The Fourth Trimester:

Off My!

Disclosure

- Alice Gong, M.D. discloses the following relationships with commercial companies:
  - Grant/Research Support from Novartis

Learning Objectives

- At the end of this presentation the participant will be able to:
  1. List some unique components of human milk and their benefits to infants.
  2. Discuss some differences in gastrointestinal microbiomes between babies fed human milk and formula.
  3. Utilize effective strategies to assist mothers and providers in overcoming some common barriers to successful breastfeeding.

AAP, 1984 Task Force on Infant Feeding

- Findings of domestic report: inconclusive that breastfeeding has a large positive effect on infant health in the US.
- Modest protective effects may exist with regard to gastroenteritis.

Bo Vahlquist, 1981

- “In all mammalian species the reproductive cycle comprises both pregnancy and breast-feeding: in the absence of latter, none of these species, man included, could have survived”
- “There is no reason to accept the premise that breastfeeding is incompatible with modern industrialized society, and every reason to believe that, with adequate supportive measures to help meet those needs, breastfeeding can retain its integral place in process of human reproduction and child development.”

Evidence established past 3 decades

- Children breastfed for longer periods have lower infectious morbidity and mortality, fewer dental malocclusions, higher intelligence than breastfed for shorter periods or not breastfed.
  - Growing evidence to suggest BF protects against overweight and diabetes later in life
- Breastfeeding benefits mothers
  - Prevent breast cancer
  - Improve birth spacing
  - Reduce risk of diabetes and ovarian cancer

AAP, 1984

Pediatrics

Bo Vahlquist, 1981

Evidence established past 3 decades

AAP, 1984

Victora et al, Lancet, 2016
### Past 3 decades (cont)

- High-income countries have shorter breastfeeding duration than low and middle income countries (only 37% exclusively BF at 6 months)
- Scaling up of breastfeeding can prevent 823,000 child deaths and 20,000 breast cancer deaths annually.
- New biological methods discovered novel mechanisms that characterize breastfeeding as personalized medicine for infants
  - Microbiome, epigenetics, stem cells

### What’s in Human Milk?

**Nutritional components**
- Non-nutrition
  - Antimicrobial factors
  - Digestive enzymes
  - Growth factors
  - Pollutants
  - Drugs
  - Allergens
  - Viruses

**Breastmilk Composition is Dynamic: Infant Feeds, Mother Responds**

- Milk composition is not constant but changes in the short term in response to milk removal by the infant.
- Changes in gene expression stimulated by milk removal facilitate secretion of lipids and active migration of cells into breastmilk.

### Nutrition

- Metabolic fuels
  - Fat – 98% triglycerides, mostly medium and long chain
  - Protein – 75% N compounds
    - Micellar caseins, β-casein (soft, flocculent curd)
  - CHO – lactose, oligosaccharides (10%)
- Water
- Fatty acids
- Amino acids
- Minerals
- Vitamins
- Trace elements

### Bioactive components (multiple activities)

- Antimicrobial factors – specific, non-specific
  - Active within breast and baby’s GI and respiratory tracts
  - sIgA, IgM, IgG; lactoferrin, lysozyme, complement C3 leukocytes, bifidus factor, lipids and fatty acids, antiviral mucins, GAGs, oligosaccharides
- Cytokines and Anti-inflammatory
  - Tumor necrosis factor, interleukins, interferon-γ, prostaglandins, α1-antichymotrypsin, α1-antitrypsin, platelet-activating factor: acetyl hydrolase
- Hormones
  - Feedback inhibitor of lactation (FI), insulin, prolactin, thyroid hormones, corticosteroids, ACTH, oxytocin, calcitonin, parathyroid hormone, erythropoietin
- Digestive enzymes
  - Amylase, bile acid-stimulating ester, bile acid-stimulating lipases, lipoprotein lipase

### Breastmilk is so much more than nutrition, containing molecules that protect the infant as well as epigenetic modulators that program development!


Bioactive components (cont)

- Growth factors
  - Epidermal (EGF), nerve (NGF), insulin-like (IGF), transforming (TGF), taurine, polyamines
- Transporters
  - Lactoferrin, folate binder, cobalamin binder, IgF finder, thyroxine binder, corticosteroid binder
- Others
  - Casomorphins, d-sleep peptides, nucleotides, DNA, RNA
- Potentially harmful
  - Viruses, aflatoxins, trans-fatty acids, nicotine, caffeine, food allergens, PCBs, DDT, dioxins, radioisotopes, drugs

Breastmilk stem cells

- Cregan et al (2007) reported breastmilk cells with stem-like properties expressing ectodermal progenitor markers.
  - Confirmed by others
  - Self-renew in 3D spheroid culture
- Express pluripotency genes
- Capable of differentiating into cells from all 3 germinal layers and replicate
- Can migrate to all end organs
- Numbers higher in colostrum and involution milk

Cells

- Maternal cells, from leukocytes to epithelial cells of various developmental stages that include stem cells, progenitor cells, lactocytes, and myoepithelial cells.
- Variations of cellular content include breast fullness, stage of lactation, health status of mother/infant dyad, permeability of basement membrane, development of breast epithelium
- Colostrum and early lactation milk has more cells than mature milk
- Infection of mother and/or infant associated with increase in milk leukocytes that decreases with resolution of infection
- Cells work synergistically with bioactive components to have direct or indirect effect to increase infant immunity.

Assessment of Growth Factors Secreted by Human Breastmilk Mesenchymal Stem Cells

- Cultured mesenchymal breastmilk stem cells had growth potential for at least 10 days and both vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) were found to be secreted from the cells without influence of cord serum in the medium.
Breastmilk Imparts the Mother’s Stem Cells to the Infant
• Hassiotou, Mobley, Geddes, Hartmann, and Wilkie, School of chemistry and biochemistry, University Western Australia
  – fate of breastmilk stem cells in the breastfed offspring.
• Mouse model
• 1-2 months after weaning, stained stem cells found in stomach, thymus, liver, pancreas, spleen, and brain
• Evidence of migration and functional integration of native milk stem cells to organs of neonate.
• Faseb, 2015

The microbiome
• Human body has 10X more bacteria than human body cells
  – 100 trillion organisms
• Bacteria has 100X more genes than human genome.

Definitions
• Microbiota – all the microbes in a given environment.
  – Microbial super organ residing symbiotically within mucosal tissues and integumentary system of human host
  – Difficulties of culturing complex samples with fastidious or un-cultur able organisms
• Metagenomics
  – Application of modern genomics technique to study of communities of microbial organisms directly in their natural environments
  – Utilize analytical instruments that define molecular signatures

Molecular Technique
• Exploits ubiquitous and evolutionarily conserved 16S rRNA
• Samples undergo DNA extraction before universal PCR primers to allow for amplification of intervening hypervariable regions.
• Amplicons are differentiated into similar groups to be classified into operational taxonomic unit
• Bacterial classification determined by open access sequence databases
• Fungal, archaean, protozoal, and viral microbiomic studies exist.

Figure 1
• First generation techniques: separation by physical means using denaturing or temperature gradient gel electrophoresis (DGGE) - fingerprinting techniques
• Next generation sequencing (NGS): combinations of enzymology, chemistry, high-resolution optics, hardware, and software engineering. Generate enormous amounts of data and sequence to great depth - examples: 454 pyrosequencing (Roche), HiSeq and bench top MiSeq (Illumina) platforms. The MiSeq was recently found to have the highest throughput and lowest error rate of its type (6).
• Third generation platforms: capable of generating reads greater than 400 base pairs, facilitating identification to species level.
• Human Microbiome project - NIH
  – Integrative Human Microbiome Project – 2nd generation
    • Pregnancy and preterm birth
    • Inflammatory bowel disease
    • Prediabetes

• Metagenomics of Human Intestinal Tract – European Commission

• Neonatal Microbiota study
  – Longitudinal prospective study assessing importance of GI microbiota in relation to NEC and sepsis

Cradle to Grave

• Constantly fine-tuning to maintain homeostatic balance with host’s immune system
• Evolution governed by:
  – Mother
  – Adaptive and innate immune system
  – Diet
  – Lifestyle
  – Medication
  – Toxin exposure
  – Illness

How does a newborn acquire microbiome?
• Evidence suggest that process occurs before delivery.
• Aagaard et al – placental microbiome profile
  – Non-pathogenic mutualistic microbiota: Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla
  – Similar to oral microbiome
  – Low numbers, large number of species
  – Theories as to meaning of placental microbiome, not always harmful, helpful in terms of priming immune system, colonization fetal gut

Shifting Paradigm: Bacteria do not always harm the fetus

• Microbiome of placentas identified bacterial community of low numbers of organisms from large number of species
  – Nonpathogenic mutualistics – phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, Fusobacteria

Possible mechanism for bacteria to reach placenta

Aagaard et al, Sci Transl Med, 2014
Abrahamsson et al, Pediatric Research, 2015
Mode of delivery

- Term C/S fecal microbiome
  - Enterobacter, Cancerogenus, Hemophilus, Staphylococcus, Streptococcus
  - Skin and oral microbes, surrounding environment
- Vaginal delivery
  - Bacteroides, Bifidobacterium, Parabacteroides, Escherichia/Shigella (earlier sample)
- Difference gradually decreased at 4 months and 12 months
- C/S infants remain more heterogeneous
- Bacteroides less prevalent or missing in C/S delivered even up to 12 months.

Breastfeeding

- Breast-fed infants receive mix of nutrients, fatty acids, lactoferrin, sigA to affect milieu of development of microbiota
- Oligosaccharides, glycoconjugates also prevent attack of enteropathogens and stimulate growth of Bifidobacterium
- Interleukin-10, EGF, TGF-B1, erythropoietin are mediators in inflammatory response against pathogens in gut.
- Microbiota – Staphylococcus, Streptococcus, Bifidobacterium, Lactobacillus
  - Enteromammary pathway
  - Genotyping mother milk and infant fecal samples identical strains.

Formula feeding

- Microbiota – E coli, Colstridium difficile, Bacteroides, Prevotella, and Lactobacillus
- Mixed feeding shifts pattern to that of formula feeding.

Intestinal development

- Human newborn intestines are immature at birth; breastmilk contains functional components for protection and maturation
- Fetal small intestine – immature epithelium, delayed enterocyte proliferation, sparse lymphoid cells
- Newborn infant ingested breastmilk – actively proliferating, mature epithelium with all subclasses enterocytes and abundance of lymphoid tissue
Symbiotic Relationship

- Microbiota benefit from warm, nutrient-rich environment of the GI tract
  - Optimal growth within stable ecosystem
- Infant benefits from microbiota activities
  - Increased digestive capacity
  - Harvesting nutrients
  - Limit nutrient resources to pathogens
  - Development of barrier function, integrity, and immune function
  - Tolerant state

Complementary Foods

- Microbial composition and function again change
  - Fewer species that degrade BM sugars to those that assist with metabolism of complex sugars and starch.
  - In western countries, levels of Bacteroides and Clostridium changes the lactobacilli and Bifidobacterium levels.
  - Related more to cessation of breastfeeding
  - By 3, intestinal microbiota is more adult-like.

Goal

- Innate and adaptive immune system interact with microbiota to establish normal digestion, gut motility, immune tolerance to foods and microbial antigens, and protection against pathogens.
- Infant’s microbiome is established and matured during first year. Early childhood is a crucial age window in disease prevention since microbial diversification and maturation occurs during this time.

Breastfeeding

- First vaccine against death, disease and poverty and is the most enduring investment in physical, cognitive, and social capacity.
- Evidence that it increases IQ scores, improves school achievement, and boosts adult earnings.
Op-Ed: Breast milk is best and free, so why is it a luxury for American moms?

- “Unfortunately, there is a sharp socioeconomic divide when it comes to breastfeeding. Studies show a distinct correlation between parents’ income and education levels and a mother’s likelihood of breastfeeding. Privilege helps a lot.”
  - many employers don’t meet their legal obligation to provide nursing mothers with breaks and a clean, private space (other than a bathroom) for pumping. Few women protest, for fear of losing their jobs.
  - Nations far less prosperous than the United States have successfully addressed breastfeeding inequity.
- Breastfeeding is an important public health issue!

Jennifer Grayson, LA Times, 7/17/2016

What is the debate?

- Breastfeeding infants is the most natural and best thing that can happen for mothers and babies.
- Still very polarizing
- Watch the news: “Parents feel pressured” “Why should mothers feel guilty if there is perfectly acceptable alternative that does not tie the mother down to her baby?” “She is showing her breast in public” “Breastfeeding is for those who cannot afford formula”

“I breast-feed because I am lucky, have choices, and am physically able to. I have a supportive husband, an incredible mother who watches her granddaughter three days a week for free, and a workplace that allows me to exercise all of my breastfeeding rights as laid out by the Affordable Care Act. (For the record, mothers, you are entitled to these things: reasonable break time to express breast milk for one year after your child’s birth each time you experience the need to express milk, and a place to pump, other than a bathroom, that is shielded from view and free from intrusion from coworkers and the public.)”

Angela Gerbes, The Stranger, 2015

2016 Lancet

- In depth series that confirm benefits of breastfeeding in fewer infections, increased intelligence, protection against obesity and diabetes, and cancer prevention for mothers.
- Reasons women avoid or stop breastfeeding:
  - medical
  - cultural
  - psychological
  - physical discomfort
  - inconvenience

Public Health issue

- Educate everyone
- Change hospital policies and actions
- Artificial feedings only when necessary
- Have support for breastfeeding mothers
- Breastfeeding medicine support
- Paid maternity leave
- Appropriate pumps and places for pumping
- Acceptance by public that breastfeeding is the norm

Breastfeeding Friendly Pediatric Practice – 10 steps

- Have a breastfeeding promotion and support policy that is communicated to all staff, including those who cover for you.
- Have all staff attend in-services or workshops that teach skills necessary to promote and support breastfeeding
- Inform all pregnant women about the benefits and management of breastfeeding:
  - Use written, non-commercial information
  - Avoid advertising for formula companies
  - Recommend families attend prenatal breastfeeding classes
  - Orders at hospital not to give commercial discharge bags from formula companies, instead give care materials that support breastfeeding
Breastfeeding Friendly Pediatric Practice

• Help mothers initiate and continue breastfeeding during hospital rounds. As part of exam in mother’s room, watch baby breastfeed to assist with latch and hold
• For the mother-baby dyad that is separated due to illness, prematurity, etc., confirm that hospital grade electric breast pump is available for expressing milk
  – Encourage mother to express at least 8 times per day
  – Teach her to avoid nipple soreness, engorgement, or other problems related to pumping
  – Prescription for breast milk may be necessary to help get mother an electric breast pump

Breastfeeding Friendly Pediatric Practice

• Advise mothers to feed babies on cue.
  – Teach behavioral feeding cues to avoid underfeeding or over-hunger with resulting infant behavior disorganization
• Avoid artificial nipple and pacifier use in newborn breastfed infants
• Have access to lactation consultants who can assist you. Refer mothers to community resources that can support her breastfeeding goals.

Massachusetts Breastfeeding Coalition

Breastfeeding Friendly Pediatric Practice

• Avoid use of sterile water, glucose water, formula for breastfed newborns unless medically indicated. Colostrum is adequate for most newborns.
• Encourage rooming in
  – Protects babies from diseases in nursery
  – Provides unrestricted contact and feeding
  – Enables mothers to become aware of baby’s needs
  – Mothers who keep babies with them get more sleep than those who send baby to nursery

Resources: Texas

![Resource Image]

References

References


References