Innovating Practice Through Research and Evidence
Topic Updates and Promising Research

Jean Rhodes, PhD, CNM, IBCLC

Since its inception in 2011 the "Innovating Practice through Research Evidence" series has highlighted important topics in the field of human milk and lactation relevant to both clinicians and mothers and their babies, e.g. milk collection and storage; CMV transmission via breastmilk; preterm infant morbidities including NEC; the impact of human milk on cognitive and neurological development; and, cost benefits of breastfeeding. However, like all fields of scientific inquiry, research in human milk and lactation is ongoing, with new discoveries continuously informing established ideas. Therefore, the purpose of this discussion is to update research findings vis-à-vis previous topics and to introduce some areas of promising, new research that have emerged in 2012.

Updates
Human Milk Collection and Storage

In the first Innovating Practice document, "Collection & Storage of Human Milk", we touched upon three aspects of human milk collection and storage: anti-bacterial properties of human milk, the Academy of Breastfeeding Medicine's revised recommendations for storage of milk for term infants, and refrigerator storage in the NICU. Since that time, Takci and associates[1] published their findings on the effects of freezing on the bactericidal activity of human milk. Their results indicated that bactericidal activity against E.coli declines significantly when milk is stored for three months at -20°C but not when stored at -80°C. Similarly, Xavier *et al*[2] found human milk total antioxidant capacity was reduced within 48 hours of refrigeration and freezing, with continuing decline over one week in the freezer at -8°C.

Highlighting the lack of consensus regarding milk storage in NICU settings, Cossey and colleagues[3] surveyed storage practices in Belgium and Luxembourg. Respondents reported on a variety of practices across 19 hospitals, including whether they performed bacterial testing of mothers' milk (47%) or if they had a milk pasteurizer on the unit (32%). Most noteworthy, two of 19 NICUs reported storing fresh and thawed human milk in the refrigerator for up to seven days. The authors concluded there is still a lack of best practice guidelines and standards however, they went on to note that staff from nine units in Belgium have since begun to discuss best practice standards.

Lastly, on the topic of human milk collection and storage, Labiner-Wolfe and Fein[4] published data from 436 to 1060 mothers about their breastmilk handling and storage practices. Numbers of participants varied depending on infant age at the time of survey – 1.5 to 9.5 months. Encouragingly, mothers generally don't store milk beyond recommended time frames; approximately 97% of mothers reported keeping expressed milk at room temperature for no more than 4 hours, with 99% reporting room temperature storage for no more than 8 hours. Eight hours is the maximum time for room temperature storage of fresh milk for term infants recommended by the Academy of Breastfeeding Medicine[5] and six hours is recommended by The Human Milk Banking Association of North America (HMBANA).[6] Milk refrigerator storage followed the same pattern: approximately 88% of women used expressed milk within 2-3 days of refrigeration, 99% within 6-8 days.

As discussed in the first Innovating Practice paper, human milk collection and storage practices are informed by numerous scientific disciplines. Inevitably, recommendations will continue to change as we discover more about milk properties and factors affecting milk quality.

CMV

The second Innovating Practice edition, "CMV Transmission and Breastmilk", started with a systematic literature review by Kurath and associates[7] that revealed short and long term consequences of postnatal CMV infection. Their findings showed that perhaps the exposure to CMV via breastmilk was not as prevalent as once thought. This subsequently led to a discussion of the risks and benefits of milk treatment measures to reduce postnatal CMV risk in preterm infants. However, despite continued publications on the topic, the issue of how to manage use of fresh mothers' milk in the NICU is still unresolved.

In 2012, Okulu and associates[8] published a neonatal case study of a very low birth weight infant with CMV, presumably, breastmilk acquired CMV. In this particular case, the infant responded well to supportive treatment and ganciclovir-based antiviral therapy. Follow-up cranial ultrasound and auditory testing were normal. Okulu et al propose considering anti-viral therapy for treatment of CMV infections but do not address human milk management in the NICU.

Bevot *et al*[9] published long-term outcomes at eight and nine years of age in 20 preterm children with breastmilk-acquired CMV compared to 21 non-CMV infected children. Three children in the non-CMV group were hearing impaired. All CMV-positive children had normal hearing with cognitive and motor functioning within normal limits, but lower than those of their control group. Beyond the need for further study, the authors made no specific clinical recommendations.

Resch – a coauthor with Kurath of the systematic review of CMV transmission[7] and letter to the editor[10] highlighted in "CMV Transmission and Breastmilk" -- published a letter responding to Bevot et al suggesting that results are still confusing and that larger studies of long-term sequelae are "urgently needed." Thus, questions raised in our previous paper on breastmilk-acquired CMV remain unresolved. What are the risks of postnatal CMV infection? Do the benefits of fresh human milk outweigh the risks for preterm infants? Are there other options to reduce or eliminate the risks?

Emerging Hot Topics

Human Milk Oligosaccharides (HMOs) and NEC

Lars Bode[11] from the University of California in San Diego has investigated the structure, function and benefits of HMOs, including their ability to nourish commensal bacteria, prevent pathogen attachment to mucosal surfaces, and modulate immune responses.[12] In recent presentations and publications, Bode explained that while 150-200 HMOs have been identified in human milk, they don't all have the same function. Furthermore, the composition of HMOs varies from woman to woman with the function of each individual HMO being very structure-specific. Bode described laboratory processes involved in isolating a single HMO, Disiayllacto-N-tetraose (DSLNT) that has been found to be the most effective HMO in reducing the risk of NEC in laboratory rats.[11]

Bode suggested human diversity and the function of DSLNT could explain why some infants receiving human milk might still develop NEC: their mothers may not produce sufficient quantities of DSLNT. Clinical application of these findings could lead to the absence of DSLNT in mother's milk becoming a biomarker for infants at risk for NEC. Bode also suggested a process for DSLNT supplementation might be possible for at-risk infants in the future.

Gut Microbiota and NEC

Joseph Neu[13] is also focused on current evidence related to the prevention of NEC and has stressed the importance of intestinal microbiota, particularly commensal bacteria, in risk reduction. Of note, Neu emphasized the role of intestinal bacterial composition on the development of a healthy immune system.

Although a sterile environment would intuitively seem to protect the infant from infection, Neu maintained current evidence supports the view that a healthy immune system develops better in an environment with pathogenic and non-pathogenic bacteria. When discussing the risk of NEC, Neu identified multiple events that can disrupt infant intestinal flora including pre- and post-natal antibiotics, the NICU environment, preterm birth, mode of delivery, immediate bathing of newborns, infant diet, probiotics and separation from mother. Although several clinical interventions to reduce the risk of NEC have been proposed – probiotics, supplemental prebiotics or combinations of both – research thus far is inconclusive as to their benefit. What is effective, according to Neu, is providing human milk to premature infants.[13,14]

Stem Cells in Human Milk

Mammary stem cells are thought to contribute to the ability of the breast tissue to grow and differentiate during pregnancy and lactation, then regress until the next gestational cycle.[15] In 2007, Cregan and associates published seminal work[16] on their discovery of stem cells in human milk.

Foteini Hassiotou and associates[16] of The University of Western Australia have described remarkable advancements in this field: research now demonstrates human breastmilk-derived stem cells, like embryonic stem cells, are pluripotent with the ability to differentiate *in vitro* into multi-lineage stem cells producing not only breast cells -- myoepithelial, ductal, and alveolar cells -- but also cells of other lineages. Hassiotou and associates are growing in culture a variety of cells from human milk stem cells including bone cells, adipose cells, cardiomyocytes, neural-like cells, hepatocytes and pancreatic cells. Furthermore, cultured cells are secreting cell-specific substances: breast alveolar cells are secreting beta-casein, lactoferrin and alpha lactoalbumin (all components of human milk); hepatocytes are secreting albumin; and pancreatic cells are secreting insulin.

At this time, the benefits of breastmilk stem cells for infants are unclear. However, the potential for stem cells to produce functioning tissues commands great interest in health care research. Hassiotou proposes human milk stem cells might be useful in regenerative medicine or in cancer research, helping identify why and how cells mutate into cancer cells. According to Hassiotou, advantages of human milk-derived stem cells are that they are plentiful and are accessible non-invasively and ethically.[15,17]

Summary

Human milk and lactation research spans the globe. The wealth, variety and depth of research underscores a common interest in the science of breastfeeding and the use of human milk for vulnerable infants. In this document, we have examined recent research related to previously explored topics and have touched on emergent discoveries in human milk research pertaining to oligosaccharides, gut microbiota and stem cells.

References

1. Takci S, Gulmez D, Yigit S, Dogan O, Dik K, Hascelik G. Effects of freezing on the bactericidal activity of human milk. *J Pediatr Gastroenterol Nutr.* Aug 2012;55(2):146-149.

2. Xavier AM, Rai K, Hegde AM. Total antioxidant concentrations of breastmilk--an eye-opener to the negligent. *J Health Popul Nutr.* Dec 2011;29(6):605-611.

3. Cossey V, Johansson AB, de Halleux V, Vanhole C. The use of human milk in the neonatal intensive care unit: practices in Belgium and Luxembourg. *Breastfeed Med.* Aug 2012;7:302-306.

4. Labiner-Wolfe J, Fein SB. How US Mothers Store and Handle Their Expressed Breast Milk. *Journal of human lactation : official journal of International Lactation Consultant Association.* Aug 28 2012.

5. ABM. ABM clinical protocol #8: human milk storage information for home use for full-term infants (original protocol March 2004; revision #1 March 2010). *Breastfeed Med.* Jun 2010;5(3):127-130.

6. Jones F. *Best practice for expressing, storing and handling human milk in hospitals, home, and child care settings.* Fort Worth, Texas: HMBANA;2011.

7. Kurath S, Halwachs-Baumann G, Muller W, Resch B. Transmission of cytomegalovirus via breast milk to the prematurely born infant: a systematic review. *Clin Microbiol Infect.* Aug 2010;16(8):1172-1178.

8. Okulu E, Akin IM, Atasay B, Ciftci E, Arsan S, Turmen T. Severe postnatal cytomegalovirus infection with multisystem involvement in an extremely low birth weight infant. *J Perinatol.* Vol 32. United States2012:72-74.

9. Bevot A, Hamprecht K, Krageloh-Mann I, Brosch S, Goelz R, Vollmer B. Long-term outcome in preterm children with human cytomegalovirus infection transmitted via breast milk. *Acta paediatrica.* Apr 2012;101(4):e167-172.

10. Kurath S, Resch B. Cytomegalovirus and transmission via breast milk: how to support breast milk to premature infants and prevent severe infection? *Pediatr Infect Dis J.* Jul 2010;29(7):680-681.

11. Bode L. The role of oligosaccharides in the protection from NEC: data from an original study. ISRHML Conference: Breastfeeding and the use of human milk. Science and practice; 9/27/2012 - 10/1//2012, 2012; Trieste, Italy.

12. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology.* Sep 2012;22(9):1147-1162.

13. Neu J. The prevention of human NEC: current evidence. ISRHML Conference: Breastfeeding and the use of human milk. Science and practice; 9/27/2012 - 10/1//2012, 2012; Trieste, Italy.

14. Neu J, Mihatsch W. Recent developments in necrotizing enterocolitis. *JPEN. Journal of parenteral and enteral nutrition.* Jan 2012;36(1 Suppl):30S-35S.

15. Hassiotou F, Beltran A, Chetwynd E, et al. Breastmilk is a novel source of stem cells with multilineage differentiation potential. *Stem Cells.* Oct 2012;30(10):2164-2174.

16. Cregan MD, Fan Y, Appelbee A, et al. Identification of nestin-positive putative mammary stem cells in human breastmilk. *Cell and tissue research.* Jul 2007;329(1):129-136.

17. Hassiotou F. Review of the current status of stem cells in human milk. ISRHML Conference: Breastfeeding and the use of human milk. Science and practice; 9/27/2012 - 10/1//2012, 2012; Trieste, Italy.