

Role of Lactoferrin in Prevention of Premature Rupture of Membranes

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ABSTRACT

Background: The development of the fetal membranes “amnion and chorion” begins with embryogenesis, although they do not have a direct role in the formation of the embryo or fetus. **Objective:** This study aimed to decrease the morbidities and mortalities resulting from premature rupture of membranes (PROM.).

Patients and methods: This was a cohort study conducted on 90 patients at Department of Obstetrics and Gynecology in Benha University Hospital and Zagazig General Hospital through the period from November 2022 to November 2023.

Results: A notable divergence was observed concerning the cervical length, with the first measurement yielding a p-value of 0.005 and the second measurement yielding a p-value of 0.01. Furthermore, a significant disparity was noted in the gestational age at the time of termination, as evidenced by a p-value of 0.029. When evaluating the effectiveness of lactoferrin (LF) in averting membrane rupture, lactoferrin demonstrated a sensitivity of 75.6%, a specificity of 57.8%, and predictive values for positive and negative outcomes of 64.2% and 70.3% respectively, albeit with a non-significant p-value of 0.163. In terms of neonatal vital signs (including pulse rate and respiratory rate) and neonatal weight, no significant differences were discerned, as indicated by p-values of 0.69, 0.545, 0.013, and 0.091, respectively.

Conclusion: Augmentation through lactoferrin supplementation presents a conceivable strategy to diminish the incidence of PROM. Additional research is imperative to substantiate the preliminary evidence, which offers a significant conceptual foundation for the prospective utilization of lactoferrin in thwarting preterm childbirth.

Keywords: Lactoferrin, PROM, neonatal outcome.

INTRODUCTION

The ontogenesis of the fetal membranes, specifically the amnion and chorion, commences concomitantly with embryogenesis, albeit without a direct involvement in the embryonic or fetal configuration. Analogous to the embryo, the initial proliferation of the disparate layers of the fetal membranes is brisk and autonomous. The amalgamation of the amnion and chorion into a unified structure transpires by the 12th week of gestation [1].

Premature rupture of membranes (PROM) denotes the breach of the fetal membranes preceding the commencement of labor, transpiring at any gestational epoch, even at the 42nd week of gestation. Consequently, it is alternatively termed "pre-labor rupture of membranes." PROM can manifest either at term or preterm (before 37 weeks of gestation). The term "prolonged PROM" is used to describe a scenario where the rupture of membranes exceeds 24 hours, a condition correlated with an augmented risk of ascending infections [2]. Histological examinations of the locus of rupture in the fetal membranes at term have revealed a region of distinct morphology, typified by a diminution in the thickness of the cytotrophoblast layer and decidua, an augmentation in the density of the connective tissue constituents of the membranes, accompanied by a disjunction in the linkages between the chorion and amnion [3].

Human lactoferrin and bovine lactoferrin exhibit a significant degree of sequence homology and exhibit analogous multifaceted functions, including antibacterial,

antiviral, antifungal, and antiparasitic properties, as well as immunomodulatory and anti-inflammatory [4].

The fetal membranes function as a protective shield against ascending infections. Upon rupture of these membranes, both the mother and the fetus become susceptible to infection and various other complications [5]. The objective of this study was to mitigate the morbidities and mortalities associated with PROM.

PATIENTS AND METHODS

This was a cohort study conducted on 90 patients at Department of Obstetrics and Gynecology in Benha University Hospital and Zagazig General Hospital from November 2022 to November 2023.

Sample size: The patients were divided into two equal groups, 45 in each group: Group A included patients who received 100 mg of recombinant human lactoferrin (rhLf) twice a day before meals and group B involved patients who received placebo medicine (control group).

Inclusion criteria: Age ranged from 20-40 years, gestational age from 30 to 34 weeks, patient with history of preterm premature rupture of membrane, patient with history of preterm labour, patient with UTI, patient with chronic cervicitis, patient with recurrent vaginal infection, single pregnancy and living fetus.

Exclusion criteria: Twin pregnancy, uterine bleeding and IUFD.

METHODS

All patients were subjected to the following: A verbal informed consent, full history taking and thorough

clinical examination including: General examination and full obstetric examination.

Preparation for endocervical swab:

We asked the patient to do the following: Avoid having intercourse for few days before the procedure and using any vaginal products or douching.

Specimen collection: Using polyester fiber-tipped cleaning swab, to remove excess blood and mucus from the cervical os. Insertion of the collection swab into cervical canal and rotate for 15-30 seconds. Withdrawing the swab carefully, avoiding contact with the vaginal mucosa. Fully insertion of the collection swab into the diluent tube. Labelling the tube with the patient information, date and time of collection to send it for microbiological examination.

Measurement of cervical length: The ultrasound apparatus utilized was a GE P8 equipped with a Convex Probe C7-3. For optimal imaging, the cervix should encompass at least 75% of the display area, with the anterior and posterior lips of the cervix demonstrating equivalent thickness. The entirety of the endocervical canal should be distinctly visible, along with both the internal and external cervical os, as well as the virtual inner os in instances where the isthmus is present (the isthmus being the lowermost segment of the uterine corpus that evolves into the lower uterine segment as the pregnancy advances). Additionally, the calipers should be accurately positioned at the internal and external os and at the virtual inner os if the isthmus is present [6].

Ethical considerations: The study was done after being accepted by The Research Ethics Committees, Benha University and Zagazig General Hospital. All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their

confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Data acquisition throughout the study was meticulously conducted, encompassing historical documentation, fundamental clinical assessments, laboratory evaluations, and measurements of outcomes. These collected data underwent initial coding, entry, and analysis utilizing Microsoft Excel software. Subsequent to this preliminary analysis, the data were exported to the Statistical Package for the Social Sciences (SPSS version 20.0) software for advanced statistical examination. Depending on the categorical nature of the data, various statistical methodologies were employed to ascertain the significance of disparities. For qualitative variables, divergences between frequencies and percentages across groups were scrutinized using the Chi-square test. For quantitative variables within parametric independent cohorts, discrepancies between means were analyzed using the T-test. All statistical tests were executed with a bidirectional approach, with a significance threshold set at $P \leq 0.05$ for significant outcomes and $P \leq 0.001$ for outcomes of high significance. The data underwent rigorous statistical scrutiny, employing an array of statistical tests and parameters, including the mean, standard deviation, Chi-square test, the T statistic, and the level of significance. The data were judged significant if the p-values were ≤ 0.05 and of high significance if $p < 0.01$.

RESULTS

In group (A), the age distribution spanned from 20 to 40 years, whereas in group (B), it ranged from 21 to 40 years. Furthermore, there were no statistically significant differences observed between the groups concerning gravidity and parity (Figure 1).

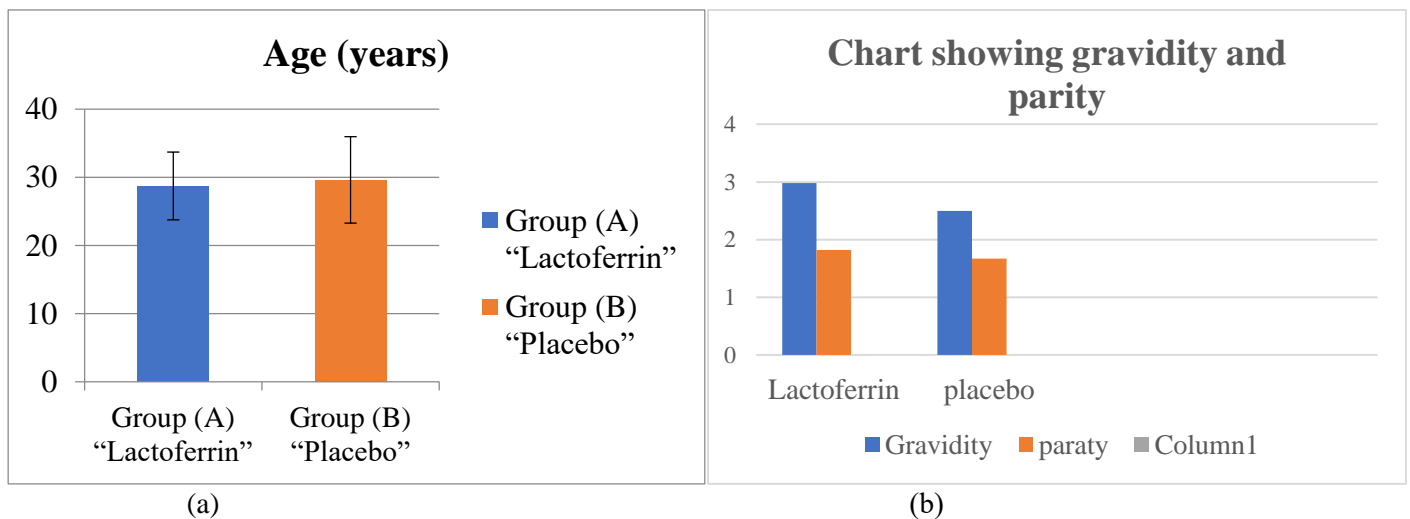


Figure (1): Demographic data of patients

There was also statistically significant lower positive second cervical swap at 34 weeks' gestation in group (A) 5 (11.1%) than in group (B) 15 (33.3%) (Figure 2)

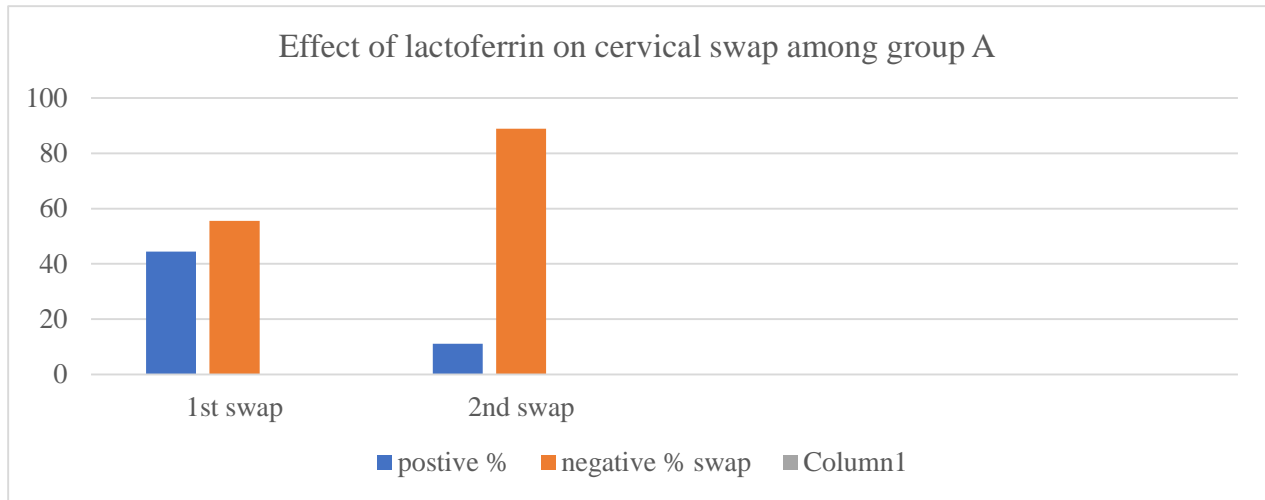


Figure (2): Evaluation of the first and second cervical swabs within the lactoferrin group.

In group A, 42.2% had CS delivery and 57.8% had VD, while in group B, 53.3% had CS, but 46.7% had VD. (Figure 3).

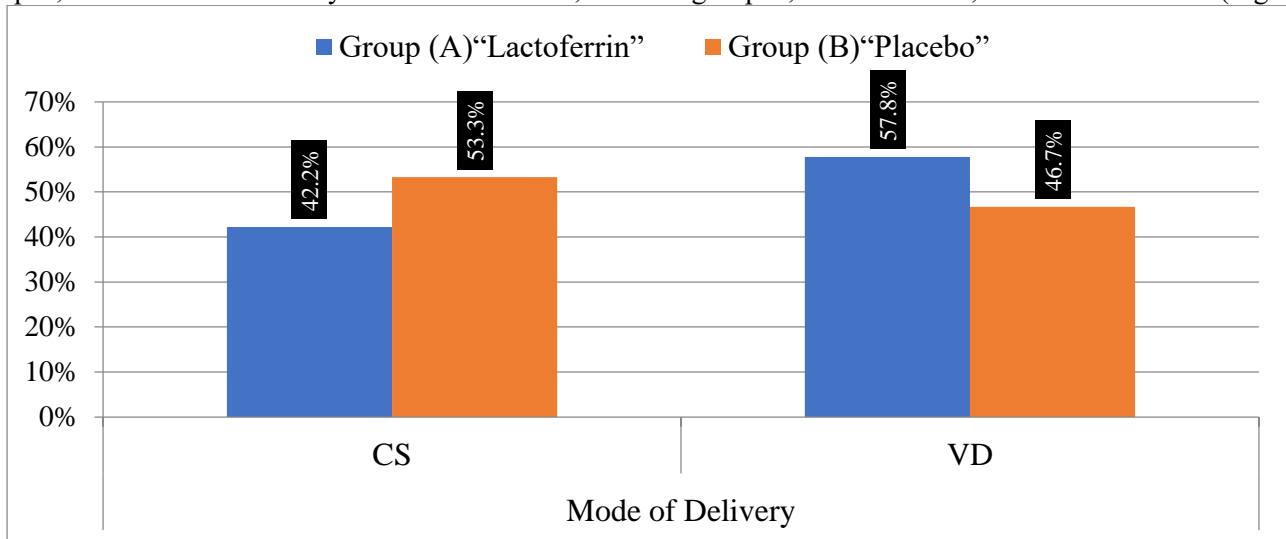


Figure 3: Comparison between two groups as regard patient's mode of delivery

Among the studied groups, there was statistically significant difference as regards cervical length with p-value 0.005 in 1st cervical length and 0.01 in the 2nd cervical length (Table 1).

Table (1): Comparison between two groups as regard patient's cervical length

	Group (A) "Lactoferrin" (n=45)	Group (B) "Placebo" (n=45)	t-Test value	P-value
1st Cervical length (mm) "30 weeks" Mean ± SD Range	31.87±8.83 20-50	27.27±6.26 17-43	2.85	0.005
2nd Cervical length (mm) "34 weeks" Mean ± SD Range	27.8±8.4 18-47	23.47±6.75 15-40	2.50	0.01

t-Independent Sample t-test for Mean ± SD; p-value >0.05 is insignificant.

Among the studied groups, there was statistically significant difference as gestational age at time termination with p value 0.029 (Table 2).

Table (2): Comparison between two groups as regard GA at termination.

GA at termination	Group (A) “Lactoferrin” (n=45)	Group (B) “Placebo” (n=45)	t-Test value	P-value
Mean ± SD	36.20 ± 1.52	35.40 ± 1.88	4.950	0.029*
Range	33- 39	30-38		

t-Independent Sample t-test for Mean ± SD; p-value >0.05 is insignificant.

No statistically significant disparities were noted in relation to neonatal vital signs (respiratory rate and pulse rate) and neonatal weight, with corresponding p-values of 0.69, 0.545, 0.013, and 0.091 respectively (Table 3).

Table (3): Comparison between two groups as regard neonatal outcomes

Neonate weight (gm)	Group (A) “Lactoferrin” (n=45)	Group (B) “Placebo” (n=45)	Test value	P-value
Mean ± SD	2851.18 ± 325.94	2674.36 ± 336.44	6.412	0.013*
Range	2121 - 3285	2054 - 3206		
Vital signs				
Pulse rate				
Mean ± SD	145.93 ± 9.34	146.73 ± 9.62	0.400	0.690
Range	125-160	130-165		
Respiratory rate				
Mean ± SD	44.67±9.55	45.82±8.45	0.608	0.545
Range	30-60	31-60		
Neonate weight (gm)				
Mean ± SD	2851.18 ± 325.94	2674.36 ± 336.44	6.412	0.013*
Range	2121 - 3285	2054 - 3206		

An evaluation of lactoferrin's effectiveness in preventing membrane rupture revealed that lactoferrin exhibited a sensitivity of 75.6%, a specificity of 57.8%, and positive and negative predictive values of 64.2% and 70.3% respectively, with a p-value of 0.163 (Table 4).

Table (4): analysis of the efficacy of lactoferrin on prevention of rupture of membranes.

	Sensitivity	Specificity	PPV	NPV	P-value
Lactoferrin	75.6%	57.8%	64.2%	70.3%	0.163

DISCUSSION

In terms of demographic data, the age range for group (A) spanned from 20 to 40 years, with a mean age of 28.71 ± 4.98 years, while for group (B), it ranged from 21 to 40 years, with a mean age of 29.62 ± 6.35 years. The comparison between the groups revealed no statistically significant differences, as indicated by a P value of 0.451. Furthermore, there were no statistically significant disparities observed between the groups concerning gravidity and parity, with respective P values of 0.281 and 0.50.

Regarding the mode of delivery in our investigation, group (A) demonstrated that 26 (57.8%) participants underwent normal vaginal delivery (NVD) and 19

(42.2%) underwent CS, while in Group (B), 21 (46.7%) experienced NVD and 24 (53.3%) underwent CS. The comparison between the groups revealed no statistically significant disparities, as indicated by a P value of 0.291.

In reference to the principal outcome of the current investigation, our findings demonstrated that the inaugural measurement of cervical length, expressed in millimeters, at the 30-week mark in group (A) spanned from 20 to 50, with an average value of 31.87 ± 8.83, while in Group (B), it extended from 17 to 43, with an average value of 27.27 ± 6.26. The intergroup comparison yielded statistically significant variances, as denoted by a P value of 0.005. Subsequent evaluation of cervical length at the 34-week juncture indicated that in group (A), the

cervical length oscillated between 18 and 47, with a mean value of 27.8 ± 8.4 . Whereas, in group (B), it fluctuated between 15 and 40, with a mean value of 23.47 ± 6.75 . The differences between the groups were deemed statistically significant, with a P value of 0.01.

There was also statistically significant lower positive second cervical swab at 34 weeks gestation in group (A) 5 (11.1%) than in group (B) 15 (33.3%), with p-value-p 0.011. Among group (A) we compared between the first cervical swab at 30 weeks gestation with 20 (44.4%) positive swab and the second cervical swab at 34 weeks' gestation with 5 (11.1%). There were statistically significant differences between groups where $P= 0.0004$

Regarding neonatal outcomes in the present study, the neonatal weight in group (A) varied between 2121-3285 grams, with a mean of 2851.18 ± 325.94 grams, while in group (B), it ranged from 2054 - 3206 grams, with a mean of 2674.36 ± 336.44 grams. There were statistically significant differences between the groups, with a P value of 0.013. In terms of NICU admissions, group (A) demonstrated that 17 (37.8%) required ICU admission, while in group (B), 25 (55.6%) required ICU admission. However, the difference between the groups was not statistically significant, with a p-value of 0.091.

The main data of our study are consistent with different studies designed to evaluate the role of lactoferrin as a protective measurement for PPRM and PTB. In a critical analysis conducted by **Otsuki *et al.***^[7], it was posited that lactoferrin could potentially function as a protective agent against inflammation in the cervical tissue of pregnant humans. The study further elucidated the antibacterial and anti-cytokine properties of lactoferrin in cervical cell lines derived from humans that are capable of producing mucus. Additionally, it was elucidated that lactoferrin possesses the capacity to mitigate the occurrence of preterm births and enhance the prognosis of preterm births induced by inflammation. Consequently, we have discerned the significance of LF in this context.

In an investigative study by **Grigor *et al.***^[8], the objective was to examine the association between serum concentrations of LF, alpha-2-macroglobulin (a2-MG), alpha-1-antitrypsin (a1-AT), and albumin, and neonatal outcomes in pregnant women experiencing PROM. Serum samples were obtained from women with PROM during the gestational period of 24-33 weeks and were juxtaposed with a control group consisting of 27 healthy women who underwent testing within the same gestational timeframe. The measurement of serum lactoferrin was conducted using ELISA techniques. The findings revealed that women with PROM, irrespective of the health status of their newborns, exhibited statistically significantly elevated serum levels of lactoferrin.

In a separate open-label clinical trial, 60 pregnant participants scheduled for amniocentesis were segmented

into three cohorts: The first group served as the control, the second group was administered lactoferrin 4 hours prior to amniocentesis, and the third group received lactoferrin 12 hours before the procedure. The TAS and OSI were evaluated in the AF samples. Additionally, the in vitro antioxidant efficacy of LF on a cellular line was assessed. The outcomes of this investigation indicated that lactoferrin led to a reduction in the OSI within the amniotic fluid, with the second group exhibiting the lowest OSI value in comparison with the control group ($P < 0.0001$). These findings align with our data, suggesting that lactoferrin mitigates oxidative stress both in vivo and in vitro. Overall, the administration of lactoferrin could be considered a promising clinical strategy as an adjunctive therapy to address pregnancy complications associated with inflammation and oxidative stress^[9].

In a clinical trial designed to investigate the potential of lactoferrin in averting preterm births attributed to cervical infections and maturation, a study was conducted between November 2009 and August 2010. This study involved 21 pregnant women, aged 22 to 36 years, who were 26 to 32 weeks into their pregnancies and afflicted with iron deficiency anemia (IDA), thereby placing them at an elevated risk of preterm delivery. The initial cohort (N=14) was administered a dosage of 100 mg of recombinant human lactoferrin (rhLf) (lactoferrina; AG-pharma®) twice daily, prior to meals, over a span of one month. In contrast, the second group (N=7) was prescribed a daily dosage of 520 mg of ferrous sulfate (Ferro-Grad®; Abbott Laboratories, USA). Following the administration of these therapeutic regimens, a transvaginal ultrasound examination was performed to evaluate the cervical length and the presence of funneling, while vaginal swabs were utilized to detect infections. Moreover, samples of cervicovaginal fluid were collected for the determination of IL-6 levels. The outcomes of this trial revealed a correlation between the oral administration of 200 mg of rhLf and the normalization of vaginal flora (evidenced by the eradication of vaginal infections) as well as a diminution in the levels of IL-6 within the cervicovaginal fluid of women who were at an increased risk of preterm delivery^[10].

In a research endeavor aimed at assessing the efficacy of vaginal lactoferrin supplementation in preventing preterm birth (PTB) among women with a history of first-trimester bacterial vaginosis and previous spontaneous PTB, a total of 847 pregnant women with a history of spontaneous PTB underwent screening for bacterial vaginosis. The findings revealed that women who received lactoferrin supplementation experienced a significantly reduced incidence of PTB before 37 weeks (25.0% versus 44.6%; $p = .02$), a higher average gestational age at delivery (37.7 ± 3.2 weeks versus 35.9 ± 4.1 weeks; $p = .01$), and a decreased rate of hospital

admission due to threatened preterm labor (PTL) (45.0% versus 70.8%; $p = .04$)^[11].

In a research investigation led by **Russo *et al.***^[12], the principal outcome demonstrated a reduction in the median risk of preterm birth by 30% due to lactoferrin. According to the findings by **Ochoa *et al.***^[13], the occurrence of sepsis-associated mortality was observed in 22 infants (10.5%) within the bovine lactoferrin cohort, as opposed to 30 infants (14.6%) in the placebo group. After adjusting for variables such as hospital and birth weight, no significant difference was noted, with a hazard ratio of 0.73 (95% CI, 0.42-1.26). Specifically, for infants with birth weights below 1500 grams, the hazard ratio stood at 0.69 (95% CI, 0.39-1.25). Over a 2-year follow-up period, growth outcomes and rates of rehospitalization were comparable across both groups, with the exception of a significantly lower incidence of bronchiolitis in the bovine lactoferrin group (rate ratio, 0.34; 95% CI, 0.14-0.86). Additionally, they revealed that the cumulative incidence of sepsis in LF group was 12/95 (12.6%) versus 21/95 (22.1%) in the placebo group, and 20% (8/40) versus 37.5% (15/40) for infants weighing ≤ 1500 grams. The adjusted hazard ratio for lactoferrin, factoring in birth weight, was 0.507 (95% CI, 0.249 to 1.034). There were four episodes of culture-proven sepsis in the LF group compared to four in the placebo group.

Based on the aforementioned data, lactoferrin is postulated to possess protective properties against infections through various mechanisms of action. One such mechanism involves the regulation of iron, which is essential for bacterial growth, primarily attributed to lactoferrin's potent iron-binding capacity. Additionally, lactoferrin has the ability to disrupt bacterial cell membranes by attaching bacterial cell membrane proteins. It also exerts an inhibitory effect on the production of inflammatory cytokines (TNF- α , IL-1 β , IL-6, and IL-8 mRNA) induced by LPS in monocytic cells by interfering with the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). Moreover, lactoferrin has been reported to regulate the differentiation and function of lymphocytes, as well as activate neutrophils, macrophages, and natural killer cells, while controlling oxidative damage^[14].

CONCLUSION

Supplementation with lactoferrin could potentially serve as a viable option to diminish the risk of PROM. However, additional research is essential to validate existing data, which offer valuable insights into the feasibility of utilizing lactoferrin for preventing preterm delivery.

Financial support and sponsorship: Nil.

Conflict of interest: Nil.

REFERENCES

1. **Moore K, Persaud T, Torchia M (2019):** Before We Are Born: Before We Are Born-E-Book: Elsevier Health Sciences, 4: 1-100.
2. **Menon R, Richardson L (2017):** Preterm prelabor rupture of the membranes: A disease of the fetal membranes. *Semin Perinatol.*, 41: 409-19.
3. **Tchirikov M, Zhumadilov Z, Winarno A *et al.* (2017):** Treatment of Preterm Premature Rupture of Membranes with Oligo-/Anhydramnion Colonized by Multiresistant Bacteria with Continuous Amnioinfusion and Antibiotic Administrations through a Subcutaneously Implanted Intrauterine Port System: A Case Report. *Fetal Diagn Ther.*, 42: 71-6.
4. **Telang S (2018):** Lactoferrin: A Critical Player in Neonatal Host Defense. *Nutrients*, 10: 9.
5. **Barinov S, Tirskaia Y, Kadsyna T *et al.* (2022):** Pregnancy and delivery in women with a high risk of infection in pregnancy. *J Matern Fetal Neonatal Med.*, 35: 2122-7.
6. **Kuusela P, Wennerholm U, Fadl H *et al.* (2020):** Second trimester cervical length measurements with transvaginal ultrasound: A prospective observational agreement and reliability study. *Acta Obstet Gynecol Scand.*, 99: 1476-85.
7. **Otsuki K, Nishi T, Kondo T *et al.* (2023):** Review, role of lactoferrin in preventing preterm delivery. *Biometals*, 36: 521-30.
8. **Grigor'eva E, Renge L, Zorina V *et al.* (2020):** Immunoregulatory and transport proteins as markers of antenatal prognosis of preterm infants' status in women with preterm prelabor rupture of membranes. *Obstetrics and Gynecology*, 4: 148-54.
9. **Trentini A, Maritati M, Rosta V *et al.* (2020):** Vaginal Lactoferrin Administration Decreases Oxidative Stress in the Amniotic Fluid of Pregnant Women: An Open-Label Randomized Pilot Study. *Front Med (Lausanne)*, 7: 555.
10. **Giunta G, Giuffrida L, Mangano K *et al.* (2012):** Influence of lactoferrin in preventing preterm delivery: A pilot study *Corrigendum in/mmr/7/4/1366*. *Molecular medicine reports*, 5: 162-6.
11. **Miranda M, Saccone G, Ammendola A *et al.* (2021):** Vaginal lactoferrin in prevention of preterm birth in women with bacterial vaginosis. *J Matern Fetal Neonatal Med.*, 34: 3704-8.
12. **Russo R, Superti F, Karadja E *et al.* (2019):** Randomised clinical trial in women with Recurrent Vulvovaginal Candidiasis: Efficacy of probiotics and lactoferrin as maintenance treatment. *Mycoses*, 62: 328-35.
13. **Ochoa T, Zegarra J, Bellomo S *et al.* (2020):** Randomized Controlled Trial of Bovine Lactoferrin for Prevention of Sepsis and Neurodevelopment Impairment in Infants Weighing Less Than 2000 Grams. *J Pediatr.*, 219: 118-25.e5.
14. **Gu Y, Hsu A, Pang Z *et al.* (2019):** Role of the Innate Cytokine Storm Induced by the Influenza A Virus. *Viral Immunol.*, 32: 244-51.