

# Do probiotics benefit new-borns and especially colic babies? A clinical opinion of the evidence

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## Purpose

Parents often present their infant for chiropractic care. In the therapeutic encounter there are often questions about the use of probiotics for their baby. There has been considerable research in the area of probiotics for infant colic. The goal of this opinion piece is to explore and interpret the research literature and help to clarify when probiotics may best serve the baby and mother.

## Background

Chiropractic offices are receiving large numbers of infant patients into their practices, and an excessively crying baby, aka infant colic, is a very common presentation. Chiropractors have a well-known effective treatment for excessive crying,<sup>1</sup> but it may not solve the problem for every baby or parents may want a different approach. Consequently, and with the increased probiotic marketing in the last few years, parents frequently ask about it. Therefore, large numbers of parents are seeking professional information on the place of probiotics for their baby's health.

However, there is a lack of clear guidance on how to advise parents regarding probiotics for their infant. Despite many randomized controlled trials and several systematic reviews, it is not always recognized that research data must be translated for the practical applications to benefit the end user, the parent and infant. In short, evidence suggests that probiotics might offer some benefits,<sup>2,3</sup> but don't tell you why and when to apply it. On the other hand, the latest Cochrane Review in 2019 reported that there is no evidence that probiotics are more effective for infant colic than placebo.<sup>4</sup>

## When should a probiotic be taken?

So why should probiotics be given to the infant? Because mode of birth, mode of feeding and the environment make a difference to baby's microbiome. Infants rely on colonization of the gut in order to complete development of the immune system and gastrointestinal tract.<sup>5</sup> Major microbial colonization of the human intestine is transmitted at birth (through the birth canal), as well as through skin to

skin contact and feeding in the first year of life and sets the stage for long-term health.<sup>6</sup> Furthermore, researchers have found that one of the main sources of infant gut bacteria is the mother's gut,<sup>7,8</sup> as they are directly transmitted by vaginal birth and breast feeding. In the mother, gut flora is transmitted to vaginal flora,<sup>9</sup> which then is seeded to the newborn by passing through the vaginal canal at birth.<sup>7</sup> Further studies have elucidated several modes of transmission (skin, oral, placental, vaginal, breastmilk) broadening our understanding of the development, maturation and stability of the fetal/infant microbiome.<sup>10,11,12</sup>

This is especially important, because in vaginally born infants, maternal strains are found to be very stable over the first year of life. In contrast, non-maternal strains are unstable and less viable and nearly always replaced by the age of 12 months.<sup>13</sup> This supports the hypothesis that the first inoculation of the infant's gastrointestinal tract with the mother's bacterial milieu is a significant introduction to bacteria and that the infant gut is seeded prior to birth with bacterial DNA. It is further supported as short chain fatty acids (SCFAs) (which play an important role in gut health and development) have been found in the amniotic fluid. It has been argued that this might be due to contamination.<sup>14</sup> However, a study done by Stinson et al. (2019) retested this hypothesis by eliminating contamination as much as possible and still found bacterial DNA in the amniotic fluid.<sup>15</sup> Since the fetus swallows amniotic fluid in the last two semesters of pregnancy, it supports the conclusion that the first colonization of the gut starts at this point. In fact, data from Stinson et al. indicated that the majority of bacterial DNA found in the fetal gut at birth is also found in the amniotic fluid.<sup>15</sup> Furthermore, not only is the amniotic fluid microbiota similar to that in the fetal gut, but also to the placental microbiota.<sup>14</sup>

Even though this data shows that the fetus is exposed to bacterial DNA prior to birth, it does not prove that the DNA comes from viable bacteria.<sup>15</sup> It might be the case that dead bacterial remnants have passed through the placenta into the amniotic fluid. Therefore, it is still unknown whether

the baby's microbiome development begins prenatally in utero or singularly, by passing through the birth canal. What is known is, that the infant microbiome is colonized no later than when going through the birth canal and that the vaginal flora is similar to the gut flora of the mother. Further, it is known that bacterial DNA by itself influences the fetal immune development,<sup>15</sup> as well as sets the stage for fetal metabolism.<sup>16</sup>

An abundance of literature has become available in the last decade highlighting the importance of the microbiome. El Aidy et al (2016) reported that the gut microbiome plays a crucial role in host physiology. Disruption of its community structure and function can have wide-ranging effects making it critical to understand exactly how the interactive dialogue between the host and its microbiota is regulated to maintain homeostasis.<sup>17</sup> Ringel-Kulka, et al (2013), then Cryan, et al (2019) described how the brain-gut axis allows bi-directional communication between the central and enteric nervous systems, linking emotional and cognitive centers of the brain with peripheral intestinal function.<sup>18,19</sup> Recent experimental work suggests that the gut microbiota have an impact on the brain-gut axis.<sup>20</sup>

Research supports that the microbiome influences metabolism, immunity, hormones and possibly even behavior.<sup>20,21</sup> Among mammals, milk constituents directly influence the ecology of the infant's commensal microbiota. The immunological and nutritional impacts of breast milk and microbiota are increasingly well understood; less clear are the consequences for infant behavior. It has been hypothesized by Allen-Blevins, et al (2015), that infant behavior likely varies as a function of their mother's milk composition interacting with the infant's neurobiology directly and indirectly through the commensal gut bacteria.<sup>22</sup>

Although the precise pathways of microbiota-hormonal signalling have not yet been deciphered, specific changes in hormone levels correlate with the presence of gut microbiota. The microbiota produces and secretes hormones, responds to host hormones and regulates expression levels of host hormones. The concept proposed by Clark, et al (2014) was, that the gut microbiota serves as a virtual endocrine organ, which arose from a number of important observations.<sup>23</sup> They cited animal studies that showed exaggerated responses to psychological stress, which normalized following colonization of the gut by *B. infantis*.<sup>23</sup> This discussion was expanded by Williams, et al in 2020.<sup>24</sup> Another study by Luoto, et al (2010) demonstrated that *L. rhamnosis* PL60 played a role in the reduction of body weight gain and white adipose tissue without any effects on food intake.<sup>25</sup> And lastly, a study showed that manipulating the microbial composition of the GI tract modulated plasma concentrations of tryptophan, an essential amino

acid and precursor to serotonin, a neurotransmitter that is known to improve mood and positive feelings.<sup>26</sup> Long term implications of the protective role in the function of many systems of the body that a healthy microbiome plays cannot be underestimated. The investigation of the microbial bioprotective role of probiotics at the intestinal level has been promoted by Garcia-Conzales et al (2020).<sup>27</sup>

The development of the functional human microbiome is also critical in the development of preventive protocols for one of the most devastating neurologic disabilities befalling children at an alarming rate (1:54 children according to the most recent CDC data available).<sup>28</sup> According to Doenyas (2018), there is accumulating evidence, which has strengthened a link between dysbiotic gut microbiota and autism.<sup>29</sup> This recent evidence implicates immune system alterations and gut microbiotic dysbiosis and its potential effects on ASD (autism spectrum disorder), susceptible genes, neurodevelopment and intestinal and blood brain barrier integrity in at least some subpopulation of individuals with ASD.<sup>30</sup>

Therefore, it cannot be emphasized enough that it is important for the mother to have a healthy gut microbiome before birth and it might be beneficial to start probiotic supplementation in the third trimester of pregnancy. To find out the need for supplementation exactly, a stool analysis should be done, although this is considered costly. Even without the stool sample findings showing imbalance, taking a pregnancy specific probiotic supplement can beneficially support a healthy floral array during pregnancy. It may prove to be a prudent recommendation especially since there is virtually no risk and there may be a powerful health benefit in the establishment of stable microbiota colonization for the infant.<sup>31,32</sup>

### The reproductive microbiome

It is customary to encourage mothers to take special care of themselves when they become pregnant. But what about her state of health before her pregnancy, let alone the state of her biological partner? In recent years the preconception and pregnancy microbiome has become the focus of several studies. Studies show that during the preconception period, the composition of the female as well as the male microbiome can interfere with reproduction as well as development of the fetus. Interestingly, the most prevalent microbiota detected in follicular fluid are *Lactobacillus* spp.<sup>33</sup> Additionally, they are associated with a better embryo quality and therefore leading to a significant higher rate of embryo transfer and successful implantation and gestation in women who undergo the complex series of procedures involved with in vitro fertilization.<sup>33</sup>

Fetal viability, growth and development are largely dependent on optimal placental function, which includes

a healthy microbiome.<sup>33</sup> A different microbiome compared with normal pregnancy outcomes has been detected in placentas of pregnancies complicated by preterm birth.<sup>34</sup> It has also been found that the bacteria found in the preterm placenta were similar to those commonly residing in the vagina.<sup>34</sup> A vaginal microbiome composed solely of *Lactobacillus* before embryo transfer is associated with a successful outcome of IVF-embryonic transfer.<sup>35</sup> This suggests that the placental microbiome is influenced by the vaginal microbiome. Additionally, the placental microbiome has a taxonomic profile that is similar to the oral microbiome and longstanding periodontal disease may cause adverse pregnancy outcomes.<sup>36</sup> This suggests a full circle where colonizing bacteria may all have originated in the oral cavity which is, no doubt, influenced by what we put in our mouth. So, logic predicts that, when planning for pregnancy, taking an array of viable healthy oral and gut bacteria by mouth (powder or liquid would be preferable, as capsules open first at the upper intestinal tract) might be the recipe for success.

What about the male? It has been revealed that semen contains strictly facultative anaerobic bacteria of which the most abundant bacteria are *Lactobacillus*, *Pseudomonas*, *Gardnella*, *Finegoldia*, *Corynebacterium* and *Staphylococcus*.<sup>37</sup> Interestingly, a positive association of a *Lactobacillus* dominated microbiome has been correlated with good semen quality due to the protective lactic acid synthesized by *Lactobacillus*.<sup>37</sup> Evidence shows that the seminal microbiome has a lower biomass, but a higher diversity compared to the vaginal microbiome and induces a significant change in the vaginal microbiome after intercourse.<sup>38</sup> So if the vaginal microbiome can be significantly changed by the seminal microbiome and since a healthy vaginal microbiome may be the deciding factor for implantation, it might be important for the male to take probiotics, especially when fertility problems arise.

### **The Cesarean-Section and the Formula-Fed baby**

Infants born by Cesarean-section or those exposed to antibiotics (administered directly or indirectly through the mother) have been found to be at increased risk of developing metabolic, inflammatory and immunological diseases, which is thought to be due to disruption of normal gut microbiota.<sup>13</sup> In a double blind, placebo-controlled randomized clinical trial, mothers were given a multispecies probiotic, consisting of *Bifidobacterium breve*, *Propionibacterium freudenreichii*, *Shermanii* JS, and *Lactobacillus rhamnosus*. Results indicated that it is possible to correct the changes due to antibiotic ingestion in microbiota composition.<sup>13,39,40</sup> It is well known that antibiotic treatment alters the composition and metabolic function of the intestinal microbiota. These alterations may contribute to the pathogenesis of necrotizing enterocolitis and antibiotic-associated diarrhea, severe and life-

threatening illnesses of the neonate.<sup>40</sup> Their result suggests that correcting a potential health issue with a non-invasive and harmless procedure like the application of scientifically tested probiotic bacteria might be worth doing for both the baby born by C-section and the one receiving antibiotics.

The microbiome in the Cesarean delivered infants has been shown to be mostly devoid of the maternally transmitted seeding classes Actinobacteria and Bacteroidia during the first months of life; species of Bacteroidia in particular were consistently missing.<sup>13</sup> The likelihood of transmitting maternal strains with vaginal birth is 0.87 compared to neonates born by Cesarean, which is 0.13. In addition to the absence of maternal strains, the Cesarean-delivered infants showed higher strain fluctuations than the vaginally born ones, particularly regarding Bacteroidia strains.<sup>13</sup> Therefore, an unstable microbiome that is easily invaded by pathogens can also be easily supplemented by probiotics (good bacteria). Supplementing with Bacteroidia would be the solution to counteract the missing bacterial strain in Cesarean born infants. Just one obstacle arises; Bacteroides are an absolute anaerobic bacteria and are currently not for sale on the market. However, pre-clinical trials indicate that Bacteroides genus is widely considered as a source of novel beneficial candidates for attenuating inflammation by regulating lymphocytes and cytokine expression, controlling metabolism and preventing cancer.<sup>41</sup> Therefore, they are in the development stage as a next generation probiotic (NGP).<sup>42</sup> Until a NGP is available, supplementing with Bifidobacteria (phylum Actinobacteria) is a viable choice, not least because Bifidobacteria comprises the largest group within a healthy infant microbiome.<sup>5</sup>

Maternal breast milk contains live, culturable bacteria including Bifidobacteria,<sup>6</sup> which are transported from the mother's gut by the enteromammary pathway to the mammary glands. The enteromammary pathway involves immune cell-mediated bacterial translocation from the mother's gastrointestinal tract into the mammary gland, where some of these bacteria are able to colonize the available niche in the baby's gut.<sup>43</sup> Even if the young intestine proved relatively unpopulated, vaginal birth, skin to skin contact, breast, even siblings, pets<sup>44</sup> and the environment<sup>45</sup> further populate it with a diversity of healthy bacteria.<sup>44,45</sup> As the baby gets older, the bacteria increase in number and colonize more of the surface area of the gut. If this natural process is interfered with, opportunistic organisms (pathological bacteria, yeast, parasites) are presented with the opportunity to populate the gut. It then becomes a matter of the infant's healthy microbiome to suppress the colonizing invaders to counteract disease. This could prove a hard fight to win. Fortunately, human milk is another avenue of stimulation of the further proliferation of mainly Bifidobacterium and numerous *Lactobacillus* strains. Bifidobacteria, the predominant probiotic microorganisms

present in the maternal and infant gut, creates an acidic environment, rich in short chain fatty acids (SCFAs) with a protective and nutritive role for the neonate’s intestines,<sup>46,47</sup> and therefore supports the baby’s immune system.<sup>13,47</sup> Subsequently, the lowering of the pH creates an ideal environment for the “good” bacteria to function, which then leads to the inhibition of inflammation, pathogens and creates an environment to naturally avert any invasion of ‘bad’ bacteria.<sup>48,49,50</sup> Furthermore, the microbiome composition of the neonate is influenced by the gestational age at birth (preterm vs term), by birth mode (vaginal vs c-section), by diet and feeding method (breast vs bottle (pumped breastmilk vs formula) and later solid food)<sup>49</sup> and environment (Hygiene Hypotheses).<sup>44,45</sup> The gut colonization continues with a shift to a solid food diet, which contains a lot of plant polysaccharides. Clostridia (phylum Firmicutes) are introduced in addition to the species Actinobacteria and Bacteroidia which are derived directly from the mother by vaginal birth.<sup>13</sup>

Table 1 shows predominant bacterial strains in the gut delineated by birth mode, feeding mode or environment.

Research has shown that a suboptimal microbiome can be changed towards a more preferable one. In recent years, the implementation of formula with added prebiotics and probiotics has been shown to modulate the microbiome composition towards that of a breast-fed infant gut flora and to stimulate the immune response.<sup>51</sup> Two things should be noted. Most of these studies were done by special interest groups, such as formula companies. Secondly, it is well-known that probiotic bacteria cannot survive in temperatures above 40° Celsius (104° Fahrenheit). Most

mothers prepare bottles with boiling water, far above those temperatures.

**Does probiotic supplementation help with infant colic?**

Table 2 (following page) shows evidence that probiotics may be helpful for infant colic in breastfed babies. The question must be asked: Why do probiotics only help infant colic in breastfed babies and not in formula fed babies? Why does the Intervention with *L. reuteri*, for example, only have a significant effect on babies being breast fed? This suggests that maybe it was the composition and beneficial bacteria of breast milk helping *L. reuteri* to proliferate and not vice versa.

If the normal development of the microbiome is interrupted due to circumstances that arise during gestation, birth and early postpartum, infants may require support to populate the gut with bifidobacteria along with lactobacillus in order to maintain the pH necessary to avoid overgrowth of pathogens. This will help normalize immune function and decrease inflammation which may reduce the infant’s discomfort. Viable Bifidobacteria are found in breast milk.<sup>52</sup> The question remains: if we supplement with an additional probiotic (*L.reuteri*), is there a “critical mass” of these two probiotic bacteria that when it is reached, the effect is to calm the irritable infant or is it because there is already a lower incidence of colic in breastfed babies, and these mild cases would resolve on their own?

Prebiotics such as human milk oligosaccharides (HMOs) are a group of important complex carbohydrates that are found in breast milk. These HMOs are important in the developing infant because they help to shape the infant’s

1. Pregnancy	- Term: - Preterm:	Decrease Faecalibacterium/ Increase Proteobacteria, Actinobacteria Decrease Bifidobacteria
2. Birth mode:	- Vaginal - C-Section:	Lactobacillus, Prevotella, Bacteroidales, Actinomycetales Increased pathogenic Microbes (ex. Staphylococcus, Clostridium)/Decreased Bacteroides, Bifidobacterium
3. Feeding mode:	- Breast milk: - Formula fed: - Solid food:	Decreased Firmicutes/ Increased Bifidobacteria ( <i>B. Infantis</i> ), Lactobacilles , <i>L. rhamnosus</i> Veillonella (18.4%) and Escherichia/Shigella (15.2%) Decreased Bifidobacteria/Increased Enterococci, Enterobacteria Streptococcus (18.64%) and Klebsiella (17.41%) Bacteroidetes increase for the digestion of innsoluble fibers
4. Diet :	- Europe: - Africa:	Decreased Bacteriodetes/ Increased Firmicutes (Clostridia, Enterococcus) Decreased Firmicutes/ Increased Bacteriodetes
5. Environment:	- Hygiene	Hypotheses, where the child for example is not exposed to unhygienic circumstances and a decrease in diversity of comensial microbes results.
Most important time period for colonization is from birth to one year of age. In C-Section, this is delayed. After the third year of age the microbiome stabilizes.		

Table 1: Predominant bacterial strains by birth mode, feeding mode or environment (most common strain in bold)<sup>22,25,45-49,56,57</sup>

gut microbiome by facilitating the selection of beneficial bacteria,<sup>50</sup> and therefore, improve the balance and function of the microbiome. Could it perhaps take more time in some vulnerable infants to reach the protective levels in the microbiome? If so, might a longer time of intervention with probiotics be required?

Colostrum, which is the first milk produced, contains the prebiotic HMOs, which promotes in vitro growth of gut bacteria like *Bifidobacterium longum* subspecies *infantis*, while suppressing growth of pathogens like *Escherichia coli* and *Clostridium perfringens*.<sup>5</sup> No one has studied whether the colic babies who were breastfed may have missed out on early colostrum due to difficulty with feeding in the first place (but later overcome). There are many confounding factors that must be examined before merely accepting the studies' findings without question. Another factor that is not controlled for in the studies is the type of birth, which, of course, is an absolute key to the health of the microbiome. The confluence of natural vaginal birth with breastfeeding optimizes the normal microbiome. Vaginally born infants receive the advantage of being colonized with *Lactobacillus*, especially *L. bifidus*.<sup>11,36</sup> These have been found in preponderance in stools of breast-fed infants and this creates an ideal environment for the "good" bacteria to develop and grow, which in turn improves the health of the infant. Another question would be: are the colicky infants in those studies more likely to have had a mechanized birth, since it is known that assisted birth is the major risk factor for colic?<sup>253</sup> That alone would explain why only some breastfed babies have colic and why a probiotic might be helpful. In the opinion of the authors, more research is indicated in this area, controlling for confounding factors.

**Which baby should take probiotics?**

Perhaps such a simple concept, as administering probiotics to an infant is not quite so simple. Confusion is created by studies using different probiotics (mono-and multispecies,

powder and capsules, different species, etc.), measuring systems, research approaches and finding variable results. This may make interpretations convoluted and difficult to follow as well as to know which probiotic to apply for which condition. Without additional knowledge about single strain development and their specific benefits in human health, probiotics in the general population are being treated as if they are all the same without taking their singularity nor interactions into consideration.

For example, two probiotic bacteria that have been shown to benefit a specific health condition, might not interact beneficially with each other if mixed together in one supplement. One probiotic supplement does not equal another. If one species demonstrated a beneficial result, another species might not when administered for the same disorder. It is even possible that when multiple species are combined in one supplement, they could potentially counteract each other's benefits. Research and discernment are required when preparing probiotic supplements. The spectrum design needs to be suitable for the pregnant woman, and the baby, respectively. Further, the probiotic must be approved by the FDA to be certain that the probiotic bacteria cannot translocate antibiotic resistance and has not been genetically altered.<sup>54</sup>

Research has found that dysbiosis (defined as imbalanced gut microbiota) is present in some full-term babies diagnosed with colic.<sup>43,55</sup> Does this automatically mean that supplementation with probiotics is the cure? Over recent years, criticism of the undefined use of the 'dysbiosis' term has been repetitively articulated. For example, there are many confounding factors, such as the maturation of the gut microbiota, the type of delivery, the type of feeding as well as prior antibiotic use.<sup>40</sup> Table 3 (following page) shows the main constituents of a dysbiotic microbiome.

A detailed history of the mother and baby needs to be

Intervention, N, Time	Result
<i>L. reuteri</i> (345 infants, 21-28 days) <sup>2,3</sup>	The probiotic group averaged less crying and/or fussing time than the placebo group at all times. The duration of crying in breast fed infants at the end of the intervention was reduced by almost 50 minutes, but only for exclusively breastfed infants.
<i>L. rhamnosus</i> (30 infants, 28 days) <sup>2,3</sup>	No significant effect was found. Breast- and formula-fed infants were included.
<i>L. casei</i> , <i>L. rhamnosus</i> , <i>S.thermophilus</i> , <i>B. breve</i> , <i>L. acidopilus</i> , <i>B. infantis</i> , <i>L. bulgari-cus</i> (50 infants, 30 days) <sup>2,3</sup>	The probiotic group averaged less crying than the placebo group at the end of the intervention. The duration of daily crying time was reduced by at least 50%. The study was only done on breast fed infants.
<i>B. animalis</i> subsp. <i>lactis</i> (80 infants, starting at less than 7 weeks of age for 28 days) <sup>58</sup>	A reduction of ≥50% of mean daily crying duration after 28 days of intervention. The mean number of daily crying episodes was also lower in the probiotic group than in the placebo. Studied on only exclusively breastfed infants.

Table 2: Effects of two mono- and one multispecies probiotic supplement to treat infant colic.

taken. That history must include these diverse factors to get a better understanding of the origin of any excessive crying. A neonate of a mother with a healthy diet, no known genetic or predisposing health problems, like allergies or neurodermatitis, vaginal birth and breast fed might

not profit significantly from probiotic supplementation. Additionally, a mechanical problem might be the cause of excessive crying and chiropractic care might be appropriate for a trial of treatment.

### Conclusion

The microbiome health of a new-born baby depends largely on the mother's health, a natural vaginal birth and breastfeeding. When one or all of these are not present, supplementation with probiotics may be an appropriate answer. We hope to make the clinician aware of the research literature, how it is interpreted and when to take a complete history to determine which babies may benefit most from supplemental probiotics. In the case of supplementation, some gut bacteria are more beneficial for pregnant women and their offspring. In general, the most beneficial ones for the neonate and colic babies appear to be Bifidobacteria (especially when not breastfed) and Lactobacillus (especially when born by Cesarean). Further research on this subject is warranted.

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| <ol style="list-style-type: none"> <li>1. Abnormal microbial exposure. Mother eating unhealthy, mother being unhealthy, parents with genetic or predisposed health issues, C-Section, bottle fed</li> <li>2. Disruption in diet, (a) infant is formula fed; (b)breastfeeding only a short time such as a few weeks, (c) less than one year of breast feeding.</li> <li>3. Medication usage, like antibiotics.</li> <li>4. Host genetics, meaning a parent having allergies, atopic skin disease, asthma, diabetes, etc.</li> </ol> |
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Table 3. Four main pathways to dysbiosis in the infant<sup>43,55</sup>

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