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Lactobacillus Rhamnosus BMX 54 Vaginal Application Usefulness as Adjuvant Therapy for Preterm Premature Rupture of Membranes (PPROM) in the Second Trimester of Pregnancy

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Abstract

Objectives: Very Preterm Premature Rupture of Membranes (PPROM) complicates about 2% - 5% of pregnancies and leads to an exponential increase in perinatal mortality and morbidity. A lot of Bacteria have been associated with PPROM. The aim of the following study was to evaluate the impact of Lactobacillus BMX 54 vaginal application added to standard of care therapy on pregnancy and neonatal outcome.

Material and Methods: This observational study includes 38 consecutive women with PPROM hospitalized in the Department of Obstetric and Gynecology of Perugia Hospital. All the women presented PPROM before of 30 week of pregnancy and have been treated with antibiotics, tocolitics therapy and bed rest: 58% of the pregnancies (22 women) added to standard of care once day vaginal Lactobacillus rhamnosus BMX 54 application while the others (42% of the pregnancies: 16 women) have been treated only with standard therapy without probiotic vaginal application

Results: The women who added to standard therapy Lactobacillus rhamnosus BMX 54 daily vaginal application from the beginning of PPROM up to the delivery shown a statistical significant increase (p = 0.04) of delivery gestational age when compared with the women treated only with standard therapy. The time course between PPROM and delivery was 26.5 +/- 27.4 days in the Lactobacillus treated group and 11.6 +/- 14.2 days in the other group (p = 0.03). CRP (C-Reactive Protein) value at delivery time was 2.37 +/- 2.2 in the lactobacillus treated group and 3.87 +/- 2.5 in the control group (p = 0.0003). 5th minute Neonatal APGAR Score was 8.8 +/-0.8 in treated group and 6.6 +/-3.7 in control group (p= 0.006) while the Hospitalization time of the newborn in Neonatal Intensive Unit Care (NICU) was 43.4 +/- 24 days in the treated group versus 61 +/- 33 days in control group (p = 0.01)

Conclusions: Lactobacillus rhamnosus BMX 54 vaginal application used as adjuvant therapy to standard treatment seems to be an useful approach to reduce PPROM complications improving neonatal and maternal outcomes

Keywords: PPROM (Preterm Premature Rupture of Membranes), Lactobacillus Rhamnosus BMX 54, B.V. (Bacterial Vaginosis)

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Introduction

Preterm Premature Rupture Of Membrane (PPROM) complicates only 2% of pregnancies but is associated with 40% of preterm deliveries and can result in significant neonatal morbidity and mortality [1-3]

PPROM is associated with lower latency period from membrane rupture to deliver and during this latency period the ascent of pathogen bacteria from the lower urogenital area could create complications such as intrauterine infections [4-8]

Ureaplasma urealyticum, Sneathia species, Escherichia coli, Chlamydia trachomatis, Mycoplasma hominis, Enterococcus faecalis and Gardnerella vaginalis are the common bacteria associated with PPROM [9].

Surprising mostly of these bacteria are responsible of Bacterial Vaginosis, the most common infective vaginal pathology of the women all over the world in which the absence of the predominant-lactobacillus species is replaced by gram– bacteria in the vagina [10].

The bacteria spread rapidly after the PPROM has occurred and colonize the surfaces of the amniotic membranes, the umbilical cord

and the fetus [11,12].

Low transplacental transfer of antibiotics is responsible for the moderate success of systemic antibiotic therapy [11,12].

Some Investigators have demonstrated that antibiotics could not eliminate the amniotic infection in 83% of PPROM cases [13].

Romero et Coll. [14] recently demonstrated that upon clinical admission, 37.3% of preterm PPROM patients delivered within 48 hours of membrane rupture and that only 32.2% of all PPROM patients had a latent phase of more than 14 days.

The three causes of neonatal death associated with PPROM are prematurity, sepsis and pulmonary hypoplasia and women with intrauterine infections deliver earlier than non-infected women and infant born with sepsis have a four times highest mortality than those without sepsis [15]

Concluding, there are evidences demonstrating an association between ascending infection from the lower uro-genital tract and PPROM [16,17]: in patients with PPROM about one-third of pregnancies have positive amniotic fluid cultures [16,17] and studies have shown that bacteria possess the ability to cross intact membranes [15-17].

Standard of care of PPROM, broad-spectrum antibiotics and antenatal corticosteroids, are routinely used in this condition, with very limited success to prevent bacteremia, chorioamniositis, funisitis and intra-amniotic infection syndrome [18]

Taking into account the role of bacteria in PPROM complications and neonatal outcomes and the partial uneffectiveness of systemic antibiotics therapy we thought to add to standard therapy (therapeutic tocolysis, systemic antibiotic and bed rest) in PPROM women a cycle of Lactobacillus rhamnosus BMX 54 (NORMOGIN) vaginal tablet application (1 tab/day from PPROM to the delivery) with the aim to control bacterial replication locally and to improve the fetal/maternal outcomes.

We have selected Lactobacillus rhamnosus BMX 54 between all the available vaginal probiotics because of its effectiveness demonstrated in a large sample size of women (more than 700) affected by Bacterial Vaginosis, in controlled and uncontrolled published clinical trials [19-24].

Materials and Methods

38 consecutive pregnancy women affected by PPROM before 30 weeks of gestational age hospitalized in the Obstetric and Gynecology department of Perugia University and Hospital departement of obstetric and gynecology between 2014 and 2015 entered in this observational trial.

The aim of this observational retrospective study was to preliminary understand if a daily vaginal tablet application of lactobacillus rhamnosus BMX 54 (NORMOGIN) was able to improve pregnancy's delivery time and newborn's score in women affected by PPROM.

All the women have been treated with PPROM standard of care therapy (antibiotics (macrolides or B-lactamic) plus Tocolytics (Atosiban and Progesterone) and bed rest during hospitalization: in 22 (patients of hospital department) of 38 PPROM pregnancies a vaginal tablet containing Lactobacillus rhamnosus BMX 54 (NORMOGIN) has been added daily to the standard therapy.

Medium Gestational Age (GA) together with cervicometria, leukocites count and CRP at the PPROM (hospitalization time) was collected for every woman.

During hospitalization time in every woman has been collected at alternate days a blood sample for the leukocites and CRP (C-Reactive Protein) detection and a medium value of every week for these values have been reported.

Delivery time, CRP and leukocites have also been collected at delivery. In every newborn fetal blood pH, BE (Basis Excess) and 1 minutes and 5 minutes APGAR score have been detected together with number of neonatal deaths, number of neonatal infections and serious neonatal complications.

Data analysis was performed with the help of the SPSS software version 17.0. Average measurements and standard deviation with their respective C I95% were calculated: Student's t test was used for independent data averages for the two groups. Analyses with p less than 0.05 were considered statistically significant.

Results

Gestational age at PPROM was homogeneous in the two different observational groups (26.19 +/- 2.6 weeks for women treated with lactobacilli too (group A) versus 26 .2 +/- 2.2 weeks for non lactobacillus treated women (group B)) with no statistically significant differences between group A and Group B (p = 0.495)(table 1).

Table. 1: Differences in Gestational Age (G.A.), Cervicometria (Cx), Leukocites counts (Leuk) and C-Reactive Protein (CRP) at PPROM in the two different groups.

	Group A (Lactobacillus rhamnosus BMX54 group + standard therapy)	Group B (only standard therapy)	Student's T-test for indipendent data
Number of women	22	16	= = =
GA at PPROM (weeks)	26.19 ± 2.6	26.2 ± 2.2	p = 0.495 NS
Cx at PPROM (mm)	24.76 ± 11	21.2 ± 9.5	p = 0.16 NS
Leuk at PPROM	11.6 ± 2.8	12.0 ± 4.3	p = 0.355 NS
CRP at PPROM	1.52 ± 2.19	2.04 ± 2.75	p = 0.243 NS

Also cervicometria, leukocites count and CRP were homogeneous in the two differential observational groups (table 1)

The pregnancy and newborn outcomes has been reported in tab 2; Gestational Age (GA) at delivery time was significant increased in group A patients (29.66 +/- 2.6 weeks) versus group B patients (28 +/-3 weeks) (p=0.04) and, obviously, also the treatment days after PPROM was significant increased in group A (21 +/- 18 days) versus group B women (11.3 +/- 13.1 days) (p = 0.04) (tab 2).

Also the number of days between PPROM and delivery was significantly higher in group A (26.5 +/- 27.4 days) versus group B (11.6 +/- 14.2) (p=0.03) while newborn outcomes as medium weight at delivery (1299.7 +/- 477 g versus 1161.2 +/- 663 g: p=0.23) and medium weight percentile at delivery (35.7 +/- 19.7 versus 42.3 +/- 15.9%: p=0.14) were not statistically difference between groups (tab 2).

APGAR scores at 1 minute and at 5 minutes were significantly increased in group A newborns versus group B newborns (APGAR 1 min: 6.85 +/- 1.79 versus 5.06 +/-3.6: p=0.02 and APGAR 5 minutes: 8.8 +/- 0.8 versus 6.6 +/-3.7: p=0.006); a statistically significative difference between groups were also shown in newborn blood pH (7.2 +/- 0.1 versus 7.07 +/- 0.19: p=0.01)), in newborns blood Base Excess (BE: 8.4 +/- 6.6 versus 14.1 +/- 0.1: p =0.01) and in newborn recovery days in Neonatal Intensive Care Unit (NICU) (43.4 +/-24 days versus 61 +/- 33 days: p=0.04) (tab 2)
Leukocites counts not significantly differ between groups during the trial observation while CRP was significantly lower in group A women during the second, third and fourth weeks (tab 3)
No newborns was died in lactobacillus treated group (0/22 = 0%) while 4 newborns died in group B women (4/16 = 25%). Also the number of newborn infections (7/22 = 31.8% versus 7/16 = 58.3%) and the number of newborn serious complications (4/22 = 18.1% versus 5/16 = 41.6%) were lower in lactobacillus treated patients versus the control group.
No drug's related adverse events have been collected during the trial.

Discussion

PPROM is associated with lower latency from membrane rupture until delivery and, consequently, it represents an important cause of perinatal morbidity and mortality [1-3].
During the latency period the ascent of pathogenic microorganisms from the lower uro-genital tract could create complications such as intrauterine infections [4-8].
One of the most common complications in PPROM women is intrauterine infection which could lead to chorioamniositis, metritis after delivery and perinatal outcome such us neonatal sepsis [1-8].
Perinatal outcomes constitute prematurity, neonatal sepsis, respiratory distress syndrome, intraventricular hemorrhage and risk of fetal and neonatal death [1-8].
Since PPROM causes definite maternal and neonatal morbidity and mortality every effort could be done in order to manage this pathology. The latency time between PPROM and delivery seems to be a Key point to improve perinatal morbidity and mortality: increase this period could help attending physicians in PPROM management.
Expectant management with antenatal antibiotics and corticosteroids administration are "the recommended standard of care" in the setting of PPROM at gestational age of less than 34 weeks [18].
In this observational retrospective clinical trial we observed that by adding vaginal probiotic (Lactobacillus rhamnosus BMX 54 – NORMOGIN) to the standard of care immediately after PPROM we obtained an increase in latency time between PPROM and delivery improving neonatal outcome.
Taking into account that no differences has been shown between the two groups of women (standard of care plus lactobacillus rhamnosus BMX 54 – group A and only standard of care treatment – group B) at PPROM time in terms of gestational age, CRP, leukocites values and cervical index it was really surprising to observe a significant increase in latency time between PPROM and delivery only adding vaginal probiotics (table 2).

Table. 2: Results – Pregnancy and newborn outcomes.

	Group A	Group B	Student's T-test for indipendent data
G.A. at delivery (weeks)	29.66 ± 2.6	28 ± 3	p = 0.04
Treatment days after PPROM (days)	21 ± 18	11.3 ± 13.1	p = 0.04
Days from PPROM after delivery (days)	26.5 ± 27.4	11.6 ± 14.2	p = 0.03
Medium newborn weight at delivery/(gr)	1299.7 ± 477	1161.2 ± 663	p = 0.23
Medium newborn percentile weight at delivery (%)	35.7 ± 19.7	42.3 ± 15.9	p = 0.14
APGAR score 1 minute	6.85 ± 1.79	5.06 ± 3.6	p = 0.03
APGAR score 5 minutes	8.8 ± 0.8	6.6 ± 3.7	p = 0.02
Newborn blood pH	7.2 ± 0.1	7.07 ± 0.19	p = 0.01
Newborn blood Base Excess (BE)	8.4 ± 6.6	14.1 ± 0.1	p = 0.01
Neonatal hospitalization days in NICU (days)	43.4 ± 24	61 ± 33	p = 0.04

More the doubling the days between PPROM and delivery (26.5 days versus 11.6 days: p=0.03) obtained only by adding lactobacillus rhamnosus BMX 54 (one vaginal tablet daily) to the PPROM standard of care was an intriguing observation especially if related to the best neonatal outcomes obtained in terms of APGAR Score after 1 and 5 minutes, neonatal blood pH and Base Excess (table 2).
Also the differences in terms of hospitalization days in Neonatal Intensive Care Unit between the two groups of newborn (43.4 versus 61 days: p=0.04) seems to encourage the use of vaginal probiotics during PPROM (table 2).
Also relevant was the observation that in group A patients no neonatal death has been detected while 4 newborns was died in group B patients: also neonatal serious complications and neonatal infections were higher in women treated only with standard of care confirming that lactobacillus rhamnosus BMX vaginal addiction to the standard of care could improve PPROM outcomes.
It's not surprising that a selected lactobacillus, that has recently demonstrated to be able to counteract Bacterial Vaginosis (BV) recurrences in more than 700 women affected by BV [20-24], could be able to improve neonatal and maternal outcome in women affected by PPROM .
Since bacteria like Ureaplasma urealyticum, Escherichia coli, Mycoplasma hominis, Enterococcus faecalis and Gardnerella vaginalis are the common bacteria associated with PPROM [9] it could be possible that by restoring vaginal ecosystem we can control gram-vaginal pathogens overgrowth in vagina and their spread up versus amniotic membranes.
CRP movements during the first weeks after PPROM in lactobacillus treated group seems to confort this idea making lactobacillus supplementation "a natural defense" against gram- bacteria

overgrowth: we also have to consider that systemic antibiotic therapy could decrease lactobacillus populations in vagina making the ecosystem more available to faecal and urogenital gram- bacteria overgrowth.

Amniotic infections related to gram- facultative pathogens could represent a "break-down" in PPROM maternal and neonatal complications making lactobacillus rhamnosus BMX 54 vaginal supplementation "a new strategy" to counteract these infections: best APGAR scores at 1 and 5 minutes together with significant differences in neonatal blood pH and Base Excess (BE) seems to correlate with this observation.

On the other hand only Lactobacillus rhamnosus has demonstrated to be able to colonize vaginal ecosystem during the 7th weeks after implantation [25] making Lactobacillus rhamnosus BMX 54 vaginal administration a reasonable approach to restore vaginal ecosystem.

A significant difference in hospitalization days in NICU (Neonatal Intensive Care Unit) and in neonatal complications also seems to demonstrate that only by restoring vaginal ecosystem by lactobacillus application we can improve neonatal outcome in PPROM women.

Obviously, this retrospective, observational study has a lot of methodological limitations: the study is not a prospective, randomized, double blind-placebo controlled clinical trial; the control group (group B) is "unblinded"; the sample size is too small and the number of patients is different in the two groups, nevertheless, the results obtained only by adding lactobacillus rhamnosus BMX 54 1 vaginal tablet daily to the standard of care are surprising in term of maternal and neonatal outcomes.

If these results will be confirmed in prospective, double blind, placebo-controlled clinical trials vaginal probiotic application could become a new interesting therapeutic strategy to control PPROM when adding to standard of care.

Table. 3: Results – Maternal Leukocites and CRP during the trial

	Group A	Group B	Student's T-test for indipendent data
Leukocites at 1st week	11.7 ± 3.1	10.1 ± 3.6	p = 0.09
Leukocites at 2nd week	10.6 ± 1.8	9 ± 2	p = 0.24
Leukocites at 3rd week	9.8 ± 2	9 ± 1.5	p = 0.28
Leukocites at 4th week	9.34 ± 3.4	9.25 ± 3.8	p = 0.48
CRP at 1st week	2.03 ± 3.9	2.59 ± 2.2	p = 0.329
CRP at 2nd week	0.92 ± 1.2	2.4 ± 2.2	p = 0.03
CRP at 3rd week	0.56 ± 0.51	1.56 ± 1.6	p = 0.05
CRP at 4th week	0.49 ± 0.43	0.57 ± 0.03	p = 0.03
CRP at Delivery	2.37 ± 2.2	3.87 ± 2.5	p = 0.0003

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