Does the use of probiotics in pregnant women with premature rupture of membranes improve the maternal and perinatal outcome? A systematic review

O uso de probióticos em gestantes com rotura prematura de membranas ovulares melhora o desfecho materno e perinatal? Uma revisão sistemática

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ABSTRACT

Introduction: Premature rupture of membranes (PROM) is a condition that affects 8–10% of all pregnancies, and contributes with 20–40% of preterm deliveries. Evidence shows that changes in the vaginal microbiota may also have a favorable impact on the decrease in the prevalence of PROM, and that expectant treatment may be an appropriate approach to reduce morbidity in these cases. **Objective:** To investigate whether the use of probiotics in pregnant women with premature rupture of ovary membranes improves the maternal and perinatal outcome. **Methods:** This is a systematic review, developed from articles published between January 2001 and August 2018, which justify the use of probiotics in pregnant women with PROMto improve maternal and perinatal outcome. **Results:** Some studies have shown a potential role of probiotics in modulating vaginal bacterial communities, reducing rates of cesarean section and PROM, and increasing the latency and weight of newborns in pregnant women with PROM. However, in other studies, there was no confirmation of changes in the vaginal microbiota from the use of oral probiotics. **Conclusion:** There are benefits in the administration of probiotics to the mother-fetus binomial. However, there are still doubts about routes of administration, choice of strains and period of use. More studies are necessary to settle them.

Keywords: probiotics; premature rupture of fetal membranes; pregnancy; microbiota.

RESUMO

Introdução: A rotura prematura de membranas ovulares é uma condição que afeta 8–10% de todas as gestações e contribui com 20–40% dos partos prematuros. Evidências mostram que mudanças na microbiota vaginal podem ter impacto favorável na diminuição de sua prevalência, e o tratamento expectante pode ser uma abordagem adequada para reduzir a morbidade nesses casos. **Objetivo:** Investigar se o uso de probióticos em gestantes com rotura prematura de membranas ovulares melhora o desfecho materno e perinatal. **Métodos:** Trata-se de uma revisão sistemática desenvolvida com base em artigos publicados no período de janeiro de 2001 a agosto de 2018, que justificam o uso de probióticos em gestantes com rotura prematura de membranas ovulares para melhorar o desfecho materno e perinatal. **Resultados:** Alguns estudos mostraram potencial atuação dos probióticos em modular comunidades bacterianas vaginais, em reduzir taxas de cesarianas e rotura prematura de membranas ovulares, além de aumentar o período de latência e peso do recém-nascido de gestantes com esse quadro. Porém, em outros trabalhos, não houve confirmação de mudanças na microbiota vaginal pelo uso de probióticos orais. **Conclusão:** Há benefícios na administração dos probióticos sobre o binômio mãe-feto, contudo ainda há dúvidas sobre vias de administração, sobre escolha das cepas e sobre tempo de uso. Mais estudos precisam ser realizados para dirimi-las.

Palavras-chave: probióticos; ruptura prematura de membranas fetais; gravidez; microbiota.

INTRODUCTION

Premature rupture of membranes (PROM) is a condition that affects 8–10% of all pregnancies, and since prematurity is the most frequent consequence of PROM[1], it acts as a cause of 20–40% of preterm deliveries[2]. When rupture occurs at an earlier gestational age, between 24–36 weeks, expectant management may be a suitable approach to reduce the morbidity associated with prematurity[3]. However, it is a difficult decision to make. On the one hand, the anticipation of labor prevents infections in the newborn; on the other hand, the prolongation of this pregnancy may allow a greater maturation of the fetus and reduce the risk of complications associated with preterm birth[2,3].

It is known that alterations in the vaginal microbiota, in the early stage of gestation, can be a predictor for abortions and for premature births[1,4]. There is also evidence that restoration of the vaginal microbiota may also have a favorable impact on the decrease in the prevalence of PROM, since there is clear association with vaginal infections[1]. Some of the probable mother-fetus contact sites that may promote infection are: amniotic fluid, placenta, fetal membranes and umbilical cord[5].

There are bacteria that are commensal and which are present in the placenta in non-pathological situations. With the development of techniques of molecular biology and bacterial genetics, it has been possible to reveal the great diversity of the microbiome of the whole reproductive tract and, in particular, the detection of bacteria in the uterine cavity by polymerase chain reaction (PCR) technique[5,6].

Satokari et al.[6] demonstrated the presence of two strains, *Bifidobacterium* and *Lactobacillus rhamnosus*, by deoxyribonucleic acid (DNA) analysis in 34 samples of placentas collected shortly after delivery. It is known that *Bifidobacterium* and *L. rhamnosus* are part of the normal intestinal microbiota in adults. The bacterial DNA of the two strains was detected in almost all placenta samples, regardless of the type of delivery. It is believed that exposure of the

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newborn to intestinal bacteria may be the beginning of the development of the infant immune system[6].

The systemic inflammatory response of the fetus varies according to the intrauterine microbiota, and occurrences of fetal injury and other sequelae of target organ damage may contribute to premature labor, as well to immunomodulation and strengthening of the immune system[4]. Groups of bacteria that cause bacterial vaginosis, for example, confer an inflammatory response in the newborn much larger than any microorganism alone, whereas *Lactobacilli* appear to have anti-inflammatory properties[4]. *Lactobacilli* protect vagina from pathogenic organisms, producing antimicrobial agents such as hydrogen peroxide and bacteriocins, compete for nutrients, adhesion to the epithelial surface and keep vaginal pH low[7]. These aspects were confirmed by Fichorova et al.[4], analyzing 25 biomarkers collected from blood and placenta samples, in which they observed that *Lactobacilli* were more related to anti-inflammatory cytokines, while pathogenic bacteria, such as *Gardnerella sp*. and other ones linked to bacterial vaginosis, were more related to inflammatory cytokines. Therefore, placental colonization of specific bacteria, such as probiotics, during the initial phase of gestation may have promising effects to prevent preterm birth and complications of prematurity[4].

A group of researchers followed 256 women during pregnancy and followed her infants in the first 24 months of life to identify whether the administration of probiotics to the mother could alter maternal and perinatal outcomes. Probiotics have been associated with reduced risk of gestational diabetes mellitus, improved immune regulation of the newborn and the intestinal barrier function, and reduced risk of intestinal infection and of childhood allergic diseases. Considering the maternal microbiota as the first contact for the development of the child's microbiota, it is important to recognize the intestine as a key organ involved in host homeostasis[8].

Probiotics seems to be beneficial to the mother-fetus binomial. However, there are still doubts about routes of administration, choice of strains and time of use.

OBJECTIVE

This review proposes to look for what there is in the scientific literature on the influence of probiotic use on the outcome of gestation in PROM.

METHODS

A systematic review of articles published from January 2001 to August 2018 was conducted in the MEDLINE, Latin American and Caribbean Health Sciences Literature (Lilacs), Cochrane Library, Highwire Stanford and Embase databases. We also searched at the Center for Reviews and Dissemination (CRD), the clinical trials, the Brazilian Registry of Clinical Trials and PROSPERO, looking for some previous systematic or ongoing systematic review on the topic. In these latter databases, no records were found. In addition, a search was made in the references used by the selected articles, of some studies that fit the criteria of inclusion and exclusion.

The search was performed using combinations of keywords: "fetal membranes, premature rupture" [MeSH Terms] (OR "fetal" [All Fields] AND "membranes" [All Fields] AND "premature" [All Fields] AND "rupture" [All Fields]) OR "premature rupture fetal membranes" [All Fields] OR "fetal" [All Fields] AND "membranes" [All Fields] AND "premature" [All Fields] AND "rupture" [All Fields] OR "fetal membranes, premature rupture" [All Fields]) AND "probiotics" [MeSH Terms] OR "probiotics" [All Fields] OR "probiotic" [All Fields].

Articles were selected by means of eligibility criteria. Priority was given to original articles published in Portuguese, Spanish and English, whose object of study were pregnant women with premature rupture of membranes and using probiotics as an intervention factor. Review articles, with themes different from the proposed objective, in languages other than those previously mentioned or published before 2001 were excluded.

Two reviewers participated in the selection of articles, according to the recommendations of the Brazilian Medical Association[9]. Initially, the studies were selected from the title. Next, a detailed analysis of the abstracts was carried out in order to restrict the selection only to those that fulfilled the inclusion criteria. All selected articles were included in the survey. All selected articles were included in the survey. If any of the studies generated disagreement among the reviewers, a third reviewer would be chosen for the evaluation and whether or not this study would be included. Finally, all selected articles make up the research sample.

RESULTS

The search initially returned 111 results, but, after applying the inclusion and exclusion criteria, 16 articles were selected. Then, after a qualitative evaluation, another 10 articles were excluded, totaling six articles included in this review (**Figure 1**). The most relevant results of each study are shown in **Chart 1**.

DISCUSSION

Rautava et al.[10] conducted a randomized, placebo controlled clinical trial of 43 women between June 2007 and November 2009. These women were divided into three groups: a placebo; a group in use of a strain of *Bifidobacterium lactis*; and a third group using *B. lactis* and *L. rhamnosus*. The objective was to verify the interaction between microbes and the maternal-fetal interface and whether

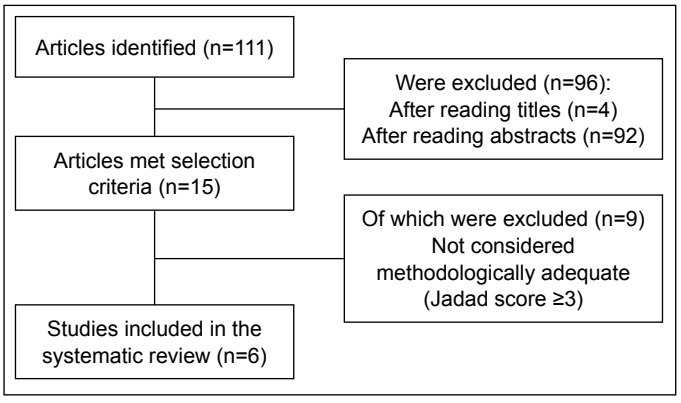

Figure 1 – Selected studies flowchart.

this could be modulated by oral administration of probiotics to mother during pregnancy, in order to bring benefits to the mother-fetus binomial. In order to analyze the composition of the microbiota, samples of amniotic fluid and placenta were collected during cesarean section. Toll like receptor (TLR) genes were identified by Array PCR. TLRs are membrane proteins that are part of the innate immune system and, through the exposure of pathogens, contribute to the signaling of inflammatory cytokines[11]. Microbial DNA was detected in the placenta samples of the three groups and in 6 of the 14 samples of amniotic fluid (43%). Maternal supplementation of probiotics was associated with statistically significant changes in the expression of the TLR gene in the placenta and in particular TLR 1 and 7, as well as reduction of TLR 4. There was also reduction of TLR 1 and 6 in the fetal gut in the group in use of *B. lactis* and *L. rhamnosus*. The authors concluded that the presence of bacterial DNA in the amniotic fluid and in the placenta affects the expression of the TLR gene in the fetal gut and that is essential for the maturation of its immune system. Besides that, the modulation of the inflammatory process, as well as the consequent signaling of TLR in the maternal-fetal unit through the use of specific strains, may result in a fetal metabolic and immunological programming.

If there are benefits in this approach, what would be the best and optimal time to do it? Several studies have tried to answer this question. In a pilot, placebo-controlled and non-randomized study conducted by Vitali et al.[12], the impact of daily oral probiotic supplementation on the vaginal microbiota and immune system of healthy women during the late gestation phase was evaluated. Twenty-seven women were followed for four weeks, between 33 and 37 weeks' gestation, divided into two groups. The intervention group used a probiotic pharmaceutical compound containing four strains of *Lactobacilli*, three strains of *Bifidobacterium* and one of *Streptococcus thermophilus*. The analysis of the vaginal microbiota before and after the intervention was performed by real-time PCR analysis; besides, inflammatory cytokines were analyzed in the vaginal fluid by Luminex immunoassay. Differences in the intervention group's microbiota compared to placebo suggest a potential role for probiotics in modulating vaginal bacterial communities. Compared to the intervention group, the placebo group had reduction of *Bifidobacterium* (related to vaginal health) and increase of *Atopobium*, mainly near the 37th week (bacteria related to bacterial vaginosis). In addition, in the probiotic group, women who previously had *Streptococcus agalactiae* positive after treatment did not present it any more. In this group, there was also greater reduction of inflammatory cytokines. Therefore, they concluded that there is stabilization of vaginal immunity during pregnancy that can be attributed to the intake of probiotics.

Other studies were similar to that of Vitali et al.[12], but with a better delineated search of more solid scientific evidence. A randomized, triple blind, placebo controlled trial published by Gille et al.[13] also sought to verify if the use of oral probiotics could maintain or restore the vaginal microbiota in healthy pregnant women. There were selected 320 pregnant woman with less than 12 weeks, and then they were divided into two groups and followed up for eight weeks. The product contained two strains *L. rhamnosus* and *Lactobacillus reuteri* showed good oral tolerability. Vaginal swabs were collected before and after treatment and the microbiota was

Chart 1 – Original studies conducted between 2001 and 2018 on the use of probiotics in premature rupture of membranes (PROM) and their outcomes.

Study	Year ofpublication	Study design	Women (n)	Method of analysis	Results
Rautava et al.[10]	2012	RCT	43	PCR array QIA amp DNA Placenta and LA samples	Bacterial DNA in LA and in the placenta affects TLR expression in the fetal gut Microbial interaction with TLR is essential for a healthy immune system
Vitali et al.[12]	2012	Pilot study, non-randomized	42	PCR, DGGE and PCR - real time Vaginal swab before and after the intervention	Probiotics reduce pro-inflammatory cytokines in the treated group Placebo group: increase of *Atopobium* (linked to BV) and reduction of *Bifidobacterium* (linked to health)
Gille et al.[13]	2016	RCT	320	Swab vaginal Nugent score	Oral probiotic did not modify the vaginal microbiota Lower prematurity rate in the treatment group
Di Pierro et al.[14]	2016	Non-randomized trial	406	Swab retal/vaginal Statistical analysis	*Streptococcus agalactiae* reduction (6%) PROM reduction (30%) Reduction of cesarean section rate (5.51 / 10.39%) Reduction of umbilical pH (0 / 6.09%)
Daskalakis et al.[15]	2016	RCT	106	GA at birth Birth weight Latency period	Treatment group: GA increase Weight gain at birth Increased latency period
Kavak et al.[16]	2014	Non-randomized trial	40	APGAR 5th min GA at bith Birth weight Latency period	Treatment group: Increase GA Increased latency Increase of APGAR 5 minutes Weight gain at birth 3 cases of chorioamnionitis in the placebo group and none in the probiotic group

RCT: randomized clinical trial; PCR: polymerase chain reaction; QIA: quantitative immune analyzer; DNA: deoxyribonucleic acid; LA: amniotic liquid; DGGE: denaturing gradient gel electrophoresis; GA: gestational age; TLR: Toll like receptor; BV: bacterial vaginosis.

classified according to Nugent score. There was no difference in the vaginal microbiota of the two groups before and after the intervention. In addition, rates of bacterial vaginosis were not significantly different, neither there was divergence in the Nugent score between the groups. However, in relation to the secondary outcomes, the prematurity rate reduced in the treatment group (3.8%) compared to the placebo group (5.0%). The authors concluded, however, that the study did not confirm in pregnant women significant changes in the vaginal microbiota with the use of oral probiotics, different outcomes from the results found in non-pregnant women[13].

Di Pierro et al.[14] conducted an open, non-randomized, placebo-controlled study in which 406 pregnant women were followed up, divided into two groups, from January to December 2015, between 30–40 weeks. The objective was to evaluate the effect of the use of oral probiotics in the last weeks of pregnancy in relation to the influence on cesarean rates, PROM and pH analysis of fetal umbilical artery. The product used was composed of two strains of *Lactobacillus*, one strain of *Bifidobacterium* and one strain of *Enterococcus faecium*. Vaginal and rectal swabs were collected between 36 and 37 weeks for *Streptococcus agalactiae* in order to perform the statistical analysis of the data already mentioned. Vaginal and rectal swabs were positive for *S. agalactiae* in 27/127 (21%) of the women in the treatment group and 76/279 (27%) in the control group. No case of PROM was identified in the treatment group against 87 (31.2%) in the control group. Regarding cesarean rates, 7 (5.51%) in the treatment group and 29 (10.39%) in the control group. And in the pH analysis of the pathological umbilical artery, no case was identified in the treatment group against 17 (6.09%) in the control group. Thus, reduction of the positivity rates for *S. agalactiae* was observed: reduction by about 30% of PROM, reduction of the cesarean section rate and reduction of the pH of the pathological umbilical artery. These data lead the authors to conclude that, despite the limitations of the study because it is not randomized and it presents heterogeneous groups, there are benefits in the use of probiotics at the end of pregnancy, besides good tolerability.

Daskalakis et al.[15] conducted a prospective, randomized study aimed to identify the efficacy of vaginal probiotics in combination with conventional treatment already used in pregnant women with premature PROM in order to prevent maternal and perinatal complications. For this purpose, 106 women were followed and divided into two groups. The analyzed variables were: gestational age at birth; duration of the latency period; birth weight; Apgar score in 1 and 5 minutes, in addition to complications related to prematurity (admission to neonatal intensive care unit, necrotizing enterocolitis and neonatal sepsis). In the intervention group, the mean gestational age was 35 weeks *versus* 32 weeks on the placebo group. The duration of the latency period was 5.6 weeks, while in the placebo it was 2.4 weeks; and birth weight 2,439.08 g *versus* 2,004.81 g, higher in the group using probiotics. Thus, the authors conclude that there are clearly benefits in the association of probiotics with the standard antibiotic regimen as a conservative treatment of PROM[15].

A study with a design similar to Daskalakis et al.[15] had already been published in 2014. Kavak et al.[16] conducted a non-randomized clinical trial from July 2011 to June 2013 with pregnant women with premature PROM (between 23 and 31 weeks). There were selected 40 pregnant women, divided into two groups, with treatment and placebo, and their data were analyzed retrospectively. The objective was to evaluate the efficacy of the use of vaginal probiotics (strain *Lactobacillus casei* and *L. rhamnosus*) in combination with conventional antibiotic treatment for patients with PROM who were on conservative treatment. The factors evaluated were: gestational age at birth, latency period and birth weight, and the Apgar score at the 1st and 5th minute. Comparing the two groups, there were statistically significant differences in the following aspects: gestational age at birth (31.5 weeks in the treated group while in the placebo group it was 28.1 weeks); latency period (41.4 *versus* 12.3 days, respectively); and birth weight (1,947 in the treatment group *versus* 1,320 g in the placebo group). Despite having a much lower sample, this study presented similar conclusions to the investigation by Daskalakis et al.[15]. The authors concluded that the addition of probiotics to this group of women reduced laboratorial infection parameters, as well as maternal and fetal adverse effects and rates.

CONCLUSION

The research has shown that there are benefits in the use of probiotics during gestation, through changes in vaginal microbiota, for maternal and perinatal outcomes. However, some studies still rely on small samples and heterogeneous scenarios. Larger and better-designed studies are needed to make possible to have a more definitive conclusion and so to be replicated for the general population. It is important to highlight that these studies allow us to guide the best way and the ideal moment to introduce this supplementation during prenatal care.

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Conflict of interests

The authors declare no conflict of interests.

REFERENCES

1. Kilbride HW, Thibeault DW. Neonatal complications of preterm premature rupture of membranes. Clin Perinatol. 2001;28(4):761-85. https://doi.org/10.1016/S0095-5108(03)00076-9
2. Genovese C, Corsello S, Nicolosi D, Aidala V, Falcidia E, Tempera G. Alterations of the vaginal microbiota in the third trimester of pregnancy and pPROM. Eur Rev Med Pharmacol Sci. 2016;20(16):3336-43.
3. Frenette P, Dodds L, Armson BA, Jangaard K. Preterm Prelabour Rupture of Membranes. Effect of Latency on Neonatal and Maternal Outcomes. J Obstet Gynaecol Can. 2013;35(8):710-7. https://doi.org/10.1016/S1701-2163(15)30861-6
4. Fichorova RN, Onderdonk AB, Yamamoto H, Delaney ML, DuBois AM, Allred E, et al. Maternal Microbe-Specific Modulation of Inflammatory Response in Extremely Low-Gestational-Age Newborns. mBio. 2011;1(2):e00280-10. https://doi.org/10.1128/mBio.00280-10
5. Solt I. The human microbiome and the great obstetrical syndromes: A new frontier in maternal-fetal medicine. Best Pract Res Clin Obstet Gynaecol. 2015;29(2):165-75. https://doi.org/10.1016/j.bpobgyn.2014.04.024
6. Satokari R, Grönross T, Laitinen K, Salminen S, Isolauri E. Bifidobacterium and Lactobacillus DNA in the human placenta. Lett Appl Microbiol. 2009;48(1):8-12. https://doi.org/10.1111/j.1472-765X.2008.02475.x

7. Barthow C, Wickens K, Stanley T, Mitchell EA, Maude R, Abels P, et al. The Probiotics in Pregnancy Study (PiP Study); rationale and design of a double-blind randomised controlled trial to improve maternal health during pregnancy and prevent infant eczema and allergy. BMC Pregnancy Childbirth. 2016;16(1):133. https://doi.org/10.1186/s12884-016-0923-y
8. Luoto R, Laitinen K, Nermes M, Isolauri E. Impact of maternal probiotic supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. Br J Nutr. 2010;103(12):1792-9. https://doi.org/10.1017/S0007114509993898
9. Camargos AF. O significado do grau de recomendação e força de evidência A da classificação da Associação Médica Brasileira. Femina. 2010;38(2):59-62.
10. Rautava S, Collado MC, Salminen S, Isolauri E. Probiotics Modulate Host- Microbe Interaction in the Placenta and Fetal Gut: A Randomized, Double-Blind, Placebo-Controlled Trial. Neonatology. 2012;102(3):178-84. https://doi.org/10.1159/000339182
11. Ferraz EG, Silveira BB, Sarmento VA, Santos JN. Receptores Toll-Like: ativação e regulacão da resposta imune. Rev Gaúcha Odontol. 2011;59(3):483-90.
12. Vitali B, Cruciani F, Baldassere ME, Capursi T, Spisni E, Valerii MC, et al. Dietary supplementation with probiotics during late pregnancy: outcome on vaginal microbiota and cytokine secretion. BMC Microbiol. 2012;12:236. https://doi.org/10.1186/1471-2180-12-236
13. Gille C, Böer B, Marschal M, Urschitz MS, Heinecke V, Hund V, et al. Effect of pro-biotics on vaginal health in pregnancy. EFFPRO, a randomized controlled trial. Am J Obstet Gynecol. 2016;215(5):608.e1-e7. https://doi.org/10.1016/j.ajog.2016.06.021
14. Di Pierro F, Parolari A, Brundu B, Nigro R. Positive clinical outcomes derived from using a proprietary mixture of selected strains during pregnancy. Acta Biomed. 2016;87(3):259-65.
15. Daskalakis GJ, Karambelas AK. Vaginal Probiotic Administration in the Management of Preterm Premature Rupture of Membranes. Fetal Diagn Ther. 2017;42(2):92-8. https://doi.org/10.1159/000450995
16. Kavak SB, Kavak E, Ilhan R, Atilgan R, Arat O, Deveci U, et al. The efficacy of ampicillin and *Lactobacillus casei rhamnosus* in the active management of preterm premature rupture of membranes remote from term. Drug Des Devel Ther. 2014;2014(8):1169-73. https://doi.org/10.2147/DDDT.S68552

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