The Neonatal Microbiome: “It’s a Small World After All”
Terry S. Johnson APN, NNP-BC  ASPPS, CLEC, MN

Behavioral Objectives
At the conclusion of this presentation the learner will be able to:
- Define the terms microbiota and microbiome.
- Discuss the role of “evolutionary discordance” on establishment of the neonatal microbiome.
- Describe the difference between a prebiotic and a probiotic and give an example of each.
- Identify the role of Human Milk Oligosaccharide (HMO) in the development of the infant microbiome and neonatal immunity.

Partial Restoration of the Microbiota Via Vaginal Microbial Transfer

“Vaginal Seeding”
The practice of swabbing down babies born by C/S with maternal vaginal fluids to facilitate the normal acquisition of the 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
- Procedure consists of:
  - Incubating sterile gauze in the vagina of mothers
  - Negative for group B Streptococcus (GBS)
  - No signs of vaginosis
  - Vaginal pH < 4.5 during the hour preceding C/S

Disclosure Statement
- I currently present and receive financial reimbursement
  - Prolacta Bioscience as an employee
  - Abbott Nutrition Health Institute (ANHI) as a presenter
- I personally developed this slide deck for strictly educational purposes and audiences
- Images & photographs used in the presentation are from publicly accessed sources
- It without bias, branding or commercial influence, and it is evidenced-based
- I will make no recommendation for the off-label use of any drug, nutritional, or device

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Partial Restoration of the Microbiota of Cesarean Born Infants via Vaginal Microbial Transfer

Within the first 2 min 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.

1,519 samples were obtained
- Anal, oral and skin sites of infants and mothers
- Six time points during the first month of life at (1, 3, 7, 14, 21 and 30 days after birth)
- Microbiome composition was characterized
- Historically relied solely upon culture methods
- Now by sequencing the V4 region of 16S rRNA gene
- Widely used to characterize taxonomic diversity present in microbial communities

• Discovery of the Microbiome

“We inherit every one of our genes, but we leave the womb without a single microbe.”

Michael Specter (2012)

• Evolutionary Biology

For the purpose of our discussion it’s just LONG PERIODS OF TIME

“Evolutionary Biology”
- For millennia woman have labored and delivered/babies –
  - At or very near term
  - With labor after/with ROM
  - Vaginally delivered
  - Remained with their mother
  - Exposed to colostrum
  - Exclusively breastfed
  - Microbiome colonizes
  - Immune system matures
  - Infant grows and develops

Transfer of the Maternal Microbiome
- First Stage (birth-1 week)
  - Infant exposed to maternal GI flora
- Role of ROM, Labor, SVD, skin-to-skin

Colonization of the Neonatal Gut
- Second Stage (1-4 weeks)
- Role of infat's diet major factor

“Evolutionary Discordance”
- In last 60+ years the complexity of pregnancy, labor, delivery, and neonatal care have changed
  - Advanced maternal age
  - Assisted reproductive Technology
  - Multiple gestation pregnancy
  - Premature delivery
  - Antibiotic exposure
  - Maternal morbidities
  - Maternal diet
  - Maternal obesity
  - Environmental toxins
  - ? Maternal microbiome

“Evolutionary Discordance”
- In last 60+ years those chances have resulted in practices that alter the infant’s microbiome
  - Preterm, near term, late term delivery
  - ? Elective/Non-Elective C/S
  - Labor or not, with/without ROM
  - Hyper-hygienic measures, antibiotics
  - ? Mother/infant contact
  - ? Exposure to colostrum
  - ? Breast milk feeding
  - ? Microbiome colonization
  - ? Immune system matures
  - ? Infant grows and develops
• Colonization of the Neonatal Gut
  • Second Stage (1-4 weeks)
    • Role of the infant’s diet is a major factor

  "In contrast to term infants, preterm infants largely acquire their colonizing gastrointestinal bacteria from the NICU environment rather than from their mothers."

• Maternal Obesity: Colostrum Microbiome

• Maternal Obesity: Milk Microbiome

• Delivery Mode: Colostrum Microbiome

• Delivery Mode: Milk Microbiome

• The Mucosal Immunologic System
  • Complex mechanical barrier and an inherent defense system
  • Integrated network of tissue, cells, and signaling molecules
  • GI tract provides largest interface with external environment
  • It is critical to host defense

At no time in life is this function more important than shortly after birth

**Components of Mucosal Immune System**

- Mucous Layer
- Epithelial cell layer
- Lamina propria
- Role in microbiome

**Mucosal Immunologic System**

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

- Insults to the Premature Microbiome/Gut
  - Immaturity of end organ system
  - Hospital environment
  - Mode of delivery
  - Hypoxic-ischemic reperfusion events
  - Luminal starvation
  - Inflammation/Infection
  - Antibiotic exposure
  - Non-human milk first
  - Altered GI Colonization

*Factors Influencing the Intestinal Microbiome and Predisposing to Feeding Intolerance and NEC*

*“Dysbiosis” probably the key event associated with the pathogenesis of NEC.*


**Neonatal Innate Immunity**

- First line of defense at birth
- Provides immediate protection at a local/cellular level
- Acts with non-specific responses
- Uses “pro-inflammatory” mechanisms
- Vasodilatation, cellular activation, microvascular permeability, coagulation
- Secondary collateral damage


**Neonatal Adaptive Immunity**

- Later developed immune response
- Requires exposure to antigens
- Amplifies with repeated exposure
- Highly specific response
- Relies on T & B cells and “memory”
- Elaborate cascade of responses
- Balanced response
- Role of human milk

"Human milk is an evolutionary wonder whereby the (1) lactating mother (2) produces a species specific (3) nutritional (4) and biologically active product that (5) confers the best health to the human offspring."

https://doi.org/10.3389/fimmu.2017.00584

Human Milk Microbiome
- Directly shapes the infant's intestinal microbiome
- Human milk oligosaccharide (HMO) drives the growth of these microbes within the gut

"This unique milieu of enhanced immune protection with diminished inflammation results from a complex interaction of anti-inflammatory and anti-oxidative factors provided by human milk to the intestine."

Evolution of Human Milk
- The "Four Biologics*” or "Immunizations**
  - Womb Milk
  - Amniotic Fluid
  - Colostrum
  - Breast Milk

"Womb Milk"
- Embryonic nutritional liquid found in placenta of mammals
- Produced by uterine gland very early in gestation
- Contains proteins/amino acids
- Nourishes the embryo during early first days of development

Womb Milk + the syncytiotrophoblast "embryotroph"

Amniotic Fluid
- Appears within 12 days following conception
- NOT water
- Amino acids, proteins, vitamins, minerals
- Hormones, and growth/trophic factors
- Immune regulatory cells, cytokines
- Human milk oligosaccharide (HMO)
- Stem cells
- Role in the Microbiome

Colostrum
- Antioxidants in colostrum and mature milk


Values are presented as Mean ± SD and * indicate significant difference in comparison with colostrums (p<0.05)
**Colostrum**

- Absorbed in OFALT structures and pass directly into the infant’s GI tract
- Antibodies, IgA
- Anti-inflammatory factors
- Anti-infective properties
- Tissue growth factors
- Anti-adhesive factors
- Oligosaccharides (2-3X)
- Antioxidants
- “Immune Therapy” NOT “Oral Care”
- DO use all of the colostrum
- Do NOT use cotton-tipped swabs

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**Human Milk Microbiome**

- A Miracle Happens
  - “Bacterial Imprinting”

**Milk**
- First originated as a meme, “Get him some milk!” or “That boy needs some milk!”
- Should be used for anyone in need of help or self-improvement.

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**Human Milk Oligosaccharides (HMOs)**

- “HMO provides the newborn with a variety of bioactive factors that promote a healthy colonization of the neonatal gut and support the maturation of the neonatal immune system.”

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**What’s In Human Milk?**

- Human Milk Contains Over 100,000 Components
  - Anti-Microbial Factors
  - Secretory Ig, IgA, lactoferrin
  - Complement C3
  - hBD-1, -2, -3
  - Other factors
  - Composers & Anti-Inflammatory
  - Tumor Necrosis Factor (TNF)
  - IL-1, -6
  - ANG
  - Prostaglandins
  - Eicosanoids
  - Growth Factors
  - Epidermal Growth Factor (EGF)
  - Other factors
  - Transporters
  - Leucine
  - Lactoferrin
  - Others
  - Lactobacillus
  - Bifidobacterium
  - Other beneficial microorganisms

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**Maternal Translocation**

- Increased Bacterial Translocation By Mother
  - Increased vascular permeability
  - Increased gut permeability
  - Higher gut pressure
  - Higher leukocyte concentration
  - Increased gut motility

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**Human Milk Evolutionary Wonder**

- Human Milk First originated as a meme, “Get him/her some milk!” or “That boy needs some milk!”
- Should be used for anyone in need of help or self-improvement.

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**Millennial Slang**

- “Get him/her some milk!”

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**Entero-Mammary Pathway**


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**Cardiovascular System**

- Progressive vasodilation
- Higher blood flow to the uterus, gut, breast
- Higher angiogenesis gut, breast
- Higher pressure on mesenteric vessels, lymph node
- Higher leukocyte concentration

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**Stomach**

- Decreased motility
- Decreased peristalsis
- Higher emptying time
- Constipation

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**Gut**

- Decreased motility
- Decreased peristalsis
- Higher emptying time
- Constipation

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**Mouth**

- Alteration of oral pH
- Modification of the oral microbiota
- Hyperemic, edematous, bleeding gums
- Trend to gingivitis

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**Goats**

- Increased Bacterial Translocation By Mother
  - Increased vascular permeability
  - Increased gut permeability
  - Higher gut pressure
  - Higher leukocyte concentration
  - Increased gut motility

---

**Rods**

- Increased Bacterial Translocation By Mother
  - Increased vascular permeability
  - Increased gut permeability
  - Higher gut pressure
  - Higher leukocyte concentration
  - Increased gut motility

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**Gall Bladder**

- Decreased motility

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**Uterus**

- Increased size
- Increased pressure on surrounding organs, blood, and lymph vessels

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**Breasts**

- Ducts/alveoli hyperplasia
- Ducts/alveoli hypertrophy
- Increase of immune cells
- Increased lymph/blood supply
- Production of precolostrum

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**Gastrointestinal System**

- Higher blood flow to the gut
- Higher angiogenesis gut
- Higher gut pressure
- Higher leukocyte concentration
- Increased gut motility

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**References**

HMOs

- Human Milk Oligosaccharides (HMOs)
  - Structurally complex, diverse sugars
  - Composed of 5 monosaccharide "building blocks"
  - HMOs are HMOs mount/composition vary over the course of pregnancy
  - Wide range of interpersonal variation in HMOs
  - Not every woman synthesizes same set of HMOs
  - "Partial" and/or "non-secretor" status
  - Composition influenced by maternal genetic factors and blood type

- Human Milk Oligosaccharides (HMOs)
  - ~150-200 HMOs present in human milk
  - 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

During Pregnancy
- Found in:
  - Cord blood
  - Amniotic fluid
  - Maternal urine
  - Colostrum
- The majority of HMO makes it to the large intestine intact which is also the primary location of beneficial microbes.

In the Infant
- Found in:
  - Enzyme breakdown
  - Low stomach pH
  - "Prebiotics"
  - Microbes release SCFA which feed gut cells
  - SCFAs facilitate production of gap junction proteins
  - Restricts pathogens entering systemic circulation
  - Provides diverse anti-inflammatory molecules

Human Milk Oligosaccharides (HMOs)
- Beneficial Role in the Neonatal Gut
  - Provide food for beneficial microbes
  - Microbes release SCFA which feed gut cells
  - SCFAs facilitate production of gap junction proteins
  - Restricts pathogens entering systemic circulation
  - Provides diverse anti-inflammatory molecules
Human Milk Oligosaccharides (HMO)
- Beneficial Role in the Neonatal Gut
  - Anti-adhesive antimicrobials
  - Serve as soluble decoy receptors
  - Prevent attachment of pathogens on mucosal/epithelial surfaces
  - Regulates immune-inflammatory processes connecting the intestine, liver, muscle, and brain (BGA)

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

Benefits of Human Milk to the Neonatal Gut

Exclusive Human Milk Diet (EHMD)
- Exclusive [Ex-SKLOO-siv, -xiv]
- Not admitting of something else or other things
- Limited to the object or objects designated
- Shutting all things from a part or share

Exclusive Human Milk Diet and the ELBW Infant

Immunonutrition

EHMD: Multicenter Retrospective Cohort Study
- Objective
  - To compare clinical outcomes in 1,587 Extremely Premature infants (birth weight <1250 g) before and after an institutional change to the use of an exclusive human milk-based diet (EHMD) including fortifiers from a diet that included cow milk-based (CMD) products (formulas and/or fortifiers)
- Method
  - Conducted at four geographically disparate hospitals: Texas, California, Illinois, and Florida
  - Each of the four hospitals reviewed charts from an equal period before and after implementing an exclusive human milk-based protocol

HMOs

"SYMBIOSIS"
- Insults Premature Gut
  - Immaturity of end organ system
  - Limited nutrition
  - Hypoxic reperfusion injury
  - Inflammatory infection
  - Antibiotic exposure
  - Altered GI colonization
- "DYSBIOSIS"
  - Benefits of HMO
  - Decreased inflammation
  - Less oxidative stress
  - Maturation of immune system
  - Maturation of gut defenses
  - Normalization of the microbiome
  - Role in Gut-Brain Axis (GBA)
  - "HOMEOSTASIS"

Benefits of Exclusive Human Milk Diet

Dictionary.com, meanings and definitions of words

The Urban Dictionary, compiled by Aaron Peckham
Study Hospital Feeding Protocols

Texas Children’s Hospital
- Prolact+ fortification initiated at 60 mL/kg/day with <1000 g BW
- Weight gain = <15 g/kg/day; fortification increased to 90 mL/kg/day
- If weight gain still = <15 g/kg/day: fortification increased to 120 mL/kg/day

Good Samaritan San Jose
- Prolact+ fortification initiated at 100 mL/kg/day with <1000 g BW
- If weight gain was deemed suboptimal by the attending doctor:
  - <1250 g BW: fortification increased to 120 mL/kg/day
  - ≥1250 g BW: fortification increased to 150 mL/kg/day

Northwestern
- Prolact+ fortification initiated at 60 mL/kg/day with <1000 g BW
- If weight gain was <15 g/kg/day: fortification increased to 90 mL/kg/day
- If weight gain was <15 g/kg/day and <1000 g BW: fortification increased to 120 mL/kg/day

Weiner Palmer Hospital
- Prolact+ fortification initiated at 120 mL/kg/day with <1000 g BW
- If weight gain was <15 g/kg/day: fortification increased to 150 mL/kg/day

Oxygen Radical Disease of Neonatology

Inflammation and Oxidative Stress

Cell/Tissue/Organ Injury

Early Human Milk Donation

EHMD: Multicenter Retrospective Cohort Study

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**EHMD group had significantly lower incidence of:**

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EFMD: Beyond Necrotizing Enterocolitis Prevention: Improving Outcomes with an Exclusive Human Milk Diet


ACOG practice advisory November 2017

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“Should a patient insist on performing the procedure herself, a thorough discussion with the patient should be held acknowledging the potential risk of transferring pathogenic organisms from the woman to the neonate.”

Partial Restoration of the Microbiota Via Vaginal Microbial Transfer

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Interesting and Oxidative Stress

Cell/Tissue/Organ Injury

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Partial Restoration of the Microbiota Via Vaginal Microbial Transfer

- ACOG practice advisory November 2017
- “Risk stratification is reasonable for such women in the form of testing for infectious diseases and potentially pathogenic bacteria.
- Serum testing for herpes simplex virus and cultures for group B streptococci, Chlamydia trachomatis and Neisseria gonorrhoea should be encouraged.

ACOG practice advisory November 2017
- “It is further recommended that the obstetrician-gynecologist or other obstetric care provider document the discussion.
- Because of the theoretical risk of neonatal infection, the pediatrician or family physician caring for the infant should be made aware that the procedure was done.”


Protect the Neonatal Microbiome!
- Human milk FIRST
  - Complete the “immunization”
  - Critical timing, dose, duration
  - HMO benefits
- Skin-to-Skin opportunities
  - Protect the “Sacred Hour”
  - Early, frequent, prolonged
  - Transitional milk benefits
- Antibiotic Stewardship
  - Maternal and Infant
  - “Be the Nurse…”
  - When, Why, Which, How Long?

Presenter
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