The First 1,000 Days of Life: The Importance and Benefits of a Healthy Infant Microbiota

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Disclosures

Gerber: Consultant

Learning Objectives

- Explain why microbial dysbiosis carries a risk of disease development in infants.
- Review how gastrointestinal bacteria can influence health outside of the GI tract.
- Provide scientific evidence for short- and long-term examples of dysbiosis and ill-health.
- Explain current evidence for the role of probiotics in treating short- and long-term diseases related to microbial dysbiosis.

Intestinal Microflora

- Are a complex ecosystem that depend on multiple host and environment factors
- A balanced intestinal ecosystem is essential for survival
- Intestinal bacteria support gut mucosal structure and function
- Intestinal bacteria are critical in inducing maturation and adequate gut barrier function and immune response (cellular and humoral)

Adult Microbiota: A Complex Ecosystem

- > 1000 species

<table>
<thead>
<tr>
<th>Location</th>
<th>CFU/g</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td></td>
<td>Eubacterium</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td>Candida albicans, Helicobacter pylori</td>
</tr>
<tr>
<td>Duodenum</td>
<td>10^4-10^5</td>
<td>Bacteroides, Clostridium, Enterobacteriaceae</td>
</tr>
<tr>
<td>Jejunum</td>
<td>10^5-10^7</td>
<td>Bacteroides, Lactobacillus, Enterococcus</td>
</tr>
<tr>
<td>Ileum</td>
<td>10^7-10^8</td>
<td>Bacteroides, Clostridium, Enterococcus, Eubacterium</td>
</tr>
<tr>
<td>Colon</td>
<td>10^10-10^13</td>
<td>Bacteroides, Clostridium, Eubacterium, Fusobacterium, Peptostreptococcus, Ruminococcus, Streptococcus</td>
</tr>
</tbody>
</table>

Speaker Introduction

- Dr. Pietzak had received her MD from SUNY Buffalo. She did her Pediatric residency and fellowship in gastroenterology and nutrition at USC/Childrens Hospital Los Angeles. She is a practicing doctor at USC for 24 years. She wrote more than 100 publications.
Intestinal Flora: A Balanced Ecosystem

- Potentially Harmful Bacteria
  - Proteus
  - Clostridia
  - Enterococci
  - E. coli
  - Lactobacilli
  - Streptococci
  - Eubacteria
  - Bifidobacteria
- Potentially Helpful Bacteria
  - Pseudomonas
  - Staphylococci
  - Bacteroides

Roles of the Intestinal Microflora

- Foster maturation and integrity of immune system
- Immune stimulatory function
- Innate immunity
- Adaptive immunity
- Protect from enteric pathogens
- Compete for nutrients
- Interfere with pathogen adherence and growth
- Secrete anti-bacterial substances which protect against pathogens
- Produce vitamins
- May help mucosal maturation

A Healthy Gut Microbiota Is Important for Maturation of Gut Barrier Function And Immune System Development

- Important phases of gut microbiota development in early life
  - First inoculation
  - Initial colonization
  - Increasing diversity

Bacterial Colonization of the Neonatal Gut – Delivery

- Colonization by bacteria affected by:
  - Route of delivery
  - Hygiene of neonatal environment
  - Maternal bacterial flora
  - Diet of the infant
- By one week of age:
  - Vaginal delivery: anaerobic bacteria, primarily Bacteroides
  - C-section: delayed colonization by anaerobes; primarily Enterobacteriaceae
- FLORA OF AN INFANT DELIVERED VIA C-SECTION MORE CLOSELY RESEMBLES THAT OF THE HOSPITAL ENVIRONMENT THAN THAT OF THE MOTHER!!!

The Gut Barrier and the Immune System

- Mucus (Mucin)
  - Keeps pathogens from invading
- Tight Junctions (between cells)
  - Modulate gut permeability
- Immune cells (GALT)
  - 70% of immune cells are located in the GI tract
  - Innate: Macrophages, White cells, Mast cells
  - Adaptive: T and lymphocytes

Vaginal delivery is a source of bacteria

Are there consequences to not being delivered vaginally?
Morbidity in Children Born via Cesarean Delivery is Higher


<table>
<thead>
<tr>
<th>Condition</th>
<th>Decreased risk</th>
<th>Increased risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>1.74</td>
<td>1.31</td>
</tr>
<tr>
<td>Gastroenteritis + Asthma</td>
<td>1.23</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Bacterial Colonization of the Neonatal Gut – Feeding

- Formula vs breast-fed infant have different early intestinal flora
- Breast-fed
  - Lactobacilli and Bifidobacteria outnumber Enterobacteriaceae 1000-fold
  - More adult pattern after weaning: fewer E. coli and Clostridium and more Bacteroides and Gram +
- Formula fed
  - flora develops quicker
  - primarily Enterobacteriaceae (E. coli)
  - iron fosters growth of more complex flora

Bacteria in Breast Milk

- Culture-dependent and independent analyses of breast milk from healthy women identify multiple species of live bacteria and bacterial DNA
- Bacteria generally isolated include:
  - Staphylococcus
  - Streptococcus
  - Enterococcus
  - Lactobacillus
  - Bifidobacterium

Breastfeeding is a source of bacteria

Are there consequences to not breastfeeding?

Breastfeeding is Associated with a Lower Risk of Developing:

Respiratory
- Upper respiratory tract infection
- Lower respiratory tract infection
- RSV bronchiolitis

Gastrointestinal
- Necrotizing enterocolitis (NEC)
- Gastroenteritis
- Celiac disease
- Inflammatory bowel disease
- Otitis media
- Type 1 and 2 diabetes
- Obesity
- Childhood leukemia
- SIDS

Morbidity in Non-Exclusively Breastfed Infants is Higher

Graph Adapted from:
Chronic Disease Prevalence 1950-2000
Decrease in infections is associated with increased immune disorders

"Modern" Lifestyle Has Decreased Exposure to Bacteria
- Cesarean sections
- Sterile infant formula
- Antibiotics
- Sterile processed food
- Decrease in naturally fermented foods
- Increased hygiene measures
- Urban life

Lower microbial exposure → Altered Intestinal microbiota → Inadequate immune response

Ingestion of Bacteria: An Old Idea!
- 1900 BC - 2000 BC
- Antibiotics
- Probiotics

In the last 100 years, we have drastically changed our ingestion of microbes and our microbial environment

Ingestion of Bacteria Proposed as Beneficial
- Elie Metchnikoff (1845-1916)
- Suggested that ingested bacteria could have positive influence on normal microflora in intestinal tract
- Hypothesized that Lactobacilli were important for human health and longevity
- Promoted yogurt and fermented foods as healthy

Probiotics
- "Pro-biotics" mean "for life"
- WHO: "Live microorganisms, which when consumed in adequate amounts, confer a health effect on the host"
- Vet: "Live microbial food supplement which affects host animal by improving microbial balance"
- Examples: Bifidobacteria, Lactobacilli, yeast
- Example: feeding probiotics to chickens lessens colonization with Salmonella
A probiotic should

• Be nonpathogenic in nature
• Be resistant to destruction by technical processing
• Be resistant to digestion by salivary amylase, gastric acid, bile and pancreatic enzymes
• Adhere to or transiently colonize intestinal and colonic epithelial tissue
• Provide a measurable benefit to the host

Teitelbaum JE and Walker A. Int'l Seminars in Pediatr Gastroenterology and Nutrition 2002;11:5-7

Common Probiotics

• Lactobacillus
  • Can adhere to gut mucosa
  • L. acidophilus, L. bulgaricus, L. rhamnosus GG
• Bifidobacteria
  • Predominant colonic flora of breast fed infant
  • B. bifidum, B. longum, B. breve, B. infantis, B. animalis
  • Both are normal intestinal flora
  • Both can be recovered in the stool after ingestion
    - suggests colonization of gut

Bacteria Identified in Human Milk with Demonstrated Probiotic Qualities

Examples:

- L. reuteri
- L. rhamnosus
- L. fermentum
- L. salivarium
- L. gasseri
- B. breve
- B. animalis
- B. longum
- B. bifidum
- B. catenulatum


Quantity of Bifidobacteria in Breast Milk Differs Between Mothers

Mean = 2.64 log cells; 90% CI: 1.85 – 1.77

Bifidobacterium Species in Breast Milk Differs Between Mothers

Outcomes of Clinical Studies in Pediatrics with Probiotic Bacteria

• Modification of intestinal microflora
• Immune support
• Prevention and treatment of atopy
• Treatment of acute diarrhea
• Prevention of acute diarrhea
• Decrease of antibiotic associated diarrhea
• Prevention of NEC
• Treatment of colic and regurgitation
• Treatment of inflammatory bowel disease
BIFIDOBACTERIA

- Anaerobic, non motile, Gram + curved rods
- Produce acids: acetate and lactate
- Growth inhibited at pH of 5.5
- Can survive intestinal digestion and appear in stool
- Constitute most of the microflora of breastfed infants

Bifidobacterium lactis

Probiotics and Immunity

Bifidobacterium lactis fed to humans can modify intestinal microflora

- Increased counts of Bifidobacteria in pts fed B. lactis supplement
- Increase in short chain fatty acids, and lower stool pH
- Decrease in Clostridia, Coliforms and Bacteroides

Mohan J Clin Microbio 2006;44:4025-403

Probiotics and Atopy

Treatment of Atopic Dermatitis
With Extensively Hydrolyzed Formula and Probiotics LGG or B. lactis x 2 months

Potential mechanisms to reduce risk of allergy:

- Improved integrity of gut barrier
- Decreased intestinal permeability
- Increased secretory IgA
- Major role in tolerance development
- Decreased TH2 related cytokines (i.e. IL 4 & 5)
- Reduced pro-inflammatory mediators (i.e. TNF-α, ECP)

Probiotics and Atopic Disease

Probiotics and Diarrhea

Probiotics in Oral Rehydration Solution for Treatment of Diarrheal Disease due to Rotavirus

Meta-analyses of Randomized Clinical Trials on Probiotic Treatment of Acute Diarrhea

Probiotics Shown to Reduce the Duration of Acute Diarrhea (in hours)

Probiotics and Risk of Acute Diarrhea

Controlled Clinical Trials

* *P<0.05 compared to incidence in control of each study
**Probiotics and Risk of Antibiotic Associated Diarrhea**

Controlled Clinical Trials

- **RR=0.32**
  - CI (0.10-1.02)
- **RR=0.52**
  - CI (0.29-0.95)
- **RR=0.47**
  - CI (0.18-1.20)
- **RR=0.29**
  - CI (0.13-0.61)
- **RR=0.96**
  - CI (0.60-1.50)

% Reduction in incidence

- Arvola 1999
- Correa 2005
- Jirapinyo 2002
- Tankanow 1990
- Vanderhoof 1999

*P<0.05

**Results of published randomized controlled trials (RCTs) have indicated that there is modest benefit of giving probiotics in preventing acute gastrointestinal tract infections in healthy infants and children.**

**...there is evidence to support the use of probiotics, specifically LGG, early in the course of acute infectious diarrhea to reduce the duration by 1 day.**

**Antibiotic Associated Diarrhea**

**...probiotics can be used to reduce the incidence of antibiotic-associated diarrhea.**

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**Probiotics and NEC**

- Is delayed
- Antibiotics
- Infection control procedures
- Washing hands and environment
- Less exposure to maternal flora
- Sterile feeds (donor breast milk is pasteurized)
- Resistant bacteria
- Yeast colonization (lines)
- The abnormal pattern of bacterial colonization in preterm infants may also contribute to the pathogenesis of neonatal necrotizing enterocolitis

**Prevention of NEC with Probiotics in Premature Infants**

- **Consistent and significant reduction in incidence**
- **Best and studied B. infantis, B. bifidum, LGG**
- In all cases given as a drug/ supplement
- **Number needed to be treated: up to 24**
- **Cost/benefit easier to justify**
- **Compliance easier to attain**
- **Vulnerable population (questionably at increased risk with probiotic use)**
Clinical Report on Probiotics and Prebiotics in Pediatrics

NEC
“There is some evidence to support the use of probiotics to prevent NEC in preterm infants with a birth weight of 1000 g or higher. However, the amount and specificity of which probiotic or mixture of probiotics to use is problematic, given the many unanswered questions from a review of the available literature. Furthermore, many of the probiotics used and cited in the literature for treatment in preterm infants are not readily available.”

Differences in Intestinal Microbiota

Infants with colic tend to have
- More coliforms (E. coli and Klebsiella)
- Less Lactobacilli
compared to non-colicky infants

Is there a role for probiotics in infant colic?

L. reuteri Supplementation Reduces Crying Time In Colicky Infants In 7 Clinical Studies

Possible Mechanisms of Action:
Probiotics and Reduced Crying in Colic

- Improvement in gut motility and function, including increased gastric emptying rate and reduced gastric residuals
- Inhibits visceral pain
- Decreasing hydrogen gas production
- Improved mucosal barrier
- Increasing counts of intestinal lactobacilli and decreasing counts of E. coli
- Anti-inflammatory effects
L. reuteri Significantly Reduces Daily Regurgitation Frequency in 4 Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Age/Population</th>
<th>Feeding Mode</th>
<th>Duration</th>
<th>L. reuteri Group Regurgitations (mean ±SD)</th>
<th>Placebo Group Regurgitations (mean ±SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indrio 2008</td>
<td>Preterm BF/FF 4 Weeks</td>
<td>2.1±0.9 (n=10)</td>
<td></td>
<td>4.2±1.1 (n=10)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Indrio 2011</td>
<td>&lt;4 Months GER FF 4 Weeks</td>
<td>1.0 (1.0‐2.0)* (n=19)</td>
<td></td>
<td>4.0 (3.0‐5.0)* (n=15)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Garofoli 2014</td>
<td>Healthy BF 4 Weeks</td>
<td>9** (n=20)</td>
<td></td>
<td>18.5** (n=20)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Indrio 2014</td>
<td>Healthy BF/FF 4 Weeks</td>
<td>2.7±1.5 (n=238)</td>
<td>12 Weeks</td>
<td>3.3±2.3 (n=230)</td>
<td>0.35</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.9±1.1 4.6±3.2 &lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: "L. reuteri offers a safe alternative to pharmaceutical intervention and may be considered in the prevention and treatment of infant regurgitation."

Probiotics and Inflammatory Bowel Disease

ETILOGIC HYPOTHESES

Probabilities of Remission with Lactobacillus GG in Addition to Standard Maintenance Therapy in Children with Crohn's Disease

<table>
<thead>
<tr>
<th>Author</th>
<th>Probiotic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klein</td>
<td>E. coli Nissle 197</td>
<td>Equal to Mesalamine</td>
</tr>
<tr>
<td>Vancan</td>
<td>E. coli Nissle 197</td>
<td>Equal to Mesalamine</td>
</tr>
<tr>
<td>Copaci</td>
<td>S. boulardii</td>
<td>Equal to Mesalamine</td>
</tr>
<tr>
<td>Ishikawa</td>
<td>Bifidobacteria Milk</td>
<td>Superior to placebo</td>
</tr>
<tr>
<td>Fedorak</td>
<td>VSL#3</td>
<td>Improvement</td>
</tr>
<tr>
<td>Prantera</td>
<td>LGG</td>
<td>Superior to placebo</td>
</tr>
<tr>
<td>Furri</td>
<td>Bifidobacteria + Fiber</td>
<td>Improvement</td>
</tr>
</tbody>
</table>

Probiotics in Crohn's Disease: Clinical Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Probiotic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malchow</td>
<td>E. coli Nissle</td>
<td>Improved</td>
</tr>
<tr>
<td>Guslandi</td>
<td>ASA +/- S. boulardii</td>
<td>Improved</td>
</tr>
<tr>
<td>Campieri</td>
<td>VSL#3 vs ASA Post-op</td>
<td>Improvement</td>
</tr>
<tr>
<td>Prantera</td>
<td>ASA vs LGG</td>
<td>Improved</td>
</tr>
<tr>
<td>Of others</td>
<td>Crohn's various</td>
<td>Mixed results</td>
</tr>
</tbody>
</table>

Probiotics in Ulcerative Colitis

<table>
<thead>
<tr>
<th>Author</th>
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<th>Result</th>
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</thead>
<tbody>
<tr>
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<td>Copaci</td>
<td>S. boulardii</td>
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<td>Ishikawa</td>
<td>Bifidobacteria Milk</td>
<td>Superior to placebo</td>
</tr>
<tr>
<td>Furri</td>
<td>Bifidobacteria + Fiber</td>
<td>Improvement</td>
</tr>
</tbody>
</table>
Ongoing Areas of Research: Fecal Microbial Transplant

- Only one FDA approved:
  - C. difficile colitis
- Inflammatory bowel disease
- Gastroenteric infections
- Irritable bowel syndrome /other functional GI
- Colorectal cancer
- Metabolic syndrome and obesity
- Liver diseases
- Allergic diseases
- Neurological diseases such as autism and MS

AAP Clinical Report on Probiotics and Prebiotics in Infants:

Statements on Probiotic Safety

- "To date, these products (probiotics and prebiotics) seem to be safe for healthy infants and children."
- "Probiotics should not be given to children who are seriously or chronically ill until the safety of administration has been established."
- "All ingredients used in infant formula must be safe and lawful—that is, food ingredients that are, to date, generally regarded as safe (GRAS)... Prebiotics and probiotics now being added to commercial infant formulas are classified as GRAS."

Prebiotics

History of the “bifidus factor” of human milk

Prebiotics HMOs: Human Milk Oligosaccharides

- Non-digestible carbohydrates that selectively stimulate the growth of certain bacteria in the GI tract, that have the potential to benefit the human host
- Prebiotics are the preferential substrate (“food”) for specific genera of bacteria (particularly bifidobacteria)
- Composed of polymers (chains) of sugars that are not absorbed, thus behave like soluble dietary fiber
  - They are fermented, producing short chain fatty acids, gas and water
  - Lower colonic pH
  - Support integrity of colonic mucosa
  - Soften stools

HMOs are the Third Largest Solid Component in Human Breast Milk

- HMO levels range between 20 and 25 g/L for colostrum and 5 and 15 g/L for mature milk
- 2’FL and LNnT are among the most abundant HMOs

Adapted from Zivkovic et al., 2011. Macronutrients, micronutrients and HMOs

- Water (about 8 g/L)
- Proteins (about 8 g/L)
- Lipids (about 40 g/L)
- Lactose (about 70 g/L)
- 2’‐Fucosyllactose (2’FL)
- Lacto-N-fucopentaose
- Lacto-N-(Neo)tetraose (LNnT)
- 3’‐Fucosyllactose
- 3’- and 6’-Sialyllactose
- Sialyllacto-N-tetraose
HMOs Can Modify the Intestinal Microbiota (Prebiotic Effect)

- HMOs selectively enhance the growth of good bacteria, specifically bifidobacteria
- Higher levels of bifidobacteria are associated with timely and proper maturation of the gut
- Some strains of bifidobacteria use HMOs as the sole source of carbon

HMOs Can Modify the Intestinal Microbiota (Decoy Effect)

- HMOs can competitively bind to pathogenic bacteria reducing their adherence to epithelial cells
- HMOs can directly interact with intestinal epithelial cells and modulate their glycan expression
- Acts as an alternative mechanism to prevent pathogen attachment to the host cell

HMOs Can Directly Influence the Immune System (Immune Effect)

- HMOs can directly modulate immune responses by affecting immune cell populations and cytokine secretion
- HMOs influence lymphocyte maturation and promote a shift in T-cell response

Factors that Influence HMO Composition in Human Breastmilk

**Genetics:**
- Two genes are well characterized: Secretor and Lewis genes which encode specific fucosyltransferases (required for addition of fucose to HMOs)

**Other factors hypothesized to impact HMO composition:**
- Maternal age, time postpartum (lactation stage)
- Geography: The environment may play a role in regulating the synthesis of HMOs
- Maternal weight and body mass index

2’ Fucosyllactose (2’FL) HMO

- The most abundant HMO in breastmilk samples
- Constitutes nearly 20-30% of total HMOs
- 80% of mothers make 2’FL in their breast milk
- They have the secretor and Lewis genes which encode specific fucosyltransferases (FUT2 positive)

Consists of 3 monosaccharides: tri saccharides: galactose and glucose (forming the disaccharide lactose) + fucose (monosaccharide)

2’FL Impacts the Gut Microbiota

- 2’FL is the most abundant HMO in the milk of secretor women, and promotes the colonization of the gut with bifidobacteria
More children (2-3 years of age) were colonized with bifidobacteria if from secretor mothers (those with higher 2'FL levels in their breastmilk) vs. non-secretor mothers.


% of Children Colonized with Bifidobacteria

Was Higher in Secretor Mothers with 2'FL in Breastmilk

Objective:
To investigate the effects of feeding formulas supplemented with the HMO 2'-fucosyllactose (2'-FL) on biomarkers of immune function in healthy term infants.

Conclusions:
Data indicate that infants fed formula supplemented with 2'-FL exhibit lower plasma and ex vivo inflammatory cytokine profiles (TNFα, IFNγ), similar to those of a breastfed reference group.

Challenges for uses of probiotics in neonates, infants and children

- Which bacterial strains to use?
- Concomitant use of prebiotics
- Stability in stomach and intestine
- Palatability and route of delivery
- Dosaging
- Safety and purity

Live probiotic bacteria are available in foods from commercial outlets and are not regulated by the FDA (except when in infant formula).

Infant Probiotic Supplements · Available in the U.S.

Probiotic Foods for Infants and Children

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dannon</td>
<td>DanActive L. casei probiotics</td>
<td>L. casei Immunitas</td>
</tr>
<tr>
<td>General Mills</td>
<td>YoPlus L. rhamnosus GG</td>
<td>L. casei and L. bulgaricus</td>
</tr>
<tr>
<td>Horizon Organic</td>
<td>YoGurt Tubes</td>
<td>L. casei and L. bulgaricus</td>
</tr>
<tr>
<td>Kraft Foods</td>
<td>LiveActive Cheese</td>
<td>B. infantis</td>
</tr>
<tr>
<td>Stonyfield Farm</td>
<td>YoBaby YOGURT yogurts and drinks</td>
<td>L. acidophilus, L. casei, L. rhamnosus</td>
</tr>
<tr>
<td>Yakult</td>
<td>Yakult (probiotic drink)</td>
<td>L. casei Shirota</td>
</tr>
</tbody>
</table>

Dose ranges between 10^8 – 10^10 per serving.

Infant Formulas with Probiotics · Available in the U.S.

<table>
<thead>
<tr>
<th>Product</th>
<th>Protein Type</th>
<th>Probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerber Good Start Gentle Pro (HMO)</td>
<td>Partially hydrolyzed whey</td>
<td>B. lactis, Bb-12</td>
</tr>
<tr>
<td>Gerber Good Start Soothe Pro (HMO)</td>
<td>Partially hydrolyzed whey</td>
<td>L. reuteri, DSM 17938</td>
</tr>
<tr>
<td>Culturelle Baby Drops/ Packets</td>
<td>Lactobacillus rhamnosus LGG and Bifidobacterium lactis BB-12</td>
<td></td>
</tr>
<tr>
<td>Culturelle Baby Grow and Thrive With Vitamin D  Packets</td>
<td>Lactobacillus rhamnosus LGG and Bifidobacterium lactis BB-12</td>
<td></td>
</tr>
<tr>
<td>Nutramigen with Enflora LGG</td>
<td>Extensively hydrolyzed casein</td>
<td>L. rhamnosus GG</td>
</tr>
<tr>
<td>Neocate Syneo</td>
<td>Amino Acid Based</td>
<td>B. breve (and FOS)</td>
</tr>
</tbody>
</table>
Infant Formulas with Prebiotics - Available in the U.S.

<table>
<thead>
<tr>
<th>Brand</th>
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<th>Prebiotic</th>
</tr>
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<tbody>
<tr>
<td>Neocate Syneo</td>
<td>Amino Acid Based</td>
<td>FOS</td>
</tr>
<tr>
<td>Gerber Good Start GentlePro</td>
<td>Partially hydrolyzed whey</td>
<td>2'FL-HMO</td>
</tr>
<tr>
<td>Gerber Good Start SoothePro</td>
<td>Partially hydrolyzed whey</td>
<td>2'FL-HMO</td>
</tr>
<tr>
<td>Enfamil NeuroPro</td>
<td>60% whey, 40% casein</td>
<td>GOS, PDX</td>
</tr>
<tr>
<td>Similac Advance</td>
<td>52% casein, 48% whey</td>
<td>2'FL-HMO and FOS</td>
</tr>
<tr>
<td>Similac Pro-Advance, Pro-Sensitive, Pro-Total Comfort</td>
<td>52% casein, 48% whey</td>
<td>2'FL-HMO and FOS</td>
</tr>
</tbody>
</table>

A "healthy" intestinal microbiota modulates important GI functions

- Improved Gut Barrier Function
- Improved Immune Response

Birth

- C-section
  - Formula feeding
  - Antibiotic use
  - Environment (hospital & sterility)

Promote

- Vaginal delivery
- Breastfeeding

Hinder

- Vaginal delivery
- Breastfeeding

Questions?