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Lactoferrin supplementation during pregnancy — a review of the literature and current recommendations

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ABSTRACT

Pregnancy is a period which requires special care and attention. Maintaining health during pregnancy helps to avoid birth related complications and is the best way of promoting a healthy birth. Besides a daily intake of folic acid, iron, iodine, vitamin D3 and A, calcium and polyunsaturated fatty-acids, as recommended by health agencies, supplementation of lactoferrin — a protein of multidirectional biological activity and proven safety of use — seems to be beneficial. A wide range of lactoferrin biological roles (including regulation of iron balance, modulation of immune responses, antimicrobial, antiviral, antioxidant, and anti-inflammatory activity) may contribute to better pregnancy and birth related outcomes.

Key words: lactoferrin; supplementation; pregnancy; lactoferrin activity

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INTRODUCTION

Lactoferrin (Lf) is an 80 kDa naturally occurring glycoprotein from the transferrin family involved in a wide range of biological functions. This multipotential protein is the most important of bioactivators in human milk and other external secretions such as saliva, tears, vaginal fluids, semen, nasal and bronchial secretions, bile, gastrointestinal fluids, urine, and neutrophil granules (15 µg in 106 neutrophils) [1, 2]. Lactoferrin concentration is particularly abundant in human milk and is lactation-stage related. The highest concentrations are found in colostrum (~7 g/L) then, significantly decrease in mature milk (2–3 g/L) [3]. By comparison, cow's milk has a relatively low concentration of protein: 1.5 mg/L in colostrum and 0.5 mg/L in mature milk [4]. Lactoferrin is a molecule characterized by a multi-directional mechanism of action — so far, studies have shown 20 different functions that it performs in mammalian organisms and research concerning new properties is still ongoing [5]. Even though lactoferrin is not included in any of the international and national recommendations to be supplemented during pregnancy, the latest studies indicate its beneficial effect on maternal and fetal health. The article gathers the researches in which lactoferrin was administered to pregnant women and may serve as a helpful contribution supporting pregnancy supplementation and preventative or adjunctive treatment in many pathological conditions as Lf reduces the risk of preterm birth, iron deficiency anemia and has a positive influence on the pregnant woman's reproductive tract microbiota [6–10]. The US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have marked lactoferrin as Generally Recognized as Safe (GRAS) for use as a food additive and dietary supplement [11, 12]. Its safety and tolerability have been confirmed by clinical studies and taking 100 mg to 4.5 g of lactoferrin a day has shown no apparent toxicity [13]. As per numerous pieces of research, human lactoferrin may be used both enteral and parenteral (i.v., s.c., body cavities washing, on skin and wounds) and bovine lactoferrin (bLf) as a species foreign protein only orally or enterally [14].

IRON DEFICIENCY ANEMIA

Anemia is one of the most frequent complications related to pregnancy, which if uncontrolled, may have serious health consequences for both mother and her offspring and directly influence the course of labor and the postpartum period. Iron deficiency anemia (IDA) is the most common

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type of anemia affecting up to 52% of pregnant women in developing countries [15]. In 2011, global prevalence of overall anemia for pregnant women was 38.2% of which 19.2% were due to iron deficiency [16, 17]. Iron deficiency anemia adversely impacts maternal and fetal well-being and is associated with increased maternal and fetal morbidity and mortality. Iron deficiency anemia increases the risk of cesarean section, preterm birth, low birth weight and intrauterine growth retardation. Affected mothers are at increased risk of developing perinatal infection, preeclampsia, and post-partum hemorrhage [18, 19]. Iron deficiency anemia may lead to fetal central nervous system disorders resulting in neurobehavioral defects such as lowered concentration and psychomotor coordination, deficiency in motor, emotional and social development. Moreover, children of mothers who developed iron deficiency anemia during pregnancy are more prone to anemia occurrence and more susceptible to infections in later development [20, 21]. Iron requirement during pregnancy increases significantly due to the development of the fetus and placenta, increasing hemoglobin and uterine muscle mass. The demand for iron in the first trimester of pregnancy is 3–4 mg/day, then increases to 7–9 mg/day in the second trimester and reaches 12–15 mg/day at the end of pregnancy [22].

Prophylaxis or treatment of anemia, if it occurs, is extremely important for the proper course of pregnancy and maternal/fetal health. Many studies have shown a beneficial effect of lactoferrin in the prevention of iron deficiency. Moreover, Lf has been suggested as a promising alternative to iron deficiency supplementation and as a preventative factor against the need of iron doses increasing [6–8]. Lactoferrin is a non-hemic iron-binding protein, a member of the transferrin family, which plays a key role in maintaining iron balance in the body and controls its physiological concentrations. Its molecule has a very high affinity to iron and binds reversibly two Fe + 3 ions. Additionally, Lf retains them in lower pH values than transferrin and can avoid proteolysis [23]. Moreover, lf by virtue of free iron sequestration controls formation of reactive oxygen species, thus protecting cells from oxidative stress [14].

Some studies conducted in groups of pregnant women with diagnosed iron deficiency anemia, have already demonstrated a significant superiority of lf when compared to iron supplements. Such conclusions were made, among others, by Italian researchers who gathered a group of over one thousand pregnant women and compared results of hemoglobin levels and ferritin concentrations after treatment with ferrous sulfate, liposomal iron, and ferric sodium EDTA enriched in lactoferrin (Lafergin). In particular, supplementation with Lafergin increases the hemoglobin levels and ferritin concentration. Furthermore, compared to control groups Lafergin indicates a longer duration of pregnancy (on average by 1.5 weeks) and higher infant weight at birth [6].

Non- heme irons formulas (sulfate, fumarate, gluconate) which are most frequently used in the treatment of anemia are moderately bioavailable and therefore administered in high daily doses. Undesirable side effects such as loss of appetite, abdominal pain, nausea, vomiting, diarrhea, and constipation are burdensome especially during pregnancy. The above — mentioned study confirmed good tolerability of Lafergin compared to iron salts. A lower incidence of gastrointestinal side effects was observed; however, no differences were found when comparing Lafergin to the liposomal iron group [6]. Similar results were obtained in clinical trials involving 300 women at different trimesters of pregnancy with diagnosed iron deficiency anemia. Ferrous sulfate (520 mg/day) or 30% iron-saturated bovine lactoferrin (bLf) (200 mg/day) were administered orally. After 30 days of treatment hemoglobin and total serum iron values were compared between the bLf group, the women treated with ferrous sulfate and untreated women. Both hemoglobin and total serum iron values were higher in the bLf treated group, independently of the trimester of pregnancy. Unlike ferrous sulfate, bLf did not result in any side effects [7].

Hepcidin is a molecule that binds the iron exporter ferroportin leading to its degradation and inhibition of iron transport from blood to cells. Lactoferrin through the downregulation of interleukin-6 (IL-6) can modulate hepcidin and ferroportin synthesis. One of the clinical trials conducted on a group of pregnant and non-pregnant women suffering from ID/IDA and treated with bovine lactoferrin or ferrous sulfate, shows that besides improving hematological parameters, bLf established iron homeostasis by decreasing serum IL-6 and increasing prohepcidin synthesis (precursor molecule of hepcidin). Ferrous sulfate instead led to an increase of IL-6 (exacerbation of inflammation), decreased prohepcidin and failed to increase hematological parameters [24].

The effectiveness of lf in the treatment of anemia was also confirmed in the study by Lepanto et al. Reduction of IL-6 and hepcidin levels by Lf, contributed to the restoration of ferritroportin-mediated iron export from cells to the blood and thus increased hematological parameters [8].

PREBIOTIC ACTIVITY

Prebiotic activity of lf on gastrointestinal and genital tract microbiota has been proved and well- documented. Lactoferrin shows both direct and indirect prebiotic activity. Lactoferrin acts directly through stimulating the growth of certain selected probiotic strains such as *Lactobacillus*, *Bifidobacterium*, and other symbiotic gut bacteria and indirectly by limiting the growth of several pathogens. Lactoferrin

also has the property of killing pathogenic microorganism without affecting probiotic bacteria [25–27].

Beneficial prebiotic activity of Lf was observed in microbiota disorders of the urogenital tract of pregnant women. As it is well known, vaginitis is an extremely common inflammation that affects women of all ages. For pregnant women, inflammations, especially those of bacterial etiology, can cause serious complications, including miscarriage, spontaneous preterm labor, premature rupture of membranes (PROM) or infection of the fetus and newborns [28–31]. Bacterial vaginosis (BV) increases the risk of preterm delivery, and the odds are twofold higher. Lactoferrin has a positive influence on the genital tract of pregnant women. Several clinical trials have shown that Lf contributes to the normalization of infection and inflammation and protects pregnant mothers against threatening complications [9, 32, 33].

One of the studies performed in a group of women with first trimester bacterial vaginosis and prior spontaneous preterm birth shows that supplementation with vaginal lactoferrin reduces the risk of preterm delivery (< 37 weeks of gestation). Outcomes were compared in women who received 300 mg of lactoferrin daily for 21 days (n = 60) with those who were not supplemented (n = 65) [9].

In another study a group of six women (5 pregnant and one non-pregnant) with a history of multiple pregnancy losses/preterm delivery and refractory bacterial vaginosis, received prebiotic Lf in a dose of 150 mg/day vaginally and 700 mg/day orally. Women started the treatment before pregnancy or from 11th–21st gestational week and continued administration of Lf until delivery. Lactoferrin significantly improved vaginal bacterial flora, *Lactobacillus* appeared after one month and became gradually dominant. Patients achieved pregnancy, delivered at term and cervical maturation related to preterm delivery was not observed [32].

The same doses of lactoferrin mentioned in the above study were administered to a 38-year-old multiparous woman with three consecutive preterm premature ruptures of membrane (pPROM) and confirmed refractory vaginitis. Lactoferrin was administered for 41 weeks starting 13 weeks before pregnancy and continued until delivery for 38 weeks. The woman achieved pregnancy and delivered a healthy infant. Lactoferrin supplementation was necessary to maintain normal vaginal microflora and resulted in the appearance of Lactobacillus after a month and its gradual dominance after three months of treatment. No Lactobacillus was detected in the vaginal discharge culture after one and three months after discontinuation of Lf administration [33].

ANTI-INFLAMMATORY ACTIVITY

The state of physiological inflammation is inherent to normal pregnancy [34]. However, pathologically elevated maternal inflammation during pregnancy is associated with adverse birth outcomes and linked to miscarriage, preterm birth, inhibition of embryo growth and preeclampsia [35]. Moreover, excessive inflammation may adversely affect programming of the fetal immune metabolic and lead to neurodevelopmental disorders [36]. Inflammatory processes result from an immune imbalance between pro- and anti-inflammatory cytokines [35]. Interleukin-6 (IL-6) plays a key role in acute phase activity and is a chief stimulator of the production of most acute phase protein [37]. IL-6, together with other pro-inflammatory cytokines, initiates the synthesis of secondary mediators, *e.g.*, cervix prostaglandins F2α (PGF2α), which contribute to the shortening of cervical length, preterm labor, preterm premature rupture of membranes (pPROM) and finally to the onset of preterm delivery [38].

Lactoferrin as a potent anti-inflammatory molecule may contribute to inflammatory suppression regulation of pro- and anti-inflammatory mediators' levels, thus improving clinical state and prolongation of pregnancy to the physiological period. Several studies demonstrated that combined oral and intravaginal lactoferrin administration may serve as prophylaxis of preterm labor by reducing IL-6 concentrations in cervicovaginal fluids and concentrations of cervicovaginal prostaglandin, which are the main activators of uterine contractions [10, 39, 40]. The study by Paesano et al. [39] showed lactoferrin effectiveness in blocking further shortening of cervical length and increasing fetal fibronectin thereby prolonging the length of pregnancy. Similar findings were reported by Locci et al. [10] in a group of 64 women at risk of preterm delivery (based on borderline cervical length and elevated cervico-vaginal IL-6) who received 300 mg of vaginal lactoferrin per day for 21 days. Sixty-four controls received no treatment. The results of the study showed a decrease in cervico-vaginal IL-6 levels and an increase in cervical length compared to the non-treated group. Moreover, regular uterine contraction and reduced cervical consistency before 37 weeks of pregnancy was found in the control group.

High concentrations of IL-6 in amniotic fluids are associated with heightened probability of miscarriage, preterm labor, and intrauterine growth retardation. One of the clinical trials on a group of women undergoing genetic amniocentesis who received 300 mg of lactoferrin intravaginally 4 or 12 hours before a procedure evaluated the concentration of 47 cytokines, chemokines, and growth factors in amniotic fluid. Among the 47 tested mediators, 24 (51.06%) were influenced by lactoferrin. 17 pro-inflammatory were down-regulated amniotic mediators whereas 7 anti-inflammatory amniotic mediators were up-regulated [40].

IMMUNOMODULATORY ACTIVITY

Lactoferrin as a natural immunomodulatory molecule has the ability to modulate and affect the response of both innate and adaptive immune system [41]. This activity is possible due to the presence of Lf receptors on a wide variety of immune cells and their capability of binding the molecule [42]. Lactoferrin plays an important role in the regulation of the innate immune response, being a first line host defence mechanism against invasive pathogens [41]. Moreover, Lf, by inducing mediators of innate response, triggers signaling pathways that impact subsequently adaptive immune cell's function [43]. Lactoferrin affects the innate immune system in a variety of ways including increasing natural killer (NK) cell activity, promoting function of neutrophils by enhancing phagocytosis, activating macrophages and limiting intracellular pathogen proliferation [44–46]. Besides the above-mentioned changes exerted on leukocytes, Lf modulates cytokines production from the leukocyte's population. In a manner dependent on the actual host's immune status, Lf may either increase or decrease pro-inflammatory cytokines production, thereby augmenting or lowering excessive immunity response [47, 48]. Lactoferrin mediates antigen presentation cell function such as APC activation, maturation, migration, and antigen presentation which directly affects lymphocyte function. Therefore, Lf is suggested to combine innate and adaptive cell function for both T and B- cells [43, 49]. Lactoferrin affects the T-cells line in a variety of ways, often related to their maturation, differentiation, and activation status. Duality of response may be developed either anti-inflammatory or stimulatory. Lactoferrin modulates and directly changes T helper Th2 and Th2 balance depending on the stimulus or antigen and increases the cytotoxicity of T-cells [50]. In addition to B lymphocytes cellular immune responses, Lf promotes maturation of immature B-cells enhancing B-cell antigen presentation [51]. Moreover, Lf can promote skin immunity and inhibit allergic responses [52]. Disturbances in the earliest stages of pregnancy and endometrial receptivity affect placental development and fetal growth with further implications of offspring phenotype and health issues in later life. Maternal tract cytokines and immune cells within the female reproductive tract play an important role in the conception, implantation, and receptivity of the endometrium. In an animal study pregnant rats were orally treated with 50 μgm/kg body weight/day of lactoferrin, starting from one week before and persisting for one week after mating. The research demonstrated that lactoferrin supplementation during pregnancy has a positive impact on some immune cytokines and parameters that may improve immune status during early pregnancy. In the present study lactoferrin increases the number of leukocytes, TNF, C-reactive protein in serum and concentrations of interleukin 1 A and interleukin 10 [53]. In human study supplementation with low doses of lactoferrin (10–20 mg) has been shown to effectively stimulate immune cell responses, thereby promoting immunity [54].

ANTIMICROBIAL ACTIVITY

Lactoferrin contributes to protection of maternal and fetal microenvironments by exerting an antimicrobial effect towards a broad-spectrum of microorganisms such as Gram-negative and Gram-positive bacteria, fungi, protozoa, and viruses both naked and enveloped [55]. Antimicrobial activity of Lf was discovered at the earliest stages and was confirmed in numerous clinical and laboratory studies. Lactoferrin antimicrobial properties are mostly due to its direct interaction with the infectious agent or by exerting a static effect by depriving microorganisms of iron, which is vital to their functioning. Lactoferrin may also work indirectly by regulating the activity of the immune cells [56]. Antimicrobial activity is exhibited both by the secretory proteins on the mucosal surfaces and those contained in the granules of neutrophils. Granulocytes are one of the most important cells in infection eradication processes. Undergoing degranulation, in which numerous enzymes (including lactoferrin) are released into the circulation or tissues affected by infection, destroys pathogens. The concentration of lactoferrin in a healthy organism does not exceed 0.5–1 μg/mL, however, it increases significantly in acute systemic or local inflammation (for example, during sepsis Lf concentration may be higher than 200 mg/mL) [57].

Antibacterial activity

The antibacterial activity of lactoferrin results mainly from its bacteriostatic mechanism of action through iron sequestration. Depriving bacteria of this important nutrient inhibits growth and downregulates their virulence expression [58]. The bactericidal mechanism of lactoferrin involves direct interaction with the bacteria cell membranes and depends on whether the bacteria are Gram positive or negative. Lactoferrin facilitates direct interaction with anionic lipid A of lipopolysaccharides (LPS) of Gram-negative bacteria. Alteration to the outer membrane permeability results in the release of LPS and consequent damage to the bacteria [59]. Lactoferrin, by affecting LPS or other surface proteins, triggers additional bacterial effect and potentiates activity of natural antibacterial such as lysozyme [60]. The positively charged molecule of Lf has the ability of binding to negatively charged molecules on the Gram-positive bacterial surface, such as lipoteichoic acid. As a result, reduction of the charge occurs facilitating the enzymatic effect of lysozyme on the underlying peptidoglycan [61]. Lactoferrin also prevents the attachment of some bacteria in the upper respiratory mucosa and intestinal tract *i.e. Haemophilus*

influenzae, enterotoxigenic *E. coli* and possesses a serine protease-like activity [62]. In an open-label cohort clinical study, bLf was administered intravaginally (in a daily dose of 300 mg/person) to pregnant women asymptomatically affected by *Chlamydia trachomatis* and showing high concentration of IL-6 in cervical fluids. After 30 days *C. trachomatis* in cervicovaginal smears and IL-6 concentration in the cervical fluid were evaluated showing 86 % of specimens negative for *C. trachomatis* and a decrease in IL-6 levels [63]. In another clinical study, administration of vaginal bovine lactoferrin resulted in a decrease of vaginal bacteria species associated with BV such as: *Gardnerella*, *Prevotella*, *Lachnospira*, *Streptococcus spp*., *Staphylococcus spp*., *Escherichia coli* [64]. As is well known, *Streptococcus agalactiae*, or Group B *Streptococcus* (GBS) is the leading infection-related cause of preterm birth, stillbirth, chorioamnionitis, funisitis, neonatal sepsis, bacteremia, and mastitis [65]. In a pregnant mouse model exposure of GBS to lactoferrin represses GBS growth and viability in a dose-dependent manner [66]. Also, a significant reduction of *S. agalactiae* was observed in vaginal smears of pregnant women after 30 days of oral lactoferrin administration [67].

Antifungal activity

The antifungal effect of Lf results from several modes of action including direct destruction of the cell membrane and wall, iron sequestration, induction of fungal apoptosis, inhibition of glucose uptake and synthesis of DNA and other proteins by fungal cells, and stimulation of host cell immune mechanisms [68, 69]. Although Lf shows activity toward human pathogenic fungi such as *Candida*, *Trichophyton*, *Aspergillus* and *Rhodotorula*, the antifungal activity of Lf may vary among the fungi genus and is species dependent. The candidacidal activity of Lf results from its direct interaction with the fungal cell surface leading to cell damage. Lactoferrin has been shown to be highly fungicidal for *C. tropicalis* and *C. krusei* with subsequently decreasing susceptibility as follows: *C. albicans* > *C. guilliermondii* > *C. parapsilosis* > *C. glabrata*, with the last one almost resistant to Lf [69]. Conversely, host defence against *Aspergillus fumigatus* is based on iron sequestration [70]. Lactoferrin acts synergistically with antifungal drugs [68, 70]. Synergism was reported i.e. with fluconazole, which combined with Lf to enhance the growth inhibitory effects of *Candida spp*. and *Candida albicans* [71]. In a study by Giunta et al. [67] 21 pregnant women received orally 200 mg of recombinant human lactoferrin, and a significant reduction of abnormal vaginal flora (71% to 15%) was observed with total reduction of *C. albicans* [67].

Antiparasitic activity

Antiparasitic activity of Lf has been shown against *Giardia lamblia*, *Entamoeba histolytica*, *Pneumocystis carinii*, *Trypanosoma cruzi*, *Toxoplasma gondii*, and *Plasmodium spp*. [72–77]. The molecular mechanism of antiparasitic activity of Lf is complex but usually involves interference with the acquisition of iron necessary for parasitic growth *e.g.*, Trypanosoma [72]. Transplacental fetus toxoplasmosis infection in pregnant women poses a risk of the classic triad of chorioretinitis, intracranial calcifications, and hydrocephalus. Lactoferrin can bind to specific membrane receptors of *Toxoplasma gondii* and inhibit its intracellular growth in the host cell. Moreover, Lf also reduces *T. gondii* infectivity [73, 74]. Lactoferrin inhibits the growth of *P. falciparum* in a dose-dependent way, and it has been suggested that the complex of Lf and iron generates oxygen-free radicals, which may cause membrane damage to both erythrocyte and parasite [75]. Studies have also shown giardicidal and amoebicidal activity of Lf. Lactoferrin can bind the lipids on the membrane of *E. histolytica* causing membrane disruption and damage to the parasite [76, 77].

Antiviral activity

Lactoferrin exhibits antiviral activity against a broad range of human and animal RNA and DNA-viruses. Depending on the virus type, Lf prevents viral entry by binding to host cell surface molecules, which are used by viruses as receptors or co-receptors, by direct binding to the viral particles or by interfering with the attachment factors. Besides inhibition of viral entry into host cells, Lf inhibits viral replication and regulates immune response after infection [78]. *In vitro* studies and clinical trials on humans have demonstrated the anti-viral properties of Lf against enveloped and naked viruses including influenza, parainfluenza, human papilloma virus, herpes simplex virus type 1 and 2, cytomegalovirus, human immunodeficiency virus, hepatitis B and C virus, respiratory syncytial virus, hantavirus, coronavirus, rotavirus, polio, and adenovirus [79]. The current COVID-19 pandemic has forced scientists around the world to search for new treatment drugs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). One of the in vitro studies conducted in 2011 showed the effectiveness of Lf in blocking SARS-CoV from invading host cells [80]. Additionally, considering the 75% genetic similarity between SARS-CoV and SARS-CoV-2, it was assumed that Lf may also inhibit the infection with the second variant of the virus and serve as a potential preventative and adjunct treatment for COVID-19 [81, 82]. In a clinical study conducted in 2020, liposomal bovine Lf was implemented among people with SARS-CoV-2 infection, showing a beneficial effect in most subjects and the reduction in the intensity of such symptoms of infection such as: headache (in all subjects), cough (in 50% of people vs 61.11% control), myalgia (44.44% vs 66.67%), fatigue/weakness (66.67% vs 94.44%) [83]. The anti-viral activity of Lf against

SARS-CoV-2 may result from the inhibition of viral binding to the host cell surface in the early phase of virus amplification in the salivary glands, pharynx, and upper respiratory tract [80]. One of the most likely mechanisms is competition for binding to glycan chains which are used by many CoVs either as receptor determinants or as attachment factors, decreasing the accumulation of SARS-CoV-2 on the host cell membrane [80, 84]. Lactoferrin may also compete for ACE2 receptor access and therefore block the initial interaction between virus and host cells [80]. Also, interaction of Lf with proteins spike (S), membrane (M), and envelope (E) present on the SARS-CoV-2 membrane is possible [85]. Pregnant women are at increased risk of severe illness from COVID-19, morbidity and mortality when compared to non-pregnant women. A symptomatic course of infection increases the risk of caesarean birth and fetal distress during active labour. SARS-CoV-2 infection in pregnancy seems to be associated with increased risks of preeclampsia, stillbirth, preterm birth, premature rupture of membrane and NICU admission [86, 87]. As Lf levels increase during SARS-CoV-2 infection, Lf might be a protective factor while inhibiting SARS-CoV-2 entry into cells [88] (Tab.1).

CONCLUSIONS

Lactoferrin is a multidirectional molecule with excellent potential for use as a preventative or adjunctive treatment in many pathological conditions. Its proven safety means that Lf can be recommended and successfully used by pregnant women not only for the prevention and treatment of anemia but also of many other pregnancy related conditions, primarily because of its antimicrobial properties.

Table 1. Clinical studies with lactoferrin performed on pregnant, in chronological orders

Researchers, Year, Country	Study participants	Intervention	Outcomes
Paesano et al. 2006; Italy [7]	Pregnant women (n = 300) with mild ID/IDA at 12th–31st weeks of pregnancy	n = 107 received 100 mg of bLf (30% iron saturated) twice a day p.o. before meals for 30 days; n = 102 received 520 mg of ferrous sulphate daily p.o. for 30 days; n = 91 refusing treatment	Increase of Hb and total serum iron (TSI); No gastrointestinal (GI) side effects
Nappi et al. 2009; Italy [89]	Pregnant women (n = 97) between 12th–36th weeks of pregnancy, suffering from IDA	n = 49 received orally 100 mg of bLf twice a day before meals for four weeks; n = 48 received 520 mg of ferrous sulfate for four weeks	Increase of Hb, TSI, serum ferritin (sFtn); Decrease of total iron binding capacity (TIBC); Lower incidence of GI side effects
Paesano et al. 2009; Italy [90]	Pregnant women (n = 143) suffering from ID/IDA	n = 60 received orally 100 mg of bLf (30% iron saturated) twice a day for 30 days; n = 50 received 520 mg of ferrous sulfate; n = 33 refusing treatment	Increase of RBC, Hb, TSI, sFtn; No side effects
	Pregnant women (n = 5) suffering of ID and IDA	n = 5 received lactoferrin for 30 days, followed by 30 days of ferrous sulfate treatment	Increase of RBC, Hb, TSI, sFtn and decrease of IL-6 following 30 days of Lf treatment; After 30 days of ferrous sulfate treatment blood values decreased and Il-6 increase
Paesano et al. 2010; Italy [24]	Pregnant women (n = 75) suffering from IDA/ID at third trimester of pregnancy	n = 30 received norally 100 mg of bLf (30% iron saturated) twice a day before meals for 30 days; n = 33 received 520 mg of ferrous sulfate for 30 days; n = 12 refusing treatment	Increase of RBC, Hb, Ht, TSI, sFtn; Decrease of IL-6 and increase of prohepcidin; Lower incidence of GI side effects
Giunta et al. 2012; Italy [67]	26–32 weeks pregnant women (n = 21) suffering from IDA and abnormal vaginal flora (AVF), at risk of preterm delivery	n = 14 received orally 100 mg of rhLf twice a day before meal for one month; n = 7 received 520 mg of ferrous sulfate once a day	Normalization of vaginal microflora (reduction of AVF from 71% to 15% in rhLf vs 71% to 57% in ferrous sulfate group); Decrease of cervicovaginal IL-6

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Table 1. cont. Clinical studies with lactoferrin performed on pregnant, in chronological orders

Researchers, Year, Country	Study participants	Intervention	Outcomes
Paesano et al. 2012; Italy [39]	Cohort of (n = 161) pregnant women in various trimester of pregnancy with ID/IDA and normal uterine cavity and subcohort of (n = 11) pregnant women with ascertained PTD threat not related to cervical and vaginal infection and PROM	Oral administration of 100 mg bLf (20% iron-saturated) twice a day, before meals, for at least 4 weeks until delivery; Additional intravaginal administration of 100 mg lyophilized bLf (20%iron-saturated) every 8 hours for 4 weeks of gestation, no longer than 37th week of gestation in subcohort	In cohort: increase of RBC, Hb, TSI, sFtn and decrease of IL-6; In subcohort: Increase of RBC, Hb, TSI, sFtn; Decrease of serum and cervicovaginal fluids IL-6, cervicovaginal PGF2α. Suppression of uterine contractility; Blocked further shortening of cervical length; Increase of fetal fibronectin thus prolonging the length of pregnancy
Locci et al. 2013; Italy [10]	Pregnant women (n = 128) at 20th–24th weeks of gestation, showing borderline cervical length (25–29 mm) and elevated cervicovaginal IL-6	n = 64 received 300 mg of bLf in vaginal tablets for 21 days; n = 64 no treatment	Decrease in IL-6 levels and an increase in cervical length
Paesano et al. 2014; Italy [91]	Pregnant women (n = 295) at 6th–8th weeks of pregnancy affected by hereditary thrombophilia (HT) suffering from ID/IDA	n = 156 received 100 mg of bLf p.o. twice a day, until delivery; n = 139 received 520 mg of ferrous sulfate once a day, until delivery	Increase of RBC, Hb, TSI, sFtn; Decrease of IL-6; Lower incidence of GI adverse effects; No maternal, fetal and neonatal adverse effects; No miscarriage in bLf group (0 vs 5 in ferrous sulfate)

Vesce et al. 2014; Italy [92]	Pregnant women (n = 60) Undergoing genetic amniocentesis at the 16th Gestational week and at risk of inflammation	n = 20 received 300 mg of bLF in vaginal tablet, once 4 h prior amniocentesis;
		n = 20 received 300 mg of bLF in vaginal tablet, once 8 h prior Amniocentesis;
		n = 20 received no treatment
			Decrease in amniotic fluid IL-6

Otsuki et al.; 2014; Japan [33]	Case report of 38-year-old multiparous woman with three consecutive preterm (pPROM) and confirmed refractory vaginitis	Vaginal suppositories (150 mg/day) and and p.o. tablets (700 mg/day) of bovine LF, starting before pregnancy and continued until delivery for 38 weeks	Appearance of Lactobacillus after a month and its gradual dominance after three months of treatment. Woman achieved pregnancy and delivered a healthy infant No *Lactobacillus* in the vaginal discharge culture after one and three months after Lf discontinuation
Cignini et al. 2015; Italy [6]	Pregnant women (n = 1143) in the first trimester of pregnancy to 39 weeks, suffering from IDA	n = 82 received bLf from Lafergin — dietary multicomponent based on ferric sodium EDTA, lactoferrin, vitamin C and B12, from 0 to the end of gestation; n = 534 received ferrous sulfate n = 527 received liposomal iron	Increase of Hb, sFtn; Higher mean birth weight and longer duration of pregnancy; Lower incidence of gastrointestinal side effects
Mehedintu et al. 2015; Romania [93]	Pregnant women (n = 307) between 12–32 weeks of pregnancy, suffering from ID/IDA	n = 119 pregnant women with ID and n = 188 patients with IDA in pregnancy received 100 mg bovine lactoferrin twice a day, before meals, for 90 days	Correction of iron deficiency; Increase of Hb and TSI; Lower incidence of GI side effects.
Rezk et al. 2016; Egypt [94]	Pregnant women (n = 200) in the second trimester of pregnancy, suffering from IDA	n = 100 received 150 mg of dried ferrous sulphate capsules once daily for eight consecutive weeks; n = 100 received 250 mg lactoferrin capsules once daily for eight consecutive weeks	Higher Hb in Lf group (2.26 ± 0.51 vs 1.11 ± 0.22 in ferrous sulfate); Less gastrointestinal side effects

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Table 1. cont. Clinical studies with lactoferrin performed on pregnant, in chronological orders

Researchers, Year, Country	Study participants	Intervention	Outcomes
Trentini et al. 2016; Italy [95]	Pregnant women (n = 111) undergoing genetic amniocentesis at the 16th–18th gestational week	n = 54 received 300 mg of bLf in vaginal tablet, once, 4 h before amniocentesis; n = 57 no treatment	Lower levels of the inflammatory markers in the amniotic fluid: PGE2, MMP-9 and TIMP-1 compared to control

Maritati et al. 2017; Italy [40]	Pregnant women (n = 60) undergoing genetic amniocentesis at the 16th gestational week	n = 20 received 300 mg of bLF in vaginal tablet, once, 4 h prior amniocentesis;
		n = 20 received 300 mg of bLF in vaginal tablet, once, 8 h prior amniocentesis;
		n = 20 untreated patients
			Down-regulation of 17 pro-inflammatory amniotic mediators with highest significance for IL-9, IL-15, IFN- γ, IP-10, TNF-α, IL-1α, MCP-3;
			Up-regulation of 7 anti-inflammatory amniotic mediators:IL-17, FGF-b, G-CSF, GM--CSF, MCP-1, IL-3, SDF-1

Otsuki, Imai; 2017; Japan [32]	n = 6 women (5 pregnant and 1 non-pregnant) with a history of multiple pregnancy losses or preterm delivery and refractory BV	BLf in vaginal suppositories (150 mg/day) and and p.o. tablets (700 mg/day), starting before pregnancy (n = 2) or from 11 to 21 weeks of gestation (n=4) until delivery	Appearance of *Lactobacillus* after a month of treatment and its gradual predominance; Women delivered healthy infants at term
Sessa et al.; 2017; Italy [63]	Pregnant women (n = 7) asymptomatically affected by *Chlamydia Trachomatis*, having high concentration of IL-6 in cervical fluids	Vaginal administration of bLf in a dose of 100 mg, every 8 h for 30 days	86% of cervicovaginal smears negative for *C. trachomatis* and decrease of IL-6 concentration in the cervical fluid; Women achieved pregnancy and delivered at term
Lepanto et al. 2018; Italy [8]	Pregnant women (n = 90) between 6–8th weeks of pregnancy, suffering from IDA of which: n = 20 with β-minor thalasemia n = 70 with heredity thrombophilia (HT) n = 20 suffering from various pathologies And non-pregnant women (n = 88) suffering from IDA	Pregnant and non-pregnant women received 100 mg of 20–30% iron — saturated bLf, p.o., twice a day; 329,7 mg of ferrous sulfate once a day as a control	In pregnant and non-pregnant women: Increase of RBC, Hb, TSI, sFtn; Decrease of serum IL-6 and hepcidin
Darwish et al. 2019, Egypt [96]	Pregnant women (n = 120) with gestational age between 14–28 weeks with confirmed clinical and laboratory evidence of IDA	n = 60 received pineapple flavored lactoferrin oral sachets 100 mg twice daily continuously for 4 weeks and health education; n = 60 received total dose infusion (TDI) of low-molecular weight iron (LMW) dextran	In both groups increase of Hb, MCH, TSI, sFtn, TIBC; Higher MCV and MCH in TDI of LMW dextran group Higher serum iron and serum ferritin in Lf group

Trentini et al. 2020; Italy [97]	Pregnant women undergoing genetic amniocentesis at the 16th gestational week	n = 20 received 300 mg of bLF in vaginal tablet, once, 4 h prior amniocentesis;
		n = 20 received 300 mg of bLF in vaginal tablet, once, 8 h prior amniocentesis;
		n = 20 received no treatment
			Lf decreased oxidative stress by lowering the levels of amniotic thiobarbituric acid reactive substances (TBARS) and increasing amniotic total antioxidant status (TAS)

Miranda et al. 2021; Italy [9]	n = 125 pregnant women with first trimester diagnosis of BV and prior spontaneous preterm birth (PTB)	n = 60 women received 300 mg/day of vaginal LF tablets for 21 days while n = 65 did not	Lower rate of preterm birth (25.0% in LF group vs 44.6% in control group) Lower mean gestational age at delivery (37.7 weeks vs 35.9 weeks) Lower rate of admission for threatened preterm labor (45% vs 70.8%)
Ali et al. 2021; Egypt [98]	Pregnant women (n = 48) at risk of PROM	n = 24 received 100 mg of recombinant human lactoferrin p.o. twice a day for 30 days; n = 24 received placebo as control	Lowered risk of PROM

IDA — Iron deficiency anemia; bLf — bovine lactoferrin; Hb — hemoglobin; TSI — total serum iron; GI — gastrointestinal; sFtn — serum ferritin; TIBC — total iron binding capacity; RBC — red blood cells; Lf — Lactoferrin; HT — heredity thrombophilia; LMW — low-molecular weight iron; PTB — prior spontaneous preterm birth; TBARS — thiobarbituric acid reactive substances; TAS — total antioxidant status; BV — bacterial vaginosis

Article informations and declarations

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Conflict of interest

The authors declare no conflict of interest.

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