The Neonatal Microbiome: “It’s a Small World After All”

Terry S. Johnson, APN, NNP-BC, CLEC, ASPPS, MN
Neonatal Nurse Practitioner
Founder, Lode Star Enterprises, Inc.
Director, Education and Professional Development
Prolacta Bioscience

Disclosure Statement

• Financial Arrangements
  – I currently present and receive financial reimbursement
  • Prolacta Bioscience
  • Abbott Nutrition Health Institute (ANHI)

• I personally developed this slide deck for strictly educational purposes and audiences
  – Images & photographs used in the presentation are from publicly accessed sources
  – It is evidenced-based
  – I will make no recommendation for any “off-label” use of any drug, nutritional, or device

The Neonatal Microbiome

• Delivery Room Practices

  More parents want ‘vaginal seeding’ for their newborns. But it might not be safe.


The Neonatal Microbiome

• “Vaginal Seeding”

  The practice of swabbing down babies born by Cesarean section with maternal vaginal bacteria


The Neonatal Microbiome

• “Partial restoration of the microbiota of cesarean born infants via vaginal microbial transfer”

  – Pilot study in which infants delivered by C-section were exposed to maternal vaginal fluids at birth
  – Consists of incubating sterile gauze in the vagina of mothers who were:
    • Negative for group B Streptococcus (GBS)
    • No signs of vaginosis
    • Vaginal pH < 4.5 during the hour preceding the C-section

  Cunnington, A. “Vaginal seeding” of infants born by caesarean section BMJ 2016; 352 doi: http://dx.doi.org/10.1136/bmj.i227 (Published 23 February 2016) Cite this as: BMJ 2016;352:i227

The Neonatal Microbiome

• “Partial restoration of the microbiota of cesarean born infants via vaginal microbial transfer”

  – Within the first 2 minutes of birth, babies were exposed to their mother’s vaginal contents by being swabbed with the gauze, starting with the mouth, then the face and finally the rest of the body

  Nature Medicine Volume: 22, Pages:250–253 Year published:(2016) DOI:doi:10.1038/nm.4039 Received
03 July 2015 Accepted 22 December 2015 Published online 01 February 2016
The Neonatal Microbiome

- “Partial restoration of the microbiota of cesarean born infants via vaginal microbial transfer”
  - A total of 1,519 samples were obtained
  - From anal, oral and skin sites of infants and mothers
  - Taken at six time points during the first month of life (1, 3, 7, 14, 21 and 30 days after birth)
  - Microbiome composition was characterized by sequencing the V4 region of 16S rRNA gene

Nature Medicine Volume: 22, Pages: 250–253 Year published: (2016) DOI: doi:10.1038/nm.4039 Received 03 July 2015 Accepted 22 December 2015 Published online 01 February 2016

The Neonatal Microbiome

- “Next Week on Masterpiece Theatre...”

The Neonatal Microbiome

- Distribution of Microbiome
  - Total Bacterial Counts (cfu/ml)
    - stomach $0-10^5$
    - jejunum $0-10^8$
    - ileum $10^3-10^7$
    - colon $10^{10}-10^{13}$

The Neonatal Microbiome

- Evolutionary Biology
  - For the purpose of my discussion today, either is OK... I just mean for as long as we have been having babies.
The Neonatal Microbiome

• Evolutionary Biology
  • For millennia women have delivered and babies have been born –
    – At term or very near term
    – With labor
    – After/with ROM
    – Vaginally delivered
    – Exclusively breastfed
    – Remained with their mother
    – Microbiome was colonized

The Neonatal Microbiome

• “Evolutionary Discordance”
  – Changing clinical practices
    • A.R.T. and G.M.K.s
    • Maternal morbidities
    • ↑ C/S deliveries, ↑ anesthesia
    • Management of ROM
    • Hyper-hygienic measures
      • ↑ Maternal/infant antibiotics
      • Limited mother/infant contact
      • ↓ Exposure to colostrum
      • ↓ Breast milk feeding
      • Microbiome altered

The Neonatal Microbiome

• Colonization of the Microbiome
  • First Stage: Birth to 1 Week of Age
    – Role of mode of delivery is major determining factor
    – Composition of infant’s evolving microbiota initially defined by mother
    – Role of ROM, labor, SVD exposes the infant to maternal GI flora
    – Establishing colonization similar to mother’s vaginal flora species
      Lactobacillus and Bifidobacterium

Am J Clin Nutr September 2012 vol. 96 no. 3 544-551

The Neonatal Microbiome

• Colonization of the Microbiome
  • Delivery Mode
    – 7 year olds in Finland;
    – n = 31 C/S and 29 SVD
    – Assessed at 7 years age
      • PE, perinatal data, fecal samples, serum total and antigen specific IgE
      – Via C/S delivery
        • ↓ strict GI anaerobes present
        • More slowly acquired diversifying microbiota

S Salminen et al (2004) GUT 53(9); 1388-1389

The Neonatal Microbiome

• Colonization of the Gut Microbiome
  • Delivery Mode
    – n = 95 SVD and 17 C/S
    – Rectal swabs at DOL 3
    – Fecal samples (1, 2, 4, 8 weeks) and at (6 and 12 months)
    – SVD had ↑ E coli colonization
    – C/S had later acquisition of many traditional gut bacteria

Alderberh et al (2006) Ped Research 59(1); 96-101

The Neonatal Microbiome

• Colonization of the Gut Microbiome
  • Second Stage: 1-4 Weeks of Age
    – Role of infant’s diet is a major determining factor
    – Human milk has lower buffering capacity - acidic milieu potentiates growth of nonpathogenic bacteria
    – Human milk (including colostrum) has specific antibodies and oligosaccharides to support growth of commensal bacteria in the infant’s gut

Am J Clin Nutr September 2012 vol. 96 no. 3 544-551
The Neonatal Microbiome

- Neonatal Gut Microbiome
- Abnormal Bacterial Colonization
  - Delivery Mode
    - Overall C/S rate ~ 33%
    - C/S infant first colonized with skin flora from care givers/environment
    - Abnormal intestinal colonization still present at 2 years age
    - C/S more frequently colonized with Staphylococcus
    - Stool samples of preterm infants < 32 wks PMA
      - mode of delivery significant impact on Bacteroides (p <0.001); not affected by antibiotics or feeding


- Colonization of the Gut Microbiome
  - Delivery Mode: Colostrum Microbiome
    - Am J Clin Nutr September 2012 vol. 96 no. 3 544-551

- Neonatal Microbiome
  - Maternal Obesity: Colostrum Microbiome
    - Am J Clin Nutr September 2012 vol. 96 no. 3 544-551

- Neonatal Microbiome
  - Delivery Mode: Milk Microbiome
    - Am J Clin Nutr September 2012 vol. 96 no. 3 544-551
The Neonatal Microbiome

• Neonatal Microbiome
  – Maternal Obesity: Milk Microbiome

Am J Clin Nutr September 2012 vol. 96 no. 3 544-551

The Neonatal Microbiome

• Colonization of the Gut Microbiome
  – Infant Antibiotics
    • ↑ Total days of antibiotics,
    • ↓ In microbial diversity
      – Colonization with Proteobacteria may be an effect of prolonged antibiotics (>7 days)
      – May lead to excessive immune and inflammatory responses and translocation of gut bacteria
      – Proteobacteria more predominant in infants with NEC


The Neonatal Microbiome

• Colonization of the Gut Microbiome
  – Infant Gender

• Prospective exploration of day-to-day changes in microbiome patterns over first 30 days in NICU and potential factors for development of microbiome
• Stable preterm infants 28 0/7-32 6/7 weeks gestation; 0-7 days old
  – Fed mother’s own breastmilk (MBM)
  – Non-mother’s breastmilk (non-MBM) (including donor milk)
  – Preterm infant formula

doi:10.1371/journal.pone.0152751 April 25, 2016

The Neonatal Microbiome

• Colonization of the Gut Microbiome
  – Placenta and Amniotic Fluid

• “Abundant, metabolically rich” microbiome in the placenta
• Commensal bacteria *E. coli* is most abundant
• Differences in microbiome of term/preterm placentas
• Role of vaginal and oral, and fetal microbiome and PTB


The Neonatal Microbiome

• Colonization of the Gut Microbiome
  – DNA V4 region of 16S RNA Gene
    • Proteobacteria most common (54.3%)
      – Shift patterns of increased Clostridium, Bacteroides, Staphylococcus, Haemophilus
      – Alpha-diversity increased significantly after birth
      – Influenced by postnatal days, feeding types, gender
      – Males began with a lower α-diversity
      – Females more likely abundant Clostridiates, lower Enterobacteriales than males
      – Infants fed MBM had greater diversity of microbiome

doi:10.1371/journal.pone.0152751 April 25, 2016

The Neonatal Microbiome

• Colonization of the Gut Microbiome
  – Placenta and Amniotic Fluid

  – Linkage of infection/inflammation/PTB
  – Bacterial colonization of placenta seen in normal pregnancies
  – These may represent “mutualism” with the host, providing resistance to pathogens
  – Microbes are within EVT cells (extravillous trophoblasts)
    • Cells are fatally derived; coated with self antigens (HLA-G)
    • Have “immune-privileged” status
    • Maintain immune tolerance of the fetus

The Neonatal Microbiome

• Colonization of the Gut Microbiome – Infant Feeding
  • Enteral diet of MOM ↓ incidence of NEC and LOS
  • Less abundant research as to type of feeding and microbiota in the preterm population
  • There is support that pasteurized DM results in ↑ microbial diversity with age, improved feeding tolerance and weight gain

Dollings MC & Brown L (2016). An integrated review of intestinal microbiota in the very premature infant. Neonatal Network. 35(4);204-215

The Neonatal Microbiome

• Colonization of the Gut Microbiome – Infant Diet
  • Variation in microbiota and organisms seen in breast fed and formula fed infants
  • Formula fed infants have less Lactobacillus and Bifidobacterium species
  • Formula fed infants have more bacterial diversity making it more similar to that of adults

The Neonatal Microbiome

• Colonization of the Gut Microbiome – Infant Feeding
  • Infants fed formula have higher intestinal molecular permeability
  • Both high intestinal permeability and translocation have been described in formula-fed babies
  • Increased predominance of facultative anaerobes in formula fed than breast fed infants
  • ?Potentially predisposing them to NEC and LOS


The Neonatal Microbiome

• Mucosal Immunologic System (MIS)
  – Provides a complex mechanical barrier and an inherent defense against pathogens that constantly threaten the human body
  – Evidence suggests that these systems do not work independently, but an integrated network of tissue, cells, and signaling molecules

The Neonatal Microbiome

- **Mucosal Immunologic System (MIS)**
  - The lining of the GI tract provides the largest interface with the external environment and is critical to host defense.

- **Epithelial Cells**
- **Mucous Secretions**

**Pulmonary**

**Gastrointestinal**

**Genitourinary**

---

Facts Influencing the Intestinal Microbiome and Predisposing to NEC

- **Dysbiosis**

**Brain-Gut Axis**

- Functional communication exists between the CNS and the GI Tract

**70% of the immune system is in the gut**

**Immune System**

---

The Neonatal Microbiome

- **“Brain-Gut Axis”**
  - Key role in early programming of health outcomes
  - Operates in a bidirectional signaling system
  - Has both top-down and bottom-up effects
  - Emerging evidence linking intestinal "dysbiosis" of microbiome in preterm infants:
    - Neurodevelopmental disease outcomes
    - Preceding late-onset neonatal sepsis and NEC

---


**The Neonatal Microbiome**

*“Brain Gut Axis”*

**The Neonatal Microbiome**

*Evolutionary Biology*

- “Human milk is an evolutionary wonder whereby the lactating mother produces a (1) species-specific (2) nutritional (3) and biologically active product that confers the best health to the human offspring”.

**The Neonatal Microbiome**

*Milk as Medicine*

- “Milk as Medicine”
  - “The major components of human milk are not primarily for nutrition, but for host defense”

**The Neonatal Microbiome**

*Evolutionary Immunobiology*
The Neonatal Microbiome

**Immunonutrition**

“The modulation of the immune and inflammatory responses in critically ill patients with the use of enteral feedings enriched with immune-enhancing ingredients”.

*Neu J & Bernstein, H Update on host defense and immunonutrients Clinics in Perinatology 29(1); 2002.*

**“Milk as a Medicine”**


---

**Human Milk Oligosaccharides (HMOs)**

- Composed of 5 monosaccharides
- HMOs mount/composition vary over the course of pregnancy
- Wide range of interpersonal variation in HMOs
- Not every woman synthesizes the same set of HMOs
- Composition mirrors maternal blood group characteristics


---

**Human Milk Oligosaccharides (HMOs)**

- HMOs are present in colostrum, early, and mature milk
- ↑HMO in colostrum
- ↑HMO in preterm infant
- ↓HMO in mature milk
- Diverse variety and large number in donor milk
- Survive pasteurization intact
- Low content in bovine/cow milk
- Recent “bio-fermentation” of 1 HMO from bovine lactose


---

**Evolutionary Immunobiology**

~70% OF THE IMMUNE SYSTEM IS LOCATED IN THE GUT

The Neonatal Microbiome

**PREbiotics**
- Non-digestible food ingredient (sugars) that promote growth of beneficial bacteria in the colon and improve the health of the host
  - Galacto-oligosaccharides,
  - Fructo-oligosaccharides,
  - Inulin lactulose, Psyllium

**PRObiotics**
- Live microorganism (bacteria or yeast) which, when administered in adequate amounts, confers a health benefit to the host

The Neonatal Microbiome

- Questions Regarding *Probiotics* in the NICU
  - Have we also looked at ↓ antibiotic usage?
  - Can we initiate practices that facilitate infant colonization with maternal flora?
    - C/S without ROM - Early skin-to-skin opportunities
  - When using probiotics in the NICU?
    - Is there evidence of best preparations?
    - Which preparation is best for neonatal population?
    - Are the probiotics of human origin?
    - Where did we get them?
    - How do we determine dosage?

The Neonatal Microbiome

- “Vaginal Seeding”
  - In the absence of evidence of benefit, or of guidelines to ensure the procedure is safe, how should health professionals engage with the increasing demand for vaginal seeding?
  - We have advised staff at our hospitals not to perform vaginal seeding because we believe the small risk of harm cannot be justified without evidence of benefit.

Cunnington, A. “Vaginal seeding” of infants born by caesarean section BMJ 2016; 352 doi: http://dx.doi.org/10.1136/bmj.i227 (Published 23 February 2016) Cite this as: BMJ 2016;352:i227

The Neonatal Microbiome

- “Vaginal Seeding”
  - However, the simplicity of vaginal seeding means that mothers can easily do it themselves. Under these circumstances we should respect their autonomy but ensure that they are fully informed about the theoretical risks.

Cunnington, A. “Vaginal seeding” of infants born by caesarean section BMJ 2016; 352 doi: http://dx.doi.org/10.1136/bmj.i227 (Published 23 February 2016) Cite this as: BMJ 2016;352:i227

The Neonatal Microbiome

- “Vaginal Seeding”
  - Parents should be advised to mention that they performed vaginal seeding if their baby becomes unwell because this may influence a clinician’s assessment of the risk of serious infection.

Cunnington, A. “Vaginal seeding” of infants born by caesarean section BMJ 2016; 352 doi: http://dx.doi.org/10.1136/bmj.i227 (Published 23 February 2016) Cite this as: BMJ 2016;352:i227
The Neonatal Microbiome

Terry S. Johnson, APN, NNP-BC, CLEC, APPS, MN

Neonatal Nurse Practitioner
Certified Lactation Educator
Associate in Society of Professionals in Patient Safety

Founder, Lode Star Enterprises, Inc.
Phone: 630.881.2606
Email: lodestar@mindspring.com