

CMV Transmission and Breastmilk



Research Overview: The Transmission of Cytomegalovirus to Preterm Infants via Breastmilk: Evidence and Issues

Breastmilk - with all of its bioactive, immunological, anti-inflammatory and nutritive components - is generally believed to be the most beneficial form of nourishment for human infants. However, breastmilk is also a mode of cytomegalovirus (CMV) transmission to infants. While term infants infected with CMV via breastmilk rarely exhibit any outward signs of illness, preterm infants infected with CMV can present with a variety of signs and symptoms, some quite serious. How to approach this clinical issue is both complex and controversial.

Women who have had CMV at some point in their lives are seropositive for CMV antibodies. During pregnancy and lactation, CMV may reactivate and be excreted in her milk and other mucosal surfaces. Infants receive CMV antibodies from their mothers during the third trimester of pregnancy such that at the time of birth, they have a form of passive immunity to the virus. Term infants are protected through breastmilk through what is thought to be a natural immunization process.¹ CMV-infected term infants will shed the virus in urine and saliva, but are generally asymptomatic for the infection.

Researchers hypothesize that preterm infants miss the transmission of maternal antibodies to CMV.² Thus, when preterm infants - who are by nature physically immature and vulnerable - acquire CMV postnatally via breastmilk, they are at greater risk than term infants of exhibiting symptoms of the disease.³

The distinctions among asymptomatic infection, symptomatic infection and a CMV sepsis-like syndrome in preterm infants have evolved through clinical studies and case reports. Asymptomatic infection is the most common scenario in term and preterm infants: the infant sheds CMV in urine and saliva but otherwise shows no signs or symptoms of illness. In studies reporting symptomatic infections in preterm infants, infants present with a variety of laboratory and/or clinical conditions. Generally, they recover spontaneously without evidence of long-term consequences.⁴⁻⁶ Of greatest concern to researchers, health care providers and parents is a CMV sepsis-like syndrome evidenced by a very small percent of preterm infants. These cases can clinically be very challenging, however most resolve spontaneously.

Strategies to reduce or prevent CMV transmission to preterm infants have focused on breastmilk treatment such as pasteurization or freezing or simply withholding breastmilk until the infant is more mature. None of these options is ideal: each option prevents the preterm infant from receiving fresh mother's milk during a time of critical growth and development. Theoretically, withholding milk may not protect against CMV exposure from other maternal secretions.

In addition, studies of human milk consistently demonstrate not all components survive heating and freezing intact.^{7,8} For example, live milk cells such as leukocytes and lymphocytes are significantly diminished or damaged by pasteurization and freezing. The emerging science of human milk illuminates promising new discoveries, e.g., live stem cells,^{9,10} antigen-specific T cells and highly-activated memory B lymphatic cells,^{11,12} which, as live cells, are vulnerable to temperature changes. In this context, the risks and benefits of freezing and pasteurizing breastmilk require additional scrutiny.

These articles present current research related to breastmilk-acquired CMV infection in preterm infants.

Through them, we will explore dominant themes in the literature and their implications for clinical practice. Article reprints are included for your review.

- **Transmission of cytomegalovirus via breast milk to the prematurely born infant: a systematic review. Kurath S, Halwachs-Baumann G, Muller W, Resch B. *Clinical Microbiology and Infection*, August 2010;16(8):1172-1178.**
- **Transmission of cytomegalovirus via breast milk in extremely premature infants. Hayashi S, Kimura H, Oshiro M, et al. *Journal of Perinatology*, Dec 16 2010:1-6**
- **Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: a prospective study. Capretti MG, Lanari M, Lazzarotto T, et al. *Journal of Pediatrics*. Jun 2009;154(6):842-848.**

References:

Stagno S, Reynolds DW, Pass RF, Alford CA. Breast milk and the risk of cytomegalovirus infection. *N Engl J Med*. May 8 1980;302(19):1073-1076.

Capretti MG, Lanari M, Lazzarotto T, et al. Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: a prospective study. *J Pediatr*. Jun 2009;154(6):842-848.

Forsgren M. Cytomegalovirus in breast milk: reassessment of pasteurization and freeze-thawing. *Pediatric research*. Oct 2004;56(4):526-528.

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.

Hamprecht K, Maschmann J, Jahn G, Poets CF, Goelz R. Cytomegalovirus transmission to preterm infants during lactation. *J Clin Virol*. Mar 2008;41(3):198-205.

Neuberger P, Hamprecht K, Vochem M, et al. Case-control study of symptoms and neonatal outcome of human milk-transmitted cytomegalovirus infection in premature infants. *J Pediatr*. Mar 2006;148(3):326-331.

Hanna N, Ahmed K, Anwar M, Petrova A, Hiatt M, Hegyi T. Effect of storage on breast milk antioxidant activity. *Arch Dis Child Fetal Neonatal Ed*. Nov 2004;89(6):F518-520.

Young FS, Heicher DA, Uemura HS, Sia CC. The effects of freezing and pasteurization on human milk. *Hawaii Med J*. Nov 1979;38(11):330-332.

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.

Fan Y, Chong YS, Choolani MA, Cregan MD, Chan JK. Unravelling the mystery of stem/progenitor cells in human breast milk. *PLoS One*. 2010;5(12):e14421.

Sabbaj S, Ghosh MK, Edwards BH, et al. Breast milk-derived antigen-specific CD8+ T cells: an extralymphoid effector memory cell population in humans. *Journal of immunology*. Mar 1 2005;174(5):2951-2956.

Schanler RJ. Editorial: CMV acquisition in premature infants fed human milk: reason to worry? *J Perinatol* 2005; 25:297-8.

Tuailon E, Valea D, Becquart P, et al. Human milk-derived B cells: a highly activated switched memory cell population primed to secrete antibodies. *Journal of immunology*. Jun 1 2009;182(11):7155-7162.

Research Overview: The Transmission of Cytomegalovirus to Preterm Infants via Breastmilk: Evidence and Issues

Clinicians and researchers have studied CMV acquisition by preterm infants via breastmilk for more than 40 years. In this collection of materials, you will find summaries with commentary on three current research articles. These materials provide an overview of breastmilk-acquired CMV infection in preterm infants and discuss implications of common management strategies.

Key Points

- CMV is a common virus present in body tissues and sheds in body fluids: saliva, urine, genital secretions, blood, and breastmilk.
- However, if acquired early in pregnancy, initial CMV infection may result in congenital infection.
- Women who have had CMV are seropositive for CMV antibodies. During pregnancy and lactation, CMV can reactivate, causing asymptomatic infection in the mother with viral shedding in her breastmilk, cervical secretions and urine. The terms seropositive, CMV-seropositive, CMV-positive, CMV- IgG-positive are interchangeable in this context.
- Infants can acquire non-congenital CMV infection during the birth process or through breastfeeding if mothers are seropositive.
- Term infants rarely show indications of breastmilk-acquired CMV infection.
- Preterm infants who acquire CMV via breastmilk may become symptomatic of the disease. The most common signs and symptoms are neutropenia, thrombocytopenia, hepatitis, elevated liver enzymes and hepatosplenomegaly.
- A sepsis-like syndrome may occur in preterm infants with breastmilk-acquired CMV. These symptoms may include apnea, bradycardia, distended abdomens and gray pallor.
- Despite a wide range of clinical symptoms, preterm infants recover from breastmilk-acquired CMV spontaneously and without long-term sequelae.
- Pasteurization eliminates CMV in breastmilk and freezing reduces viral load. Both processes affect milk components so are not recommended for usual feeding practices.

Concluding Remarks

The survival very premature infants presents challenges in neonatal care that did not exist forty years ago. The majority of reports of acute and serious CMV illness are clinical cases of extremely low birth weight infants born before 28 weeks gestation. Kurath and colleagues point out it is often difficult to distinguish between complications related to prematurity and complications from CMV infection. The research evidence suggests the actual risk of severe, symptomatic CMV infection is very low, even in very immature, tiny infants. All three studies in this collection, along with many others, support this conclusion.

At the time postnatal CMV came of interest, techniques for milk pasteurization were well established; thus, they were logical interventions for study and practice in response to CMV in breastmilk. Since that time, human milk science has expanded exponentially. Science supports the greater potential benefits from fresh milk compared to frozen or pasteurized milk and that this competes with the previous desires to reduce CMV transmission with heat and cold treatment of milk. This new information, along with current clinical and outcome data on CMV infection in preterm infants, obligates a more comprehensive analysis of the risks and benefits of temperature treatments on human milk. As such, the intentional withholding of fresh breastmilk from preterm infants deserves further attention.

Shifting focus away from breastmilk related interventions, two of the three authors presented in these materials suggested more study of prophylactic immunoglobulin therapy. Not everyone will agree this is the right approach or even necessary. Undoubtedly, additional research is needed before clinicians and researchers come closer to a consensus on the issue of CMV transmission via breastmilk.

Notes

Research Summary

Prepared by Jean Rhodes, PhD, CNM, IBCLC

Transmission of Cytomegalovirus Via Breast Milk to the Prematurely Born Infant: A Systematic Review.

Kurath S, Halwachs-Baumann G, Muller W, Resch B. *Clinical Microbiology and Infection*, August 2010; 16(8): 1172-1178.

Background:

The purpose of this systematic review was to analyze data related to breastmilk-acquired CMV infection in preterm infants, including long-term sequelae of infection.

The authors analyzed 26 prospective studies published between January 1966 and December 2008 of CMV transmission in preterm and/or low birth weight infants. Studies analyzed varied in design, infant inclusion criteria, and laboratory evidence of CMV infection. In some studies, breastmilk was pasteurized or frozen prior to feedings, in others it was given fresh. Four studies were case reports and five studies focused long-term outcomes. The remaining studies were clinical research of CMV transmission in hospitalized preterm infants.

Results:

- In CMV-seropositive mothers who were expressing milk for their preterm infants:
 - A median of 87% had CMV detected in their breast milk.
 - A median of 20% of their infants acquired CMV. The range of infection was 5.7-58.6%.
 - A median of 3.7% of their infants had laboratory and/or clinical indices of infection (mean 9.3%, range 0-34.5%). The most common signs and symptoms were neutropenia, thrombocytopenia, hepatitis, elevated liver enzymes and hepatosplenomegaly.
- Sepsis-like symptoms due to CMV infection occurred in a median of 0.7% of these preterm infants (mean 3.5, range 0-13.8%). Sepsis-like symptoms were not specifically identified.
- The risks of neurosensory or developmental delays were generally low and not significant.

Conclusions

- The risk of CMV acquisition and clinical symptomatology is low.
- When mothers are CMV-seropositive and infants are less than 32 weeks, the authors recommended an individualized clinical approach to breastmilk feedings rather than a generalized approach such as routine pasteurization or freezing.

Addendum

Kurath S, Resch B. Cytomegalovirus and transmission via breast milk: how to support breast milk to premature infants and prevent severe infection? *Pediatric Infectious Disease Journal*, Jul 2010; 29(7): 680-681.

Kurath and Resch published a follow-up letter to the editor in *Pediatric Infectious Disease Journal* in which they proposed a clinical algorithm in cases of CMV-seropositive mothers who wish to provide breastmilk to their preterm infants. The algorithm starts with a stable preterm infant. If parental consent is obtained, fresh breastmilk is given and the infant is tested weekly for CMV. If an infected infant exhibits signs and symptoms of CMV infection, fresh breastmilk feedings are withheld until the infant is greater than 32 weeks.

Commentary

In this systematic review, Kurath *et al* examined the short and long-term outcomes of preterm infants who become infected with CMV via maternal breastmilk. As with most meta-analyses or systematic reviews, studies evaluated varied in methodology, testing procedures, populations and outcomes. The purpose of this review was to combine multiple studies to get a broader perspective on the problem of breastmilk-acquired CMV in preterm infants. As is common in this type of research, statistical results are reported as ranges of occurrence but also as medians and means. When wide variations in ranges occur, median values are usually more accurate indicators of the data as they buffer the effects of outliers or extremes.

This analysis of multiple studies suggests the majority of women of childbearing age are CMV-positive and more than three-quarters of CMV-seropositive women shed the virus in their breastmilk. Of preterm infants receiving breastmilk from CMV-positive mothers, CMV infection occurs at a rate of approximately 20%. A small percent (median rate of 3.7% or mean of 9.3%) of preterm infants of breastfeeding seropositive mothers will develop symptomatic CMV infection. Symptoms vary widely in terms of severity and can include one or more of the following: hepatitis, pneumonia or pneumonitis, neutropenia, thrombocytopenia, elevated liver enzymes, hepatosplenomegaly, gray pallor, fever and hyperbilirubinemia.¹⁻⁴ The most commonly reported single symptom is neutropenia alone without other indications of illness.^{5,6} In studies reporting symptomatic infections in preterm infants, the infants generally recover spontaneously without evidence of long-term consequences.^{1,5,7,8,9}

Of greatest concern to researchers, health care providers and parents is a severe CMV sepsis-like syndrome evidenced by a very small percent of preterm infants. This analysis by Kurath and colleagues suggests a median of less than 1% of preterm infants of CMV-positive mothers will demonstrate symptoms of severe infection. (Of note, Kurath *et al* do not report any deaths in infants with breastmilk-acquired CMV.) In a separate review of multiple clinical and case studies, Hamprecht *et al*⁷ reported very similar results and infant outcomes. Their total sample size was more than 1000 infants. Unlike Kurath *et al*, they identified two infant deaths in one study by Cheong,¹⁰ both deaths in infants with NEC and CMV.

In 1998, Vochem *et al*¹¹ identified in five preterm infants with breastmilk-acquired CMV a pattern of more acute illness now known as sepsis-like symptoms or syndrome. These infants exhibited apnea, bradycardia, distended abdomens and gray pallor. Later studies reported similar and additional clinical findings in infected infants, many of whom were extremely low birth weight.^{1,2,5,10} However, despite the severity of sepsis-like infections, almost all infants recovered and were discharged home.^{1,5}

After publication of their systematic review, Kurath and Resch (both physicians) published a letter to the editor¹² proposing an algorithm for individualized clinical management of CMV in preterm infants. Their

algorithm outlines a management protocol based on clinical and laboratory surveillance. If an infant becomes positive for CMV and is symptomatic, mother's milk is stopped until 32 weeks. But some important questions go unanswered: what symptoms of CMV infection warrant intervention and what are infants missing when fresh breastmilk is withheld?

Hamprecht K, Maschmann J, Jahn G, Poets CF, Goelz R. Cytomegalovirus transmission to preterm infants during lactation. *J Clin Virol.* Mar 2008;41(3):198-205.

Kerrey BT, Morrow A, Geraghty S, Huey N, Sapsford A, Schleiss MR. Breast milk as a source for acquisition of cytomegalovirus (HCMV) in a premature infant with sepsis syndrome: detection by real-time PCR. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* Mar 2006;35(3):313-316.

Schleiss MR. Acquisition of human cytomegalovirus infection in infants via breast milk: natural immunization or cause for concern? *Reviews in medical virology.* Mar-Apr 2006;16(2):73-82.

Yeager AS, Palumbo PE, Malachowski N, Ariagno RL, Stevenson DK. Sequelae of maternally derived cytomegalovirus infections in premature infants. *The Journal of pediatrics.* Jun 1983;102(6):918-922.

Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. *Lancet.* Feb 17 2001;357(9255):513-518.

Maschmann J, Hamprecht K, Dietz K, Jahn G, Speer CP. Cytomegalovirus infection of extremely low-birth weight infants via breast milk. *Clin Infect Dis.* Dec 15 2001;33(12):1998-2003.

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.

Miron D, Brosilow S, Felszer K, et al. Incidence and clinical manifestations of breast milk-acquired Cytomegalovirus infection in low birth weight infants. *J Perinatol.* May 2005;25(5):299-303.

Neuberger P, Hamprecht K, Vochem M, et al. Case-control study of symptoms and neonatal outcome of human milk-transmitted cytomegalovirus infection in premature infants. *J Pediatr.* Mar 2006;148(3):326-331.

Cheong JL, Cowan FM, Modi N. Gastrointestinal manifestations of postnatal cytomegalovirus infection in infants admitted to a neonatal intensive care unit over a five year period. *Archives of disease in childhood. Fetal and neonatal edition.* Jul 2004;89(4):F367-369.

Vochem M, Hamprecht K, Jahn G, Speer CP. Transmission of cytomegalovirus to preterm infants through breast milk. *The Pediatric infectious disease journal.* Jan 1998;17(1):53-58.

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.

Research Summary

Prepared by Jean Rhodes, PhD, CNM, IBCLC

Transmission of Cytomegalovirus Via Breast Milk in Extremely Premature Infants.

Hayashi S, Kimura H, Oshiro M, Kato Y, Yasuda A, Suzuki C, Watanabe Y, Morishima T, and Hayakawa M. *Journal of Perinatology*, June 2011; 31 (6): 440-445.

Background:

The purpose of this study was to evaluate prospectively the rate of CMV infection in extremely preterm infants.

CMV testing included the use of real-time polymerase chain reaction (PCR) assays to identify CMV DNA in breastmilk, serum and urine. This molecular biology technique is the gold standard for CMV detection. Genetic testing was also used to link specific cases of CMV transmission from mother's breastmilk to infant.

Of the 25 mothers enrolled, 21 were CMV-seropositive; 4 were CMV-seronegative.

Twenty-seven infants with birth weights <1000 grams and/or gestational ages <28 weeks were followed. The median gestational age was 26.4 weeks and the median birth weight was 802 grams.

Mothers stayed in the hospital for 5-7 days after delivery. During this time, all infants were given fresh - not frozen - colostrum and milk. After the mothers were discharged, all infants were fed frozen-thawed breast milk for at least one month. If a mother's milk was not available, frozen donor milk was used. Each milk donor was paired with one infant in need of milk.

Breast milk from mothers and donors, infant serum and infant urine samples were collected every 2 weeks and screened for CMV infection using PCR assays.

Results:

- Twenty-two infants received CMV DNA-positive breastmilk.
- Infants of seronegative mothers received only CMV DNA-negative milk, including donor milk.
- 100% of CMV-seropositive mothers had detectable CMV DNA in their breast milk. CMV was not detected in the milk of any seronegative mothers.
- In the first week postpartum, 35% of colostrum samples contained CMV DNA.
- CMV shedding in breastmilk increased at two weeks postpartum, peaked at 4 to 6 weeks, then declined until undetectable by 10-12 weeks.
- One of the 22 CMV exposed infants (4.3%) became CMV-positive. He displayed no clinical symptoms except for a transient elevation of liver enzymes. His mother's milk was verified as the source of CMV by genetic study using CMV UL144 gene.

Conclusions:

- Use of frozen-thawed breastmilk may decrease, but does not eliminate, the rate of breastmilk-acquired CMV infection.

Commentary

Shortly after CMV was first reported in breastmilk in 1967,¹ researchers began publishing reports of breastmilk freezing and pasteurization. In 1982 Friis and Andersen reported freezing breastmilk significantly reduced the virus in breastmilk.² The same year Dworsky *et al*³ investigated the effects of pasteurization and freezing on CMV in breastmilk. In the Dworsky study, CMV was destroyed completely by Holder pasteurization (heat treatment at 62°C for 30 minutes) but only reduced by freezing. The authors expressed concerns, just as important today as it was then, about the effects of temperature treatment on the immunological properties of human milk.

Breastmilk pasteurization and freezing studies increased in the literature in response to reports of preterm infants with breastmilk-acquired CMV infection. In an attempt to preserve the beneficial properties in human milk, Hamprecht *et al* compared the effects of Holder pasteurization, short-term pasteurization (5 seconds at 72°C) and freezing at -20°C on CMV-positive breastmilk.⁴ Both methods of pasteurization destroyed the CMV, but breastmilk enzymes were also significantly reduced. The authors recommended more study to find the pasteurization temperature at which CMV could be inactivated and breastmilk preserved. Study continues on pasteurization⁵ but as Hayashi points out, it is not always practical in a clinical setting.

However, freezing breastmilk is common practice in neonatal intensive care units. Breastmilk is often stored in the freezer for later use or per protocol to reduce CMV transmission. Like Hayashi *et al*, other researchers have found lower rates of breastmilk-acquired CMV transmission in infants fed primarily frozen breastmilk. In Taiwan - where 95% of mothers are seropositive for CMV and all breastmilk is frozen before use - Jim *et al*⁶ found a 15% CMV transmission rate by seropositive mothers to their preterm infants. In Japan - another country where breastmilk is routinely frozen - Yasuda⁷ reported 10% CMV transmission via breastmilk with no infant exhibiting clinical symptoms.

While freezing breastmilk seems to reduce CMV transmission, its effects are not entirely benign. The notion that “freezing breastmilk preserves the biochemical and immunologic quality of the milk...”(p. 529)⁴ is often taken out of context and repeated. However, this assertion is not consistent with current evidence regarding important breastmilk components and properties. For example, freezing breastmilk kills or significantly reduces cellular components, including macrophages and lymphocytes.⁸ Freezing also reduces antioxidants⁹ and immunoglobulins IgG, IgM and IgA.⁸ This information along with recent breastmilk discoveries such as stem cells^{10,11} and the specific immunologic actions of breastmilk lymphoid T and B cells^{12,13} should be included in discussions about freezing, pasteurizing or withholding breastmilk for preterm infants.

Diosi P, Babusceac L, Nevinglovschi O, Kun-Stoicu G. Cytomegalovirus infection associated with pregnancy. *Lancet*. Nov 18 1967;2(7525):1063-1066.

Friis H, Andersen HK. Rate of inactivation of cytomegalovirus in raw banked milk during storage at -20 degrees C and pasteurisation. *Br Med J (Clin Res Ed)*. Dec 4 1982;285(6355):1604-1605.

Dworsky M, Stagno S, Pass RF, Cassady G, Alford C. Persistence of cytomegalovirus in human milk after storage. *The Journal of pediatrics*. Sep 1982;101(3):440-443.

Hamprecht K, Maschmann J, Muller D, et al. Cytomegalovirus (CMV) inactivation in breast milk: reassessment of pasteurization and freeze-thawing. *Pediatric research*. Oct 2004;56(4):529-535.

Czank C, Prime DK, Hartmann B, Simmer K, Hartmann PE. Retention of the immunological proteins of pasteurized human milk in relation to pasteurizer design and practice. *Pediatric research*. Oct 2009;66(4):374-379.

Jim WT, Shu CH, Chiu NC, et al. Transmission of cytomegalovirus from mothers to preterm infants by breast milk. *The Pediatric infectious disease journal*. Sep 2004;23(9):848-851.

Yasuda A, Kimura H, Hayakawa M, et al. Evaluation of cytomegalovirus infections transmitted via breast milk in preterm infants with a real-time polymerase chain reaction assay. *Pediatrics*. Jun 2003;111(6 Pt 1):1333-1336.

Young FS, Heicher DA, Uemura HS, Sia CC. The effects of freezing and pasteurization on human milk. *Hawaii Med J*. Nov 1979;38(11):330-332.

Hanna N, Ahmed K, Anwar M, Petrova A, Hiatt M, Hegyi T. Effect of storage on breast milk antioxidant activity. *Arch Dis Child Fetal Neonatal Ed*. Nov 2004;89(6):F518-520.

Fan Y, Chong YS, Choolani MA, Cregan MD, Chan JK. Unravelling the mystery of stem/progenitor cells in human breast milk. *PLoS One*. 2010;5(12):e14421.

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.

Tuaille E, Valea D, Becquart P, et al. Human milk-derived B cells: a highly activated switched memory cell population primed to secrete antibodies. *Journal of immunology*. Jun 1 2009;182(11):7155-7162.

Sabbaj S, Ghosh MK, Edwards BH, et al. Breast milk-derived antigen-specific CD8+ T cells: an extralymphoid effector memory cell population in humans. *Journal of immunology*. Mar 1 2005;174(5):2951-2956.

Research Summary

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Very Low Birth Weight Infants Born to Cytomegalovirus-seropositive Mothers Fed with Their Mother's Milk: a Prospective Study.

Capretti MG, Lanari M, Lazzarotto T, et al. *Journal of Pediatrics*, June 2009; 154(6):842-848.

Background

The purpose of this observational, prospective study was to evaluate the risk of CMV transmission in very low birth weight (VLBW) infants fed fresh breastmilk. The sample consisted of 68 mothers and their 80 infants. All infants were ≤ 32 weeks and <1500 grams with a mean gestational age of 29.0 weeks and a mean birth weight of 1125 grams.

All infants received only fresh mother's milk for at least the first month of life. No donor milk was used. Mothers were tested for CMV status, their breastmilk and infants' urine were tested weekly for CMV. CMV identified in milk and urine was genotyped to confirm CMV transmission from mother to infant.

Per the neonatal unit's policy for infants < 28 weeks, 19 infants received three doses of prophylactic IgM-enriched intravenous immunoglobulin (IVIGMA), beginning at birth. All 19 of these infants had CMV-positive mothers.

Results:

- Fifty-three of 68 mothers (78%) were CMV-seropositive. Of these 53 seropositive mothers, 21(40%) had CMV detected in their milk by viral culture.
- No infants who received CMV negative milk became CMV infected.
- CMV transmission occurred in 9 of 26 infants (35%) fed CMV-positive milk. One infant had no signs or symptoms of illness. Five infants had neutropenia and/or elevated bilirubin levels but no sepsis-like symptoms. Three infants (11.5%) had mild sepsis-like illness. All CMV infected infants recovered.
- There was no difference between infected and non-infected infants in morbidity, length of stay or outcomes at discharge.
- Of the 19 infants < 28 weeks treated at birth with IVIGMA, 1 developed CMV infection.

Conclusions:

- Breastmilk-acquired CMV infection was asymptomatic, self-limited and without sequelae in stable VLBW infants who did not have chronic illness. However, CMV infection could worsen the clinical course of pre-existing illnesses such as chronic lung disease.
- Administration of IVIGMA to infants <28 weeks may have reduced risk of CMV transmission via breastmilk.
- The authors concluded, "...the potential benefits of fresh human milk seem to outweigh the risks related to CMV transmission in most VLBW infants" p.847.

Commentary

This study by Capretti *et al* supports the use of fresh breastmilk for preterm infants from CMV-positive mothers. Study infants acquired CMV infection from their mothers at rates comparable to those in other research reports and the disease course was generally mild: all infected infants had positive outcomes with no neurosensory deficits at two years. Miron *et al*¹ in 2005 reported similar outcomes in a study of 70 preterm infants fed fresh breastmilk from sero-positive mothers. These authors reported a 5.7% infection rate with all infants recovering.

Unlike Miron *et al*, the study by Capretti and associates included an additional variable - immunoglobulin therapy. The neonatal unit's treatment policy for infants less than 28 weeks included IVIGMA therapy at birth. IVIGMA contained variable titers of anti-CMV antibodies. Nineteen study infants less than 28 weeks received IVIGMA; only one developed CMV. In comparison, three of five infants less than 28 weeks who did not receive IVIGMA became infected with CMV. Capretti *et al* hypothesized the administration of IVIGMA may have helped the very preterm infants compensate for the lack of maternal antibodies they would have received in utero if they had delivered at term.

This is not the first mention in the literature of immunoglobulin therapy to prevent breastmilk CMV transmission: a thin thread of this idea runs through the literature from beginning to end. As early as 1983, Yeager *et al*² recommended the administration of CMV immunoglobulin to preterm infants if the connection between the lack of maternal antibodies and CMV infection could be confirmed. In a later report, Mosca and associates³ used intravenous immunoglobulins in preterm infants less than 34 weeks receiving CMV-positive breastmilk. In their study, five of 20 exposed infants were CMV infected, but none had any clinical signs or consequences of infection. Lastly, in their 2010 letter presented in a previous summary, Kurath and Resch concluded, "passive immunization with either HCMV monoclonal antibodies or immune globulins might be a case of debate for high-risk low birth weight infants"⁴ (p.680). Clearly, more research is needed on this topic.

Capretti *et al* concluded that the benefits of giving fresh breastmilk outweigh the risks of CMV infection in most preterm infants. For the past 40 years, when interventions for breastmilk-acquired CMV were proposed, they have centered on treating or withholding breastmilk. The discussion of immunoglobulin therapy could shift the focus from treating breastmilk to treating the infant. The more we understand about breastmilk-acquired CMV and breastmilk itself, the closer we come to a comprehensive appreciation of all the relevant issues and options.

Miron D, Brosilow S, Felszer K, et al. Incidence and clinical manifestations of breast milk-acquired Cytomegalovirus infection in low birth weight infants. *J Perinatol.* May 2005;25(5):299-303.

Yeager AS, Palumbo PE, Malachowski N, Ariagno RL, Stevenson DK. Sequelae of maternally derived cytomegalovirus infections in premature infants. *The Journal of pediatrics.* Jun 1983;102(6):918-922.

Mosca F, Pagni L, Barbi M, Binda S. Transmission of cytomegalovirus. *Lancet.* Jun 2 2001;357(9270):1800.

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.

Notes

Additional Cytomegalovirus Resources

1. Alford C. Breast milk transmission of cytomegalovirus (CMV) infection. *Adv Exp Med Biol.* 1991;310:293-299.
2. Ballard RA, Drew WL, Hufnagle KG, Riedel PA. Acquired cytomegalovirus infection in preterm infants. *American journal of diseases of children.* May 1979;133(5):482-485.
3. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988-2004. *Clin Infect Dis.* Jun 1 2010;50(11):1439-1447.
4. Bhatia P, Narang A, Minz RW. Neonatal cytomegalovirus infection: diagnostic modalities available for early disease detection. *Indian J Pediatr.* Jan 2010;77(1):77-79.
5. Bryant P, Morley C, Garland S, Curtis N. Cytomegalovirus transmission from breast milk in premature babies: does it matter? *Archives of disease in childhood. Fetal and neonatal edition.* Sep 2002;87(2):F75-77.
6. Buxmann H, Falk M, Goelz R, Hamprecht K, Poets CF, Schloesser RL. Feeding of very low birth weight infants born to HCMV-seropositive mothers in Germany, Austria and Switzerland. *Acta Paediatr.* Dec 2010;99(12):1819-1823.
7. Buxmann H, Miljak A, Fischer D, Rabenau HF, Doerr HW, Schloesser RL. Incidence and clinical outcome of cytomegalovirus transmission via breast milk in preterm infants \leq 31 weeks. *Acta paediatrica.* Feb 2009;98(2):270-276.
8. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol.* Jul 2010;20(4):202-213.
9. Capretti MG, Lanari M, Lazzarotto T, et al. Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: a prospective study. *J Pediatr.* Jun 2009;154(6):842-848.
10. Cheong JL, Cowan FM, Modi N. Gastrointestinal manifestations of postnatal cytomegalovirus infection in infants admitted to a neonatal intensive care unit over a five year period. *Archives of disease in childhood. Fetal and neonatal edition.* Jul 2004;89(4):F367-369.
11. Chiavarini M, Bragetti P, Sensini A, et al. Breastfeeding and transmission of cytomegalovirus to preterm infants. Case report and kinetic of CMV-DNA in breast milk. *Ital J Pediatr.* 2011;37:6.
12. Curtis N, Chau L, Garland S, Tabrizi S, Alexander R, Morley CJ. Cytomegalovirus remains viable in naturally infected breast milk despite being frozen for 10 days. *Archives of disease in childhood. Fetal and neonatal edition.* Nov 2005;90(6):F529-530.
13. de Cates CR, Gray J, Robertson NR, Walker J. Acquisition of cytomegalovirus infection by premature neonates. *J Infect.* Jan 1994;28(1):25-30.
14. Diosi P, Babusceac L, Nevinglovschi O, Kun-Stoicu G. Cytomegalovirus infection associated with pregnancy. *Lancet.* Nov 18 1967;2(7525):1063-1066.
15. Doctor S, Friedman S, Dunn MS, et al. Cytomegalovirus transmission to extremely low-birthweight infants through breast milk. *Acta paediatrica.* Jan 2005;94(1):53-58.
16. Dworsky M, Stagno S, Pass RF, Cassady G, Alford C. Persistence of cytomegalovirus in human milk after storage. *The Journal of pediatrics.* Sep 1982;101(3):440-443.
17. Dworsky M, Yow M, Stagno S, Pass RF, Alford C. Cytomegalovirus infection of breast milk and transmission in infancy. *Pediatrics.* Sep 1983;72(3):295-299.
18. Embil JA, Macdonald JM, Scott KE. Survey of a neonatal population for the prevalence of cytomegalovirus. *Scand J Infect Dis.* 1975;7(3):165-167.
19. Fischer C, Meylan P, Bickle Graz M, et al. Severe postnatally acquired cytomegalovirus infection presenting with colitis, pneumonitis and sepsis-like syndrome in an extremely low birthweight infant. *Neonatology.* Jun 2010;97(4):339-345.
20. Forsgren M. Cytomegalovirus in breast milk: reassessment of pasteurization and freeze-thawing. *Pediatric research.* Oct 2004;56(4):526-528.
21. Friis H, Andersen HK. Rate of inactivation of cytomegalovirus in raw banked milk during storage at -20 degrees C and pasteurisation. *Br Med J (Clin Res Ed).* Dec 4 1982;285(6355):1604-1605.
22. Gessler P, Bischoff GA, Wiegand D, Essers B, Bossart W. Cytomegalovirus-associated necrotizing enterocolitis in a preterm twin after breastfeeding. *Journal of perinatology : official journal of the California Perinatal Association.* Feb 2004;24(2):124-126.
23. Goelz R, Hamprecht K. Breastfeeding and HCMV-infection in the preterm infant. *Gesundheitswesen.* Mar 2008;70 Suppl 1:S22-24.
24. Goelz R, Hihn E, Hamprecht K, et al. Effects of different CMV-heat-inactivation-methods on growth factors in human breast milk. *Pediatric research.* Apr 2009;65(4):458-461.
25. Granstrom M, Leinikki P, Santavuori P, Pettay O. Perinatal cytomegalovirus infection in man. *Arch Dis Child.* May 1977;52(5):354-359.
26. Hamele M, Flanagan R, Loomis CA, Stevens T, Fairchok MP. Severe morbidity and mortality with breast milk associated cytomegalovirus infection. *Pediatr Infect Dis J.* Jan 2010;29(1):84-86.
27. Hamprecht K, Goelz R, Maschmann J. Breast milk and cytomegalovirus infection in preterm infants. *Early human development.* Dec 2005;81(12):989-996.

28. Hamprecht K, Maschmann J, Jahn G, Poets CF, Goelz R. Cytomegalovirus transmission to preterm infants during lactation. *J Clin Virol.* Mar 2008;41(3):198-205.
29. Hamprecht K, Maschmann J, Muller D, et al. Cytomegalovirus (CMV) inactivation in breast milk: reassessment of pasteurization and freeze-thawing. *Pediatric research.* Oct 2004;56(4):529-535.
30. Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. *Lancet.* Feb 17 2001;357(9255):513-518.
31. Hamprecht K, Vochem M, Baumeister A, Boniek M, Speer CP, Jahn G. Detection of cytomegaloviral DNA in human milk cells and cell free milk whey by nested PCR. *J Virol Methods.* Feb 1998;70(2):167-176.
32. Hamprecht K, Witzel S, Maschmann J, et al. Rapid detection and quantification of cell free cytomegalovirus by a high-speed centrifugation-based microculture assay: comparison to longitudinally analyzed viral DNA load and pp67 late transcript during lactation. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* Dec 2003;28(3):303-316.
33. Hamprecht K, Witzel S, Maschmann J, Speer CP, Jahn G. Transmission of cytomegalovirus infection through breast milk in term and preterm infants. The role of cell free milk whey and milk cells. *Advances in experimental medicine and biology.* 2000;478:231-239.
34. Hayashi S, Kimura H, Oshiro M, et al. Transmission of cytomegalovirus via breast milk in extremely premature infants. *Journal of perinatology : official journal of the California Perinatal Association.* Dec 16 2010.
35. Hayes K, Danks DM, Gibas H, Jack I. Cytomegalovirus in human milk. *The New England journal of medicine.* Jul 27 1972;287(4):177-178.
36. Hsu ML, Cheng SN, Huang CF, et al. Perinatal cytomegalovirus infection complicated with pneumonitis and adrenalitis in a premature infant. *J Microbiol Immunol Infect.* Dec 2001;34(4):297-300.
37. Iwanaga M, Zaitso M, Ishii E, et al. Protein-losing gastroenteropathy and retinitis associated with cytomegalovirus infection in an immunocompetent infant: a case report. *Eur J Pediatr.* Feb 2004;163(2):81-84.
38. Jim WT, Shu CH, Chiu NC, et al. High cytomegalovirus load and prolonged virus excretion in breast milk increase risk for viral acquisition by very low birth weight infants. *The Pediatric infectious disease journal.* Oct 2009;28(10):891-894.
39. Jim WT, Shu CH, Chiu NC, et al. Transmission of cytomegalovirus from mothers to preterm infants by breast milk. *The Pediatric infectious disease journal.* Sep 2004;23(9):848-851.
40. Johnson SJ, Hosford-Dunn H, Paryani S, Yeager A, Malachowski N. Prevalence of sensorineural hearing loss in premature and sick term infants with perinatally acquired cytomegalovirus infection. *Ear Hear.* Oct 1986;7(5):325-327.
41. Kerrey BT, Morrow A, Geraghty S, Huey N, Sapsford A, Schleiss MR. Breast milk as a source for acquisition of cytomegalovirus (HCMV) in a premature infant with sepsis syndrome: detection by real-time PCR. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* Mar 2006;35(3):313-316.
42. 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.
43. 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.
44. Lambeth TM. Cytomegalovirus infection. Evaluation and management in neonates. *Adv Nurse Pract.* Feb 2006;14(2):43-45.
45. Lanari M, Capretti MG, Lazzarotto T, et al. Cytomegalovirus infection via mother's milk: could distinct virus strains determine different disease patterns in preterm twins? *New Microbiol.* Jan 2008;31(1):131-135.
46. Lawrence RM. Cytomegalovirus in human breast milk: risk to the premature infant. *Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine.* Summer 2006;1(2):99-107.
47. Lawrence RM, Lawrence RA. Given the benefits of breastfeeding, what contraindications exist? *Pediatr Clin North Am.* Feb 2001;48(1):235-251.
48. Lee HC, Enright A, Benitz WE, Madan A. Postnatal cytomegalovirus infection from frozen breast milk in preterm, low birth weight infants. *The Pediatric infectious disease journal.* Mar 2007;26(3):276.
49. Luck S, Sharland M. Postnatal cytomegalovirus: innocent bystander or hidden problem? *Arch Dis Child Fetal Neonatal Ed.* Jan 2009;94(1):F58-64.
50. Marshall BC, Koch WC. Antivirals for cytomegalovirus infection in neonates and infants: focus on pharmacokinetics, formulations, dosing, and adverse events. *Paediatr Drugs.* 2009;11(5):309-321.
51. Maruyama K, Koizumi T, Hirato J. Cytomegalovirus infections associated hemophagocytic lymphohistiocytosis in a premature infant. *Pediatr Int.* Dec 2006;48(6):648-650.
52. Maschmann J, Hamprecht K, Dietz K, Jahn G, Speer CP. Cytomegalovirus infection of extremely low-birth weight infants via breast milk. *Clin Infect Dis.* Dec 15 2001;33(12):1998-2003.

53. Maschmann J, Hamprecht K, Weissbrich B, Dietz K, Jahn G, Speer CP. Freeze-thawing of breast milk does not prevent cytomegalovirus transmission to a preterm infant. *Archives of disease in childhood. Fetal and neonatal edition.* Jul 2006;91(4):F288-290.
54. Meier J, Lienicke U, Tschirch E, Kruger DH, Wauer RR, Prosch S. Human cytomegalovirus reactivation during lactation and mother-to-child transmission in preterm infants. *J Clin Microbiol.* Mar 2005;43(3):1318-1324.
55. Minamishima I, Ueda K, Minematsu T, et al. Role of breast milk in acquisition of cytomegalovirus infection. *Microbiol Immunol.* 1994;38(7):549-552.
56. Miron D, Brosilow S, Felszer K, et al. Incidence and clinical manifestations of breast milk-acquired Cytomegalovirus infection in low birth weight infants. *J Perinatol.* May 2005;25(5):299-303.
57. Miura CS, Miura E, Mombach AB, Chesky M. The prevalence of congenital cytomegalovirus infection in newborn infants at an intensive care unit in a public hospital. *J Pediatr (Rio J).* Jan-Feb 2006;82(1):46-50.
58. Morgan MA, el-Ghany el SM, Khalifa NA, Sherif A, Rasslan LR. Prevalence of cytomegalovirus (CMV) infection among neonatal intensive care unit (NICU) and healthcare workers. *Egypt J Immunol.* 2003;10(2):1-8.
59. Mosca F, Pugni L, Barbi M, Binda S. Transmission of cytomegalovirus. *Lancet.* Jun 2 2001;357(9270):1800.
60. Mussi-Pinhata MM, Yamamoto AY, do Carmo Rego MA, Pinto PC, da Motta MS, Calixto C. Perinatal or early-postnatal cytomegalovirus infection in preterm infants under 34 weeks gestation born to CMV-seropositive mothers within a high-seroprevalence population. *The Journal of pediatrics.* Nov 2004;145(5):685-688.
61. Neuberger P, Hamprecht K, Vochem M, et al. Case-control study of symptoms and neonatal outcome of human milk-transmitted cytomegalovirus infection in premature infants. *J Pediatr.* Mar 2006;148(3):326-331.
62. Numazaki K. Human cytomegalovirus infection of breast milk. *FEMS Immunol Med Microbiol.* Jun 1997;18(2):91-98.
63. Numazaki K, Chiba S, Asanuma H. Transmission of cytomegalovirus. *Lancet.* Jun 2 2001;357(9270):1799-1800.
64. Omarsdottir S, Casper C, Akerman A, Polberger S, Vanpee M. Breastmilk handling routines for preterm infants in Sweden: a national cross-sectional study. *Breastfeed Med.* Sep 2008;3(3):165-170.
65. Omarsdottir S, Casper C, Zwegyberg Wirgart B, Grillner L, Vanpee M. Transmission of cytomegalovirus to extremely preterm infants through breast milk. *Acta paediatrica.* Apr 2007;96(4):492-494.
66. Paryani SG, Yeager AS, Hosford-Dunn H, et al. Sequelae of acquired cytomegalovirus infection in premature and sick term infants. *The Journal of pediatrics.* Sep 1985;107(3):451-456.
67. Peckham CS, Johnson C, Ades A, Pearl K, Chin KS. Early acquisition of cytomegalovirus infection. *Arch Dis Child.* Aug 1987;62(8):780-785.
68. Perlman JM, Argyle C. Lethal cytomegalovirus infection in preterm infants: clinical, radiological, and neuropathological findings. *Ann Neurol.* Jan 1992;31(1):64-68.
69. Prosch S, Lienicke U, Priemer C, et al. Human adenovirus and human cytomegalovirus infections in preterm newborns: no association with bronchopulmonary dysplasia. *Pediatr Res.* Aug 2002;52(2):219-224.
70. Rawls WE, Wong CL, Blajchman M, et al. Neonatal cytomegalovirus infections: the relative role of neonatal blood transfusion and maternal exposure. *Clin Invest Med.* 1984;7(1):13-19.
71. Reyes C, Pereira S, Warden MJ, Sills J. Cytomegalovirus enteritis in a premature infant. *J Pediatr Surg.* Nov 1997;32(11):1545-1547.
72. Reynolds DW, Stagno S, Hosty TS, Tiller M, Alford CA, Jr. Maternal cytomegalovirus excretion and perinatal infection. *The New England journal of medicine.* Jul 5 1973;289(1):1-5.
73. Richter D, Hampl W, Pohlandt F. Vertical transmission of cytomegalovirus, most probably by breast milk, to an infant with Wiskott-Aldrich syndrome with fatal outcome. *Eur J Pediatr.* Nov 1997;156(11):854-855.
74. Rosenthal LS, Fowler KB, Boppana SB, et al. Cytomegalovirus shedding and delayed sensorineural hearing loss: results from longitudinal follow-up of children with congenital infection. *Pediatr Infect Dis J.* Jun 2009;28(6):515-520.
75. Schanler RJ. CMV acquisition in premature infants fed human milk: reason to worry? *J Perinatol.* May 2005;25(5):297-298.
76. Schleiss MR. Role of breast milk in acquisition of cytomegalovirus infection: recent advances. *Current opinion in pediatrics.* Feb 2006;18(1):48-52.
77. Schleiss MR. Acquisition of human cytomegalovirus infection in infants via breast milk: natural immunization or cause for concern? *Reviews in medical virology.* Mar-Apr 2006;16(2):73-82.
78. Schleiss MR, McVoy MA. Overview of congenitally and perinatally acquired cytomegalovirus infections: recent advances in antiviral therapy. *Expert Rev Anti Infect Ther.* Jun 2004;2(3):389-403.
79. Sharland M, Khare M, Bedford-Russell A. Prevention of postnatal cytomegalovirus infection in preterm infants. *Archives of disease in childhood. Fetal and neonatal edition.* Mar 2002;86(2):F140.

80. Srinivasjois RM, Kava MP, Thomas A, Rao SC. Cytomegalovirus-associated ileal stricture in a preterm neonate. *J Paediatr Child Health*. Jan 2008;44(1-2):80-82.
81. Stagno S, Pass RF, Dworsky ME, et al. Congenital cytomegalovirus infection: The relative importance of primary and recurrent maternal infection. *The New England journal of medicine*. Apr 22 1982;306(16):945-949.
82. Stagno S, Reynolds DW, Pass RF, Alford CA. Breast milk and the risk of cytomegalovirus infection. *N Engl J Med*. May 8 1980;302(19):1073-1076.
83. Stranska R, Schuurman R, Toet M, Verboon-Macielek M, de Vries LS, van Loon AM. Application of UL144 molecular typing to determine epidemiology of cytomegalovirus infections in preterm infants. *J Clin Microbiol*. Mar 2006;44(3):1108-1110.
84. Stronati M, Lombardi G, Di Comite A, Fanos V. Breastfeeding and cytomegalovirus infections. *J Chemother*. Oct 2007;19 Suppl 2:49-51.
85. Takahashi R, Tagawa M, Sanjo M, et al. Severe postnatal cytomegalovirus infection in a very premature infant. *Neonatology*. 2007;92(4):236-239.
86. Tegtmeier GE. The use of cytomegalovirus-screened blood in neonates. *Transfusion*. May-Jun 1988;28(3):201-203.
87. Terry NE, Fowler CL. Cytomegalovirus enterocolitis complicated by perforated appendicitis in a premature infant. *J Pediatr Surg*. Aug 2006;41(8):1476-1478.
88. van der Strate BW, Harmsen MC, Schafer P, et al. Viral load in breast milk correlates with transmission of human cytomegalovirus to preterm neonates, but lactoferrin concentrations do not. *Clin Diagn Lab Immunol*. Jul 2001;8(4):818-821.
89. Vochem M, Hamprecht K, Jahn G, Speer CP. Transmission of cytomegalovirus to preterm infants through breast milk. *The Pediatric infectious disease journal*. Jan 1998;17(1):53-58.
90. Vollmer B, Seibold-Weiger K, Schmitz-Salue C, et al. Postnatally acquired cytomegalovirus infection via breast milk: effects on hearing and development in preterm infants. *The Pediatric infectious disease journal*. Apr 2004;23(4):322-327.
91. Welsh JK, Arsenakis M, Coelen RJ, May JT. Effect of antiviral lipids, heat, and freezing on the activity of viruses in human milk. *The Journal of infectious diseases*. Sep 1979;140(3):322-328.
92. Welsh JK, May JT. Anti-infective properties of breast milk. *The Journal of pediatrics*. Jan 1979;94(1):1-9.
93. Willeitner A. Transmission of cytomegalovirus (CMV) through human milk: are new breastfeeding policies required for preterm infants? *Adv Exp Med Biol*. 2004;554:489-494.
94. Wu J, Tang ZY, Wu YX, Li WR. Acquired cytomegalovirus infection of breast milk in infancy. *Chin Med J (Engl)*. Feb 1989;102(2):124-128.
95. Yasuda A, Kimura H, Hayakawa M, et al. Evaluation of cytomegalovirus infections transmitted via breast milk in preterm infants with a real-time polymerase chain reaction assay. *Pediatrics*. Jun 2003;111(6 Pt 1):1333-1336.
96. Yeager AS. Transmission of cytomegalovirus to mothers by infected infants: another reason to prevent transfusion-acquired infections. *Pediatr Infect Dis*. Jul-Aug 1983;2(4):295-297.
97. Yeager AS, Palumbo PE, Malachowski N, Ariagno RL, Stevenson DK. Sequelae of maternally derived cytomegalovirus infections in premature infants. *The Journal of pediatrics*. Jun 1983;102(6):918-922.
98. Young FS, Heicher DA, Uemura HS, Sia CC. The effects of freezing and pasteurization on human milk. *Hawaii Med J*. Nov 1979;38(11):330-332.
99. Yow MD, White NH, Taber LH, et al. Acquisition of cytomegalovirus infection from birth to 10 years: a longitudinal serologic study. *J Pediatr*. Jan 1987;110(1):37-42.



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