Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS

Probiotics in the NICU: Evidence and Controversies
✦ Course Transcript ✦

Overview

Neonatologist, **Teresa del Moral MD, MPH, PhD,** is a professor of clinical pediatrics at the University of Miami Leonard M. Miller School of Medicine, with a special interest in neonatal resuscitation, nutrition, and probiotic use. In this presentation, Dr. del Moral reviews the importance of premature infant dysbiosis and the manipulation of the intestinal microbiota using probiotics. She discusses the controversies that exist with the use of probiotics in the population of premature newborn infants, as well as the potential to reduce the risk of necrotizing enterocolitis.

Target Audience

This activity was developed for neonatologists, pediatricians, nurses, advanced practice clinicians, dietitians, and other healthcare providers with an interest in newborns, infants, and children.

Learning Objectives

At the conclusion of this activity, participants should be better able to:

- Recognize the prevalence and impact of dysbiosis in premature infants and its association with morbidity
- Assess the clinical impact of manipulating the intestinal microbiota in premature infants based on the latest evidence.

Faculty

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The estimated time to complete the activity is 1.0 hour.

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This activity was released on January 31, 2022 and is eligible for credit through January 31, 2024.

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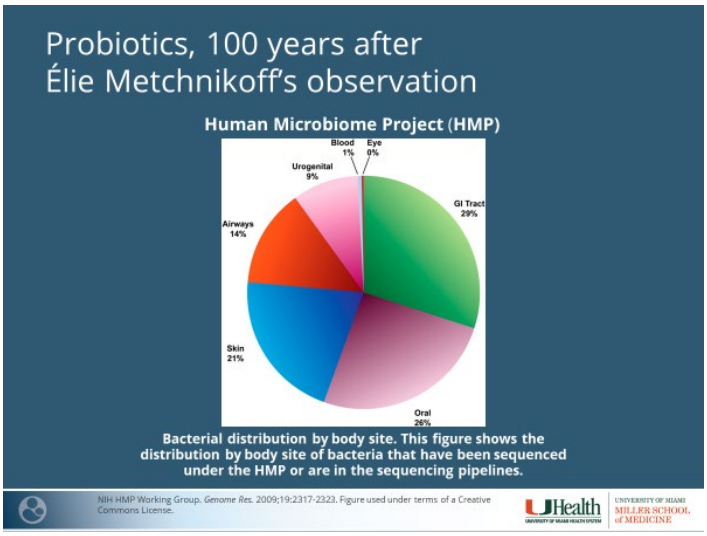
Probiotics in the NICU: Evidence and Controversies

Editor's Note: This is a transcript of a live presentation on November 7, 2021. It has been edited and condensed for clarity.

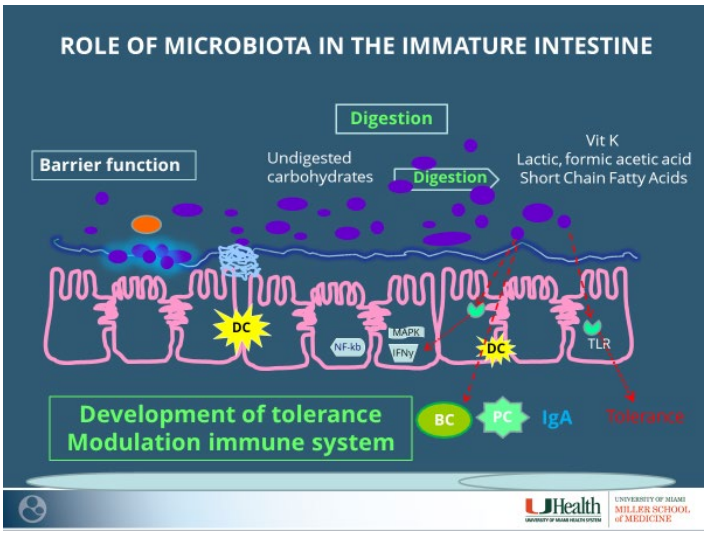
PREMATURE INFANT DYSBIOSIS

Teresa del Moral, MD, MPH, PhD: The topic I am going to talk about today is Probiotics in the NICU: Evidence and Controversies. The points that we are going to touch on are the premature infant dysbiosis and the manipulation of the microbiota with probiotics: what is the evidence and what are the controversies, still, in terms of the use of probiotics in premature infants.

It's more than 100 years now that Elie Metchnikoff made the observation that the ingestion of a light bacteria was associated with prolonged life. We know now that these organisms, this microbiome, are an important part of our body, and a relevant part of the scientific literature. It was in 2008 when the NIH promoted the Human Microbiome Project to try to better define these bacteria that are part of our body and the implication in health and disease.[1]



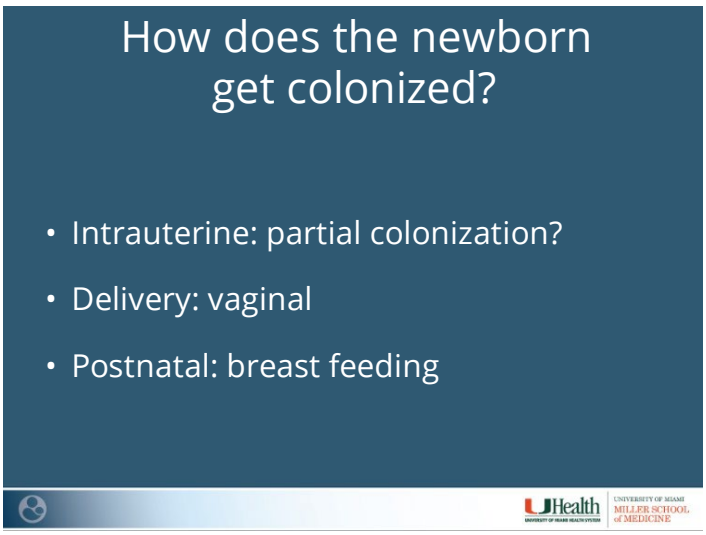
Beside the fact that there this organism is throughout our body, the most relevant part, the most important part is in the GI [gastrointestinal] tract. And in the GI tract, they have important biological functions. This biological function starts with forming a safe barrier of the intestinal mucosa to prevent the translocation of the pathogens. Also, these bacteria, the bacteria that will do the digestion of the oligosaccharides that are present in breast milk, these oligosaccharides are the nutritive for bacteria. Through the digestion and fermentation of these oligosaccharides, it will produce vitamins, lactic acid, and short-chain fatty acids, which are very important for neurodevelopment.



On the other side, these bacteria will interact with enterocytes through a mechanism that has been called crosstalk. And through this mechanism, it's going to promote the maturation of the immune system. That means that the development of tolerance and also the modulation of the immune system, through the

modulation of inflammatory markers, and also interaction with B cells, and increase of IgA.

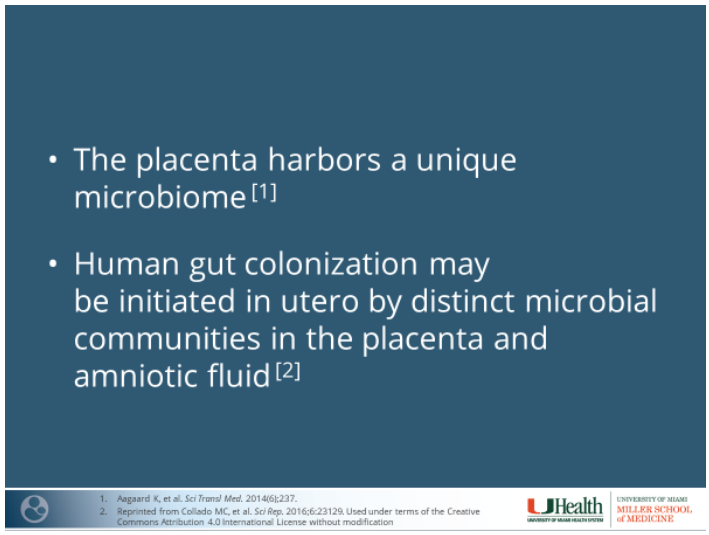
This is an important part of the function of this microbiome in the intestine. This is very important, specifically in the first weeks or months of life. But how do the newborn infants acquire those bacteria? There are three main mechanisms, which include the intrauterine, the delivery through the vaginal channel, and also the postnatal, which is mainly through the breast milk.



Slide 3

In the last few years, the idea that the placenta and the amniotic fluid are sterile, when there is no infection has been challenged, because there is a study from 2014, in which they found bacteria in the placenta that is very similar to the bacteria, which is in the mouth of the mother.[2] A few years later, Dr. Collado found that there was some bacteria that was found in amniotic fluid.[3] It was found in the placenta, and it's similar to the bacteria that is later found in the breast milk, in the first few days of life, in the meconium of the newborn.

But this idea that the placenta, that the colonization may start in prenatal life, the data are not consistent, so there is still no definitive confirmation.



Slide 4

After that, the pass through the vaginal channel is one of the important ways to colonize the newborn. This study shows babies who were born by vaginal delivery, they have bacteria—which is the red one—that is closer to the bacteria that is found in the vagina.[4] [For] these babies who were born vaginally, the bacteria are closer to those found in the vagina, versus the babies who were born by C-section in which the bacteria [the babies] are colonized with is closer to the bacteria found in the skin on the mother.

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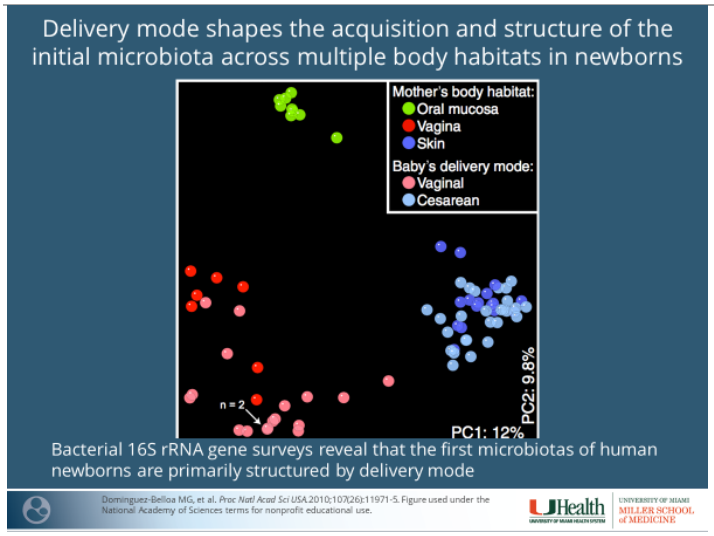
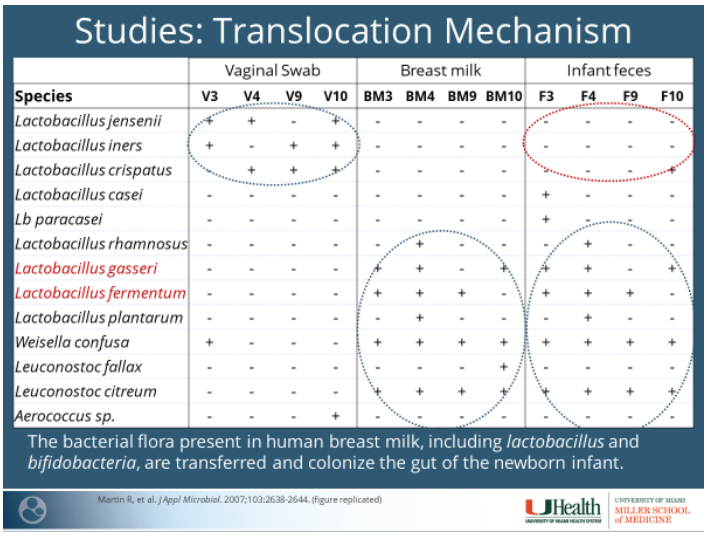


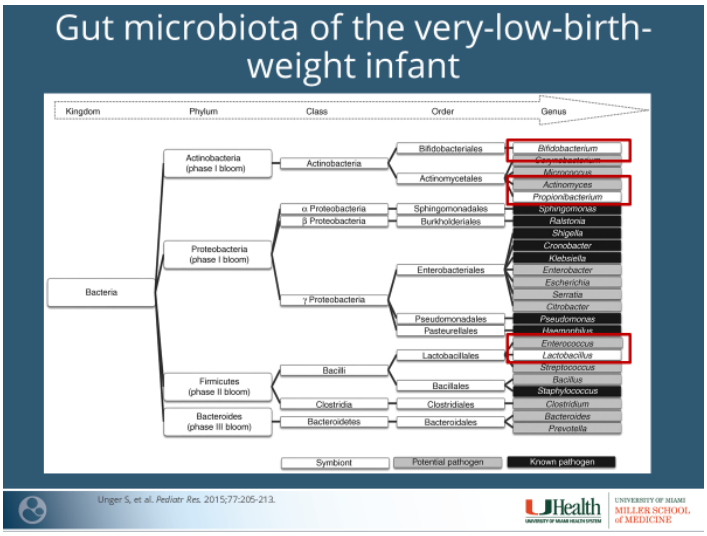
Slide 5

After birth, the main source of colonization is the breast milk. These are fecal samples of different newborns in which the bacteria that is found in these fecal samples is the same bacteria that was found in the breast milk of the mothers and also, some of these bacteria was found in the vagina of the mother.[5]



Slide 6

With that, the newborn ends with a very diverse colonization that is classified, like in this graph, in which the main symbiotic bacteria are the Bifidobacterium and the lactobacilli, which are marked. There are also bacteria that are potentially pathogens, and those marked in black are the bacteria that are pathogens.



Slide 7

But what happens with the premature baby? The premature baby, yes, because the baby is often born by C-section. Also, because they are in a different environment to be with the mother, they don't go with the mother, they stay in our units, and they're exposed to that environment. Also, often they get antibiotics in the first few days of life, and the diet is not the breast milk diet from their mother.[6] We try to give breast milk, but often they don't get as much breast milk as we would like.

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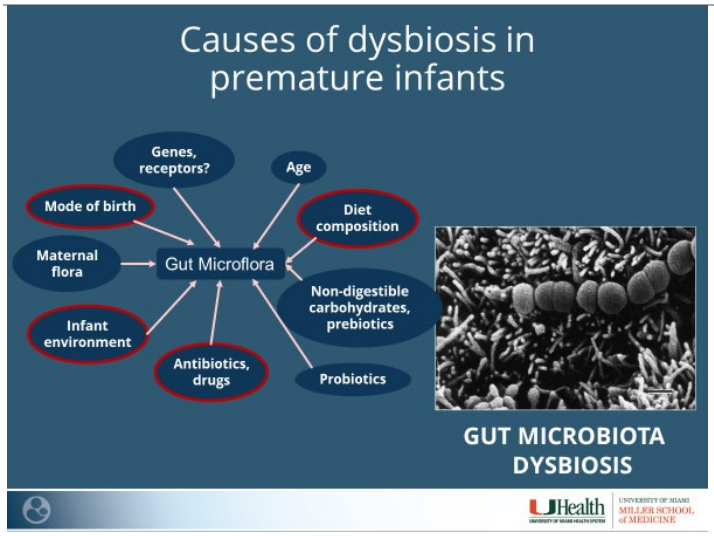

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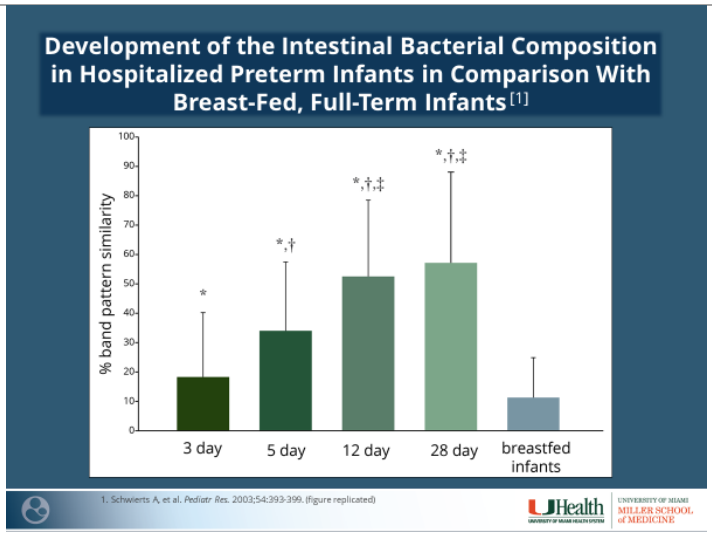
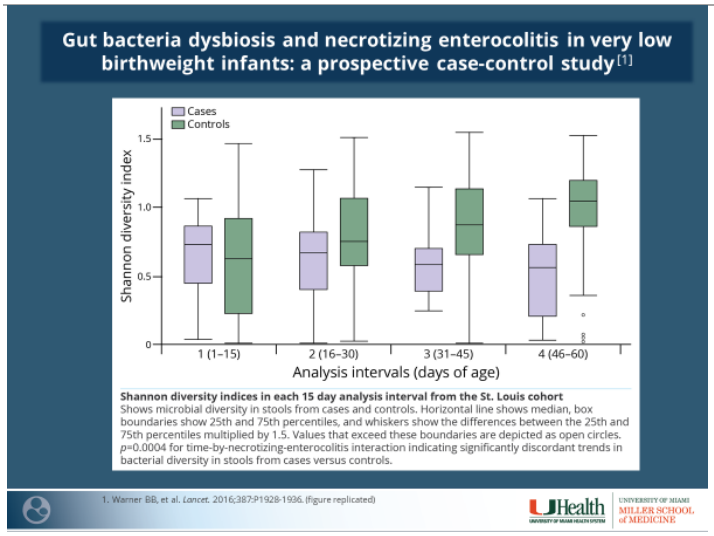


Slide 9

So, that causes changes in this colonization. This is one of the studies we chose that represents these changes in colonization.[7] This is a graph in which the bars represent the bands of similarity in terms of the intestinal bacteria. The babies who are breast fed full term—which is the last column—the similarity is low, so the diversity is very high. You see, compared with the babies who were premature babies, and three days of life, and through the first weeks until day 28 of life, the similarity is much higher, meaning that diversity is very low. So, that's one of the characteristics of the colonization of the full-term baby: they have a very diverse colonization.

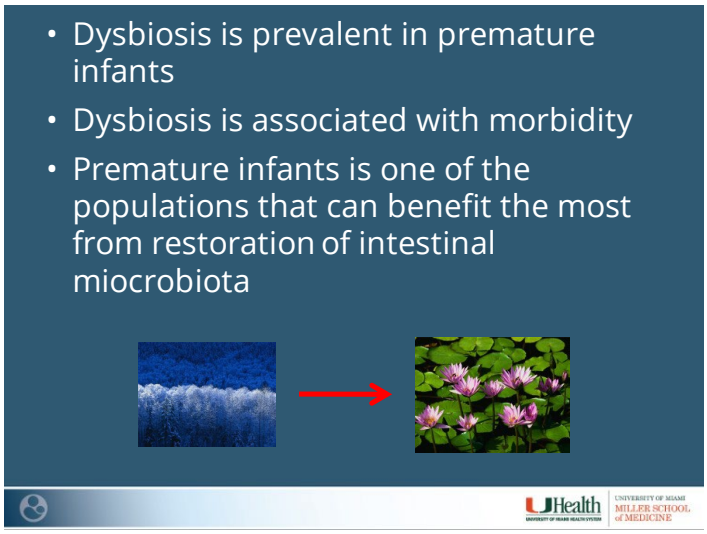
But this is not only dysbiosis in the babies. Also, this has some clinical implication, as shown in this study in which the bars represent what is called the Shannon Index, which is exactly the opposite of the previous one. This is an index that measures diversity, and we see on the red or pink bars that represent the cases, and the green bars, the controls. And this is a cohort study in which babies who developed necrotizing enterocolitis were compared with babies who did not develop necrotizing enterocolitis. [8] So, you see, in the control [group], the diversity goes up over the weeks, while in the babies who developed necrotizing enterocolitis, the diversity decreases over time.

Probiotics in the NICU: Evidence and Controversies



Slide 10

These are two of the many studies that show, that confirm the fact that in premature babies, dysbiosis is prevalent in premature infants. Also, dysbiosis in these premature infants is associated with morbidity. That makes this population, one of the populations who can benefit the most of trying to revert dysbiosis and to use the probiotics to stabilize the intestinal microbiota.



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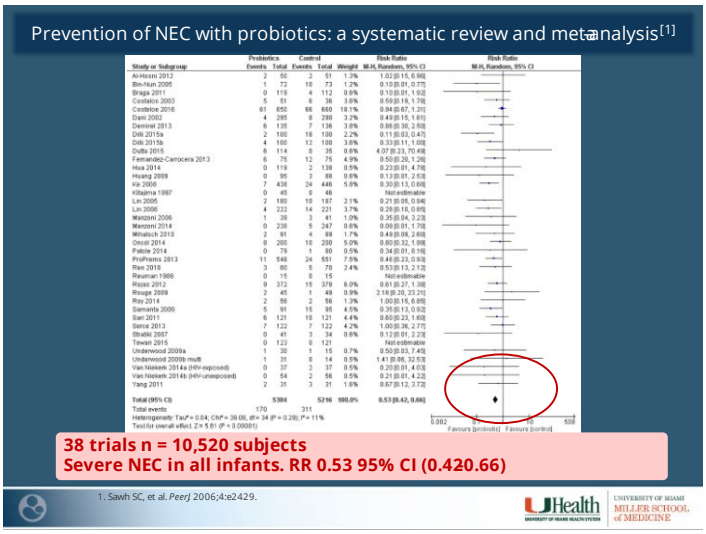
Manipulation of the Microbiota—Probiotics Evidence

Because we know in the last few years, and probably since 2005, that was the first randomized clinical trial in which probiotic was used in premature infants, and [the study] showed very drastic and impressive changes in terms of decreasing necrotizing enterocolitis, decreasing mortality, and decreasing sepsis.

There have been many studies. There are 38 represented in this meta-analysis, in which—including more than 10,000 premature infants—showed that the use of probiotics decreases the risk of necrotizing enterocolitis.[9]



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Not only necrotizing enterocolitis, but 29 of these 38 randomized, controlled trials, they also looked at the outcome of mortality, with more than 9,000 infants included, [and] also showed that the use of probiotics decreased the risk of mortality.

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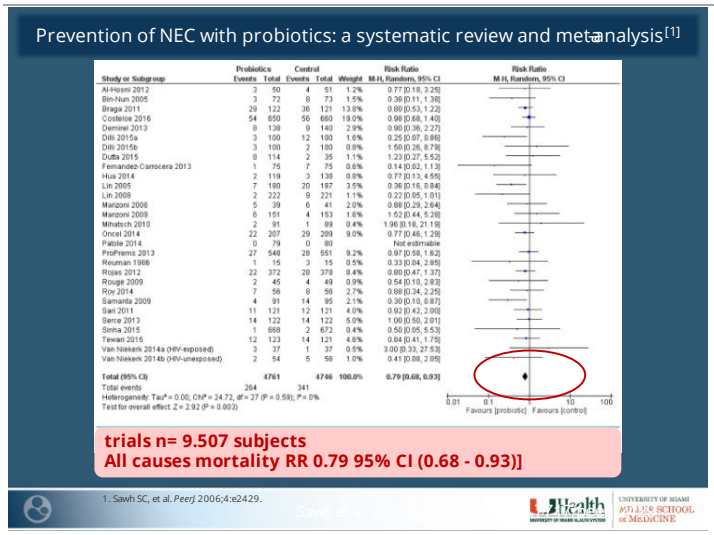

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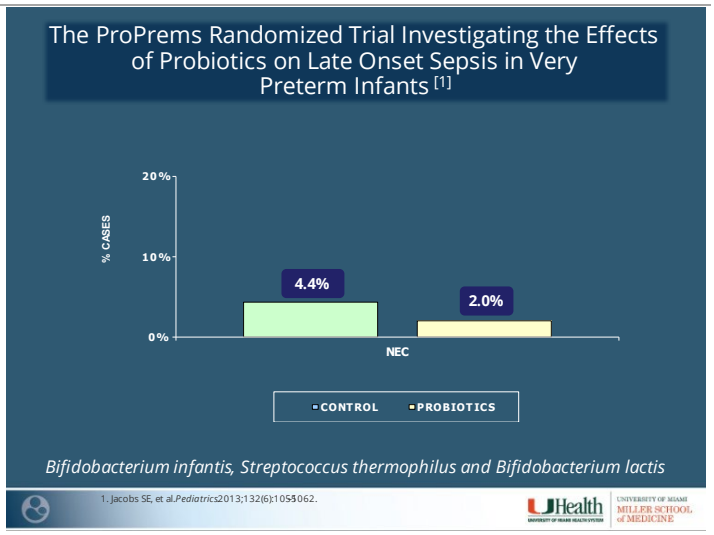

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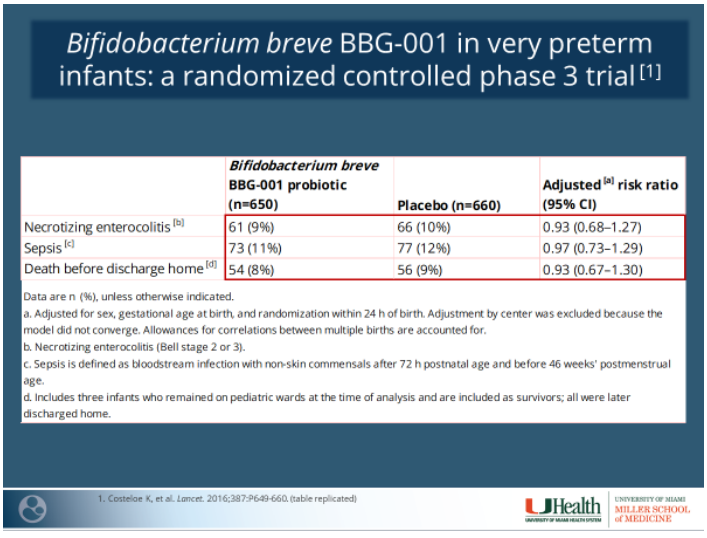
We're going to look in more detail at two of the larger randomized, controlled trials that were included in that meta-analysis. One of them is the study that was done in Australia, in which the study was targeting the decrease of nosocomial sepsis. The use of probiotic that included Bifidobacterium infantis, streptococcus thermophilus, and Bifidobacterium lactis.[10] They did not show any difference in the incidence of risk of sepsis, but they showed a decrease on the risk of necrotizing enterocolitis. And this is important because this was a population in which the prevention of necrotizing enterocolitis was optimized. This is a population in which they were receiving breast milk in more than 90 percent of the babies. And we see the incidence of necrotizing enterocolitis really low [compared with] other parts of the world. The authors claim that this intervention will be more effective or more impactful in areas where the rate of necrotizing enterocolitis is high.

The other important study was the study that was done in England. This study is a randomized, controlled trial with a similar population of babies, less than 1500 grams in, which they use only a Bifidobacterium, Bifidobacterium breve.[11] They show that the use Bifidobacterium breve, when the analysis was done by intention-to-treat analysis, there was no difference in the incidence of necrotizing enterocolitis, sepsis, or mortality.



	Bifidobacterium breve BBG-001 probiotic (n=650)	Placebo (n=660)	Adjusted [a] risk ratio (95% CI)
Necrotizing enterocolitis [b]	61 (9%)	66 (10%)	0.93 (0.68–1.27)
Sepsis [c]	73 (11%)	77 (12%)	0.97 (0.73–1.29)
Death before discharge home [d]	54 (8%)	56 (9%)	0.93 (0.67–1.30)

Data are n (%), unless otherwise indicated.
a. Adjusted for sex, gestational age at birth, and randomization within 24 h of birth. Adjustment by center was excluded because the model did not converge. Allowances for correlations between multiple births are accounted for.
b. Necrotizing enterocolitis (Bell stage 2 or 3).
c. Sepsis is defined as bloodstream infection with non-skin commensals after 72 h postnatal age and before 46 weeks' postmenstrual age.
d. Includes three infants who remained on pediatric wards at the time of analysis and are included as survivors; all were later discharged home.

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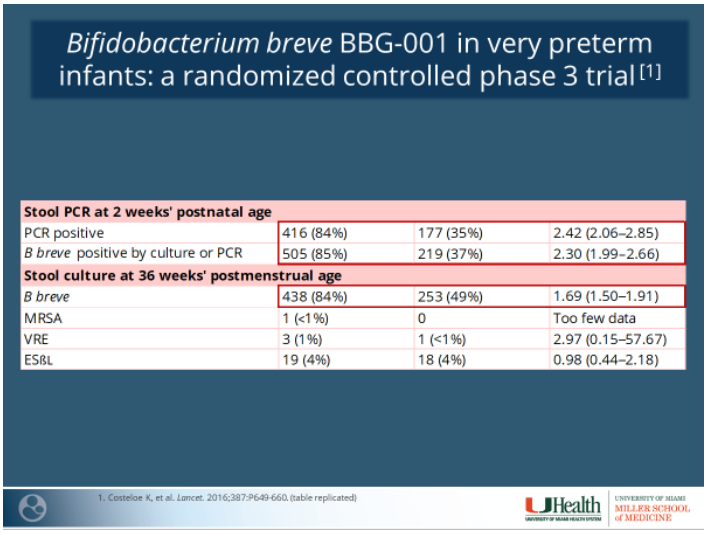
But this is the only randomized, controlled trial in which they checked the stools. They checked the fecal samples in the control and the study group at two weeks by PCR and also by culture, and they rechecked it at 36 weeks by culture. And what they found…there was a cross contamination in 37% of the stools at two weeks. And 49% of the babies who were in the control group had acquired the probiotic or the bacteria that was intended to be the intervention.



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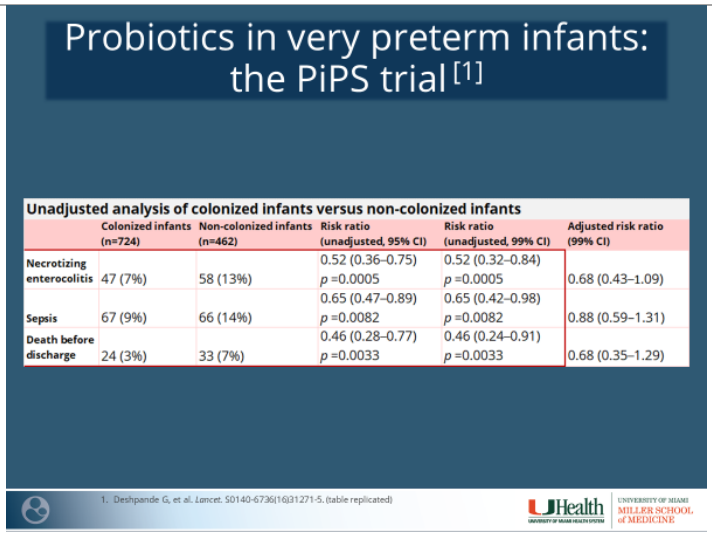
After that, not the same authors, but Dr. Deshpande decided to do an analysis of the data based on the babies who were in the group that was colonized, comparing with the group that was not colonized. [12] Doing this analysis, they found there was a decrease in the incidence of necrotizing enterocolitis, a significant decrease in the rate of sepsis, also seen as statistically significant in the rate of death or mortality.



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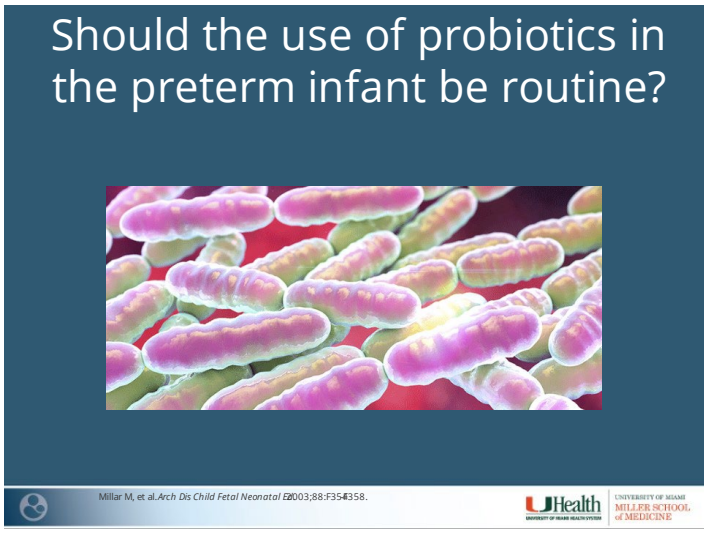
So, we have a meta-analysis with 38 clinical trials. We have two large clinical trials showing, directly or indirectly, that the use and the intervention to use probiotics in newborn decreases the incident of necrotizing enterocolitis. So, should we be using probiotics as a routine in premature infants?[13]



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This is a survey from the United States. Five hundred NICUs were asked whether they use probiotics [in VLBW infants] or not. Seventy [said] they use [them], which means 14 percent

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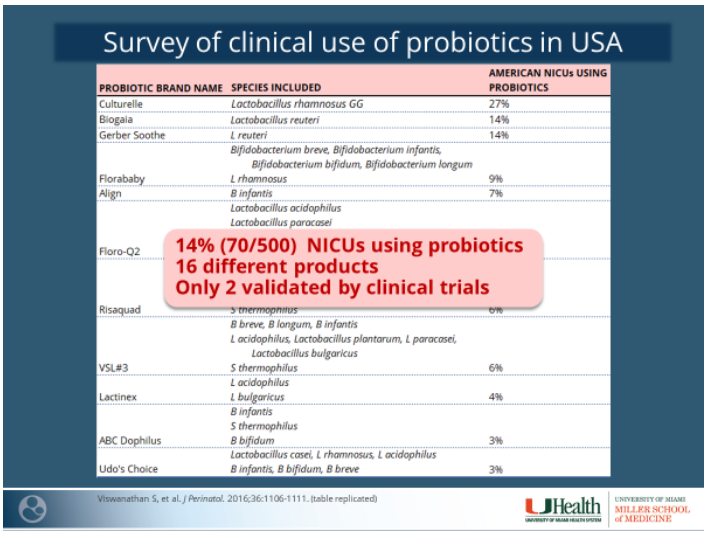
of the units use probiotics, 8 percent of these units use the probiotics in selected—not in a population—but in selected cases, and 5 percent [give them] as a standard.[14] But, when we look at what kind of probiotic they use, they use 16 different products, and only two of those different products were validated, where there was literature showing or evidence that these probiotics were going to have clinical benefits.



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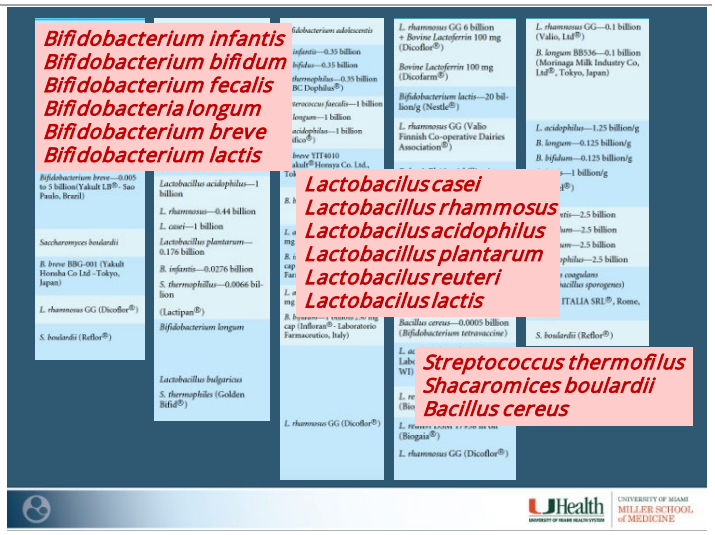
So, what happened when we collected the list of bacteria that has been used in those 13 clinical trials? We see that there is a list: six different Bifidobacterium, six different lactobacilli, and some other bacteria that are not Bifidobacterium or lactobacillus. All the clinical trials, most of them, the probiotic used was the probiotic that was available in the area where the study was done. There is no or little rationale why, in terms of mechanisms, of why this probiotic was used or what the mechanisms were or anticipated clinical benefits of these probiotics.



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Manipulation of the Microbiota—Probiotics Controversies

Strain Specific Effects

Because we are talking of probiotic like a generic thing, as if it's all the same, but really do we know if all these probiotics are the same?

This is one of the concerns, one of the first issues we have to think about when we decide, or we think about probiotics, is whether the probiotics all have the same effects.

This is the first meta-analysis in which they think they were clever enough to separate the studies that were using bifidobacteria, [and] the one using the two lactobacillus bifidobacteria and lactobacillus.[15] They found in the three cases there was a decrease of the risk of necrotizing enterocolitis, but only in the cases in which the lactobacillus was included, there was a decrease in mortality, which suggests that there are additional effects beyond the necrotizing enterocolitis that may be beneficial

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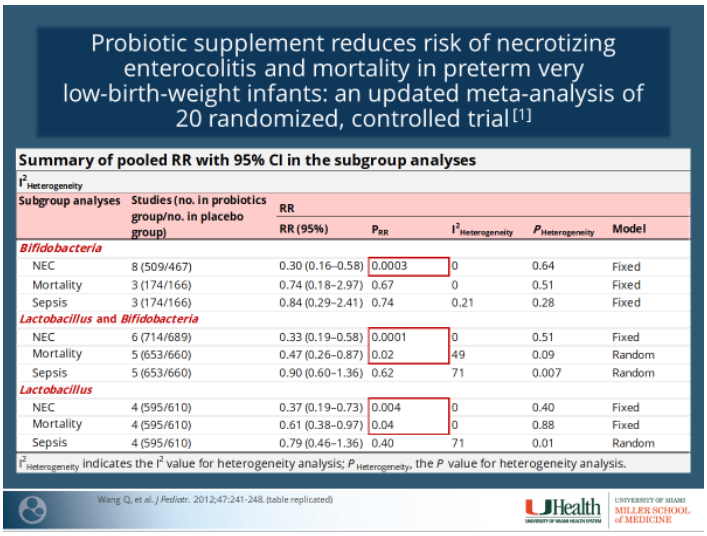
for the babies, and [this] is based on the use of lactobacillus.



Subgroup analyses	Studies (no. in probiotics group/no. in placebo group)	RR (95%)	P_{RR}	$I^2_{Heterogeneity}$	$P_{Heterogeneity}$	Model
Bifidobacteria						
NEC	8 (509/467)	0.30 (0.16–0.58)	0.0003	0	0.64	Fixed
Mortality	3 (174/166)	0.74 (0.18–2.97)	0.67	0	0.51	Fixed
Sepsis	3 (174/166)	0.84 (0.29–2.41)	0.74	0.21	0.28	Fixed
Lactobacillus and *Bifidobacteria*						
NEC	6 (714/689)	0.33 (0.19–0.58)	0.0001	0	0.51	Fixed
Mortality	5 (653/660)	0.47 (0.26–0.87)	0.02	49	0.09	Random
Sepsis	5 (653/660)	0.90 (0.60–1.36)	0.62	71	0.007	Random
Lactobacillus						
NEC	4 (595/610)	0.37 (0.19–0.73)	0.004	0	0.40	Fixed
Mortality	4 (595/610)	0.61 (0.38–0.97)	0.04	0	0.88	Fixed
Sepsis	4 (595/610)	0.79 (0.46–1.36)	0.40	71	0.01	Random

$I^2_{Heterogeneity}$ indicates the I^2 value for heterogeneity analysis; $P_{Heterogeneity}$, the P value for heterogeneity analysis.

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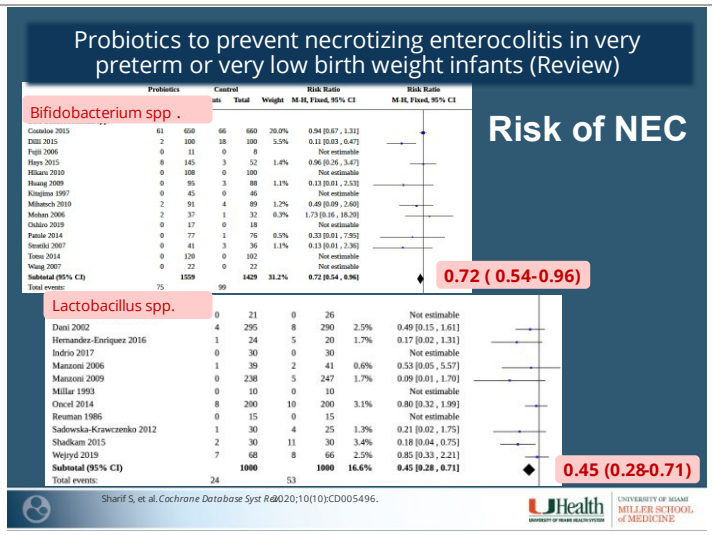
This was in 2012 [Wang et al], and last year there was another review of the use of probiotics in which they also did the same thing. They categorized the effects based on the species of probiotics.[16]

So, when we look at all the studies, and when they use Bifidobacterium, we found that there was a decrease in the risk of necrotizing enterocolitis, which it was, it reaches statistical significance. When they use the lactobacillus, there's also a decrease in the risk of necrotizing enterocolitis.[16]



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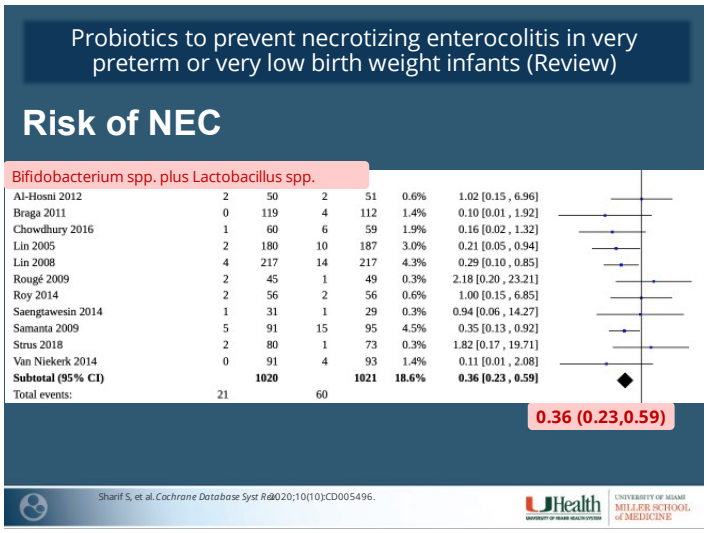
When they look at the studies in which they use the two types of bacteria, there was even more of a significant decrease in the risk of necrotizing enterocolitis.[16] That suggests, probably, if one of them individually is good, probably, the addition of the two may be more beneficial.



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But at the same time, when they look at the studies in which saccharomyces or bacillus was used, there was no statistical significance, and there was no decreased risk of necrotizing

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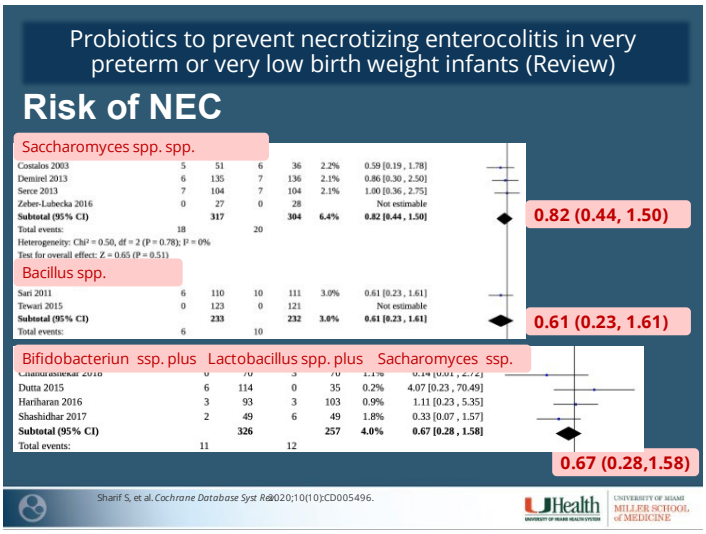
enterocolitis. And this is even more, I will say, shocking or interesting—the fact that in the last group, they looked at the studies in which Bifidobacterium and lactobacillus were used, but additionally they had saccharomyces, and then the benefits we saw in the previous meta-analysis, they are not here anymore. So, the addition of saccharomyces decreased the beneficial effect of the lactobacillus and Bifidobacterium.



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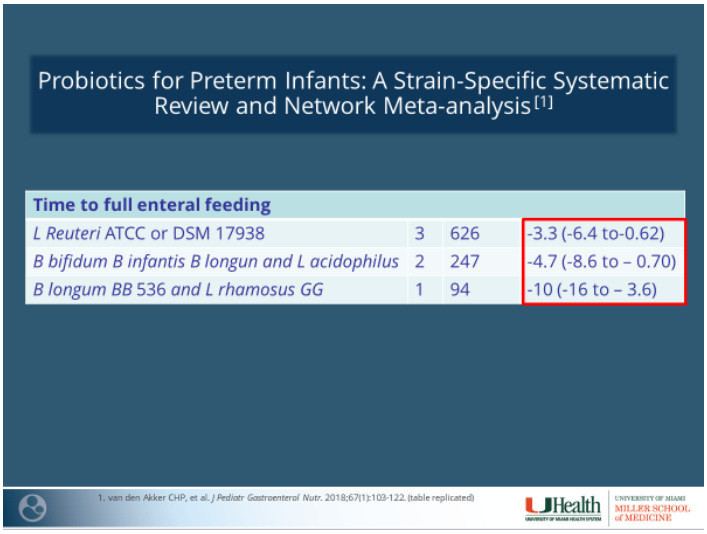
Other Possible Effects

That's one of the concerns. The other is we are focused on all these studies. We're mainly focused on the prevention of necrotizing enterocolitis, but if we think of other functions and other roles of the microbiota, maybe there are other possible beneficial effects on the newborns.

In this meta-analysis, in one of the studies I just showed, they also analyzed how many of these bacteria in which they studied were—they looked at the time to full [enteral] feeding—so, how the bacteria will benefit in terms of full tolerance? And there are three studies with Lactobacillus reuteri and two with Bifidobacterium infantis longum and acidophilus, and one with Bifidobacterium longum and rhamnosus in a total of six studies, with total of almost 1,000 babies.[17] And what they show [is a] decrease time to full feed. So, there are basic studies that show that some of these Bifidobacterium increase the intestinal motility. There may be additional benefits beyond the prevention of necrotizing enterocolitis.



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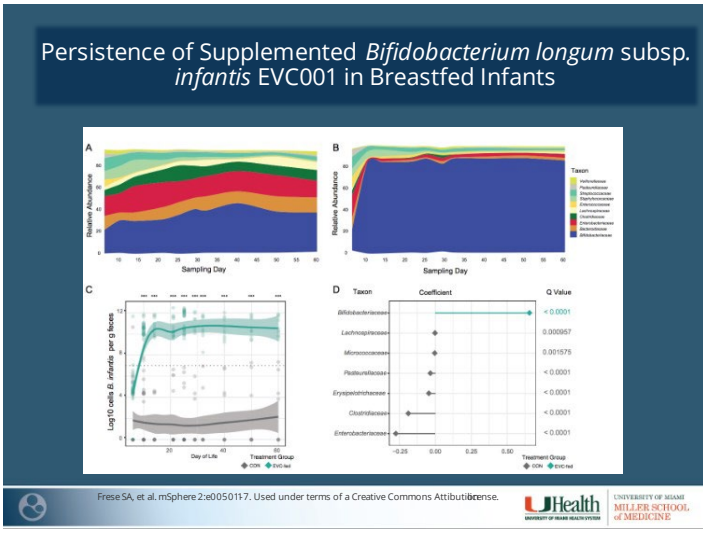
Mechanism of Action

The other thing is most of the studies were based… or were using a probiotic that was available in the area, but there was—with many of these probiotics—there is not basic research to sustain the randomized, clinical trials. There is no specific understanding of what the mechanisms are and why these probiotics may work.

In that sense, the University of California, Davis is doing a very good job leading the development of research, focusing on just one bacterium, which is the *Bifidobacterium longum*,

so the species infantis, with the idea that this is one of the predominant colonizers of the newborn, of the full-term newborn. Thinking that this physiologic colonization, and also, these bacteria are one of the major users of the oligosaccharides that are in the breast milk.

This is one of the studies. When they supplemented with bifidobacteria and they found, this is the study group and the control, and they found that bifidobacteria is predominant in the fecal samples of these babies.[18]



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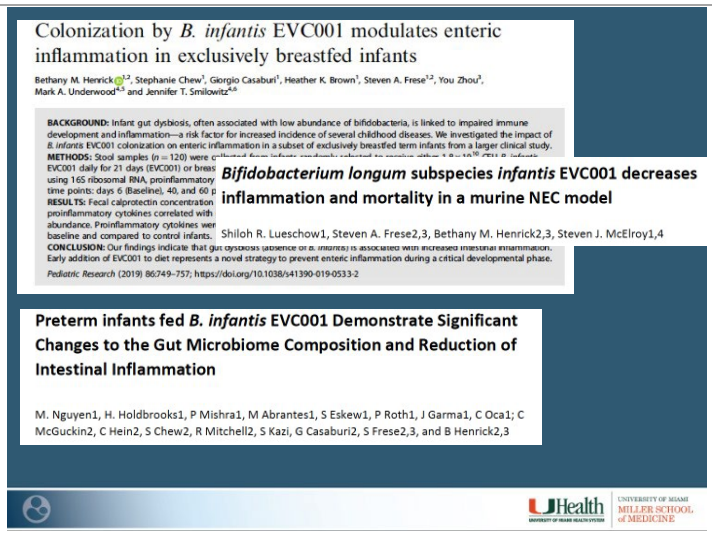
And additionally, there are several studies that we are not going to go into detail because of the time, but all the studies trying to understand the basic mechanism, and why these bifidobacteria work in the intestine, and also promotes and decreases inflammation in the intestine, which could be the basis for more clinical benefits.[19,20,21]



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Safety and Regulations

The other issue is safety. And in terms of safety, there are only a few reports reporting sepsis due to the bacteria that was used as probiotics, but none of the clinical studies showed sepsis as an adverse event. The sepsis that was described are mostly in patients who were immunodeficient.

But there is some concern in terms of long-term safety. This is a study published in Turkey, in which they started to use a probiotic.[22] I have to say it was a probiotic with many, many bacteria in which the labeling didn't quantify the amount of bacteria in that probiotic. But after a few months, they found that they had an epitome of enterococcus vancomycin-resistant. They looked at the two groups, and they looked at the retrospective, and they found the babies who had developed the resistance, there were 80 percent that were exposed to the probiotic versus 20 percent in the control group.

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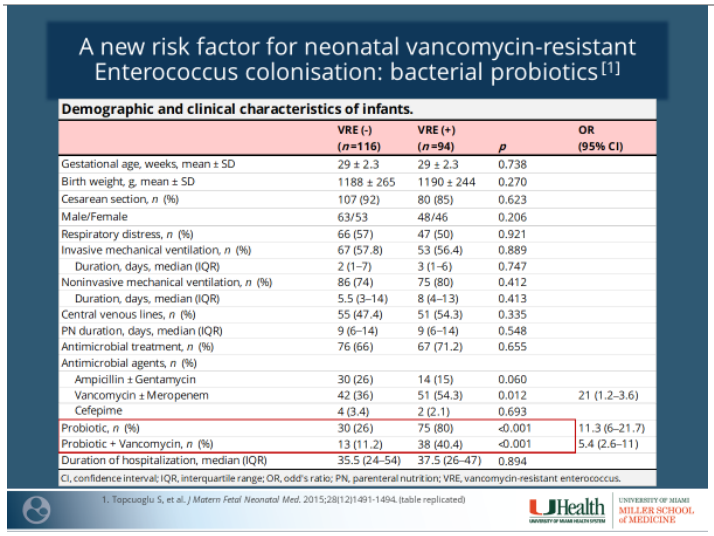

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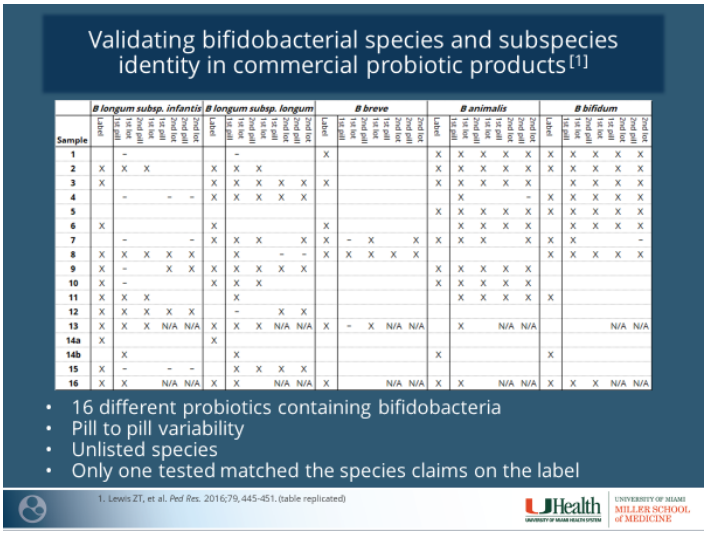
So, safety is an issue, but also the quality of the probiotic. This is a study in which an independent lab decided to test by PCR, the commercial probiotics. They tested 16 different probiotics, and they found there was some variability, pill-to-pill variability, in the 16.[23] They found that there was lot-to-lot variability in many of them. They found that there were species that were not listed on the label. And there was only one probiotic that really matched the species that was claimed on the label.



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This is important, not only in terms of safety, but if we think that many of the randomized, controlled trials were done with this kind probiotics, maybe we are missing something. Maybe the effects are even more potent because the probiotics that were given were not really what we were thinking was given.

That's why regulations are important. We need to know that all the probiotics that are available in the USA are regulated. Unlike dietary supplements, which is what is called GRAS [Generally Recognized as Safe], which are given as a supplement, and they are generally recognized as safe, but there are no standards of production, and there is no other regulation.

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In terms of trying to regulate the use of probiotics, in 2002 there was the International Scientific Association for Probiotics and Prebiotics in which they tried to better define the strain designated as probiotics. They tried to promote the use of probiotic that have been demonstrated to be efficient and also, they care about the safety of the probiotics.

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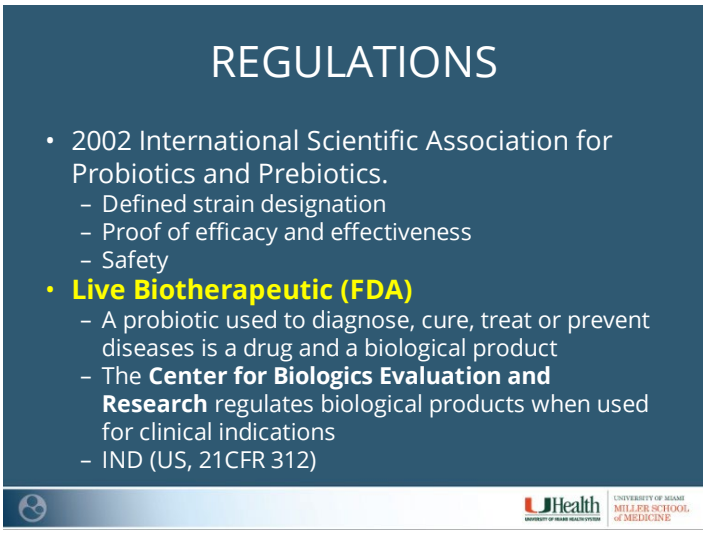
In the United States, a probiotic that is used as a diagnosis to cure, treat, or prevent diseases should be considered a drug, and, because it's a live product, is considered live—it's called a biotherapeutic. And that's regulated by the FDA, not the drug, but the Center for Biological Evaluation and Research. This probiotic needs an IND [investigational new drug] to be developed as a medication, as a pharmaceutical.



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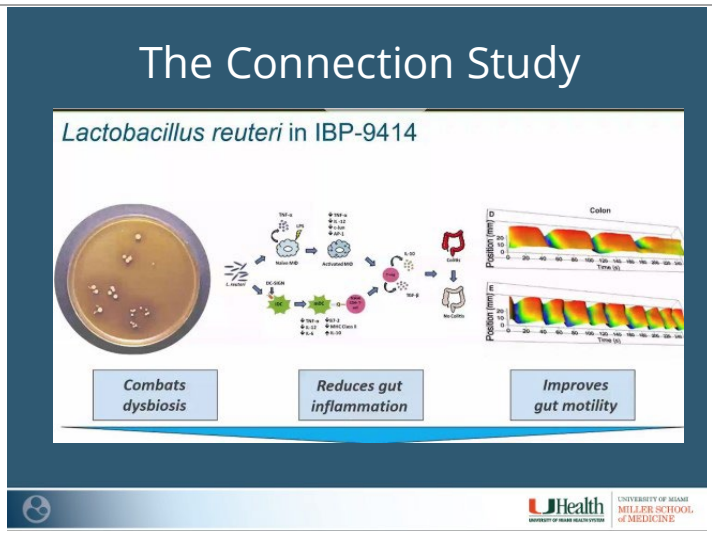
Based on all these comments and concerns, there is an ongoing randomized clinical trial, which is FDA regulated, and it's using a *Lactobacillus reuteri*. This Lactobacillus included research that shows it combats the dysbiosis, so it changes the microbiota. It has some important functions, so action in terms of reducing inflammation, and also has been shown to promote intestinal motility.



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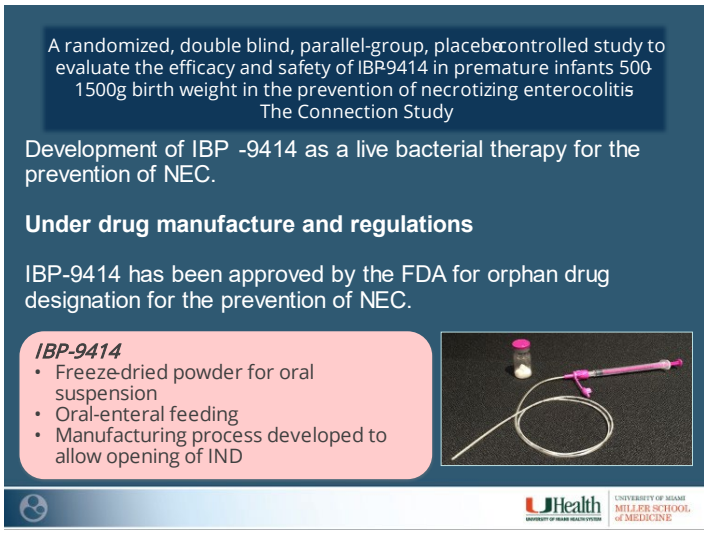
This is developed under FDA regulation and is also being done in Europe. The product is lyophilized, which is prepared right before administration to the patients.



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This is the phase 3. There was a phase 2 in which four cohorts of patients, starting with a bigger patient, between one kilo and two kilos, and less than one kilo and two doses were tested.[24] There were no differences in terms of adverse events or major adverse events in any one of the cohorts compared with the controls.

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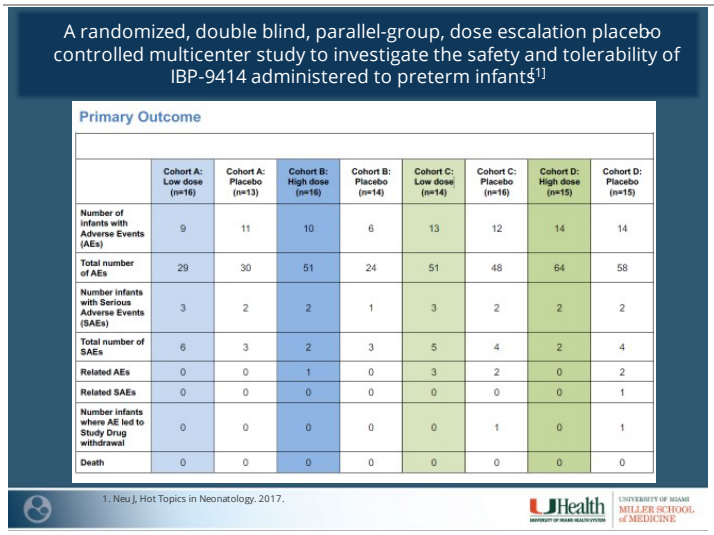

	Cohort A: Low dose (n=16)	Cohort A: Placebo (n=13)	Cohort B: High dose (n=16)	Cohort B: Placebo (n=14)	Cohort C: Low dose (n=14)	Cohort C: Placebo (n=16)	Cohort D: High dose (n=15)	Cohort D: Placebo (n=15)
Number of infants with Adverse Events (AEs)	9	11	10	6	13	12	14	14
Total number of AEs	29	30	51	24	51	48	64	58
Number infants with Serious Adverse Events (SAEs)	3	2	2	1	3	2	2	2
Total number of SAEs	6	3	2	3	5	4	2	4
Related AEs	0	0	1	0	3	2	0	2
Related SAEs	0	0	0	0	0	0	0	1
Number infants where AE led to Study Drug withdrawal	0	0	0	0	0	1	0	1
Death	0	0	0	0	0	0	0	0

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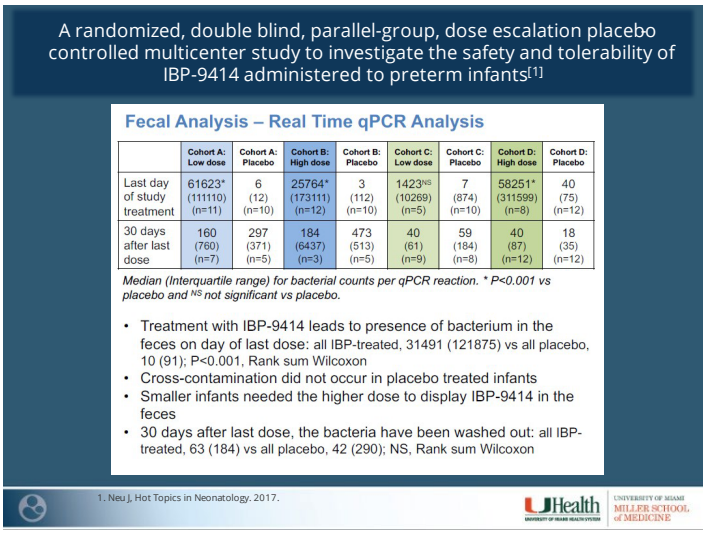
Also, the stools were tested, and there was no cross contamination between the controls and the intervention groups.



	Cohort A: Low dose	Cohort A: Placebo	Cohort B: High dose	Cohort B: Placebo	Cohort C: Low dose	Cohort C: Placebo	Cohort D: High dose	Cohort D: Placebo
Last day of study treatment	61623* (111110) (n=11)	6 (12) (n=10)	25764* (173111) (n=12)	3 (112) (n=10)	1423^{NS} (10269) (n=5)	7 (874) (n=10)	58251* (311599) (n=8)	40 (75) (n=12)
30 days after last dose	160 (760) (n=7)	297 (371) (n=5)	184 (6437) (n=3)	473 (513) (n=5)	40 (61) (n=9)	59 (184) (n=8)	40 (87) (n=12)	18 (35) (n=12)

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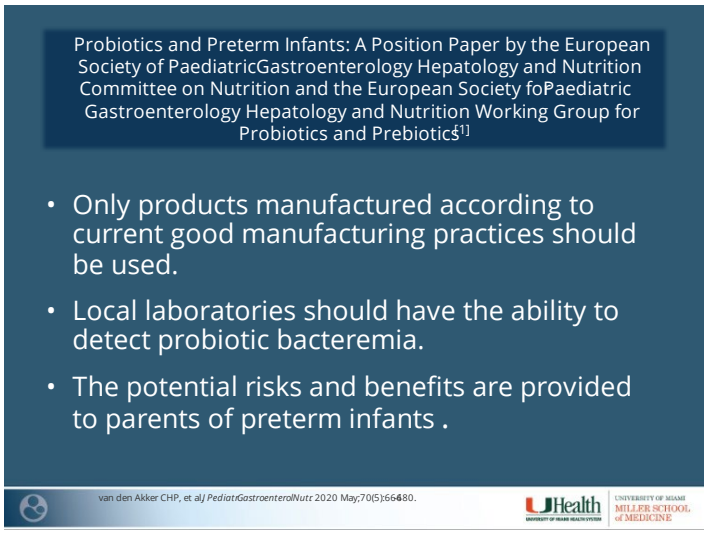
Having reviewed this, I want to finish just with the recommendation from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition Committee and the Committee of Nutrition in terms of what are the recommendations in the use of probiotics.[25] So, in Europe, they are a bit more liberal. They recommend only using products that are manufactured according to the good-manufacture practices. They recommend that, if a hospital is using probiotics, to have the capability to detect sepsis produced by that probiotic. So, to be able to grow from culture the product that is given to the babies, and also to advise the parents. They may even talk about consent, in terms of information, in terms of what are the benefits or side effects or risk of probiotics.



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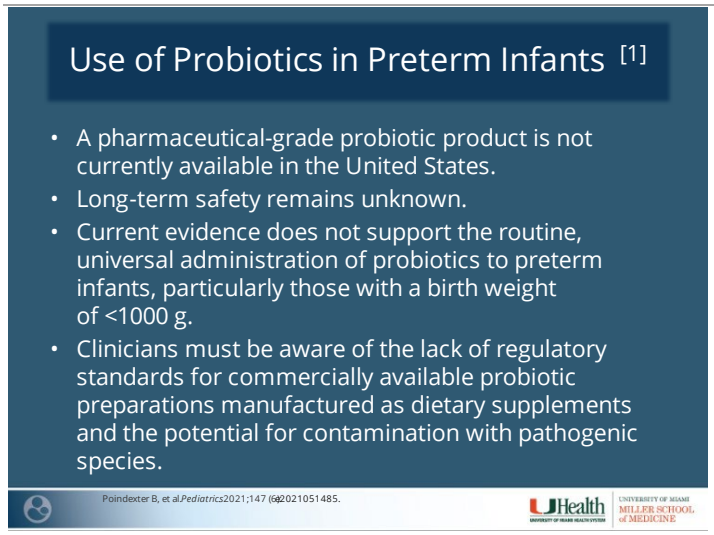
In the United States, the American Academy of Pediatrics Committee on Fetus and Newborn, they just also released a statement in 2021 in which they are more conservative.[26] They claim that there is not a pharmaceutical probiotic available at this point. There remain long-term safety unknowns that we need to investigate. And they claim that, at this point, there is no support for the routine use of probiotics, especially in babies less than one kilo, because on those babies the beneficial effects were less evident.

Probiotics in the NICU: Evidence and Controversies



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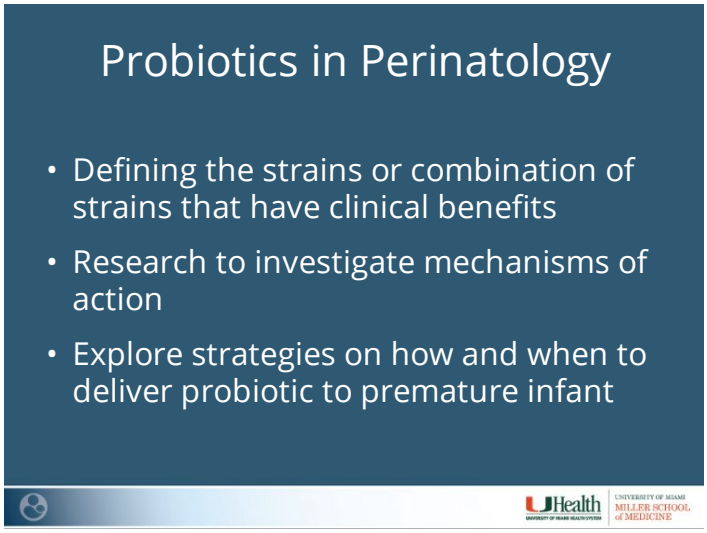
Also, they claim that the clinicians should be aware that the commercial probiotics that sometimes are used, they are only manufactured as dietary supplements, and they are at risk of contamination with other pathogens.

Next Steps

I think we have a lot of work here in terms of better defining the strains or combinations of strains that may have clinical benefits for our population of premature newborn babies. We need to promote the research to investigate mechanism of action in which we base the clinical benefits of the clinical trials. And we need to explore strategies of how and when to deliver the probiotics to the premature infant.

Maybe there is an opportunity to give the probiotic and colonize the breast milk of the mother who is giving [this] to the babies. Discussion is needed whether how long do we need to give the probiotics to change or reverse the dysbiosis. Thank you very much.



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Abbreviations

ESPGHAN	The European Society for Pediatric Gastroenterology Hepatology and Nutrition	**NICU**	neonatal intensive care units
GI	gastrointestinal	**NIH**	National Institute of Health
GRAS	Generally Recognized as Safe	**VLBW**	very low birth weight
IND	investigational new drug	**VRE**	vancomycin-resistant enterococcus
NEC	necrotizing enterocolitis		

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