The status of probiotics supplementation during pregnancy

Noroyono Wibowo,[1] Johanes C. Mose,[2] Made K. Karkata,[3] Bangun T. Purwaka,[4] Herman Kristanto,[5] Maisuri T. Chalid,[6] Yusrawati,[7] Makmur Sitepu,[8] Juneke J. Kaeng,[9] Nuswil Bernolian,[10] Damar Pramusinto,[1] Rima Irwinda[1]

[1] Department of Obstetric and Gynecology, Faculty of Medicine, Universitas Indonesia, Ciptomangunkusumo Hospital, Jakarta, Indonesia

[2] Department of Obstetric and Gynecology, Faculty of Medicine, Padjajaran University, Bandung, Indonesia

[3] Department of Obstetric and Gynecology, Faculty of Medicine, Udayana University, Bali, Indonesia

[4] Department of Obstetric and Gynecology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia

[5] Department of Obstetric and Gynecology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

[6] Department of Obstetric and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

[7] Department of Obstetric and Gynecology, Faculty of Medicine, Andalas University, Padang, Indonesia

[8] Department of Obstetric and Gynecology, Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia

[9] Department of Obstetric and Gynecology, Faculty of Medicine, Sam Ratulangi University, Medan, Indonesia

[10] Department of Obstetric and Gynecology, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

ABSTRAK

Probiotik telah sejak lama diketahui fungsinya dalam dunia medis. Salah satu aplikasinya adalah penggunaan probiotik selama kehamilan. Studi terbaru menunjukkan bahwa suplementasi probiotik prenatal *dinilai aman dan berguna. Pada review ini, kami menyorot fungsi probiotik pada ibu hamil. Beberapa* strain *probiotik terbukti mampu mencegah kelahiran prematur dan preeklampsia pada ibu yang mendapatkan suplementasi* prenatal*, mengurangi kemungkinan dermatitis atopi meski tidak mampu mencegah kejadian asma dan sesak. Mekanisme yang terkait dengan fungsi ini masih belum diketahui secara pasti, namun diperkirakan sangat berkaitan dengan regulasi sel T.*

ABSTRACT

Probiotics have been known for their use in medical field for quite a long time. Strong evidences are now available for the use of probiotics in clinical setting. One of the current issues on this topic is the use of probiotics in pregnancy. Recent studies showed that probiotics may be safe and beneficial for prenatal supplementation. In this review, we highlighted several proven use of probiotics supplementation in pregnant women. A few selected strains of probiotics showed promising outcome to prevent preterm labor and preeclampsia, and to reduce atopic eczema but not asthma and wheezing, in offspring of women who had prenatal probiotics supplementation. The mechanism of action responsible for this effect is closely related to the regulation of T cells, although the exact pathways are not defined yet.

Correspondence author: **Noroyono Wibowo**, wibowonoroyono@yahoo.com

The role of microorganisms in food has been known for a long time. The first person to suggest the role of microorganism in food spoiling was A. Kircher, in 1658, who examined decaying bodies, meat, milk, and other substances and said the process was caused by "worms" invisible to the naked eye.[1] But it was not until 1837 that L. Pasteur showed that microorganisms caused the souring of milk and used heat for the first time to prevent food from spoiling.[1]

Today, the established roles of microorganisms in food can be divided into three groups[2,3] (1) pathogenic microorganisms that cause infection or food poisoning, (2) saprophytic microorganisms that play role in biodegradation and food spoiling, and (3) beneficial microorganisms used in fermentation process and to maintain healthy digestive tract.[4]

Among the beneficial microorganisms is a group named probiotic. The original observation of the role of probiotics to promote human health was underwent by a Russian scientist Eli Metchnikoff, who suggested that "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" in 1907.[5]

The number of microbes along human digestive tract is tremendous, sizing up to 100 trillion microorganisms.[6] These microbes are of distinct phenotypes and each specific site can carry different type, from 56 phenotypes in mouth to 195 in colon. They are responsible to break down ingested polysaccharides, including the indigestible plant-derived pectin.[7]

In 2001, World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO) defined probiotics as "living microorganisms which when administered in adequate amounts confer a health benefit on the host".[8] Probiotics consist of yeast or bacteria, especially lactic acid bacteria.[4,9] The most well-known members of this group are *Lactobacillus*, *Bifidobacterium* and *Streptococcus*. They exhibit strain-specific differences on resistance against acid and bile, ability to colonize the gastrointestinal tract and clinical efficacy.[9] The possible mechanism of actions includes inhibition of pathogenic bacteria, production of useful metabolites or enzymes, enhancement of mucosal function, and modulation of host immune responses.[8,9]

Some modern definitions on probiotics include a preventive or therapeutic action of probiotics. Charteris, et al[10] defined probiotics as 'microorganisms, which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition'. Since probiotics have also been found to be effective in treatment of several gastrointestinal disorder such as acute infectious diarrhea,[11] antibiotic-associated diarrhea,[12] and irritable bowel syndrome,[13] they are also considered as therapeutic agents.[14] Probiotics are also associated with risk reduction in developing atopic sensitization in children,[15] lower incidence of gestational diabetes mellitus[16] and preeclampsia,[17] and fewer case of necrotizing enterocolitis in preterm infants[18], if given during prenatal period.

Despite the common mechanism of actions proposed, the beneficial effects of probiotics vary and can be strain-specific.[19] One strain of probiotic can have different clinical effect to a certain disease, compared to other strain of probiotics. For example, a study by Wagner, et al[20] found that four different strains of probiotics showed different efficacy and great diversity of immune effect in preventing colonization and sepsis with *Candida albicans* in mice. Therefore, generalization of probiotics effects without solid evidence should be avoided.

This literature review is aimed to address the possible benefits of probiotics and to recommend their usage in the field of obstetric.

Definition
The term probiotic was first introduced by Lilly and Stillwell[21] in 1965 to describe 'substances secreted by one microorganism which stimulates the growth of another'. In 1989, Fuller[22] highlighted that probiotics were living matter by describing them as 'a living microbial food supplement which beneficially affects the host animal by improving its microbial balance'. Newer definitions of probiotics include the possible preventive or therapeutic effect for the host because recent publications stated that probiotics are proven to be effective in treating gastrointestinal disorders.[10]

Other terms that are closely related to probiotics are prebiotics and synbiotics. Prebiotics are indigestible food ingredient that are advantageous to the host by selectively promote the growth or activity of beneficial enteric bacteria.[23] Meanwhile, synbiotic is the combination of prebiotics and probiotics designed to improve the survival of ingested microorganisms and the colonization of the intestinal tract.[24]

To be considered as probiotics, microorganisms have to own several characteristics. They must be resistant to gastric acid and bile, safe and confer health benefit for the host[5], and have the ability to colonize the intestinal tract and ward off the pathogenic bacteria.[25] Furthermore, any probiotics must be able to endure the manufacturing process and the long shelf-life.[26,27]

In newer publications, The International Scientific Association for Probiotics and Prebiotics[8] stated that there are several categories of living microorganisms for human. The first one is not probiotics. Products (foods, drinks, etc) claimed as "containing living and active culture" with following criteria: contains any food fermentation microbes in minimum 1×10^9 colony-forming unit (CFU) of microorganisms per serving, are not probiotics. Although these products contain living microbes, that do not imply they possessed the probiotic activity.

Meanwhile, the second group are the probiotics group. There are three different subgroups: supplement without health claim, supplement with health claim, and probiotic drug. The general criteria for all subgroup are every product must contain safely proven species with sufficient corroborating evidence, appropriate for human use, have proof of delivery of viable probiotics at efficacious dose, and (for drugs) meet the criteria for drug regulation.

Mechanism of action
There are several microorganisms that have been widely known as probiotics. *Bifidobacterium, Lactobacillus*, and *Saccharomyces* are the most well-known probiotics bacteria. Their mechanisms of actions are very strain-specific and case-specific.[19] Probiotics major mechanism of actions consisting of these following actions: secretion of anti-microbial substances, competitive adherence to mucosa and epithelium,

strengthening the gut epithelial barrier, and modulation of the immune system.[28]

Lactobacillus can modulate the regulation of genes encoding adherence junction protein, such as: E-cadherin and β-catenin, while also influence the phosphorylation of adherence junction proteins.[28] *Lactobacillus casei* exhibited an ability to restore mucosal integrity by enhancing expression and redistributing tight junction proteins of the zonula occludens and protein kinase C. Two isolated and purified peptide secreted by *Lactobacillus rhamnosus GG* demonstrated anti-apoptotic activity and therefore limit the epithelial damage.[28] Another mechanism is the ability to promote mucous secretion to improve barrier by increasing mucin production mediated by the upregulation of MUC2, MUC3, and MUC5AC in HT29 cells.[28]

Lactobacillus and *Bifidobacterium* also promote mucous adhesion mediated by protein, saccharide moieties, and lipotechoic acid. *Lactobacillus reuteri* produces an adhesin called MUB (mucus-binding protein).[28] *Bifidobacterium animalis subsp. Lactis* and *Bifidobacterium bifidum* facilitate the colonization of human gut by degradation of extracellular matrix of cells.[28] *Lactobacillus* and *Bifidobacterium* also have been shown to inhibit a broad range of pathogens, such as *E. coli, Salmonella, H. pylori, L. monocytogens,* and rotavirus by competing for available nutrients and mucosal adhesion sites. These bacteria can also modify their environment to make it less suitable for other competitors.[28]

Antimicrobial substances produced by probiotics are called bacteriocins, which mostly are organic acid (acetic and lactic acid) that can inhibit Gram-negative bacteria.[28] They mediate pathogens killing by destruction of target cells by pore formation and/or inhibition of cell wall synthesis. Other than that, probiotics can also interact with immune system and modulate their response via pattern recognition receptors such as toll-like receptors and nucleotide oligomerization domain (NOD)-like receptors.[28]

Safety
The safety of probiotics has been questioned for quite a long time. The issues addressed in this topics[29] are disease occurrence and the possibility of infection, toxic and metabolic effect, antibiotic-

resistance transfer between gut microflora, and immunological adverse events.[19,30] Few cases have shown the possibility of adverse events although the correlation is still doubted.

Infection

The concern regarding infection came from the probable transmigration of bacteria and several reported cases on bacteremia or sepsis attributable to probiotic strains. Theoretically, the risk is very low since probiotics are not selected among the pathogenic microorganisms. The estimated risk was less than one per one million users for *Lactobacillus* and one per 5.6 million users for *Saccharomyces boulardii* and even less in general healthy population.[31]

It has been linked to the probiotics' ability to adhere to human gut and in turn induce transmigration. Syndman stated that probiotics had no better adhesive properties to human gut compared to clinical strain and animal models suggested that probiotics actually reduced the translocation of other bacteria.[29]

In their review article, Boyle, et al[19] listed 12 cases of bacterial sepsis and 24 cases of fungal sepsis related to probiotics use in human. Most of the cases were identified using pulse-field gel electrophoresis (PFGE) of different substances, deoxyribonucleic acid (DNA) sequencing using polymerase chain reaction (PCR), or antibiotic resistance measurement. *Lactobacillus rhamnosus GG*, *Bacillus subtilis*, and *Saccharomyces boulardii* were the most frequently used probiotics in those studies. All of the cases had risk factors, such as immune compromise condition, critical illness, use of central venous catheter (CVC), use of broad spectrum antibiotics, or impaired intestinal barrier, unless it was not stated in the original paper.

Although there were some cases of infection with the use of *Lactobacillus* or *Bifidobacterium*, Sanders, et al[32] argued that no gene has been associated with pathogenicity in these microbes. Owing to the fact that different strains of *Lactobacillus* can also be found on normal human's gut, the clinical isolate from blood still does not conclude that the use of these specific strains of probiotics can cause infection in population at risk.

Metabolic effect

One of metabolic effects related to the use of probiotics is the production of D(-)-lactic acid and the probability of developing lactate acidosis.[33] D(-)-lactic acid is a compound derived from methylglyoxal metabolic pathway that is also a specific bacterial metabolite produced by gut microbes, especially *Lactobacillus spp.*[34] When the number of this particular species rises, the intraluminal pH rises. The acid state of gut creates a favorable environment for *Lactobacillus spp.* and not for other species. If the consumption of simple chain carbohydrate increases, *Lactobacillus spp.* will produce more lactic acid which may result in lactic acidosis.[35]

The lactic acidosis had been reported in patients with short bowel syndrome, with or without the use of probiotics.[35-37] Neurologic sequelae can follow this condition, such as ataxia, slurred speech, memory loss, even loss of consciousness. One of the cases of lactic acidosis[36] triggered by probiotics use a combination of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to reduce diarrhea on two year-old post gut resection infant. Lactic acidosis occurred after three years eight months after surgery and four months after probiotic use.

In other study[38] on healthy term infants, use of *Lactobacillus paracasei* was not associated with increase of lactic acid in blood. There were 88 infants, aged up to 72 hours, involved in this study. Half of the subjects were feed with formula containing probiotics while the rest were feed with non-probiotic containing formula. The subjects were followed up until day 168. There was no increase in level of lactic acid and no adverse events were associated with the use of probiotics in these infants. There is no study reporting probiotic-induced lactic acidosis in otherwise healthy individual.

Antibiotic resistance

Another concern regarding probiotic safety is the potential antibiotic-resistance transfer between probiotics and gut microbiome, particularly pathogenic bacteria.[19,29] It has been established that human gut acts as reservoir for antibiotic resistant genes called resistome.[39] The so-called genes are natural reactions from gut bacteria to protect antibiotic-producing bacteria from their own products and to increase their chance of survival.[39] The problem rise when these genes are transferred horizontally and vertically to pathogenic bacteria.

In a study of microbes found in chicken feces,[40] lactic acid bacteria were found to be most sensitive to penicillin, amoxicillin, chloramphenicol, and ampicillin, while it is more resistant to gentamycin, suplhametoxazole, kanamycin and streptomycin.

The resistance genes, originated from mobile elements such as plasmids, transposons, and integrons, are spread by horizontal gene transfer (HGT).[41,42] In lactic acid bacteria, these genes encode the resistance to tetracycline, chloramphenicol, erythromycin, and macrolide.[29,41] Tetracycline resistance genes are the most abundant in lactobacilli, at least 11 genes had been identified, including genes coding for ribosomal protection proteins and efflux pumps.[41] One chloramphenicol resistance gene had been identified in *L. acidophilus*, while four different erythromycin and three macrolide resistance genes had been found in several *Lactobacillus species*.[41] Conjugation appears to be the most prevalent mechanism[42] and a transfer from gram-positive enterococci to lactobacilli and lactococci can take place in animal gut and *in vitro*, and vice-versa, including transfer to *Staphylococcus*.[41]

Vancomycin resistance in lactic acid bacteria is characterized as intrinsic phenomenon caused by chromosomal mutation. The D-alanine/D-alanine terminal for vancomycin binding was replaced by either D-lactate of D-serine, therefore prevent the vancomycin binding process.[41] There had been evidence of possible *in vivo* vancomycin resistant gene, *vanA*, transfers from an *Enterococcus* strain to *L. acidophilus*[32] although there was no evidence of plasmids and genes related to this resistance found in *Lactobacillus rhamnosus GG* from hybridization or PCR.[29]

Safety in pregnancy

Probiotics use in pregnancy is deemed safe in general, as shown in a recent meta-analysis[43] of eight high-quality studies in more than 1,500 pregnant women. Most of the subjects were in 32-36 weeks of pregnancy, although one study was done in first trimester. The analysis compared the use of *Lactobacillus sp.* alone or in combination with *Bifidobacterium sp.* with placebo. The result showed no significant difference in outcomes: caesarean section rate, birth weight, and gestational age between two groups. Malformation as an outcome was reported in one study and it stated that there was no malformation found in probiotic group while three malformation cases were found in placebo group.

Another recent review[31] of eight different studies following the meta-analysis showed that there was no adverse pregnancy outcome related to the use of probiotics. Most of the included randomized placebo-controlled trials compared *Lactobacillus sp.* alone, or in combination with *Bifidobacterium sp.* or *Propionibacterium sp.*. Most of the studies agreed with the proceeding meta-analysis and reported no difference in gestational age, birth weight, or rate of Caesarean section. Luoto, et al[16] reported lower incidence of gestational diabetes mellitus in probiotic group and significantly lower birth weight and shorter birth length of newborns from the aforementioned group. Allen, et al[44] reported that there was no significant adverse event related to probiotic supplement on pregnancy, childbirth, and newborn's general health until six months of life.

Another concern regarding safety for use in pregnancy is the possibility of disruption of T helper one (which exhibit pro-inflammatory activity – Th1) and T helper two cells (which exhibits anti-inflammatory activity – Th2) ratio.[19] Throughout pregnancy, there is a shift from Th1 response to Th2 response that induces maternal tolerance and suppression of immune system.[45,46] This response is shown as reduction in the percentage of interferon-γ (IFN-γ) and Tumor Necrosis Factor-α (TNF-α)-secreting T helper cells, and also the increased production of interleukin-4 (IL-4) produced by Th2.[45] IFN-γ indirectly promotes Th1 differentiation by upregulating IL-12 receptor while at the same time inhibiting Th2 growth. IL-4 is the main cytokine to promote growth and differentiation of naive T cells to Th2, thus inhibiting differentiation to Th1 cells.[45,47] Increased Th2 response is linked with fetus viability *in utero*, while surge of Th1 response is associated with recurrent spontaneous abortion and preeclampsia.[45,46] There is evidence that links the use of probiotics with the changes of Th1/Th2 equilibrium. In patients with severe traumatic brain injury (TBI), probiotics were able to reverse the Th2 polarization response to the Th1/Th2 equilibrium.[48] Probiotics' ability to cause Th1 polarization may theoretically put the fetus in danger, if this mechanism occur in pregnancy. Although for now, there is still no evidence to support this claim, thus it still remains a theory.

Clinical evidence in obstetric field
Prevention of preterm delivery
Preterm delivery is a global problem. According to WHO, the estimated number of preterm delivery is 15 million per year and about one million of those babies die annually.[49] It is the leading cause of newborn death in the world, ranging from 5% to 18% of babies born, across 184 countries.[49]

Several important risk factors for preterm delivery had been named, such as history of preterm birth, twins pregnancy, ethnicity, maternal age <18 years, and genitourinary or intrauterine infections.[50] Numerous evidences have reported an association between infection/inflammation and preterm birth. One of the consistent observed causes is the evidence of chorioamnitis, which affects 20-70% placentas of preterm born babies. Positive membrane culture is detected in 30-60% of those patients.

Probiotics, particularly lactobacilli, are a potential breakthrough way to prevent preterm birth that act to restore vaginal lactobacilli count. A study by Vitali, et al[51] showed that supplementation of *Lactobacillus spp* (*L. paracasei, L. plantarum, L. acidophilus, L. delbrueckii subsp. Bulgaricus*), *Bifidobactetium spp* (*B. longum, B. breve, B. infantis*), and *S. thermophilus* can alter the cytokine and chemokine response in vaginal mucous.

A 2007 meta-analysis study[52] on three trial of medium quality showed that there was no benefit from supplementation of probiotics to prevent very preterm birth (<32 weeks) (RR = 0.65; 95% CI = 0.03-15.88) and preterm birth (<37 weeks) (RR = 3.95; 95% CI = 0.36-42.91). The confidence intervals of these two findings are very wide and therefore possess no statistical significance. Reports of the studies included in this analysis mostly focused on laboratory evidence of infection (lactobacillus count, type of abnormal vaginal flora, vaginal fluid pH, presence of clue cells, etc) rather clinical findings regarding infection or preterm labor. Therefore, the writers of this meta-analysis could neither support nor oppose the use of probiotics in pregnancy to prevent preterm delivery.[52]

The more recent publications on the same topic, on the other hand, suggested that probiotics may play a role in preventing preterm labor. A study by Myhre, et al[53] involving 18,888 women all across Norway in Norwegian Mother and Child Cohort from 2002 to 2007 showed that probiotics reduces incidence of preterm labor. The subjects were asked to complete two questionnaires in gestational week 15 and in gestational week 17-22. They were asked about their probiotic milk consumption. Answers were divided into yes or no; yes were stratified into low and high intake. Of all subjects, 950 experienced preterm delivery. The risk for developing preterm labor in subjects consuming probiotic milk was lower than those who didn't (OR = 0.857; 95% CI = 0.741-0.992; p = 0.038). Furthermore, the risk was lower in groups who consume high intake of milk (OR = 0.820; 95% CI = 0.681-0.986; p = 0.035) compared to no intake.

In women taking probiotics supplementation, the level of chemokine Eotaxine, which exerts pro-inflammatory activity, decreased from week 33 to 37, compared to control.[51] Meanwhile, the level of anti-inflammatory cytokine and chemokine, interleukin (IL)-4 and IL-10, in probiotic group stayed in the same concentration from week 33 to 37, while it declined in the control group.[51] The researcher hypothesized that:[51] (1) probiotics counteracted the decrease of anti-inflammatory cytokine levels in control group, and (2) probiotics induced the decrease of pro-inflammatory cytokine in probiotics group.

Another paper by Yang, et al[54] stated that injection of supernatant of *L. rhamnosus GR-1* (GR-1 SN) in pregnant mice can lower preterm birth by 43%. The study found that GR-1 SN decrease the production of several pro-inflammatory cytokine and chemokine, such as IL-1β, -6, TNF-α in maternal plasma, myometrium, and amniotic fluid. Furthermore, maternal plasma progesterone also reduced significantly in mice given the GR-1 SN supernatant injection. Past studies have shown that SR-1 GN stimulate release of IL-10, in human monocytes, mouse macrophages, and human trophoblast cells,[55] through the janus kinase/ signal transducer and activators of transcription (JAK/STAT) and mitogen-activated protein kinases (MAPK) pathway.[56] The upturn of this cytokine respectively suppress TNF-α.[56] This also support the hypothesis that probiotics may contribute to a reduction in overall systemic inflammation and keeping it at a subtreshold level to avoid progesterone-induced labor.

Prevention of preeclampsia

Probiotics have been long known to have antihypertensive effect. A recent meta-analysis of 14 studies showed that probiotics have a blood pressure-lowering effect in general population.[57] The studies included in this analysis originated from Japan and Europe with respondents' age ranging from 35 to 75 years old. After taking probiotic fermented milk intervention, reduction in systolic-blood pressure was reported. The net systolic changes ranging from -1.5 to -12.4 mmHg (mean: -3,10; 95% CI = -4,63-(-)1,56; p = 0,193), while the mean of net change for diastolic blood pressure were -1.09 (95% CI = -2.11-(-)0.06; p = 0,153).

Theoretically, probiotics can prevent eclampsia since it prevents inflammation both systemically and locally.[51,56] A study by Brantsæter, et al[17] showed that probiotics may actually link to reduction of preeclampsia incidence. This study was a part of Norwegian Mother and Child Cohort Study. A total of 33,399 nulliparous pregnant women were included in the study and were asked to complete two sets of questionnaire in 15 weeks and 17-22 weeks of pregnancy, including food frequency questionnaire asking about milk consumption. Among the subjects, 1,755 women (5.3%) developed preeclampsia. In crude model, consumption of probiotics was associated with the reduced risk of all sub-type of preeclampsia (early-late, mild-severe), but after adjustment, probiotic use was only associated with the severe type of preeclampsia (OR = 0.79; 95% CI = 0.66-0.96). The consumption of probiotic milk then was divided into four criteria: no, low, moderate, and high intake (median of intake: low 13.2 mL/day, moderate 28.5 mL/day, and high 200 mL/day). The incidence of preeclampsia was lower in group with higher consumption (5.6% in no intake group, 4.1% in high intake group). The calculated risk of preeclampsia was also reduced among high consumers (OR = 0.61; 95% CI = 0.43-0.89).

Prevention of eczema in offspring

For several years, probiotic supplementation to pregnant women has been linked to reduction in incidence of eczema in their offspring. The evidences were mixed, some stated that there was no link while the others said that there was reduction in number of new cases. In a 2011 meta-analysis, Doege, et al[58] on systematic review claimed that there was a significant risk reduction for atopic eczema in children aged 2-7 years old whose mother received supplementation during pregnancy. Seven studies were included, involving a total of 2,800 pregnant women in this analysis. The result showed that the risk reduction for atopic eczema was significant when the lactobacilli were used as the probiotics agent (RR = 0.82; 95% CI = 0.71-0.96) rather than probiotic mixture (RR = 0.92; 95% CI = 0.83-1.02). It concluded that the use of lactobacilli, if taken as monotherapy, in pregnancy may be beneficial in preventing incidence of infant eczema.

A more recent meta-analysis by Pelucchi, et al[59] identified 18 publications based on 14 trials and decided on two outcomes: atopic dermatitis and immunoglobulin E (IgE)-associated atopic dermatitis. The study found that there was a 20% reduction in incidence of atopic dermatitis and IgE-associated atopic dermatitis in women who had probiotic supplementation during pregnancy. The analysis reported an risk ration (RR) of 0.79 (95% CI = 0.71-0.88) for atopic dermatitis in the treatment groups. As for the IgE-associated atopic dermatitis, the RR was 0.80 (95% CI = 0.66-0.96) and consistent throughout random-effect model. Another outcome in this analysis was the effect of probiotic treatment on disease severity. Out of 18, 11 studies reported the disease severity as an outcome and 9 of 11 stated that there was no difference found between treatment and placebo groups, and there was no. Relation to dose or type of supplementation. They were also unable to determine whether the effect in those studies was limited into one specific strain, because the data availability was limited. However, they agreed with previous study that the use of lactobacilli, specifically *L. rhamnosus GG,* was associated with a RR of 0.74 (95% CI = 0.61-0.90). It was also worth noting that several studies in this meta-analysis performed intervention to only pregnant mothers while other studies also gave supplementation to the babies. Therefore, it was inconclusive whether the results of these studies were solely resulted from supplementation for mothers.

A large-scale cohort study in Norway (a part of The Norwegian Mother and Child Cohort Study),[60] reported slight reduction in relative risk for atopic eczema in offspring of women who consumed probiotic milk during pregnancy. The study

included 40,614 children born in 2003 to 2009. Among them, 12.2% had symptoms of atopic eczema by six months of age, 13.6% had current atopic eczema at 18 months of age. If only the mother received probiotic milk supplementation, when their child reach six month, there was a small reduction of atopic eczema (RR = 0.94; 95% CI = 0.89-0.99), but then it was no longer found in 18 months (RR = 1.00; 95% CI = 0.95-1.05). However, if both mother and child took the supplementation (for child even after six months old), there was a slight reduction in atopic eczema risk (RR = 0.93; 95% CI = 0.86-1.00) although it was bordering insignificance.

Another challenge on the topic is finding the possible mechanism of how supplementation in pregnant mothers can affect their offspring, in terms of atopic eczema incidence. Several mechanisms were proposed to explain this phenomenon. The conventional approach is that probiotics modulate intestinal microbiota composition or directly stimulate its immune system. In allergy pathogenesis, type one and two T helper cells play important roles. Allergic disorders are associated with a shift of the Th1/Th2 balance towards a Th2 response, and in turns promotes the secretion of IL-4, IL-5, IL-9, IL-13, and IL-31, also increase the IgE production.[15] In newborns, their immune systems are not fully developed, and tend to be skewing toward Th2 trend to prevent *in-utero* rejection. However, Th2 is responsible to stimulate B cells to produce more IgE, which can activate mast cells and cause allergic symptoms. Exposure to microbes in early life can help reverse this trend and promote the development of Th1 via activity of Th3.[61] Th3 will release transforming growth factor (TGF)-β that modulates the activity of B cells. As a result, B cells suppress their production of IgE and promote production of IgA. IgA will act as allergen exclusion system and will reduce the contact between immune system and antigen. Probiotics can also modulate the toll-like receptors and proteoglycan recognition proteins of enterocyte, leading to activation of enteric dendritic cells and a Th1 response, thus inhibiting Th2 activity.[15]

Regulatory T cells (Treg) role is also thought to play a role in preventing allergy sensitization.[62] Antigen-specific Treg (CD4+CD25+foxp3+) that secrete anti-inflammatory cytokines IL-10 and/or TGF-β has the potential to suppress the production of IgE and Th1/Th2 proliferation.[61-63] Furthermore, recent advances stated that homeostasis of mothers' Treg, Th1 and Th2 may influence their children's allergy condition.[63] With the fact that mother's immune cells can cross the placenta, this may be the possible mechanism of eczema prevention by probiotic supplementation. Other possible mechanism is that prenatal probiotic supplementation modulates the maternal vaginal and intestinal microbiota, and provides important colonizing inoculum for the newborns and thus affecting colonization of their intestinal system.[64] Furthermore, there is also new evidence that gut microbiota acquired during early postnatal period is required for the development of Treg.[62]

Prevention of wheezing in offspring

Numerous murine model experiments proved that probiotic use, both perinatal or not, can prevent airway inflammation and hyperactivity.[65-68] The mechanism underlying this property is closely related to the Th1/Th1/Treg activity to prevent eczema, with TGF-β as the main actor.[65,66]

Despite these evidences, recent meta-analysis showed that probiotics property to prevent wheezing and asthma was still debatable.[69] The analysis included 20 clinical trials published between 2003 and 2013, enrolled a total of 4,866 infants. Different species and strains of probiotics were tested, alone or in combination. The probiotics used were: four *Bifidobacterium spp* and six *Lactobacillus spp*. Outcomes observed in these studies included wheezing, asthma, and lower respiratory tract infection. The median range of follow-up among these trials was 24 months (ranging from four months to eight years). Nine trials involving 3,257 children reported asthma as an outcome, and from data analysis, it was found that there was no significant difference of asthma incidence between subjects who received probiotics and those who did not (RR = 0.99; 95% CI = 0.81-1.21). Nine other studies, including 1,949 children, with wheezing as their outcome also reported that the incidence between two groups were similar (probiotics vs placebo: 35.0% vs 31.1%, RR = 0.97; 95% CI = 0.87-1.09). This result was also supported by another meta-analysis by Elazab, et al[15] who stated that early life (prenatal and postnatal) use of probiotics

does not protect against asthma or wheezing (RR = 0.99; 95% CI = 0.88-1.12). A cohort study[60] in Norway was also in agreement with two other meta-analyses. Early life probiotic milk intake (perinatal or postnatal) does not affect the incidence of asthma in child. Both perinatal supplementation to mothers only and supplementation to mothers and children peri- and post-natally do not alter the incidence of asthma among the children (mother only supplementation: RR = 0.96 (95% CI = 0.85-1.08 *vs* mother and child supplementation: RR = 1.07; 95% CI = 0.95-1.19). Therefore, for now, the evidence to support perinatal probiotic use to prevent wheezing or asthma in offspring is still lacking.

In conclusion, the current evidences support the claim that probiotic is safe for general population and pregnant women, although some cautions should be used for immunocompromised population. There was no adverse effect of probiotics on pregnancy outcomes including gestational age, birth weight, malformation and complication of pregnancy. Probiotics, especially *Lactobacillus spp.* and *Bifidobacterium spp.*, were proven to reduce the incidence of preterm labor and preeclampsia in pregnant women and eczema in their offspring. However, present evidences failed to prove that probiotics were beneficial to prevent wheezing and asthma in the offspring of mothers given supplementation of probiotics. The mechanism underlying all these effects was closely related to regulation of T helper and T regulatory cells. Further studies are needed to determine the effective dosage of probiotics supplementation in order to produce these protective effects.

Acknowledgments

Conflicts of Interest
The authors affirm no conflict of interest in this study.

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