of infants helps to reduce the possibility of neonatal infectious diseases, hyperbilirubinemia, diabetes, etc., and helps to promote brain development (3). In addition, breast milk also has a large number of bioactive substances, such as growth factors, immunoglobulins, oligosaccharides, and chemokines, which not only contribute to the development of the neonatal intestine and other organs but also play an antioxidant and anti-infective role (4). Since epidemiological studies have confirmed that breastfeeding can significantly reduce the likelihood of infectious diseases in newborns within 6 months and reduce neonatal mortality, many scholars have begun to pay attention to the cellular immune function of breast milk (5).

Although with the continuous popularization of breastfeeding knowledge to pregnant and lying-in women and the implementation of relevant promotion measures, more and more pregnant and lying-in women in China choose breastfeeding, and the breastfeeding rate is increasing, there is still a large gap between the rate of exclusive breastfeeding of newborns and the duration of feeding within 6 months and the ideal standard (6). Whether pregnant women choose breastfeeding is influenced by many factors and is a fragile and variable behavior, such as maternal disease during pregnancy, preterm delivery, low birth weight, cesarean section, feeding experience, maternal education, family economic status, work reasons, and family social support (7). Many hospitals in China have begun to open exclusive courses for pregnant women in the department of obstetrics and gynecology to carry out perinatal health education in various forms. It mainly includes the benefits of breastfeeding on pregnant women and newborns, the lack of artificial feeding, perinatal breast care, correcting the wrong concept of breastfeeding, guiding pregnant women to take the correct and labor-saving breastfeeding posture and nipple contact posture, popularizing the characteristics of lactation, timely dealing with lactation deficiency or breast pain (8).

100 primipara were divided into two groups. One group was given breastfeeding health education and the other group was given routine admission health education. The final intervention effect was compared to explore the

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necessity of breastfeeding health education. In addition, the immune cytokines of breast milk were detected to explore its immune function to provide a reference value for the scientificity of breastfeeding.

Materials and Methods

Study subjects

The 100 primiparas who delivered in the department of obstetrics and gynecology of The Second Affiliated Hospital of Xingtai Medical College from June 1, 2020, to June 1, 2022, were selected as the study subjects. They were randomly divided into two groups according to whether health education was performed before delivery, the control group (50 cases): routine health education after admission; the test group (50 cases): receiving prenatal breastfeeding health education based on the control group. Inclusion criteria: infants were all born at term; singletons; parturients who delivered for the first time; parturients aged 20 to 40 years; parturients accompanied by family members; a single score of breastfeeding self-efficacy scale ≤ 3 points (as long as one < 3 points). Exclusion criteria: pregnant women have serious medical and surgical complications and can't perform breastfeeding, so continuing breastfeeding may cause increased maternal burden and aggravate the condition; parturients suffering from mental diseases and other diseases that can't cooperate with the investigator; parturients insisting on refusing to breastfeed; newborns suffering from diseases that can't be breastfed.

All pregnant and lying-in women and their families signed the informed consent form, and the trial process was approved by the ethics committee of The Second Affiliated Hospital of Xingtai Medical College Hospital.

Health education

Control group: routine health education after admission.

From 24 weeks to 26 weeks of gestation: preaching on breastfeeding-related knowledge; giving brochures on breastfeeding-related knowledge; giving a university curriculum with courses on fetus-related courses, and pregnant women chose whether to participate on their own.

At 27 weeks and 29 weeks of gestation: the necessity of exclusive breastfeeding, early contact, and early sucking within 6 months was publicized to pregnant women; problems such as blood glucose or nutrition in the perinatal period were treated in a timely manner; regular visits were made to check the learning progress of pregnant women and instructing them to listen to lessons.

From 30 weeks to 32 weeks of gestation: pregnant women were instructed to learn feeding posture; pregnant women were informed of breastfeeding teaching time in the outpatient department, and pregnant women chose whether to participate in it by themselves; regularly visit, checking the learning progress of pregnant women, and instructing them to listen to the class.

From 33 weeks to 34 weeks of gestation: publicizing the necessity of feeding as needed and guiding pregnant women to ensure adequate milk; regularly visiting and checking the learning progress of pregnant women and instructing them to listen to lessons.

At 34 weeks of gestation and above: health education on breastfeeding knowledge was carried out for pregnant women; feedbacking on the results of breastfeeding knowledge learned during pregnancy; checking the learning progress of pregnant women and instructing them to listen to lessons.

Test group: complementary measures based on the control group.

(i) Establishment of breastfeeding teaching class: The teaching class staff was composed of maternal and child specialist nurses, and all members were required to have very rich obstetric professional knowledge and nursing-related experience to be able to design breastfeeding health education programs based on self-efficacy information. It could also use multimedia, sitcom performance, and other forms of pre-employment training, training content including breastfeeding and self-efficacy-related knowledge, and maternal psychological counseling.

(ii) Assessment: The scores of the breastfeeding self-efficacy scale for expectant mothers were statistically analyzed by maternal and child specialist nurses and the difficulties that occurred in the concentration of breastfeeding for pregnant women were analyzed and solutions were given.

(iii) Plan: According to the evaluation results of the scale, personalized behavioral goals were developed, and pregnant women and their families were encouraged to participate in the development of goals. The overall goal was to improve the breastfeeding self-efficacy of pregnant women, through prenatal breastfeeding health education to help postpartum breastfeeding, increasing the duration of continuous exclusive breastfeeding. The phased goals were: at 34 ~ 35 weeks of gestation, helping pregnant women and their families master the relevant knowledge and methods about breastfeeding; at 36 weeks of gestation, training common problems to improve the processing ability of pregnant women and their families; at 37 weeks of gestation, pregnant women and their families were required to be able to master breastfeeding knowledge and improve their self-care ability.

(iv) Implementation: Breastfeeding health education was carried out three times a week for 30 minutes from 34 weeks of gestation. According to the content of education, the time and frequency of the course were designed; direct experience: multimedia or prosthesis model was used for lectures, including the correct milking and milk storage methods, neonatal feeding knowledge, and methods to breastfeeding after work. Specialist nurses guided pregnant women to experience breastfeeding personally on the spot to master the correct methods, and timely encouraged pregnant women. Breastfeeding knowledge brochures were distributed after class, and the last learning content was fed back before the next course to help pregnant women consolidate breastfeeding knowledge and skills; substitution experience: researchers and specialist nurses would select pregnant women with special characteristics in breastfeeding and communicate with pregnant women at breastfeeding seminars. Mothers with successful breastfeeding experiences, mothers of premature infants, mothers with depressed nipples, and some mothers who believed that they lacked breast milk in the early stage were chosen to share successful experiences and failed lessons during breastfeeding with pregnant women. Pregnant women and their families were encouraged to communicate with them, and pregnant women were guided to observe others' breastfeeding; verbal persuasion: instructors needed to pay attention to observing changes in maternal psychological

status, often communicated with pregnant women, and guided and encouraged pregnant women to establish confidence in exclusive breastfeeding; social support: a good supportive relationship was established between medical staff and patients, and network technology was used to strengthen communication. In addition, it was also necessary to ask family members to accompany and participate in the process of breastfeeding, to strengthen communication between family members and help mothers to obtain effective social support while also mitigating the negative breastfeeding impact of family factors.

Outcome measures after intervention

(i) Breastfeeding knowledge questionnaire: A breastfeeding knowledge questionnaire (9) was used, including two aspects of breastfeeding knowledge and skills, a total of 17 items, each question was a single choice question, with a correct answer of 1 point and a total score of 0-17 points, and a higher score indicated more breastfeeding knowledge mastered. Breastfeeding knowledge at 34 and 37 weeks of gestation was investigated using this questionnaire.

(ii) Breastfeeding Self-Efficacy Scale Short Form (BSES-SF) (10): The scale has 14 questions, including two dimensions: skill dimension and inner dimension, and nine questions in skill dimension, mainly measuring the degree of confidence in breast milk feeding skills, and five questions in inner dimension, mainly measuring the attitude and belief towards breast milk feeding. All items started with "I always could" and were scored on a 5-point scale (1 to 5 representing not confident at all ~ always confident), with a forward score, ranging from 14 to 70. This scale was used to evaluate breastfeeding self-efficacy levels at 34 weeks of gestation, 37 weeks of gestation, one week, four weeks, and eight weeks postpartum in both groups.

(iii) According to postpartum feeding methods, can be divided into three types: exclusive breastfeeding: newborns eat breast milk only; exclusively breastfed infants supplement vitamins, inorganic salts, and use drugs when necessary, but still belong to exclusive breastfeeding. Mixed feeding: milk replacer in addition to breast milk. Artificial feeding: All are fed with milk substitutes. The feeding methods of the two groups were observed within one week, four weeks, and eight weeks after delivery.

Immunocytological test

The number of T lymphocytes (CD3+, CD4+, and CD8+ cells) and the levels of cytokines (IFN-γ, IL-8) secreted by activated mononuclear cells in breast milk at different stages were measured from the perspective of cellular immunity.

Methods: Preparation of mononuclear cell suspension: human milk was diluted with Hanks solution, centrifuged at 4,000 r/min for 20 min, and degreased. After the supernatant was removed, it was washed with Hanks solution 3 times, then mononuclear cells were separated with lymphocyte separation solution, and finally, the cell concentration was adjusted to 2 × 109/L~3 × 109/L with a complete culture medium.

Detection of CD3+, CD4+, and CD8+ cells: A few isolated mononuclear cell pellets were taken to make smears, and it was fixed in pure acetone, and CD3, CD4, and CD8 positive cells were detected by alkaline phosphatase anti-alkaline phosphatase (APAAP) bridging enzyme labeling.

Detection of IFN-γ and IL-8: Mononuclear cell suspensions were stimulated with mitogen, and the supernatants were collected after 48 h of culture at 5% carbon dioxide and 37°C, and the levels of IL-8 were measured by double-antibody sandwich ELISA; the supernatants were collected at 72 h of culture, and the levels of IFN-γ were measured by double-antibody sandwich ELISA.

Statistical methods

SPSS 22.0 software was used to process and analyze the data. The enumeration data were expressed as a rate (%), and the measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$). The difference between the two groups was compared by t-test, and the continuous index between the two groups was compared by analysis of variance. $P < 0.05$ was considered statistically significant.

Results

Maternal general information

According to statistics, the mean age of the test group was (28.6 ± 5.1) years, the mean gestational age at delivery was (38.4 ± 1.3) weeks, the mean age of the control group was (28.4 ± 3.7) years, and the mean gestational age at delivery was (37.8 ± 1.6) weeks. There was no significant difference in delivery mode, expected feeding mode, and expected feeding time between the two groups ($P > 0.05$) (Table 1).

Feeding knowledge score comparison

Before the intervention, the maternal feeding knowledge

Table 1. Basic information about pregnant women.

Basic information		Test group (50 cases)	Control group (50 cases)
Age (years)		28.6±5.1	28.4±3.7
Gestational age at delivery (weeks)		38.4±1.3	37.8±1.6
Expected feeding mode (cases)	Artificial feeding	0	0
	Mixed feeding	38	42
	Exclusive breastfeeding	12	8
Expected feeding time (cases)	1 month postpartum	20	18
	1 to 6 months postpartum	22	26
	More than 6 months postpartum	8	6
Mode of delivery	Spontaneous delivery	33	30
	Cesarean section	17	20

score was (11.7 ± 2.4) points in the test group and (11.6 ± 2.8) points in the control group, and there was no significant difference between the two groups (P > 0.05). After a period of intervention, the maternal feeding knowledge score in the test group was (17.3 ± 2.4) points, which was significantly higher than that in the control group (14.1 ± 2.9) points, and the difference between the two groups was statistically significant (P < 0.05) (Figure 1).

Feeding self-efficacy score comparison

Before the intervention, the kill dimension score was (28.7 ± 5.2) points, the inner dimension score was (18.1 ± 2.2) points, and the total score was (28.7 ± 5.2) points in the test group; the skill dimension score was (29.2 ± 4.8) points, the inner dimension score was (46.8 ± 7.4) points, and the total score was (47.5 ± 7.4) points in the control group. The differences between the two groups had no statistical significance (P > 0.05) (Figure 2).

After the intervention, the maternal skill dimension was scored, and the results showed that at 37 weeks of gestation, the scores of pregnant women in the test group were (30.3 ± 4.1) points, and the scores of pregnant women in the control group were (29.8 ± 3.8) points, and there was no significant difference between the two groups (P > 0.05); Score results at one week postpartum showed the scores of pregnant women in the test group were (31.2 ± 4.4) points, and the scores of pregnant women in the control group were (30.3 ± 4.6) points, and there was no significant difference between the two groups (P > 0.05); Score results at four weeks postpartum showed the score of pregnant women in the test group was (33.8 ± 3.9) points, and the score of pregnant women in the control group was (31.5 ± 5.5) points, and there was a significant difference between the two groups (P < 0.05). Score results at eight weeks postpartum showed the scores of pregnant women in the test group were (37.4 ± 4.2) points, and the scores of pregnant women in the control group were (32.6 ± 5.1) points, and there was a significant difference between the two groups (P < 0.05) (Figure 3).

After the intervention, the psychological dimensions of pregnant women were scored. The results showed that at 37 weeks of pregnancy, the scores of pregnant women in the test group were (18.8 ± 2.5) and those in the control group were (18.5 ± 2.7). There was no significant difference between the two groups (P > 0.05); One week after delivery, the scores of pregnant women in the test group were (19.6 ± 3.2) points and those in the control group were (18.9 ± 4.1) points. There was no significant difference between the two groups (P > 0.05); Four weeks after delivery, the score of pregnant women in the test group was (22.4 ± 3.5) points, and that of pregnant women in the control group was (19.2 ± 3.7) points. The difference between the two groups was statistically significant (P < 0.05); The scoring results at eight weeks after delivery showed that the pregnant women in the test group were (24.3 ± 3.6) points and the pregnant women in the control group was (19.5 ± 3.3), with a statistically significant difference between the two groups (P < 0.05) (Figure 4).

After statistics, the total score of feeding self-efficacy of pregnant women in the test group at 37 weeks of pregnancy and one week after delivery was not significantly different from that of pregnant women in the control group (P > 0.05); The total score of feeding self-efficacy in the test group at four weeks and eight weeks after delivery

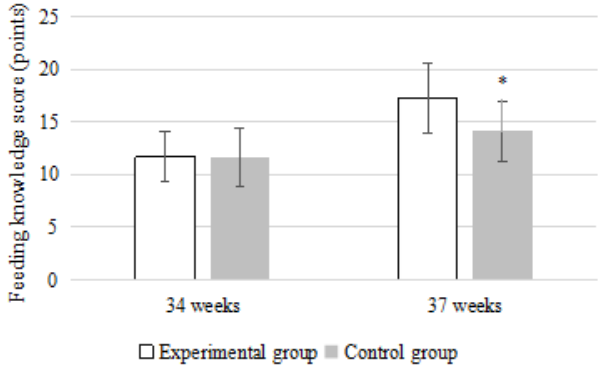

Figure 1. Comparison of breastfeeding knowledge scores between the two groups. (* indicates statistically significant difference compared to the control group, *P* < 0.05).

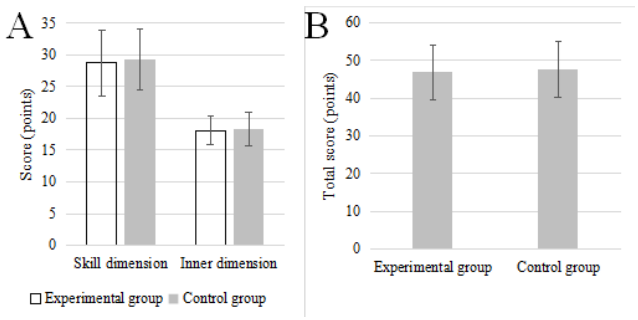

Figure 2. Comparison of maternal feeding self-efficacy scores between the two groups before intervention. (A is a skill and inner dimension score, and B is the total score).

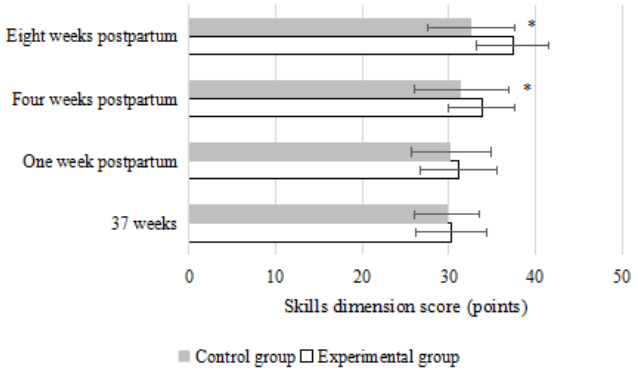

Figure 3. Comparison of scores of maternal feeding skills between the two groups after intervention. (* indicates that the difference is statistically significant compared with the control group, *P* < 0.05).

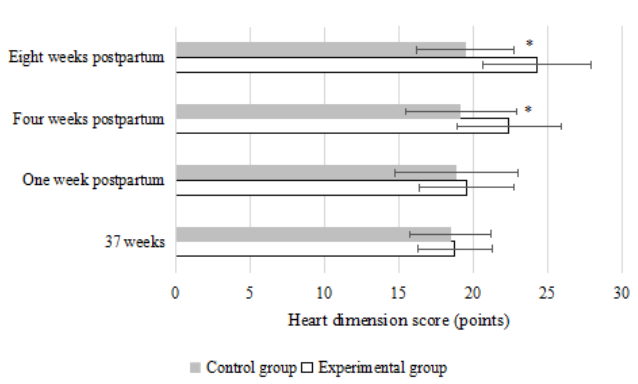

Figure 4. Comparison of scores of feeding inner dimension between the two groups after intervention. (* indicates that the difference is statistically significant compared with the control group, *P* < 0.05).

was significantly higher than that in the control group ($P < 0.05$) (Figure 5).

Comparison of exclusive breastfeeding between the two groups

After the intervention, the statistical results showed that one week after delivery, 47 pregnant women in the test group chose exclusive breastfeeding and 33 in the control group, with a statistically significant difference ($P < 0.05$); At four weeks after delivery, 45 pregnant women in the test group chose exclusive breastfeeding, while 25 in the control group, and the difference was statistically significant ($P < 0.05$); At eight weeks after delivery, 42 pregnant women in the test group chose exclusive breastfeeding and 22 in the control group, with a statistically significant difference ($P < 0.05$) (Figure 6).

Immunocytological test results

The results of T lymphocyte detection showed that CD3+ accounted for (57.8 ± 4.2)%, CD4+ accounted for (31.5 ± 3.7)%, CD8+ accounted for (26.2 ± 2.4)%, and CD4+/CD8+ accounted for (1.2 ± 0.3) in colostrum; CD3+ accounted for (43.3 ± 4.1)%, CD4+ accounted for (24.6 ± 5.4)%, CD8+ accounted for (21.3 ± 2.5)%, and CD4+/CD8+ accounted for (1.4 ± 0.3) in transitional milk; CD3+ accounted for (35.8 ± 5.4)%, CD4+ accounted for (20.7 ± 4.9)%, CD8+ accounted for (16.3 ± 2.6)%, and CD4+/CD8+ accounted for (1.4 ± 0.4) in mature milk. Compared with colostrum, the proportion of each index gradually decreased, and the difference was statistically significant ($P < 0.05$) (Figure 7). The results of IFN-γ and IL-8 content detection showed that IFN-γ was (1.4 ± 0.4) μg/L and IL-8 was (1.4 ± 0.4) μg/L in the colostrum stage; IFN-γ was (0.5 ± 0.2) μg/L and IL-8 was (0.4 ± 0.2) μg/L in the transition stage, which were significantly lower than those in colostrum stage, and the difference was statistically significant ($P < 0.05$); IFN-γ was (0.4 ± 0.1) μg/L and IL-8 was (0.3 ± 0.2) μg/L in the mature stage, which was slightly lower than that in a transition stage, but the difference was not statistically significant ($P > 0.05$) (Figure 8).

Discussion

Intervention measures were selected at 34 weeks of gestation, based on foreign studies showing that intervention at 34 weeks of gestation could significantly increase breastfeeding self-efficacy levels at four and eight weeks postpartum while breastfeeding self-efficacy levels at four and eight weeks postpartum were positively correlated with the duration of postpartum breastfeeding (11). In addition, at 34 weeks of gestation, pregnant women have received health education in prenatal clinics and at least 5 fetal university courses, mastered a certain knowledge of breastfeeding and were able to correctly judge their breastfeeding self-efficacy level. Some studies have pointed out that postpartum intervention single score ≤ 3 points, self-efficacy theory intervention can significantly improve the maternal breastfeeding self-efficacy level and improve the breastfeeding rate (12). Therefore, in this experiment, it chose to intervene in those with a single item ≤ 3 on the breastfeeding self-efficacy scale at 34 weeks of gestation.

It has been shown that correct breastfeeding knowledge can help primipara solve the problem of breastfeeding (13). However, access to correct breastfeeding knowl-

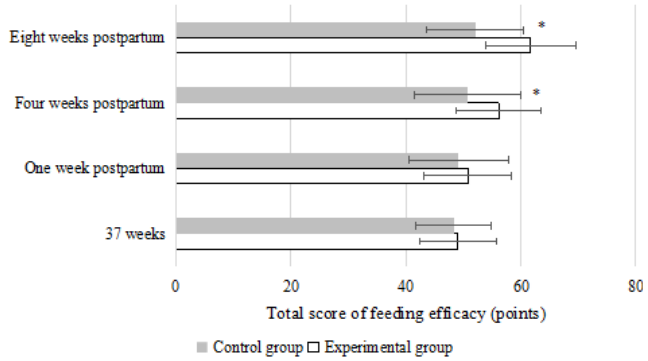

Figure 5. Comparison of total scores of self-efficacy after the intervention. (* indicates that the difference is statistically significant compared with the control group, $P < 0.05$).

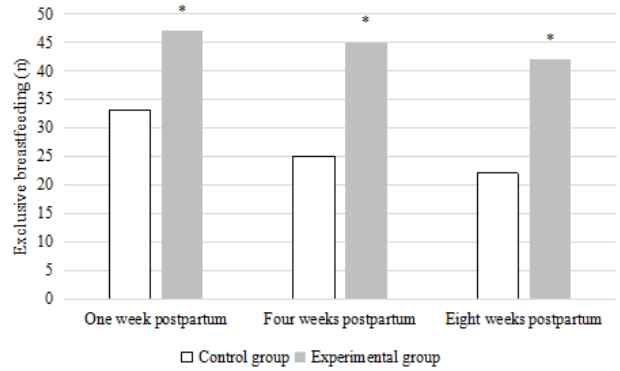

Figure 6. Comparison of exclusive breastfeeding between the two groups. (* indicates statistically significant difference compared to the control group, $P < 0.05$).

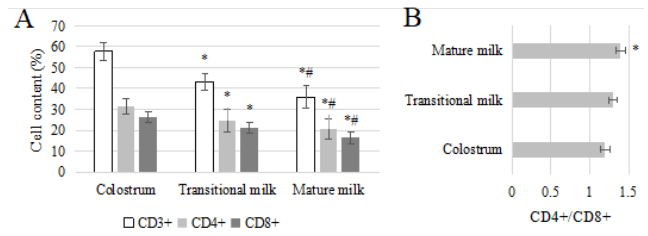

Figure 7. T lymphocyte results at different periods. (A for T lymphocyte subsets; B for CD4+/CD8+ ratio; * indicates significant difference compared with colostrum, $P < 0.05$; # indicates significant difference compared with transitional milk, $P < 0.05$).

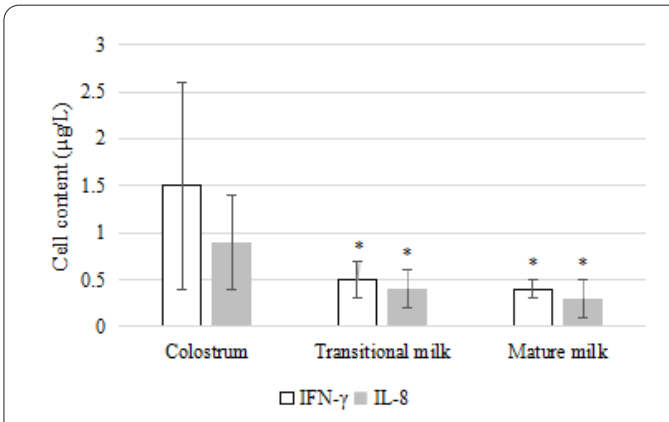

Figure 8. Detection results of IFN-γ and IL-8 at different periods. (* indicates statistically significant difference compared to colostrum, $P < 0.05$).

edge for primiparous women is not very ideal, and only 41.1% of primiparous women obtain breastfeeding-related knowledge from medical staff (14). The results of this experiment showed that there was no significant difference in maternal feeding knowledge scores between the two groups before intervention ($P > 0.05$). After a period of intervention, maternal feeding knowledge scores in the test group were significantly higher than those in the control group ($P < 0.05$). Health education has exerted the expertise of maternal and child specialist nurses, and the content changed the previously instilled education methods, which can let pregnant and lying-in women and their families fully express their ideas, and conventional outpatient breastfeeding health education does not have these. In addition, the results of this experiment showed that after the intervention, the total feeding self-efficacy scores of the test group were significantly higher than those of the control group at four weeks postpartum and eight weeks postpartum, and the difference was statistically significant ($P < 0.05$). This shows that maternal breastfeeding self-efficacy levels increase over time, which is consistent with a number of domestic and foreign studies (15,16). Now the exclusive breastfeeding rate is generally low in China, and the exclusive breastfeeding rate will be affected by a variety of factors with a continuous downward trend in time (17), which is consistent with the results of this experiment.

Epidemiological studies on the relationship between infant feeding methods and infectious diseases have shown that breastfeeding can effectively reduce the prevalence of intestinal and respiratory tract infections in children and prevent neonatal urinary tract infections. This protective effect of breast milk is closely related to the immune function of breast milk (18). Breastfeeding not only provides infants with rich nutrients but also transmits a large number of immunocompetent cells and secreted cytokines to infants. It has been shown that the production of cytokines in colostrum is more pronounced and is especially important for the protective function of newborns (19). The results showed that there was a lot of CD3+, CD4+, CD8+, IFN-γ, and IL-8 factors in the milk during the colostrum stage, which showed a gradually decreasing trend. This confirms that breast milk, especially colostrum, contains many immunocompetent cells and has the effect of secreting cytokines. Previous studies have shown that IFN-γ can activate antiviral immune cells, thereby protecting the neonatal intestine against viral invasion (20); IL-8 activates neutrophils and accumulates them to the infected sites for phagocytosis, thereby enhancing neonatal anti-infective ability (21).

The results showed that breast milk, especially colostrum, contained many immune cytokines and had a protective effect on newborns, so it was necessary to do a good job in breastfeeding health education; health education for pregnant and lying-in women helped to increase maternal breastfeeding knowledge and thus improve breastfeeding rate. Due to the limitation of conditions, the subjects are pregnant and lying-in women in The Second Affiliated Hospital of Xingtai Medical College, so the applicability of health education measures may have some limitations; in addition, only the breast milk immune function is measured to explore the impact on newborns, which needs to be further improved. In conclusion, breast milk is beneficial to protect the health of newborns, and it is necessary to carry out health education for pregnant and lying-in women and improve the breastfeeding rate, which has the value of clinical promotion.

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