EVIDENCE SUMMARIES OF INDIVIDUAL REPORTS IDENTIFIED THROUGH A SYSTEMATIC REVIEW OF HIV-FREE SURVIVAL BY INFANT FEEDING PRACTICES FROM BIRTH TO 18 – 24 MONTHS

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Contents

Systematic review of HIV-free survival by infant feeding practices from birth to 18 -24 months

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DESCRIPTION OF STUDIES

After screening 1323 citations, 68 potentially relevant studies were identified. After reviewing the 68 complete articles for the studies we determined that 52 papers (seventeen randomized clinical trials, seventeen observational prospective cohort studies, and 18 secondary papers that reported outcomes relevant to this review) met the inclusion criteria for this review (Figure 1 and 2).

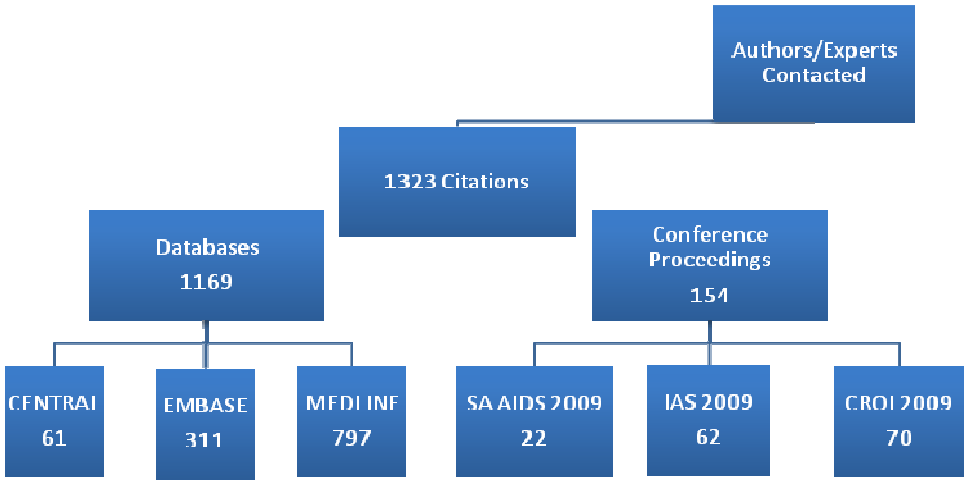

Figure 1: Electronic databases and conferences searched

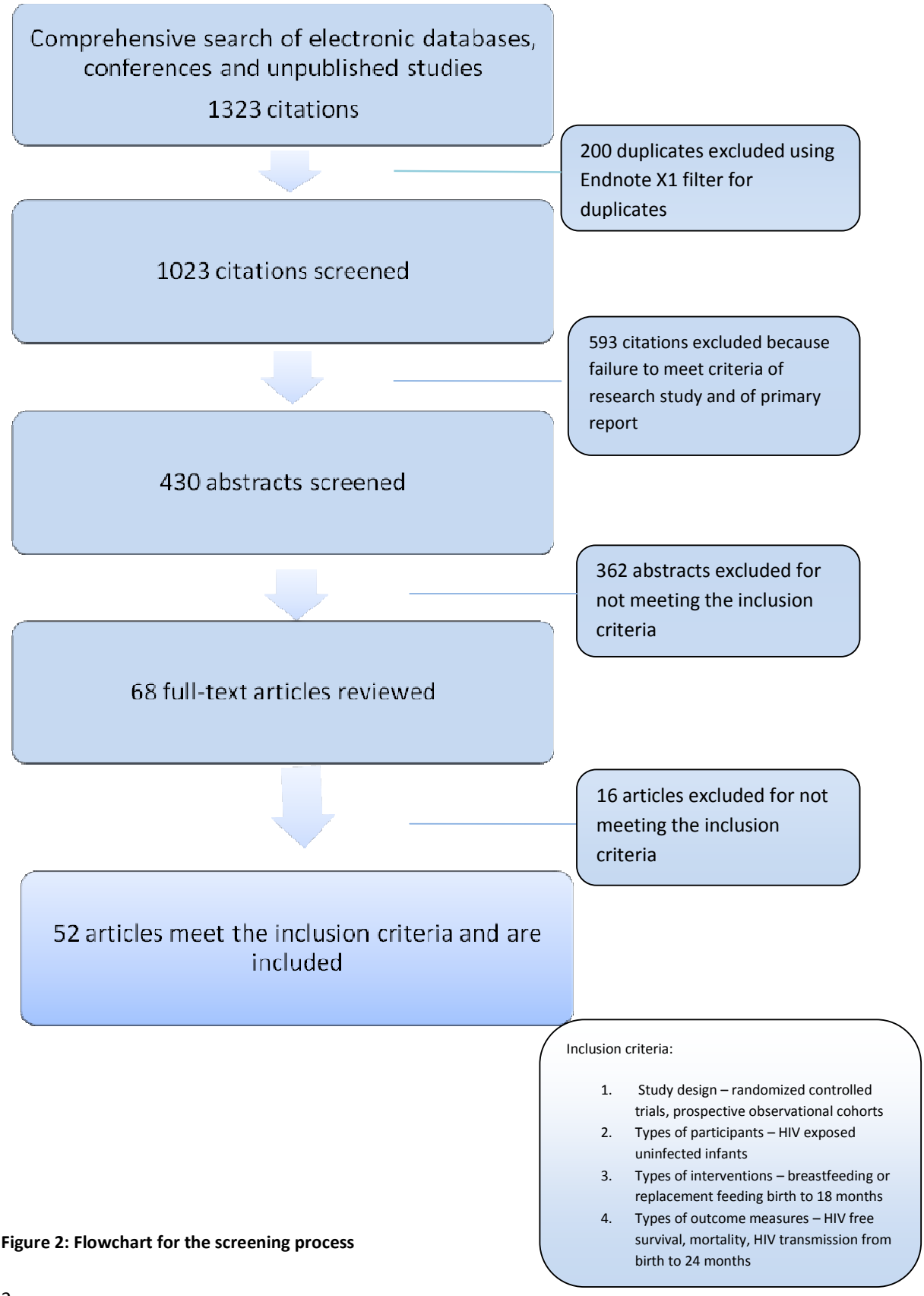

Figure 2: Flowchart for the screening process

CHARACTERISTICS OF INCLUDED STUDIES

RANDOMIZED CONTROLLED TRIALS

ZEBS

Kuhn 2008 (1)

Methods	**Study design**: randomized controlled trial comparing exclusive breastfeeding for 4 months with abrupt weaning to standard practice of continued breastfeeding for a longer period.(1) **Duration of enrolment:** May 2001 to September 2004 **Study setting:** two antenatal clinics in Lusaka, Zambia **Follow-up:** Infants were followed up at birth and 1 week and then at 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21 and 24 months. Observed length of follow-up: 24 months Lost to follow-up: 190/1435 (13.2%) women lost to follow-up before pregnancy outcome was ascertained 160/1163 (13.8%) infants lost to follow-up before randomization 48/481 (10%) of infants in the intervention group lost to follow-up before 4 months 19/477 (4.0%) of infants in the control group were lost to follow-up before 4 months 31/326 (9.5%) of infants in the intervention group were lost to follow-up between 4 and 24 months 34/353 (9.6%) of infants in the control group were lost to follow-up between 4 and 24 months. **Definition of feeding practice:** "Exclusive breastfeeding for the first 6 months" – not further defined by the study Breastfeeding duration was defined as the time from birth until the exact age that breastfeeding cessation was first reported. **Kuhn 2007 (2)** Non-exclusive breastfeeding (non-EBF) was defined as non-breast milk substances (including water) other than prescribed medicines given within the past 24 hours or at least once per week in the past week or since the last visit. **HIV testing:** Infants were tested for HIV-1 DNA infection by heel prick sample using the polymerase chain reaction (PCR).Positive results were confirmed in two or more samples, if available. Confirmation by retesting was conducted if only one blood

	sample was available. Amplification of the beta-globin gene was performed to rule out false negative test results due to an inadequate sample. Blood samples were tested in the United States as infant diagnostic facilities were not available in Zambia at the time the study was conducted. Maternal blood samples were tested at enrolment for CD4 and CD8 cell counts (FACSCount, BD Biosciences). **Analysis:** Child deaths, HIV-free survival, HIV transmission, and breastfeeding duration were treated as time-to-event variables and were analyzed with Kaplan-Meier methods and log-rank tests. HIV transmission was determined as the midpoint between the last negative and the first positive PCR test. Data was censored at the time of the last negative test in order to determine an analysis of death among uninfected children. An intention to treat analysis was carried out. **Kuhn 2007(2)** Non-EBF was treated as a time dependent covariate altered from EBF to non-EBF irreversibly the first instance anything other than breast milk was reported on the structured questionnaire. Age-specific hazard rates of postnatal transmission were investigated using actuarial life tables. Event time was censored at the age (in days) when all breastfeed ceased plus 42 days or at the time of the first positive test. Event time was calculated in months by dividing by 30.417.
Participants	**Sample size:** The total number of participants enrolled was 3216 women and 3276 infants. Infants were randomized at birth. 3106 of 3276 infants assigned an intervention were included in the primary analysis. 2389 of 3106 infants reached the HIV infection endpoint or were HIV-uninfected and were under follow-up or completed follow-up. **Eligibility:** HIV-infected pregnant women < 38 weeks gestation were recruited from antenatal clinics that offered voluntary HIV testing and counselling and single-dose nevirapine prophylaxis. Women could volunteer if they intended to breastfeed for any length of time, accepted treatment with nevirapine and agreed to be randomly assigned to the intervention or control group. **Exclusions:** women with severe pregnancy complications (eg pre-eclampsia), previous caesarean delivery, and HIV-related conditions requiring hospitalization. **Baseline characteristics:** The median CD4 cell count was 325 cells/mm^3 and 332 cells/mm^3 in the intervention and control group respectively. The median maternal plasma viral load was approximately 36,000 - 41,000 copies/ml. Two-thirds of participants in the intervention and control group were not eligible for antiretroviral therapy. Mean age of participants in the intervention and control group was 26.1 and 26.2 respectively. Almost eighty-four percent of participants in the intervention group and 85.3% in the control group were married. Approximately half of the women in the control and intervention group were not schooled or had only primary school education.

Interventions	Participants were randomly assigned to an intervention and control group. In the intervention group women were encouraged to exclusively breastfeed for 4 months and then to wean abruptly, or as quickly as possible. Women were counselled on abrupt cessation of breastfeeding from the 2-month session. In addition, a 3-month supply of infant formula and fortified weaning cereal was provided. Extensive counselling was provided to make replacement feeding as safe as possible. Women in the control group were encouraged to breastfeed exclusively up to 6 months, then gradually introduce complementary feeds, and continue breastfeeding for as long as the women chose (standard practice). Women in the control group were not provided with food. **Breastfeeding duration:** in the intervention group 69.0% of women stopped breastfeeding at the end of 5 months– 68.8% within 2 days, 25.1% within 2-7 days, and 6.1% in >7 days In the control group 7.4% of women ceased breastfeeding at the end of 5 months and 34.2% by the end of 12 months. Median duration of breastfeeding: intervention group 4 months (interquartile range [IQR] 4-14); control group 16 months (IQR 11 -19) (P<0.001)
Outcomes	Outcomes assessed were HIV-free survival and child mortality. In the study population there was no significant difference between the intervention and control groups in terms of HIV-free survival at 24 months according to intention-to-treat analysis (68.4% and 64.0% respectively, P=0.13). Similar child mortality rates were seen between the intervention and control group with 23.9% in the intervention group and 24.6% in the control group (P=0.96). In a subgroup analysis of uninfected infants who were still breastfed at 4 months, there was no significant difference in HIV-free survival at 24 months between the intervention and control group (83.9% and 80.9% respectively; P=0.27). From 4 to 24 months, the postnatal HIV transmission rate was similar in both groups (6.2% in the intervention group and 8.8% in the control group, P=0.19). Furthermore, the mortality rate among uninfected children was not significantly different between the intervention and control group (10.7% and 11.7% respectively, P=0.71) during the same period. **Morbidity and mortality** Diarrhoea associated morbidity and mortality was assessed in 593 HIV-uninfected singleton infants who were alive and still breastfeeding at 4 months. Data was collected on any episodes of diarrhoea since the last visit based on maternal report, and any hospitalizations or deaths related to diarrhoea were recorded. Intention-to-treat analyses based on actual feeding practices were carried out using regression methods. From birth to 4 months diarrhoea occurred in <10% of infants increasing to 10.3% at 4.5 months and 40.4% at 9 months. The risk of diarrhoea was 1.8 times

	(95% CI, 1.3 – 2.4) greater in the intervention group between 4 to 6 months. Based on actual practice, infants who were weaned had a 3 times (95% CI, 2.3 – 4.0) greater risk of diarrhoea between 4 to 6 months. At 4 to 6 months, infants who were weaned and infants who were not exclusively breastfed had a 3.5 and 2.5 times greater risk of diarrhoea respectively (95% CI, 2.6 – 4.7 and 95% CI, 1.7 – 3.8) compared to exclusively breastfed infants. During the first 6 months of life, infants who were weaned were also more likely to be hospitalized for or die from illness related to diarrhoea. The associations remained significant after adjusting for confounders. **Kuhn 2007(2)** **HIV transmission hazard rates per breastfeeding month in the control group (n=365)** 5-8 months: 0.00995 (12/365) (95% CI, 0.0043 – 0.0155) 9 – 12 months: 0.01237 (12/365) (95% CI, 0.0053 – 0.0194) 13 – 16 months: 0.0039 (3/365) (95% CI, 0 – 0.083) 17 – 20 months: 0.0066 (3/365) (95% CI, 0 – 0.0140) 21 – 24 months: 0.0074 (1/365) (95% CI, 0 – 0.0218) **Fawzy 2009 International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention (abstract)(3)** Diarrhoea associated morbidity and mortality was assessed in 593 singletons who were HIV-uninfected, alive and still breastfeeding at 4 months. Diarrhoeal episodes since the last visit were assessed by maternal report and diarrhoeal-related deaths and hospitalization were determined. Intent to treat analyses was conducted. The abstract does not report how the rates were calculated and censoring events. There was < 10% of diarrhoea in the first 4 months, increased thereafter from 10.3% at 4.5 months to 40.4% at 9 months, and then declining to 23,2% at 24 months. The risk of diarrhoea was 1.8 times greater (95% CI, 1.3 – 2.4) between 4-6 months in the intervention group compared to the control group. Based on actual practice, infants who were weaned had a 3 times greater risk (95% CI, 2.3 – 4.0) of diarrhoea between 4 to 6 months. When compared to exclusively breastfed infants, infants who were weaned and infants who were non-exclusively breastfed had a 3.5 times (95% CI, 2.6 – 4.7) and 2.5 times greater risk (95% CI, 1.7 – 3.8) of diarrhoea between 4 to 6 months respectively. Children who were weaned were 4.9 times (95% CI, 1.4 – 17.4) more likely to be hospitalized for or die from diarrhoeal illness during the first 6 months of life. The associations persisted after adjusting for confounders.

Notes	At the time of the study initiation, PMTCT programmes were beginning to be established. Hence, only 6 women started antiretroviral therapy before delivery and 107 women before 24 months (55 in the intervention arm and 52 in the control arm). Censoring of maternal and infant follow-up once therapy was initiated showed no significant differences in the HIV-free survival rate (84.5% in the intervention group and 80.9% in the control group, P=0.20) Kuhn 2009 (4)

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Yes	Use of computer algorithm with a randomized permuted-block design within each site
Allocation concealment?	No	"Participants were informed of their assignment at the next visit to ensure sufficient time for preparation"
Blinding?	No	The study was not blinded
Incomplete outcome data addressed?	Yes	"Home visit teams tracked the participants who did not return for appointments. Information about children's deaths was sought from the hospital and clinic records and from interviews with caretakers and health care personnel. The circumstances of all deaths were reviewed to identify the causes of death."
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

MASHI

Thior 2006 (5)

Methods	**Study design** 2 x 2 factorial randomized controlled trial Setting: District hospitals in the southern region of Botswana in 1 city, 1 town and 2 large villages Duration of enrolment: 27 March 2001 - 29 October 2003 **Follow-up:** Infants were followed up at birth, 1, 4, 7, 9, 12, and 18 months Observed length of follow-up: 18 months Loss to follow-up: 7 months and 18 months Formula fed and 1 month zidovudine (formula fed) - 16 (2.7%) infants at 7 months and 53 (9%) infants at 18 months were lost to follow-up. Breastfed and 6 months zidovudine (breastfed plus zidovudine) - 25 (4.3%) infants at

	7 months and 53 (9.0%) infants at 18 months were lost to follow-up. **HIV testing** Infants were tested at birth, 1, 4, 7, 9, and 12 months with DNA polymerase chain reaction (PCR) (Amplicor HIV-1 test; Roche Diagnostic Systems), and at 18 months by ELISA (Ortho-AB Capture, Ortho Clinical Diagnostics; Murex HIV 2.0, Abbott-Murex). **Analysis** Infant HIV-1 infection rates were compared between birth and 1 month using Fisher's exact test. The Kaplan-Meier method was used to estimate infection rates beyond 1 month of age. Risk factors were determined by using Cox proportional hazards modelling. Censoring for HIV infection was death of an infant. For HIV-free survival censoring was the time of event of HIV infection or death, whichever occurred first. Censoring of infants lost to follow-up was at their last negative HIV test and for infant mortality was the time of the last study visit. To decrease reverse causality, time-varying events that occurred during the month before the at-risk time estimation were excluded. Intrapartum or early breastfeeding transmission was defined as occurring in infants who had a negative DNA PCR at birth (≤ 15 days after birth) and their first positive DNA PCR test result at the 1 month visit (obtained ≤ 45 days after birth). Late breastfeeding MTCT was defined as occurring in infants who had a negative DNA PCR result at the 1 month visit and a positive test at any point thereafter.
Participants	HIV-infected pregnant women attending antenatal clinics were eligible for the study. The number of women who were enrolled in the study was 1200. **Eligibility:** pregnant women between 33 and 35 weeks of gestation, having a positive HIV-1 enzyme linked immunoabsorbent assay (ELISA) on 2 separate samples, age 18 years or older, having levels of haemoglobin ≥ 80g/l, absolute neutrophil count of ≥1000 cells/mm, alanine aminotransferase and aspartate aminotransferase at ≤10 times the upper limit of normal, and creatinine ≤ 1.5mg/dl (132.6µmol/l), and not having known intolerance to zidovudine or nevirapine. **Sample size:** There were 591live first-born infants in the formula-fed group and 588 infants in the breastfed plus zidovudine group. **Baseline characteristics:** The median maternal age was 26.78. Sixty-eight per cent of mothers had secondary level education and were comparable in both groups. Fifty-five per cent of women had a tap in the yard, 74% had a private latrine/house, 24% had electricity, and 30% had a refrigerator in the home. The median baseline maternal CD4 count was 366cells/$mm^3$and median plasma viral load 4.35 log10 copies/ml. The median birth weight of the infants was 3.1kg. The baseline characteristics were not significantly different between the two groups (P>0.05) except for sanitation facilities.

Interventions	Infants were randomized to one of two groups, either to receive breastfeeding with six months of infant zidovudine prophylaxis (breastfed plus zidovudine), or to receive formula feeding with one month of infant zidovudine prophylaxis (formula fed). All infants received infant zidovudine syrup (4mg/kg/12 hours) from birth until one month of age. The infant zidovudine was discontinued in all infants in the formula-fed group. In the breastfed plus zidovudine group zidovudine prophylaxis continued from 1 to 2 months of age at 4mg/kg/8hours and from 2 to 6 months of age at a dose of 6mg/kg/8 hours while breastfeeding continued. **Maternal ARV**: Zidovudine 300mg orally 12 hourly was administered to all mothers from 34 weeks gestation and during labour. Mothers and infants were randomized to receive wither single-dose nevirapine or placebo during labour in part 1 of the perinatal intervention. During the initial study era, infants were randomized to receive either single dose NVP or placebo based on their mothers' assignment. After 17 months the study design was modified with all infants subsequently receiving single dose nevirapine. **Counselling on feeding modality:** Mothers were instructed to start and complete weaning between 5 to 6 months of age. Free infant formula was provided from 5 to 12 months of age to allow for safe weaning. Mothers randomized to the formula-fed group received free formula for 12 months. All mothers were counselled about how to prepare formula safely and were administered high-protein foods for infants from 6 to 12 months of age.
Outcomes	**Cumulative HIV infection or death rate from birth** **Overall at 7 months:** formula fed 12.5% (73/530), breastfed plus zidovudine 12.9% (74/543) (P=0.86; 95% CI for the difference, --4.2 – 3.5%) **Overall at 18 months:** formula fed 13.9%% (80/493), breastfed plus zidovudine 15.1% (86/483) (P=0.60; 95% CI for the difference, --5.3 – 2.9%) **Cumulative HIV infection rate from birth** Overall at 7 months: formula fed 5.6% (32/526), breastfed plus zidovudine 9% (51/541) (P=0.04; 95% CI for the difference, -6.4% to -0.4%) 18 months: formula fed 6.0% (33/493), breastfed plus zidovudine 9.5% (53/481) (P=0.02; 95% CI for the difference, -6.7% to -0.5%) Excluding infants HIV-free at 1 month: estimated cumulative proportion of HIV infection between 1 and 7 months was 0.6% in the formula fed group and 4.5% in the breastfed group (confidence intervals were not presented).

	Cumulative infant mortality rate from birth Overall at 7 months: formula-fed 9.3% (54/559), breastfed plus zidovudine group 4.9% (28/575) (95% CI for the difference 1.5 – 7.4) (P=0.003). Overall at 18 months: formula-fed 10.7% (62/512), breastfed plus zidovudine group 8.5% (48/529) (P=0.21, 95% CI for the difference, -1.2 – 5.6%) The difference between the groups decreased beyond 7 months of age such that the time-to-mortality distribution at 18 months was not significantly different (P=0.21) The results of the trial showed that breastfeeding plus zidovudine was associated with lower rate of mortality at 7 months but both interventions had similar HIV-free survival at 18 months. **Infant mortality:** The total number of infant deaths after birth was 114, 63 of 591 (10.7%) from the formula-fed group compared to 51 of 588 (8.7%) from the breastfed plus zidovudine group. **Infant morbidity:** 7 months: the rate of grade 3 or higher signs or symptoms in the formula-fed group (17.6%), breastfed plus zidovudine group (13.1%), (P=0.03). Hospitalization formula-fed group (20.3%), breastfed plus zidovudine group (15.6%) (P=0.04). **Shapiro 2009(6)** **Analysis** Beyond 1 month of age, Kaplan-Meier methods of analysis were used to calculate infection rates. **Risk of mother to child transmission by age interval and mother-to-child transmission** 4-6 months: breastfed plus zidovudine 6/547 (1.1%); formula fed 1/537 (0.18%) 7-24 months: breastfed plus zidovudine 3/547 (0.5%); formula fed 0/537 (0%) Total (1-24 months): breastfed plus zidovudine 24/547 (4.4%), formula fed 2/537 (0.4%) **Risk factors for MTCT of HIV-1 via breastfeeding after > 1 month of breastfeeding (women who did not transmit HIV versus women who transmitted HIV)** Electricity in the home, %: 23 versus 8 (adjusted HR 0.14, 95% CI, 0.02-1.02;

	P=0.05) Refrigerator in the home, %: 31 versus 25 (unadjusted HR 1.26, 95% CI, 0.50 – 3.21; P = 0.62) Median maternal baseline CD4 count, cells/mm^3 : 376 versus 225 (adjusted HR 0.88; 95% CI 0.77 – 1.01; P=0.06) Exclusive breastfeeding during the first 5 months, %: 22 versus 13 (unadjusted HR 0,74, 95% CI, 0.27 – 2.00, P=0.55) Non-formula liquid during the first 5 months %: 70 versus 88 (unadjusted HR 2.70, 95% CI,0.92 – 7.94; P=0.07) Received solids through 5 months, %: 53 versus 63 (unadjusted HR 1.01, 95% CI, 0.42 – 2.41; P=0.98) Infant pneumonia through 6 months, %: 8 versus 21 (unadjusted HR 1.64, 95% CI, 0.38 – 7.19; P=0,51) Infant diarrhoea through 6 months, %: 31 versus 33 (unadjusted HR 0.53, 95% CI, 0.15 – 1.90; P = 0.33) Infant anaemia through 6 months, %: 24 versus 46 (unadjusted HR 1.43, 95% CI, 0.59 – 3.45; P = 0.43)
Notes	Era 1: infants randomized to receive single-dose nevirapine or placebo on the basis of their mothers' assignment. After 17 months (era 2) all infants received single-dose nevirapine

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Yes	Randomization to mode of feeding was generated as follows: "Centralized randomization to both part 1 and part 2 groups occurred at study enrolment (34 weeks' gestation), using permuted blocks of size 8 within each site."
Allocation concealment?	Unclear	Allocation concealment was probably not carried out.
Blinding?	No	The study was not blinded
Incomplete outcome data addressed?	No	Tracing of participants lost to follow-up not addressed
Free of selective reporting?	Unclear	Some post-randomization selection bias reported
Free of other bias?	Yes	No problems apparent

Nduati 2000 (7)

Methods	**Study design:** randomized controlled trial **Duration of enrolment:** November 1992 to July 1998 **Setting:** antenatal clinics in Nairobi, Kenya **Blinding**: Patient: No Provider: physician investigators were blinded to outcome data during the study **Definition of feeding practice:** Not clear. Exclusive breastfeeding defined as no intake other than breast milk. "policy recommendations for breastfeeding by HIV-infected women issued by WHO or UNAIDS were discussed" **Follow-up:** Planned length of follow-up: 24 months Observed length of follow-up: median time for both groups 24 months (P=0.88) Lost to follow-up: 68/401 (17%) infants lost to follow-up before 2 years **Analysis** Intention-to-treat analysis: carried out Kaplan-Meier techniques for analysis of survival were not carried as the data violated 2 assumptions. Instead, a method for estimating joint distribution of death and HIV infection, which takes into account informative censoring, interval censoring and imperfect diagnostic test specificity at birth was developed to approximate timing of transmission and HIV-1free survival. Censoring rules regarding HIV rate and survival: in a standard Kaplan-Meier analysis, infants who tested HIV-1 negative several months before death would be censored at time of last negative HIV-1 test. The study assumed a strong correlation between death and HIV-infection status therefore death status provided information about infection status. Thus censoring was informative in this study.
Participants	**Sample size**: Number of women: 2315 HIV-1 seropositive, of whom 425 randomized Number of infants: 401 infants **Inclusion criteria:** HIV-1 seropositive pregnant women who were residents of Nairobi, Kenya and had access to municipal water **Maternal and infant antiretroviral therapy**: none administered

	Baseline characteristics between breastfeeding group and formula feeding group Median maternal age: 23 years in both groups (P=0.79) Married: 158/212 (75%); 167/213 (78%) (P=0.35) No of years education: 8 (0-17); 8 (0-16) (P=0.62) No with refrigerators: 9/212 (4%); 12/213 (6%) (P=0.51) Absolute CD4 count (x 10^6 /l): 399; 415 (P=0.35) When CD4 count was obtained: 32 weeks Median plasma viral load (copies/ml): 42360 (range, 112-2 483 750) Median birth weight (kg) of infants: 3.2 in both groups (P=0.35) Feeding practice: Number breastfed from birth 197 Number formula fed from birth 204
Interventions	There were 212 women assigned to breastfeed and 213 women assigned to the formula feeding group. 401 mother-infant pairs included in the analysis; 197 assigned to the breastfeeding group, 204 assigned to formula feeding group Median duration of breastfeeding: 17 months (range, < 1weeks to > 24 months) Duration of breastfeeding: 189 women complied with breastfeeding. 95% breastfed until 3 months of age 90% breastfed until 6 months of age 80% at 12 months 47% at 18 months 23% at 24 months Women counselled: in pregnancy and after delivery on safe preparation of formula feeds. Women randomized to the formula group were given free dried formula milk.
Outcomes	**HIV-free survival** 80/197 (40.6%) in the breastfeeding group were dead or infected with HIV by 24 months of age 58/204 (28.4%) in the formula feeding group were dead or infected with HIV by 24 months of age The percentage of infants who were dead or HIV-infected was significantly greater in the breastfeeding arm compared to the formula arm (P=0.02) **Cumulative probability HIV infection** 6 months: breastfeeding group 28.0% (95% CI, 21.7-34.2%) (n=175) formula feeding group 15.9% (95% CI, 9.6-22.2%)(n=169) (P=0.009) 12 months: breastfeeding group 32.2 (95% CI, 25.6 – 39.0%) (n=165)

	formula feeding group 18.2% (95% CI, 11.9 - 25.5%) (n=161)(P=0.003) 24 months: breastfeeding group 36.7% (95% CI, 29.4-44.0%) (n=142) formula feeding group 20.5% (95% CI 14.0-27.0%) (n=128) (P=0.001) **Mortality** 84/401 (20.9%) infants died in the study 45/197 (22.8%) in the breastfeeding group 39/204 (19.1%) in the formula feeding group **Cumulative mortality rate** 6 months: breastfeeding group 8.8% (95% CI, 4.8 – 12.7%) (n=175) formula feeding group 10.8% (95% CI, 6.6 – 15.1%)(n=180) (P=0.48) 12 months: breastfeeding group 16.7% (95% CI, 11.4 – 22.0%) (n=151) formula feeding group 15.4% (95% CI, 10.4 – 20.4%) (n=162) (P=0.71) 24 months: breastfeeding group 24.4% (95 CI, 18.2-30.7%) (n=114) formula feeding group 20.0% (95% CI 14.4-25.6%) (n=121) (P=0.30) Cumulative mortality rate HIV uninfected infants (Mbori-Ngacha 2001) 24 months: breastfeeding group 8.1%, formula feeding group 10% (P=0.59) Cumulative mortality rate HIV-infected infants Mbori-Ngacha 2001) 24 months: breastfeeding group 46.0%, formula feeding group 40.2% (P=0.41) **Morbidity (Mbori-Ngacha 2001)(8)** 371/401 live-born, singleton or first-born twins infants were included in the analysis of morbidity and mortality Incidence of diarrhoea (history of diarrhoea since the last visit) The incidence of diarrhoea increased with age and peaked at 9 to 12 months (~225 per 100 person-years in the breastfeeding group versus ~210 per 100 person-years in the formula feeding group). The incidence of diarrhoea was similar in the formula and breastfeeding group over the 24 month follow-up. 24 months: Breastfeeding group: 149 per 100 person years Formula feeding group: 155 per 100 person years (HR, 0.9, 95% CI, 0.7-1.1) Incidence of diarrhoea in HIV-uninfected children

	Breastfeeding group: 140 per 100 person-years Formula feeding group: 150 per 100 person-years (HR, 0.9, 95% CI 0.7-1.2, P=0.43) Incidence of diarrhoea in HIV-infected children Breastfeeding group: 247 per 100 person-years Formula feeding group: 241 per 100 person-years (HR, 1.1, 95% CI, 0.7-1.9,P=0.73) Incidence of pneumonia during the first 24 months Breastfeeding group: 62 per 100 person-years Formula feeding group: 62 per 100 person-years (HR, 0.9, 95% CI, 0.7-1.3 ,P=0.74) Incidence of pneumonia HIV-infected children Breastfeeding group: 150 per 100 person-years Formula feeding group: 188 per 100 person-years (HR, 1.2, 95% CI,0.8-1.9 ,P=0.33) Incidence of pneumonia HIV-uninfected children Breastfeeding group: 45 per 100 person-years Formula feeding group: 50 per 100 person-years (HR, 0.9, 95% CI,0.6-1.4 ,P=0.73) Nutritional status Overall and in the first 6 months of life, children in the breastfeeding group had a better nutritional status (P=0.6 and 0.003 respectively). After adjusting for HIV status, overall and in the first 6 months children in the breastfeeding arm had better nutritional outcomes than children in the formula feeding arm (P=0.4 and 0.002 respectively. Among HIV-infected children malnutrition occurred 29% in the formula group versus 14% in the breastfeeding group (P=0.12). Among HIV-uninfected children, malnutrition occurred in 7% of children who were breastfed compared to 11% of children who were formula fed (P=0.19)
Notes	

Item	Judgement	Description
Adequate sequence generation?	Yes	Computer generated block randomization at 32 weeks
Allocation concealment?	No	Not applicable

Blinding?	No	Study physicians were blinded to outcome data to ensure masking of interim results
Incomplete outcome data addressed?	Yes	"A nurse visited women in their homes...following a failed clinic appointment. Women who had left Nairobi were traced to their rural homes."
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Unclear	Non-adherence to assigned intervention with only 142/204 (70%) of women in the formula group (P<0.001) complying with the intervention. "Because more than one quarter of women in the formula arm admitted to non-compliance with feeding modality, our estimated breast milk transmission rate is an underestimate."

KESHO BORA

De Vincenzi 2009 (9)

Methods	**Study design:** randomized controlled trial comparing triple-antiretroviral (ARV) prophylaxis during pregnancy and breastfeeding to short-ARV prophylaxis to prevent mother-to-child transmission of HIV-1 (MTCT) **Duration of enrolment:** June 2005 to August 2008 **Setting:** antenatal clinics in Burkina Faso, Kenya and South Africa **Blinding**: Patient: no Provider: were masked to study outcomes **Definition of feeding practice** Feeding practices were defined according to WHO/UNICEF guidelines as follows: Exclusive breastfeeding for 5½ months followed by weaning over two weeks Replacement feeding (not defined fully in the abstract) **HIV testing** The method of HIV testing of mother-infant pairs was not reported in the abstract. **Follow-up:** Planned length of follow-up: 18 months (available in June 2010) Observed length of follow-up: 12 months Cumulative 12 month follow-up in the triple arm was 92.2% and in the short arm 90.5%

	Analysis Cumulative HIV infections and infant deaths were estimated with Kaplan-Meier analyses. Log rank test at 12 months stratified by study centre and intention to breastfeed.
Participants	**Sample size**: There were 882 women eligible for enrolment. Of these 824 participated in the study – 413 in the triple antiretroviral (ARV) arm and 411 in the short arm. Number of infants: 402 live born infants were randomized to the triple arm and 403 to the short arm. **Eligibility:** HIV-infected pregnant women with CD4 count between 200 and 500 cells/$mm^{3,}$ **Exclusions:** CD4 < 200 or CD4 >500 (these women were part of an observational cohort in Kenya and Burkino Faso), haemoglobin, co morbid illness, neutrophil, and alanine transaminase **Baseline characteristics** were similar between the two groups Median maternal age: 27.4 in both groups Primigravida, %: Triple arm 18.1 , short arm 17.8 At least primary education, %: Triple arm 85.7, short arm 84.4 Employed, %: Triple arm 32.7, short arm 27.7% Married or regular partner, %: Triple arm 95.2, short arm 97.1 Mean CD4 count (cells/mm^3) at enrolment: 335 in both groups ARVs > 6 weeks before delivery, %: Triple arm 56.4, short arm 62.3 Caesarean section, %: Triple arm 11.3, short arm 12.3 Male infants, %: Triple arm 50.5, short arm 47.4 Infants delivered < 37 weeks, %: Triple arm 13.4, short arm 10.9 Birth weight < 2500g, %: Triple arm 11.2, short arm 7.7 Ever breastfed, %: Triple arm 76.4, short arm 78.2 Mean duration of breastfeeding (weeks): 21.4 in both arms Exclusive breastfeeding from birth to 3 months: Triple arm 47.5%, short arm 45.6%
Interventions	Intervention: Triple therapy comprised of zidovudine (ZDV), lamivudine (3TC), and ritonavir boosted lopinavir (LPV/r) was administered to the mother at 28-26 weeks pregnancy to a maximum of 6 months post partum. Control: women were prescribed ZDV from 28 to 36 weeks gestation until labour, and then given ZDV, 3TC and single-dose Nevirapine (sd-NVP) at the onset of labour with a postnatal tail of ZDV and 3TC until one week postpartum.

	Infants ARVs: all infants were given sd-NVP within 72 hours and 1 week of ZDV post delivery. Women counselled: in pregnancy and after delivery about safe replacement feeding practice and exclusive breastfeeding for 5½ months with weaning for 2 weeks according to WHO/UNICEF guidelines. Women were allowed to choose method of feeding and supported in their choice.
Outcomes	**Cumulative HIV infection rate – ever breastfed- all infants – triple versus short arm** 6 months: 5.0% (15) ; 8.8% (27) 12 months: 5.9% (17); 10.2% (30) (P=0.0064) **Cumulative HIV infection rates– all infants – triple versus short arm** 6 months: 4.9 (19) (95% CI, 3.1 – 7.5); 8.5 (33) (95% CI, 6.1 – 11.1) 12 months: 5.5(21) (95% CI, 3.6 – 8.4); 9.5 (36) (95% CI, 6.9 – 13.0) (P=0.039) Risk reduction at 12 months 42% in the triple arm compared to the short arm **Kaplan-Meier cumulative deaths– all infants – triple versus short arm (as reported on poster)** 6 months: 4.6 (18) (95% CI, 2.9 – 7.2); 5.9 (23) (95% CI, 3.9 – 8.7) 12 months: 6.3 (24) (95% CI, 4.3 – 9.3); 10 (37) (95% CI, 7.3 – 13.6) (P= 0.086) Risk reduction at 12 months 37% in the triple arm **Kaplan-Meier cumulative HIV infections or deaths – all infants – triple versus short arm (as reported on poster)** 6 months: 8.3 (33) (95% CI, 6.0 – 11.5); 12.6 (50) (95% CI, 9.7 – 16.3) 12 months: 10.4 (40) (95% CI, 7.7 – 13.9); 16.3 (62) (95% CI, 12.9 – 20.5) (P= 0.022) Risk reduction at 12 months 36% in the triple arm Subgroup analysis of HIV infection rates based on maternal CD4 count between 200 to 350 cells/mm^3 showed a cumulative HIV infection rate of 5.5% (12) and 6.1% (13) in the triple arm compared to 10.5%(23) and 11.1% (24) in the short arm at 6 months and 12 months respectively (P=0.044).
Notes	Not an intent-to- treat analysis, no information regarding formula feeding group

Item	Judgement	Description
Adequate sequence generation?	Yes	Computer generated randomization was by drug regimen not by feeding practice
Allocation concealment?	No	Not applicable to feeding practice
Blinding?	No	Study physicians were masked to interim outcome status of participants
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	Unclear	Abstract only, full paper awaited
Free of other bias?	Yes	No problems apparent

BAN

Chasela 2009 (10) International AIDS Society Conference (abstract)

Methods	**Study design:** randomized controlled trial 2x 3 factorial design **Duration of enrolment:** March 2004 to February 2009 **Setting:** Lilongwe, an urban setting in Malawi, **Definition of feeding practice:** Exclusive breastfeeding for 24 weeks followed by 4 weeks of weaning. Exclusive breastfeeding is not defined in the study abstract. **Follow-up:** Observed length of follow-up: 28 weeks Lost to follow-up: not reported in the abstract **Analysis** Analysis was conducted using Kaplan-Meier methods to calculate the cumulative risk of HIV infection or death at 28 weeks among infants who were free of HIV-1 one week after birth. Rates were compared using the log-rank test, stratified by nutritional supplement. Turnbull methods will be used to adjust the estimate for interval censored data.
Participants	**Sample size**: There were 3752 women who were screened for enrolment. 2790 women were followed up at delivery, of which 2367 women were randomized. **Eligibility:** HIV-1 infected antiretroviral naive, pregnant women, with CD 4 >250 cells/mm^3 and haemoglobin >7g/dl, who elected to breastfeed their infants, and infants with birth weight >2000g were eligible to enrol in the study. **Baseline characteristics** were similar between the two groups

	Median maternal age: MHAART 26; INVP 25; control 26 (P=0.7) BMI <17, %: MHAART 71; INVP 14; control 36 (P=0.21) Median CD4 count (cells/mm ^{3}): MHAART 428; INVP 440; control 442 (P=0,16) Mean birth weight of infants; g: MHAART 2998; INVP 3009; control 3008 (P=0.83)
Interventions	**Intervention:** all women and infants received single dose Nevirapine (sd-NVP) and 7 days of zidovudine (ZDV) and lamivudine (3TC). The 2 intervention groups were the maternal HAART group and the infant NVP group. Each intervention group was compared to the control group. **Maternal HAART (MHAART)** 851 mother-infant pairs were randomized to receive maternal HAART (ZDV, 3TC, and NVP). In February 2005, NVP was changed to nelfinavir (NFV) due to evidence related to the toxicity of NVP in women with CD4 > 250 cells/mm^3. In addition, (NFV) was changed to ritonavir-boosted lopinavir (LPV/r) in January 2006. Maternal HAART was initiated in breastfeeding HIV-1 infected women within 1 week of delivery and continued through 28 weeks. Interventions will be continued until breastfeeding cessation, but not longer than 28 weeks. **Infant nevirapine (INVP):** 848 infants received NVP syrup 2mg/kg through 28 weeks of age. **Control:** 668 mother-infant pairs were randomized to an "enhanced" control arm consisting of ZDV+3TC+sd-NVP intrapartum and 1 week of ZDV and 3TC postnatally. Duration of breastfeeding: all women were counselled to exclusively breastfeed their infants for 24 weeks followed by weaning over 4 weeks. (The abstract does not state how mother-infant pairs were handled if breastfeeding was continued beyond 28 weeks). **Nutritional intervention:** Mothers who had a haemoglobin >7g/dl were randomized to receive either high calorie micronutrient fortified nutritional supplement or no supplement.
Outcomes	**Risk of HIV transmission** 28 weeks: control 6.4%; MHAART 3.0% (P=0.003); INVP 1.8% (P<0.0001) MHAART versus INVP (P = 0.1203) **Risk of HIV transmission or death** 28 weeks: control arm 7.6%; MHAART 4.7% (P= 0.03); INVP 2.9% (P<0.0001) MHAART versus INVP (P = 0.0698) Denominators not reported in the abstract.
Notes	In March 2008, DSMB closed recruitment to the control arm due to inferior efficacy of

	ARVs in this group. The sample size in the control group is smaller than the two intervention groups.

Item	Judgement	Description
Adequate sequence generation?	Yes	Permuted block randomization
Allocation concealment?	Unclear	"The group size will vary randomly between 6, 12 and 18 in order to prevent discovery of the allocation scheme by study researchers"
Blinding?	Yes	Laboratory staff did not have knowledge of intervention or control status of the participants
Incomplete outcome data addressed?	Yes	"In anticipation of dropout, a slightly larger than necessary study sample will be randomized..It will be necessary to examine carefully the apparent causes of unexpected missing data and patient dropout. If indicated auxiliary analyses designed for non-ignorable missing data will be performed to gauge implications."
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

MmaBana

Shapiro 2009 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention (abstract) (11)

Methods	**Study design:** randomized controlled trial comparing highly active antiretroviral therapy regimens for virologic efficacy and the prevention of mother-to-child HIV transmission among breastfeeding women in Botswana **Duration of enrolment:** not reported in the abstract **Setting:** 4 clinical sites in Botswana **Definition of feeding practice:** The study abstract does not define exclusive breastfeeding. **Follow-up:** Planned length of follow-up: 24 months Observed length of follow-up: 6 months Lost to follow-up 95% of women and 97% of infants followed to 6 months or death.

	Analysis The analysis was not described in the abstract.
Participants	**Sample size**: Number of women: There were 730 women enrolled in the study – 285 women were randomized to Arm A, 275 to Arm B, and 170 women in the observational group Number of infants: not reported in the abstract **Eligibility:** HIV-1 infected pregnant women who elected to breastfeed their infants. **Baseline characteristics** were similar between the two groups. There were no differences in the socio-demographic characteristics of the group in terms of educational level, income, and electricity in home. Clinical status in terms of haemoglobin level and hepatitis B were similar. Median maternal age: Arm A 26; Arm B 25; observational 29 Median baseline CD4 count (cells/mm ^{3}): Arm A 398; Arm B 403; observational 147 Median plasma viral load (copies/ml): Arm A 13 300; Arm B 9 100; observation 51 700 Median HAART duration prior to delivery: 11 weeks randomized arms; 13 weeks in the observation arm Feeding practice: 97% of women initiated breastfeeding 93% of women exclusively breastfed until the time of weaning
Interventions	**Intervention:** Arm A: women with CD4 > 200 cells/ mm ^{3} were administered antenatally between 26 and 34 weeks Trizivir (Abacavir, Zidovudine, and Lamivudine) through planned weaning until 6 months postpartum. Arm B: women with CD4 > 200 cells/ mm ^{3} antenatally between 26 and 34 weeks Kaletra (Ritonavir-boosted Lopinavir) and Combivir (Zidovidune and Lamivudine) 6 months postpartum. Observational arm: women with CD4 <200 cells/mm ^{3} NVP and Combivir to women from 18-34 weeks gestation. **Infant ARV:** all infants received single-dose NVP followed by Zidovudine for 1 month Duration of breastfeeding: 71% of women breastfed > 5 months and <1% beyond 6 months. Women counselled: in pregnancy to exclusively breastfeed with rapid weaning before the 6 month visit.
Outcomes	**HIV infections:** Breastfeeding: Arm A 2/283 (0.7%); Arm B 0/270; observational 0/56

	Overall at 6 months: Arm A 5/283 *(1.8%); Arm B 1/270 *(0.4%); observational 1/156 (0.6%) *There was no significant difference between the two maternal HAART arms. Overall 1% transmission through 6 months (95% CI, 0.5% - 2.0%). P-value for differences in proportion between Arm A compared to Arm B = 0.53
Notes	

Item	Judgement	Description
Adequate sequence generation?	Unclear	Conference abstract reviewed
Allocation concealment?	Unclear	Conference abstract reviewed
Blinding?	Unclear	Conference abstract reviewed
Incomplete outcome data addressed?	Yes	Almost complete follow-up with 5% of women and 3% of infants lost to follow-up at 6 months
Free of selective reporting?	Unclear	Conference abstract reviewed
Free of other bias?	Unclear	Conference abstract reviewed

PEPI

Kumwenda 2008 (12)

Methods	**Study design**: randomized controlled trial (PEPI) of three antiretroviral prophylaxis regimens: single-dose nevirapine (NVP) plus 1 week of zidovudine (ZDV) (control), control plus daily extended prophylaxis with NVP (extended NVP), NVP plus ZDV (extended dual prophylaxis) until 14 weeks of age **Duration of enrolment:** April 2004 to December 2007 **Setting:** health centres in Blantyre, Malawi **Follow-up:** Follow-up of infants at delivery, 14 weeks, 6,12, 18 and 24 months Observed length of follow-up: 24 months Lost to follow-up: At 9 months: Control group 146; extended NVP group 147; extended NVP and ZDV arm 128 **Analysis** An intention-to-treat analysis was carried out. Kaplan-Meier analyses were used to

	calculate the time until the first HIV-1 positive test (either confirmed or presumptive with data censored at time of time of death in infants without a positive HIV-1 test), the time to death, and the time to death or the first positive HIV-1 test (whichever occurred first) according to study arm.
Participants	**Sample size**: There were 3216 women enrolled in the study. There were 3276 enrolled and randomized in the study, of which 3016 were included in the primary analysis. One thousand and eighty eight infants were randomized to the control group, 1099 to the extended NVP arm, and 1089 infants to the extended NVP and ZDV arm. **Eligibility criteria:** HIV-1 infected women pregnant women or women who gave birth within the previous 24 hours at one of the study clinics, >18 years (women< 18 years could be enrolled if they consented and permission was given by a guardian), resident of the study area, were willing to return to the study clinics for up to 24 months post delivery, and intended to breastfeed. **Exclusions:** infants with life-threatening conditions that required immediate care. **Maternal ART:** women were categorised according to the CD4 status as ineligible (CD4 ≥250 cells/µL), HAART eligible, treated (CD4 < 250 cells/µL and received HAART), and HAART eligible, untreated (CD<250 cells/µL but no HAART received)
Interventions	Arm 1: single -dose nevirapine (sdNVP) and 1 week of zidovudine (ZDV) (control) Intervention: Arm 2: infants were randomized to receive control and extended daily NVP from 7 days until 14 weeks of age; Arm 3: control and extended daily NVP and ZDV from 7 days of age until 14 weeks of age. Women were counselled in pregnancy and after delivery to breastfeed exclusively for 6 months with weaning after 6 months. Frequency of breastfeeding (%) 6 months: Control arm 66.9% exclusively breastfed (EBF); 23.7% mixed breastfed (MBF); 9.4% no breast feeding (NBF) Extended NVP arm: 64.3% EBF; 25.4% MBF; 10.3% NBF Extended NVP and ZDV arm: 61.1% EBF; 29.3% MBF; 9.3% NBF 9 months: Control arm: 2.9% EBF; 29.1% MBF; 68.0% NBF Extended NVP arm: 2.7% EBF; 24.2% MBF; 73.1% NBF Extended NVP and ZDV arm:1.6% EBF; 27.6% MBF; 70.8% NBF

	15 months: Control arm: 0.5% EBF; 18.9% MBF; 80.6% NBF Extended NVP arm: 1.9% EBF; 12.4% MBF; 85.6% NBF Extended NVP and ZDV arm:0.2% EBF; 17.8% MBF; 81.9% NBF
Outcomes	**Kaplan-Meier HIV infection rate of infants uninfected at 6 weeks** 9 months: control 10.6% (9/548) (95% CI, 8.7 -12.8); extended NVP arm 5.2% (51/593) (95% CI, 3.9 – 7.0) (P <0.001); extended NVP and ZDV arm 6.4%(61/589) (95% CI, 4.9 – 8.3) (P=0.002). **Taha 2009 CROI (13)** **Transmission rate per 100 person years (N=2318 uninfected infants at 14 weeks)** HAART eligible, untreated: 10.5 per 100 person years (52/494.4) (unadjusted HR 7.86 – 13.79)(reference group) HAART eligible, treated: 2.1 per 100 person-years (6/288.1) (adjusted HR 0.21, 95% CI 0.09-0.48) HAART ineligible: 3.7 per 100 person years (72/1067.9) (adjusted HR 0.35, 95% CI, 0.25 – 0.50) **Mortality** 9 months: control 8.9% (71) (95% CI 7.1 – 11.1); extended NVP arm 6.8% (55) (95% CI, 5.2 – 8.7); extended NVP and ZDV 6.3% (95% CI, 4.8 – 8.2) There were no statistically significant differences in the mortality between the study arms. **Kafulafula 2009(14)** The objective of the study was to assess the frequency of gastroenteritis and gastroenteritis-associated mortality with early weaning in HIV-1 exposed children in two randomized clinical trials (PEPI and NVAZ) in Blantyre, Malawi. Methods for both PEPI and NVAZ are described in this review. Statistical analysis

	HIV positive infants born to HIV-infected women in both trials were excluded from this analyses which is concerned with HIV-uninfected infants only. In this analysis data is combined from the two treatment arms in NVAZ and the three treatment arms in PEPI. Age-specific frequency of overall gastroenteritis (GE) and serious GE events reported or identified as occurring since the last scheduled visit were assessed in both trials in infants who were HIV-uninfected at the particular study visit. The GE frequencies and GE-associated hospitalization frequencies were evaluated sd interval probabilities with intervals including 0-6 weeks, 7 weeks – 3 months, and 4-6, 7 – 9, and 10 – 12 months. An infant was counted only once at each age interval and the cross-sectional data only included infants with negative HIV tests at or after the last visit in the interval. Kaplan-Meier methods were used to calculate the probability of death with censoring at the time of HIV infection, loss to follow-up or exit from the trial. **Frequency of reported gastroenteritis** **PEPI** Birth to 6 weeks: 0.6% (11/1750) 7 weeks – 3 months: 1.6% (25/1635) 4-6 months: 4.1% (63/1546) 7-9 months: 9.0% (129/1427) 10-12 months: 5.6% (70/1246) **NVAZ** Birth to 6 weeks: 0.3% (5/1469) 7 weeks – 3 months: 1.9% (25/1302) 4-6 months: 6.4% (79/1239) 7-9 months: 9.2% (107/1168) 10-12 months: 8.2% (88/1097) After 6 months of life the probability of GE associated hospitalization was significantly greater in the PEPI than NVAZ. From 7-9 months the frequency of GE-associated hospitalizations was 2.9% (41/1427) in the PEPI study versus 0.1% (1/1158) in the NVAZ study (P<0.001). From 10 to 12 months the frequency of GE-associated hospitalizations was 1.6% (20/1246) in the PEPI study versus 0.2% in the NVAZ (2/1097) study (P < 0.001)

	GE mortality PEPI: Of the 2035 uninfected infants at birth, there were 72 GE-related deaths of 251 uninfected infants who died (28.7%) NVAZ: Of the 1810 uninfected infants at birth, there were 17 GE-related deaths of 106 uninfected infants (16.0%) who died **Cumulative GE-associated mortality** 6 months: PEPI 5 per 1000; NVAZ 3 per 1000 9 months: PEPI 19 per 1000; NVAZ 7 per 1000 Over 12 months GE associated mortality in HIV-uninfected infants was significantly greater in the PEPI study versus the NVAZ study (P=0.0002)
Notes	No information from risk factor analysis in which infant feeding was related to risk of transmission and/or death.

Item	Judgement	Description
Adequate sequence generation?	Yes	Randomization was according to antiretroviral prophylaxis regimens and not by infant feeding modality and is therefore not relevant to this review.
Allocation concealment?	No	Allocation concealment is not addressed by the study
Blinding?	No	The study was not blinded
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	Unclear	Infant practices were not well reported
Free of other bias?	Unclear	

SWEN 2008

Bedri 2008 (15)

Gupte 2009 (16) 5th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention

Methods	**Study design:** two-arm randomized controlled trial comparing single-dose nevirapine (sd-NVP) to 6 week extended dose **Duration of enrolment:** 2002 to 2007. The 3 studies were initially designed to be independent clinical trials. Investigators later decided to coordinate the study procedures, primary outcomes and treatment regimens. **Setting:** antenatal and delivery facilities in Addis Ababa, Ethiopia, Pune, India and Kampala, Uganda **Definition of feeding practice** Feeding practices were defined according WHO/UNICEF guidelines. Exclusive breastfeeding was defined as infants who were breastfed only and never received any substances except study drugs and other medicines (including liquid vitamins and minerals). **HIV testing** Infant HIV-1 status was determined by DNA polymerase chain reaction (PCR) or quantitative HIV-1 RNA PCR. In Ethiopia, HIV detection was determined by RNA PCR tests. Initial positive RNA PCR test was confirmed by a second test at a subsequent visit. In India, determination of HIV infection status was by using an externally validated in-house HIV DNA PCR assay (positive result was confirmed by an HIV-1 RNA PCR test at the next visit with Roche Amplicor HIV-1 Monitor test, version 1.5). In Uganda, infants were tested for HIV infection with Roche Amplicor DNA PCR test confirmed by repeat test at the next study visit. RNA PCR tests were considered positive if the viral load was > 5000 copies/ml. Infected at birth was defined as positive HIV DNA PCR result within days of birth in all three countries. An infant was classified as HIV infected if two independent HIV PCRs were reactive at different time points or if one test was reactive and there were no subsequent infant samples available for testing. Timing of transmission was classified as *in utero* (48 hours), neonatal (< 1 month) and beyond 1 month. **Follow-up:** Infants were followed up at birth, and at 1, 2, 4, 6, 10, 14 weeks and 6, 9, and 12 months of life. Planned length of follow-up: 12 months Observed length of follow-up: 12 months Lost to follow-up At 6 months of age data was available on 928 (94.1%) of infants in the single-dose

	NVP group and 831 (92.2%) of infants in the extended NVP arm **Analysis** Intention-to-treat analysis was carried out. The risk ratio for cumulative transmission was calculated. The overall risk ratio was an inverse variance-weighted mean of the separate countries' risk ratios. Kaplan-Meier methods of analyses were used to calculate mortality, and the earliest of HIV infection or death (complement of HIV-free survival). Censoring of infants in Kaplan-Meier analyses of time-to-event (HIV detection) occurred at the earliest of document official study truncation, last HIV determination, or death. Cox regression models were used to evaluate the relationship between timing of transmission and mortality.
Participants	**Sample size**: There were 2067 women who delivered and were randomized. Number of infants: 2024 liveborn infants with at least one specimen tested before 6 months were randomized – 1047 infants in the sd-NVP group and 977 infants in the extended dose NVP group. 1887 infants were excluded in the analysis. The modified intention-to-treat population consisted of 986 infants in the sd-NVP group and 901 infants in the extended NVP group. **Eligibility:** HIV-infected pregnant women attending antenatal and delivery facilities, who intended to breastfeed their infants and provided informed consent for the study **Baseline characteristics** were similar in the intention-to-treat population with regards to proportion of caesarean section deliveries, maternal CD4 count, and plasma viral load. Overall, 3.0% (n=30) of women in the sd-NVP arm and 3.6% (n=32) of women in the extended dose NVP group received HAART. There were 53.7% male infants in the sd-NVP arm and 48.3% (n=529) in the extended dose NVP arm (n=435). By 6 months of age, 31.7% (276/871) of infants in the sd-NVP arm received "any breastfeeding" compared to 31.3% (251/801) of infants in the extended dose NVP arm. There were 27.6% (61/221) of exclusively breastfed infants in the sd-NVP arm compared to 27.7% (57/206) in the extended dose NVP arm.
Interventions	Arm 1 (control): Nevirapine (NVP) 200mg was given to women in labour and 2mg/kg to infants following delivery Arm 2: Control and infant NVP 5mg from 7 days through 6 weeks of age Women received infant feeding counselling during pregnancy
Outcomes	**Risk of death or HIV transmission** overall* 6 months: sd-NVP arm 11.58%(113) versus extended NVP arm 8.05% (74) (RR 0.73, 95% CI, 0.55 – 0.97; P=0.03): HIV free survival 6 months: sd-NVP arm 88.42%(113) versus extended NVP arm 91.95% (74) *Overall 6 month estimates are inverse variance-weighted means of country specific

	risks and risk ratios **HIV transmission risk** *overall 6 months: sd-NVP arm 8.98%(87) versus extended NVP arm 6.91% (62) (RR 0.80, 95% CI, 0.58 – 1.10; P=0.16) Of 93 infected infants at 12 months 55 (59%) were infected beyond 1 month(Gupte 2009 5th IAS) **Mortality** *overall 6 months: sd-NVP arm 3.61%(37) versus extended NVP arm 1.12% (16) (RR 0.47, 95% CI, 0.26 – 0.87; P=0.02) 1 month -12 months: 6% (95% CI, 2-16%) (Gupta 2009 5th IAS) The Kaplan-Meier estimate of mortality was 2% (95% CI, 1-4%) among HIV uninfected infants and 17% (95% CI, 10-28%) among HIV-infected infants. There were no results regarding HIV infection or HIV-free survival by infant feeding practice.
Notes	

Item	Judgement	Description
Adequate sequence generation?	Yes	Randomisation was according to drug regimen not by infant feeding practice
Allocation concealment?	Unclear	"Study products were administered by pre-filled amber oral syringes in Ethiopia and Uganda and by opaque dropper bottles in India, after training of mothers by study pharmacists. A number of specific study procedures were implemented to introduce some degree of masking of study staff and care givers. Specifically, none of the study investigators or staff, with the exception of the study pharmacists and a member of the central data management team, had access to the randomization assignments for study participants." Study pharmacists had knowledge of the random assignments and had direct contact with the mothers therefore allocation may not have been sufficiently concealed.
Blinding?	No	"This study was not blinded"
Incomplete outcome data addressed?	Yes	"Inflating for losses to stillbirth, mortality to 6 months, and loss to follow-up (about 16% in total) we arrived at 982 enrolled women in each arm, for a total of 1964 pairs of women and infants across all three countries".
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

DITRAME ANRS STUDY GROUP

Dabis 1999 (17, 18)

Methods	**Study design:** randomized, double blinded, controlled trial comparing a maternal short regimen of oral zidovudine with placebo**.** **Duration of enrolment:** September 1995 and February 1998. Stopped by DSMB **Setting:** Abidjan, Cote d'Ivoire, and Bobo-Dioulasso, Burkina Faso **Blinding**: Patient: Yes Provider: Yes Assessor: Yes **Definition of feeding practice:** Not defined **HIV testing:** day 180 specimens were systematically processed for DNA or RNA PCR. 9-15 month specimens were screened with HIV ELISA tests. The diagnosis of HIV infection was defined on the basis of one positive DNA PCR. Absence of infection was defined as a negative diagnosis 60 days or more after complete cessation of breastfeeding. **Follow-up:** Planned length of follow-up: visits at 1 week postnatal, 45 days, 90 days and then 3 monthly till 15 months. Planned for 2 years. Observed length of follow-up: 15 months Lost to follow-up • 14 of 421 (3.3%) women lost to follow-up before delivery • 11 of 406 (2.7%) infants were lost to follow-up at birth **Analysis** Intention-to-treat analysis carried out for liveborn children. Date of diagnosis if positive PCR before 45 days was the date of the first positive HIV PCR. If positive PCR after 45 days, then diagnosis of HIV infection was taken as the midpoint between last negative and first positive result. The probability of diagnosis of HIV infection, infant death and the probability of breastfeeding at a given age was calculated with Kaplan Meier method, and comparisons were made with the log-rank test. The censoring event was 60 days after weaning for definitively uninfected children and the date of last negative test for children categorised as provisionally uninfected.
Participants	**Sample size**: Number of women: 431 enrolled Number of infants: 401 livebirths were included in the analysis after random

	selection of one livebirth in 6 pairs of twins. There were 200 liveborns in the ZDV group and 201 in the placebo group (236 in Abidjan and 165 in Bobo Dioulasso). **Exclusions:** severe anaemia, neutropaenia, abnormal liver function, and sickle cell disease **Baseline characteristics** were similar between the two groups Median maternal age: ZDV arm 25; 24 in placebo (p=0.61) Median CD4 count (cells/mm ^{3}): ZDV arm 568; 535 in placebo (p=0.70) When CD4 count was obtained: enrolment Median plasma viral load: not reported Maternal education level: not reported Gender of infants: not reported Gestational age (weeks): 39.5 weeks in the ZDV arm, 39 weeks in the placebo (p=0.11) Median weight of infants: 2915g in the ZDV arm, 2860g in placebo (p=0.21) **Feeding practice** 6 months probability of being breastfed for the 388 children that commenced breastfeeding was 90.7% in the ZDV group and 93.8 in the placebo group 15 months probability of being breastfed was 45.6% and 40.1% respectively, without any difference between the treatment arms. Number formula fed from birth- 9 children in the zidovudine group and 4 in the placebo group replacement fed from birth.(p=0.16)
Interventions	Intervention: Zidovudine (ZDV) 500mg or 600mg daily starting at 36-38 weeks gestation, a single loading dose of ZDV 500mg or 600mg at the beginning of labour, and a 7 day post-partum treatment of 500mg to 600mg to the mother. The infant did not receive any treatment. Control: matching placebo intrapartum at 36-38 weeks, at the onset of labour, and postpartum for 1 week. Duration of breastfeeding: Feeding practices were reported at each visit. Duration of formula feeding: not reported Women counselled: in pregnancy/after delivery: at enrolment into study
Outcomes	**Probability (%) of HIV infection** 6 months: ZDV 17.7% (33/141) (95% CI, 12.1 – 23.3); placebo 27.4% (52/126) (95% CI, 20.2– 33.9)

	9 months: ZDV 18.3% (34/102) (95% CI, 12.6 – 24.0); placebo 30.6% (57/92) (95% CI, 23.8 – 37.4) 12 months: ZDV 20.1% (36/71) (95% CI, 14.0 – 26.2); placebo 30.6% (57/59) (95% CI, 23.8 – 37.4) 15 months: ZDV 21.5% (37/51) (95% CI, 14.9 – 28.1); placebo 30.%6 (57/46) (95% CI, 23.8 – 37.4) **HIV transmission** 6 months: 18% (95%CI 12.4-23.5) in ZDV arm 27.5% (95%CI 21.1-33.9%) in placebo arm 15 months: 21.5% (95%CI 14.9 to 28.1) in ZDV arm 30.6% (95%CI 23.8 to 37.4%) in placebo arm
Notes	

Item	Judgement	Description
Adequate sequence generation?	Yes	For the study intervention and not for feeding practice. Block randomisation stratified by study centre was used.
Allocation concealment?	Yes	Allocation concealment not relevant for feeding practice. Sequentially numbered sealed packages were prepared by independent pharmacy
Blinding?	Yes	Double-blinded
Incomplete outcome data addressed?	No	
Free of selective reporting?	Unclear	
Free of other bias?	Yes	

PETRA

PETRA study team 2002 (19)

Methods	**Study design:** randomized, double-blind, placebo controlled trial **Duration of enrolment:** June 1996 to January 2000 **Setting:** Tanzania (1 large public hospital), South Africa (2 large public hospitals) , Uganda (1 large public hospital and 1 semi-private hospital) **Blinding**: Patient: Yes Provider: Yes Assessor: Yes **Definition of feeding practice:** not defined. Ever breastfed or never breastfed **HIV testing:** samples collected from infant and mother at week 1, 3 and 6, month 3, 6, 9, 12, 15 and 18. **Infant:** Cell pellets or plasma or both were tested using HIV DNA PCR (Amplicor) or NASBA respectively. Two positive tests were required for confirmation of HIV infection. At months 15 and 18, ELISA testing was done. Positive results were confirmed by a second ELISA or western blot. **Follow-up:** Planned length of follow-up: 18 months Observed length of follow-up: 18 months Lost to follow-up at x weeks: **Analysis** Intention-to-treat analysis: of those enrolled before 18 Feb 1998 Censoring rules regarding HIV rate and survival: a child was considered negative when either 15 or 18 ELISA was negative. The last negative test and the first positive test were determined. The age of HIV infection was considered to be interval-censored. Turnbull analysis, allowing for left censored, right censored and interval censored times.
Participants	**Sample size**: Number of women -23273 screened, 1797 HIV infected women enrolled in the PETRA trial. Of these 1457 randomized women gave birth before 18 February 1998. Regimen A: Mothers – 366 (380 infants; 14 twins) Regimen B: Mothers – 371 (382 infants; 11 twins) Regimen C: Mothers – 368 (377 infants; 9 twins)

	Placebo : Mothers – 352 (362 infants; 10 twins) There were more women enrolled in Kampala Uganda than in other study sites (p=0.002) compared with women enrolled before the discontinuation of the placebo. The main analysis thus focuses on women randomized before 18 Feb 2009. **Eligibility** – 18yrs, HIV infected, before 36 weeks pregnancy, laboratory eligibility criteria **Baseline characteristics** were similar between the two groups A B C Placebo Median maternal age(years): 26 26 26 26 Median CD4 count (cells/mm ^{3}): 445 475 440 435 When CD4 count was obtained: enrolment at 36 weeks Median plasma viral load: no data reported Maternal education level: not reported Gender of infants:(%male) 53 54 50 50 Gestational age (weeks): Median weight of infants(kg): 3.1 3.1 3.1 3.1 Feeding practice: Number breastfed from birth1081(74% breastfed) Number formula fed from birth: assumed to be remainder
Interventions	Intervention: Regimen A- Oral Zidovudine (ZDV) 300mg and Lamivudine(3TC) 150mg 12 hourly from 36 weeks antenatal, intrapartum dose of ZDV 300mg 3hourly and 3TC 12 hourly until delivery and 7 days post-partum dosing of mothers with ZDV 300mg and 3TC 150mg 12 hourly and infants with ZDV 4mg/kg and 3TC 2mg/kg 12 hourly Regimen B - Intrapartum ZDV 600mg and 3TC 150mg at the onset of labour, then ZDV 300mg 3hourly and 3TC 12 hourly until delivery and 7 days post-partum dosing of mothers with ZDV 300mg and 3TC 150mg 12 hourly and infants with ZDV 4mg/kg and 3TC 2mg/kg 12 hourly Regimen C – Intrapartum ZDV 600mg and 3TC 150mg at the onset of labour, then ZDV 300mg 3hourly and 3TC 12 hourly until delivery Control: Placebo Recruitment into placebo arm stopped from 18 February 1998 **Duration of breastfeeding**: with median period of 28 weeks (IQR 7-59 weeks). In South Africa, 53% of patients breastfed for a median of 7 weeks, whereas in East Africa 97% of women breastfed for a median of 45 weeks. Duration of formula feeding: not reported Women counselled: in pregnancy/after delivery – no feeding counselling reported

Outcomes	**Turnbull HIV-free survival (Interval-censored survival analysis)** 18 months: Group A 18.9(95%CI12.7-27.3), Group B 23.8%(15.6-34.5), Group C 25.2%(16.9-35.8), Placebo 25.6(19-33) ever breastfed 18 months: Group A 21.6(14.1-31.7); Group B 24%(15.9-34.5), Group C 27.8%(17.3-41.4), Placebo 28.2%(19.2-37.1) **HIV transmission** 18 months: Group A15%(95%CI 9-23); Group B 18%(12-26); Group C 20%(13-30); placebo 22%(16-30) **Mortality** 18 months: Group A 10.1%; Group B 14.2%; Group C 12.8%; Placebo 13.4%
Notes	

Item	Judgement	Description
Adequate sequence generation?	Yes	Randomization was by block randomisation by drug regimen not feeding practice
Allocation concealment?	Yes	The study medication was packaged by the manufacturer according to the randomisation list. "All steps following the preparation of the study batches were masked." The allocation concealment was not relevant to this review
Blinding?	Yes	Feeding practice was not blinded
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	Unclear	Feeding practice not defined. In the ever breastfed group – transmission and mortality data not reported at 18 months.
Free of other bias?	Yes	

Vit A

Coutsoudis 2001 (20)

Methods	**Study design** prospective cohort study of mother-infant pairs enrolled in a randomized intervention trial of Vitamin A to reduce mother-to-child transmission of HIV-1. **Duration of enrolment:** July 1995 to April 1998 **Setting:** antenatal clinics at two hospitals in Durban, South Africa: King Edward VIII and McCords Hospital **Blinding**: not applicable **Definition of feeding practice: "**Exclusive breastfeeders" were defined as those who exclusively breastfed for 3 months or longer, or up to the time of loss to follow-up or death of the child. "Mixed breastfeeders" were all other breastfeeders, who had never breastfed exclusively, or had breastfed exclusively for periods of less than 3 months **HIV testing** Plasma samples before infants reached 9 months of age were tested using a quantitative assay of HIV viral RNA using polymerase chain reaction (PCR; Roche Molecular Systems, Branchburg, NJ, USA). After 9 months, HIV samples were tested for HIV antibodies (Abbott Laboratories, Chicago, IL, USA). HIV infection was designated as 2 or more positive PCRS and/or antibody positive at 15 months of age. Negative HIV status was designated as never had a positive PCR and at least 1 negative PCR 1 month after breastfeeding was ceased or antibody negative 6 weeks after cessation of breastfeeding. **Follow-up:** Follow-up was at 1 and 6 weeks, and 3, 6, 9, 12, and 15 months. All children were not seen each time point which has a bearing on the main analysis. If children were still breastfed at 15 months an additional sample of blood was taken for HIV testing 6 weeks after cessation of breastfeeding. Observed length of follow-up: 15 months Lost to follow-up: 75/631 (12%) singleton infants lost to follow-up before feeding practices could be determined. **Analysis** Analysis was restricted to singleton infants with at least one HIV result. All analyses were carried out using Kaplan-Meier life tables or Cox proportional hazards models because the period of follow-up varied for all mother-infant pairs (due to infant death, loss to follow-up and requirements for ascertaining HIV-infection status varied by infant feeding practice). Children who did not ever test positive and who were

	breastfed were censored if the last HIV-1 test was performed 6 weeks after cessation of all breastfeeding. Failure to censor would have led to underestimates of HIV transmission attributable to breastfeeding, especially in this study population which had a large proportion of women who breastfed for a shorter duration.
Participants	**Sample size**: 661/728 eligible women were enrolled in the study. Singleton, live born infants totalled 631. 551 mother-infant pairs included in the analysis. **Inclusion:** HIV-1 infected pregnant females between 28-32 weeks of gestation enrolled in a Vitamin A randomized controlled trial. No women received antiretroviral therapy. **Exclusions:** twin pregnancies, absence of infants' HIV-1 test result **Maternal antiretroviral:** no women in the study received any antiretroviral therapy, as none was available at the time of the study **Baseline characteristics** Mean maternal age, mean CD4 count, plasma viral load gender of infants, birth weight of infants, parity, mode of delivery, and duration of rupture of membranes were similar between the never breastfed, exclusive breastfed and mixed breastfed groups. There were some significant differences between the socio-demographic characteristics of the never breastfed and exclusively breastfed groups. Completed high school: 77/153 (50.3), 30/117 (25.6), 97/273 (17.4) (P<0.05) Employed: 68/155 (43.9), 37/118 (31.4), 100/275 (36.4) (P<0.05) Electricity in the home: 121/154 (78.6), 67/117 (57.3), 195/274 (71.2) (P<0.05) Feeding practice: Number breastfed from birth: 394/551 (71.5%) Number formula fed from birth: 157/551 (28.5%)
Interventions	Intervention: never breastfed, exclusively breastfed and mixed fed. Mean duration of all breastfeeding: 6 months (95% CI, 4.75-7.5) Women counselled: in pregnancy about the risks of transmission associated with breastfeeding and benefits of breastfeeding. Women who chose to breastfeed were counselled to breastfeed exclusively due to the risk of HIV transmission. Recommendations from UNAIDS, WHO and UNICEF were followed. Women who chose to formula feed were not provided with free formula and women who chose to breastfeed were not provided with nutritional support.
Outcomes	**Cumulative probability of HIV infection (no P values reported)** Never breastfed children (n=157); mixed breastfed children (n=276) , exclusively breastfed children (n= 118) 6 months: never breastfed 0.194 (95% CI, 0.136 – 0.260), exclusively breastfed 0.194 (95% CI, 0.125 – 0.274), mixed breastfed 0.261 (95% CI, 0.205 – 0.319)

	12 months: exclusively breastfed 0.221 (95% CI, 0.245 – 0.0.307, mixed breastfed 0.333 (95% CI, 0.253 – 0.415) 15 months: exclusively breastfed 0.247 (95% CI, 0.160 – 0.344) mixed breastfed 0.359 (95% CI, 0.267 – 0.451) **Mortality** < 3 months: 7/157 (4.5%) never breastfed and 15/394 (3.8%) of ever breastfed children **Morbidity (Coutsoudis 2003a)(21)** Only infants followed up to 9 months were included in this analysis of morbidity to avoid loss to follow-up bias. This reduced the bias relating to reverse causality where infants who died were less likely to have been breastfed. Second twins were excluded from the analysis. Sample size: Of 738 mother-infant pairs, 21 women defaulted before or at delivery, 49 children died during follow-up and 305 were lost to follow-up, 213 (70%) before 3 months of age. 363 children were included in this analysis of morbidity (completers). Assessment of growth/ weight gain Overall, median weight gain was early in life at 8.3% between 1 and 6 weeks of age. Median weight gain and variation in percentage weight gain decreased with increasing age. The median percentage weight gain per week among breastfed infants ranged from 0.3% after 18 months of age to 8.1% in the first 6 weeks of life. In children who were never breastfed, the median percentage weight gain ranged from 0.4% after 18 months of age to 8.4% in the first 6 weeks. There were no differences in the overall growth pattern between male and female infants and between ever - and never- breastfed infants. **Morbidity according to breastfeeding status** Overall, there were 437 illness episodes in 245 children. Incidence of morbidity was not associated with feeding practice. 77 (32%) breastfed infants had ≥ 2 episodes of illness compared to 45 (36%) of infants who never breastfed (OR 0.85, 95% CI, 0.53 – 1.38, P=0.48) Of the HIV – infected children who had at least one episode of illness (n=62), children who were never breastfed were more likely to have ≥ 3 illness episodes

	than those who were ever breastfed (OR 4.05, 95% CI, 0.91 – 20.63, P=0.05). In the first 2 months of life, infants who were never breastfed were more likely to have an illness episode (40%) compared to the breastfed group (26%) (OR 1.91, 95% CI 1.17- 3.13, P=0.006) Of the HIV-uninfected children, those who were never breastfed in the first 2 months of life were more likely to have an illness episode compared to those children who were ever breastfed (OR 2.02, 95% CI, 1.16 – 3.51, P = 0.008). In the first 4 months of life, infants who were never breastfed were more likely to have an illness episode than infants who were ever breastfed (OR 1.45, 95% CI, 0.92 – 2.30, P = 0.09)
Notes	In the analysis of morbidity data, the sample size in each group was small so results should be interpreted with caution.

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	Yes	"As duration of follow-up varied for all mother-child pairs (because requirements for determining HIV-status vary by infant feeding practices, and because of infant deaths and loss to follow-up) all analyses were based on Kaplan-Meier life tables or Cox proportional hazards models"
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	Possible effect of confounding factors related to transmission and non-EBF, and children being tested at different ages

PEP

Gray 2005 (22)

Methods	**Study design:** Multicentre, randomized, open-label clinical trial comparing single-dose nevirapine with 6 weeks of zidovudine (ZDV) to the infant **Duration of enrolment:** October 2000 - September 2002 **Setting**: 3 hospitals in South Africa - Chris Hani Baragwanath Hospital (Soweto), Coronation Hospital (Johannesburg), and Mowbray Hospital (Cape Town) **Definition of feeding practice** At 12 weeks infant feeding was defined as either exclusive breastfeeding or as "breast milk exposure". Infants who were classified as "breast milk exposure" consisted of infants who were exclusively breast fed, mixed fed and ever breastfed. Feeding practice was ascertained at all visits and was used to categorize infants. Exclusively formula fed infants was defined as infants who were formula fed from randomization or were exposed to < 2 days of breast milk. Women enrolled in the study were counselled on infant feeding according to South African guidelines. Women who opted to breastfeed were encouraged to breastfeed exclusively for 3 to 6 months. **HIV test** Infants were classified as HIV infected if two successive blood samples tested positive for HIV-1 DNA by PCR. Infants who had one documented HIV positive test result and were subsequently lost to follow-up were classified as infected. Infants who were infected by day 1 or before day 10 were classified as being infected *in utero*. Postuterine (intrapartum or early postpartum) infection was defined as infants who had tested negative at birth and positive at ≥ 10 days. A child was classified as uninfected if the 6 week or later result was negative in the absence of breastfeeding. If an infant was breastfed, an HIV test was carried out 1 month after cessation of breastfeeding. The breastfed infants were classified as uninfected if this sample was non-reactive. **Follow-up** Mother-infant pairs were followed up for 6 months or until 1 month after cessation of breastfeeding. Planned length of follow-up: 12 weeks Observed length of follow-up: 12 weeks Lost to follow-up at 12 weeks: Total sample: 216 (20%)

	Analysis Kaplan- Meier methods of analysis were used to calculate and compare HIV-1 transmission rates, using the log rank test. The HIV-free survival time for infants who became infected within 12 weeks was defined as the date of birth to the date of the first reactive PCR sample. For infants not infected by 12 weeks, HIV-free survival was defined as the date of birth to the date of the last negative PCRsample. Censoring of survival time occurred when the survival time was more than 100 days. An intention-to-treat analysis was carried out. Deaths with "unknown date of death" were included in the endpoint analyses.
Participants	**Sample size:** There were 1530 HIV-infected women eligible for the study. There were 1051 infants randomized into study (533 in the ZDV arm and 518 in the NVP arm). **Eligibility:** HIV-infected women accepting postpartum voluntary counselling and testing. **Exclusions**: infants were excluded if they were preterm weighing < 1200g, requiring ventilation, unable to take oral medication, or with congenital abnormalities **Baseline characteristics** were similar between the nevirapine arm and the zidovudine arm. The median maternal age in both arms was 25.0. The median CD4 count was 448.5-480.5 x 10^6cells/l and median plasma viral load was 21 800copies/ml. The median weight of infants and mean time of drug ingestion was similar in both arms. There was no difference in gender or feeding practices between the two arms.
Interventions	Infants of women who had consented to participate were randomized to receive either a single dose of nevirapine (10mg/ml oral suspension at a dose of 2mg/kg) within 24 hours of delivery or zidovudine (10mg/ml at a dose of 4mg/kg administered 12 hourly for 6 weeks).
Outcomes	**Cumulative HIV transmission rate** 12 weeks: NVP arm 14.3%; ZDV arm 18.1% (log rank test, P=0.4) **Postnatal HIV-1 transmission rate (Infants tested negative at birth and positive at ≥ 10 days of age)** 12 weeks: NVP arm 7.9% (24/476) (95% CI, 4.6 – 11.2) ; ZDV arm 13.1%(41/491) (95% CI, 8.9 – 17.3) (log rank test, P=0.06) **Infant mortality:** 24 infants (3.4%) died before 100 days (13 in the ZDV arm and 11 in the NVP arm, log-rank test P=0.8). No deaths were as a result of the ZDV or NVP. By 12 weeks of age, there were 4 breastfed infants who died (2.2%) compared to 19 (3.8%) formula-fed infants. There was no increased mortality in the formula-fed group (log-rank test, P=0.2)

	Effect of breastfeeding: Overall, when breastfed and formula-fed infants were compared in the treatment arms, the additional infection rate at 12 weeks was 14.8% in the breastfed group versus 9.4% in the formula-fed group (log-rank test, P=0.007). Within the treatment arms, the additional infection rate in the breastfed infants in the ZDV arm was 20.6% versus 11.1% in infants who were not breastfed. (log rank test, P=0.004). The additional infection rate in the breastfed infants in the NVP arm was 9.9% versus 7.3% (log-rank test, P = 0.3) in infants who were not breastfed Breastmilk exposure was identified as a risk factor for HIV transmission at 12 weeks of age (multivariable OR, 2.2; 95% CI 1.3-3.8)
Notes	

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Yes	"Computer-generated random allocations; enrolled babies were sequentially assigned the next study number". Infants were randomized according to PEP regimen not on feeding modality
Allocation concealment?	Yes	"Allocation to the study arm was provided to the study nurses in sequentially labelled non-transparent sealed envelopes that were only opened after informed consent was obtained."
Blinding?	No	Patients and providers were not blinded - open label trial
Incomplete outcome data addressed?	Yes	"Attempts to minimise follow-up loss included three or more home visits to trace non-attending participants. Any information obtained regarding ill-health or mortality obtained from the given address was used"
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

HIVNET 024

Chasela (23)

Methods	**Study design:** secondary data analyses to assess risk factors for late postnatal transmission of HIV-1 through breastfeeding of the HIVNET 024 double-blind randomized clinical trial (trial of antibiotics to prevent chorioamnionitis-associated perinatal HIV-1 transmission and preterm birth) **Duration of enrolment:** **Setting:** four sites in three African countries – Blantyre and Lilongwe, Malawi, Dar es

	Salaam, Tanzania; and Lusaka, Zambia **Definition of feeding practice:** **HIV testing** Maternal HIV status was determined by HIV-1 RNA (Roche Amplicor Monitor RNA Assay, version 1.5, Branchburg, NJ) on plasma sample. HIV-1 diagnostic testing was site-specific; either a rapid test or enzyme-linked immunosorbent assay/ western blot tests. All positive tests were confirmed with repeat tests. Infant HIV status was determined on dried blood spots (DBS). In Malawi and Zambia HIV-1 RNA was ascertained using NASBA technology (bioMerieux NucliSens QL). In Tanzania HIV-1 RNA, Roche Amplicor Monitor RNA Assay, version 1.5 was used. Positive results were confirmed by retesting with the same DBS or a subsequent sample. Late postnatal transmission (breastmilk transmission) was defined as infants with negative HIV-1 RNA assays at birth and at 4-6 weeks of age followed by positive HIV-1 RNA tests through the 12 month visit. HIV-1 uninfected infants were classified as those with negative enzyme immunoassay (EIA) at 12 months of age, or infants with negative HIV-1 RNA assay results throughout the period of follow-up. **Follow-up:** Infants were followed up at birth (within 48 hours), at 4-6 weeks, and at 3, 6, 9, and 12 months. Planned length of follow-up: Observed length of follow-up: 12 months Lost to follow-up: not presented **Analysis** Timing of HIV transmission was estimated to be the midpoint between the last negative test and the first positive HIV-1 RNA test (after the 6 week and at or before the 12 month visit). Censoring of the event-time was at the date of the last negative test if the infant did not test positive at or before the 12 month visit. Kaplan-Meier methods of analyses were used to estimate the proportion of infants' breastfeeding at different time points, infant survival, late postnatal transmission of HIV-1, and maternal survival.
Participants	**Sample size**: There were 2292 of 2659 women enrolled, of which 2052 women delivered live-born infants. Number of infants: 1317 infants with negative HIV-1 diagnostic assay at six weeks of age and continued to breastfeed beyond this time. **Eligibility:** HIV-1 infected mothers with CD4 > 250 cells/uL and infant birth weight ≥2000 grams. **Exclusions:**

	Maternal ART/ARV: NVP 200mg oral at the onset of labour **Infant ARV**: nevirapine 2mg/kg within 72 hours of delivery **Baseline characteristics** Maternal age 21-29: 840 (63.8%) CD4 count < 200 cells/mm ^{3}: 207 (15.7%) When CD4 count was obtained: 36 weeks Plasma viral load > 10 000 copies/ml: 60% Maternal education 4-9 years: 818 (62.1%) Married/living with partner: 1203 (91.3%) No electricity in the home: 808 (61.4%) No running water in the home: 776 (58.9%) Male infants: 681(51.7%) Gestational age <37weeks: 268 (20.3%) Birth weight of infants < 2500g: 117 (8.9%) **Proportion breastfeeding at different time points (Kaplan-Meier analysis)** 3 months: Blantyre 98.4% (95% CI, 96.5 – 99.3); Lilongwe 98.6% (97.2 – 99.3); Lusaka 98.7% (97.0 – 99.4); Dar es Salaam 49.2% (43.5 – 54.6) 6 months: Blantyre 93.0% (95% CI, 89.8 – 95.2); Lilongwe 97.0% (95.2 – 97.4); Lusaka 95.8% (93.4 – 97.4); Dar es Salaam 8.6% (5.6 – 12.4) 9 months: Blantyre 85.6% (95% CI, 81.4 – 89.0); Lilongwe 92.9% (90.2 – 94.8); Lusaka 85.8% (81.9 – 89.0); Dar es Salaam 2.5% (1.0 – 5.2) 12 months: Blantyre 80.8% (95% CI, 75.9 – 84.8); Lilongwe 86.5% (82.9 – 89.4); Lusaka 76.8% (72.1 – 80.9); Dar es Salaam 0%
Interventions	Intervention: Control: Duration of breastfeeding: Duration of formula feeding: Women counselled: in pregnancy about the risks and benefits of breastfeeding. Replacement feeding and other interventions to prevent breastfeeding transmission were not implemented as part of this study.
Outcomes	**Proportion of infants with HIV-1 infection** 84 of 1317 infants became infected between 6 weeks and 12 months of age

	6 months: Blantyre 1.5% (95% CI 0.5 – 3.8); Lilongwe 3.1% (1.9 – 5.3); Lusaka 7.6% (95% CI 5.3); Dar es Salaam 4.3% (1.6); overall 4.1% (95% CI, 3.2 – 5.4) 9 months: Blantyre 6.7% (95% CI, 4.3,) Lilongwe 4.4% (2.8 – 6.7); Lusaka 8.5% (95% CI 6.0); overall 6.1% (95% CI, 4.9 – 7.5) 12 months: Lilongwe 4.9% (3.2 – 7.4); Lusaka 10.5% (95% CI 7.7); overall 6.9% (95% CI, 5.6 – 8.5)
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	Randomization was by permuted block randomisation to antibiotic or placebo but randomization to feeding practice was not conducted
Allocation concealment?	Unclear	Not reported
Blinding?	Yes	Double blind study but feeding practice cannot be blinded
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	Yes	
Free of other bias?	Yes	

HIVNET 012

Jackson 2003 (24)

Methods	**Study design**: randomized controlled trial comparing single-dose nevirapine (sd-NVP) to zidovudine **Duration of enrolment:** November 1997 to April 1999 **Setting:** Kampala, Uganda **Definition of feeding practice:** Median duration of breastfeeding reported but no definition of breastfeeding was provided by the study. **HIV testing** Maternal HIV status was determined with quantitative plasma HIV-1 RNA (Roche HIV-1 Amplicor Monitor, Indianapolis, USA) before entry, at delivery, and at 7 and 6 weeks after delivery. Infant were determined as HIV-infected with qualitative HIV-1 RNA PCR carried out at age 1-3 days, 6 weeks, 14 weeks and 12 months. An HIV-1 antibody test was conducted at 18 months of age. The HIV-1 infection status of the infants was

	defined as a positive HIV-1 RNA PCR assay confirmed by either an HIV-1 RNA PCR assay or HIV culture on a second blood specimen. If an infant was HIV-1 infected HIV-1 RNA levels were quantified on subsequent samples. If the infant had died with only one positive RNA assay, the infant was categorised as infected with HIV-1. **Follow-up:** Planned length of follow-up: 5 years Observed length of follow-up: 18 months Lost to follow-up 8/645 (1.2%) women were lost to follow-up before delivery 2/619 (0.32%) firstborn infants lost to follow-up before HIV-1 test **Analysis** Intention-to-treat analysis carried out according to drug regimen. In the case of multiple pregnancies, only the firstborn infant was included. Kaplan-Meier methods of analysis were used to calculate the primary endpoints of HIV-free survival and HIV-infection at 6-8 weeks, 14-16 weeks, and 18 months. The timing of HIV transmission was defined as the time to the first positive HIV-1 assay. Time to death or first positive HIV-1 assay was used for the definition of HIV-free survival for the Kaplan-Meier method. For these analyses, all other infants were censored with follow-up time set to the last negative test. Cox regression was used to determine relative risks and confidence intervals for the two primary endpoints and to allow for adjustment of confounding variables.
Participants	**Sample size**: there were 645 of 2144 eligible women randomly assigned treatment - 313 were randomly assigned to regimen A (NVP), 313 to regimen B (ZDV), and 19 to placebo. Number of infants: 617 infants included in the analysis of HIV-free survival **Eligibility:** HIV-1 infected pregnant women **Baseline characteristics** were similar between the two groups Maternal age (median IQR) (years): ZDV 25 (22 -28); NVP 24 (21- 27) (P=0.12) CD4 count (cells/µL) at pre-entry (median [IQR]): ZDV 426 (244- 634); NVP 459 (289 – 636) (P=0.26) HIV-1 RNA (copies/ml, median [IQR] at pre-entry): ZDV 27800 (8700 – 74 552); 25 247 (6427 – 85 972) (P = 0.73) Caesarean section: ZDV 41 (13.9%); NVP 34 (11.5%) (P=0.38) Female infants: ZDV 156 (51.0%); NVP 154 (49.7%) (P=0.75) Birth weight of infants (g, median,[IQR]): ZDV 3200 (2900 – 3500); NVP 3100 (2800 – 3400) Breastfeeding rates (95% CI)

	At birth: ZDV 98.7 (97.4 – 100); NVP 99.3 (98.5 – 100) (P=0.40) At 12 months: ZDV 36.1 (30.2 – 41.9); NVP 31.2 (25.9 – 36.9) (P = 0.243) At 18 months: ZDV 14.6 (10.1 – 19.2); NVP 10.0 (6.1 – 13.9) (P = 0.134)
Interventions	Intervention: women were randomized at more than 36 weeks gestation to regimen A, B or the control group. Regimen A: maternal nevirapine 200mg orally at the onset of labour. Newborns were administered 2mg/kg nevirapine within 72 hours of delivery regimen B: maternal zidovudine 600mg orally at the onset of labour, then 300mg 3 hourly until delivery Newborns received zidovudine 4mg/kg orally 12 hourly for 1 week Placebo group: 19 women (no further information provided) No information provided regarding infant feeding counselling
Outcomes	**Probability of HIV-1 infection** 12 months: ZDV 23.9 (70/210) (95% CI, 19.0 – 28.8); NVP 15.3 (46/246) (95% CI, 11.2 – 19.4) (P = 0.0083) 18 months: ZDV 25.8 (75/199) (95% CI, 20.7 – 30.8); NVP 15.7 (47232) (95% CI, 11.5 – 19.8) (P =0.0023) **Probability of HIV infection or death** 12 months: ZDV 28.6 (86/210) (95% CI, 23.5 – 33.7); NVP 19.4 (59/246) (95% CI, 14.9 – 23.8) (P =0.0076) 18 months: ZDV 30.7 (92/199) (95% CI 25.5 – 36.0); NVP 20.7 (63/233) (95% CI, 16.2 – 25.3) (P =0.0048) **HIV-free survival for infants uninfected by HIV-1 at 6-8 weeks** 18 months: ZDV 92.2% (n=209); NVP 92.5% (n=241) **Survival for infants infected by HIV-1 at 6-8 weeks** 18 months: ZDV 59.3% (n=35); NVP 59.9% (n=23) **Morbidity (Onyango-Makumbi 2009)(25)**

	The paper published by Onyango-Makumbi et al reports on morbidity data from the HIVNET 012 trial and the HIVIGLOB/NVP trial (one arm of the SWEN trial). The objective of this study was to assess serious gastroenteritis risk and mortality associated with early cessation of breastfeeding in infants. For the purposed of this review, the methods for study will be discussed under HIVNET 012 (methods for HIVIGLOB/NVP trial are described under SWEN). Statistical analyses concentrated on the rates and timing of gastroenteritis events that resulted in hospitalization or death and all cause mortality among HIV-uninfected exposed infants in the HIVIGLOB/NVP and HIVNET 012 trials. Kaplan-Meier estimates were used to determine median duration of breastfeeding in order to take into account censoring. Each child contributed to events and person-time when uninfected. Children were censored from the risk set for all time within a specified age band if found to be HIV-infected during that time. Comparison of cumulative mortality between the two trials was based on the corresponding Kaplan-Meier based cumulative hazards estimates of mortality over the follow-up period. Gastroenteritis was defined as the passage of 3 or more loose or watery stools within 24 hours with or without vomiting. Gastroenteritis associated and overall mortality rates were calculated using the overall number of events divided by the group respective child-months over an 18 month period. Breastfeeding cessation occurred earlier in HIVGLOB/NVP (median 4.0 months) compared to HIVNET 012 (median 9.3 months) **Rates of serious gastroenteritis:** HIVIGLOB/NVP: 8.0 per 1000 child-months HIVNET 012 3.1 per 1000 child months (P<0.001) **Overall gastroenteritis-related death rates** HIVIGLOB/NVP: 0.6 per 1000 child months (95% CI, 0.2 – 1.2) HIVNET 012: 0.1 per 1000 child-months (95% CI, 0.1 – 0.5)
Notes	

Item	Judgement	Description
Adequate sequence generation?	Yes	Computer generated randomization of the drug regimens in permuted blocks of 12. Infants were not randomized according to feeding practices.

Allocation concealment?	No	Allocation concealment not applicable to the review of infant feeding
Blinding?	No	Blinding was not applicable to the review of infant feeding
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	No	Although the study is a randomized controlled trial, feeding practices were collected regarding whether infants were breastfed or not and when they stopped. Exclusive breastfeeding, mixed feeding and exclusive feeding groups were not reported.
Free of other bias?	Yes	No problems apparent

NVAZ

Taha 2007 (26)

Methods	**Study design:** observational cohort of breastfed infants previously enrolled in 2 randomized controlled trials of antiretroviral prophylaxis (nevirapine-zidovudine [NVAZ] studies) in Malawi. **Setting**: six clinics in Blantyre, Malawi **Duration of enrolment:** April 2000 to January 2002 **Definition of feeding practice** Feeding was classified as exclusive or mixed feeding. The study does not give a definition of these feeding practices. **HIV testing** Infants were classified as HIV infected if one HIV RNA test was reactive or two ELISA tests and western blot test was reactive ≥ 18 months. Infants were classified as HIV-uninfected if HIV RNA tests were non-reactive after 6 to 8 weeks or if HIV ELISA tests were negative ≥ 18 months. **Follow-up** Mother-infant pairs were followed up at 1, and 6-8 weeks, then at 3, 6, 9, 12, 15, 18, and 24 months. Maternal-infant information was collected at each visit detailing breastfeeding status and type of feeding (exclusive or mixed). Loss to follow-up: Birth to 1.5 months Total sample: 189 (9.45%) **Analysis** Late postnatal transmission (LPT) was defined as occurring between 6 to 8 weeks and 24 months in infants who were breastfed (hence infant HIV RNA non-reactive at 6 to 8 weeks and infected at a subsequent visit). Infants with indeterminate HIV tests were excluded from the analysis. The estimated timing of HIV transmission

	was the midpoint between the last negative and the first reactive HIV test. Follow-up of infants was censored at the date of weaning. Kaplan-Meier methods of analysis was used to calculate HIV-free survival, cumulative risk of late postnatal transmission and interval hazards obtained for infants not already infected who became infected at 1.5 – 6, 6-12, 12 – 18, and 18 – 24 months.
Participants	Two thousand infants were enrolled in the NVAZ studies. 1256 infants were included in this longitudinal analysis. **Eligibility criteria:** women who were HIV infected with infants who were singleton, not preterm and did not have congenital abnormalities (eg low Apgar score or any conditions that necessitated admission to the intensive care unit). **Baseline characteristics** were similar between the two groups Mean maternal age, SD: NVP+ ZDV 25.2; NVP 24.9 Mother able to read: NVP+ ZDV 140 (25.2%); NVP 116 (21.2%) Electricity in the home: NVP+ ZDV 132 (24.0%); NVP 134 (24.3%) Mean plasma viral load, log_{10}: NVP+ ZDV 4.50 (0-79); NVP 4.55 (0 -85) Maternal education level: Gender of infants: Gestational age (weeks): Infant birth weight , kg: NVP + ZDV 3.09; NVP 3.06 Breastfed at 1 week: NVP + ZDV 473; NVP 457 at 6-8 weeks: NVP + ZDV 491; NVP 475
Interventions	Infants were randomized to one of two groups. Infants received either single dose of oral nevirapine (NVP) (2mg/kg), or NVP (2mg/kg) and oral zidovudine (ZDV) (4mg/kg) 12 hourly for 1 week. Mothers of these infants were administered intrapartum NVP if they presented early for delivery (early presenters). If mothers presented later for delivery (late presenters) they were not provided with NVP. None of the women received antiretroviral treatment while breastfeeding. Median duration of breastfeeding: exclusive 2 months; mixed feeding 12 months
Outcomes	Of the 1256 infants included in the longitudinal analysis, 98 infants were HIV-infected and 1158 were not infected with HIV. At 24 months of age the cumulative risk of late postnatal transmission from 6-8 weeks to 24 months was 9.68% (95% CI 7.80%-11.56%). Among the 1256 infants included in this analysis and not infected with HIV, at 6 to 8 weeks the interval risk (hazard) of HIV infection was 1.22% (95% CI 0.61%-1.83%) from 1.5 to 6 months. The risk of HIV infection increased to 4.05% (95% CI 2.89%-5.19%) from 6-12 months of age. The risk of infection was 3.48% (95% CI

	2.24%-4.70%) between 12-18 months and 1.27% (95% CI 0.33%-2.19%) from 18-24 months. The cumulative risk of late postnatal transmission between 6 to 8 weeks and 24 months of age was 9.68% (95% CI 7.80%-11.56%). Overall, the majority of late postnatal transmission of HIV occurred after 6 months of age (87.4%). The probability of HIV-free survival of infants not infected at 6 weeks of age was 98.7% at 6 months (95% CI 98.1%-99.3%), 94.6% at 12 months (95% CI 93.2%-95.9%), 90.2% at 18 months (95% CI 88.4%-92.0%) and 87.4% at 24 months (95% CI 85.3%-89.6%).
Notes	

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	Yes Unclear	Randomization was by drug regimen "Computer generated random allocations (permutated blocks of 10 with 1:1 allocation) were produced, stratified by clinic". Randomisation by mode of feeding was not conducted..
Allocation concealment?	Yes	"To ensure allocation concealment, randomization instructions were provided to study nurses in sequentially numbered, sealed envelopes that were not opened until the woman had given consent to participate, and their babies were deemed eligible for the study." Allocation concealment is not applicable to mode of feeding.
Blinding?	No	Patients and providers were not blinded according to feeding practice
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

ZVITAMBO

Iliff 2005 (27)

Methods	**Study design:** randomized controlled trial (ZVITAMBO) to measure the impact of single dose postpartum Vitamin A supplementation **Duration of enrolment:** November 1997 to 29 January 2000 **Setting:** maternity clinics and hospitals in Harare, Zimbabwe **Definition of feeding practice:** Exclusive breastfeeding (EBF): classified according to the WHO definition. An exception to the definition was the allowance of one lapse in the exclusivity of breastfeeding at 1 of 3 time points only if the non-breast milk item consumed was a non-milk liquid.

	Predominant breastfeeding (PBF): the infant's predominant source of feeding was breast milk, with non-milk liquids ingested according to the mothers' report at all 3 time points, or at 2 of 3 time points. Mixed breastfeeding (MBF): the infant was given breast milk and either non-human milks, such as infant formula or cows' milk, or solid or semi-solid foods, or both, according to the mothers' reports at one or more time points **HIV testing** HIV testing was carried out once patient contact was complete. The last available plasma sample was tested with GeneScreen ELISA for samples collected at ≥18 months of age. Plasma samples collected from infants' ≤ 18 months were tested using cell pellets by prototype Roche Amplicor version 1.5 qualitative PCR assay (Roche Diagnostic Systems). The infant was classified as HIV-uninfected if the last available sample was negative. No further testing was conducted. For samples that were HIV-reactive, the 3 month pellet was used. If this sample was positive, the baseline and 6 week sample was tested. If these samples were negative, samples were tested in order moving forward through time until 2 successive samples tested positive for HIV. **Follow-up:** Observed length of follow-up: 24 months Lost to follow-up: After birth: 128/4495 (2.8%) mother-infant pairs **Analysis** Turnbull methods were used to calculate postnatal transmission (PNT) in infants who were PCR negative at their 6 week visit (42 days). The 18 month probability of survival was estimated using Kaplan-Meier methods. Cox proportional hazards models were used to determine the effect of early breastfeeding pattern on PNT, or PNT and death, with or without adjustment for confounding variables. Censoring rules regarding HIV rate and survival: censoring of infants who never had a positive HIV test result was done at the age of the last negative test. Infants' whose mothers died or ceased to breastfeed were censored 60 days after the mother's date of death or after breastfeeding was stopped respectively.
Participants	**Sample size**: 4495/14 110 mothers included in the study Number of infants: 2870 infant enrolled, of whom 2060 were included in this analysis Eligibility criteria: mother resident of Harare following delivery, absence of acute, life-threatening illness in mother or infants, singleton infant, and birth weight of ≥ 1500g. **Exclusions:** pregnant women who were HIV-negative or indeterminate HIV status at baseline, infants who died < 6 weeks, or alive at 6 weeks with unknown PCR

	status or positive PCR status. **Baseline characteristics** Women in the EBF group were more likely to be older (P=0.02) and unemployed (P=0.004). Mean weight of infants in the EBF group was higher than infants in MBF and PBF group (P=0.08). Women were more likely to be included in the EBF group after counselling and education intervention was implemented (P<0.0001). Feeding practice: breastfed from birth 2060 EBF 156/2060 (7.5%) PBF 490/2060 (23.8%) MBF1414/2060 (68.6%)
Interventions	Intervention: mother-infants pairs were randomly assigned to one of four Vitamin A treatment groups within 96 hours of delivery. Duration of breastfeeding: 99.1% of mothers breastfeeding at 6 months 94.0% of mothers breastfeeding at 12 months 59.1% of mothers breastfeeding at 18 months Women counselled: little information available on HIV transmission and feeding practices at the commencement of this study; new WHO/UNICEF/UNAIDS guidelines implemented in 1998, women in the study then counselled on risks and benefits of feeding alternatives
Outcomes	**Postnatal HIV transmission among infants uninfected at 6 weeks (EBF n=156; PBF n=490; MBF n=1414)** 6 months: overall 3.9% (95% CI, 3.0-4.7); EBF 1.31% (95% CI 0.00-3.29); PBF 3.03% (95% CI, 1.56 – 4.75); MBF4.40% (95% CI, 3.30 – 5.52) 12 months: overall 7.7% (95% CI, 6.6-9.3); EBF 3.42% (95% CI, 0.70 – 6.82), PBF 7.29% (95% CI, 4.95 – 9.76); MBF 8.41% (95% CI, 6.83 – 10.23) 18 months: overall 12.1% (95% CI, 10.5 – 14.0), EBF 6.94% (95% CI, 2.03 – 12-89), PBF 8.56% (95% CI, 5.47 – 11.63); MBF 13.92% (95% CI, 11.63 – 16.26) 68.2% of late postnatal transmission occurred after 6 months.
Notes	At the commencement of this study little information was available on HIV transmission through breastfeeding. In June 1998 new feeding guidelines were published by WHO/UNICEF/UNAIDS recommending that HIV-infected mothers should be counselled about the risks and benefits of infant feeding alternatives. The ZVITAMBO study was altered to allow for a 24-hour turnaround time of HIV-test results. Study nurses were trained to counsel mothers on safe replacement

	feeding practices.

Item	Judgement	Description
Adequate sequence generation?	Yes	Participants were randomly assigned by computer generated permuted blocks of 12 to the Vitamin A regimen not to feeding practice
Allocation concealment?	Yes	Allocation concealment was not relevant to the review of infant feeding
Blinding?	No	Feeding practice was not blinded
Incomplete outcome data addressed?	Yes	Home visits were conducted to trace participants who did not come for the scheduled study visit.
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

MICRO

Fawzi 2002 (28)

Methods	**Study design**: analysis of data on timing and correlates of HIV-transmission from a randomized controlled trial to examine the effects of vitamin A and other vitamin supplements on mother-to-child transmission of HIV-1 and other clinical outcomes (Fawzi 2002b) **Study setting**: public antenatal clinics in Dar es Salaam, Tanzania **Duration of enrolment**: April 1995 - follow-up continued for 2 years **HIV testing** Infants were classified as HIV positive if DNA polymerase chain reaction (PCR) at any point on peripheral blood mononuclear cell specimen was positive or plasma specimen by ELISA if the infant was ≥ 18 months of age was reactive and confirmed by western blot testing. An infant was HIV uninfected if the blood sample obtained at the last visit was negative for HIV-1 DNA, or if a plasma specimen obtained at the last visit from a child ≥ 18 months was negative by HIV ELISA. **Follow-up** Lost to follow-up: • 28 of 1078 (2.6%) HIV-infected pregnant women who were lost to follow-up at delivery. • 107 of 898 (11.9%) live-born infants were lost to follow-up at 6 weeks. **Analysis**

	The age of the first positive sample and the age of the previous sample if any were used to calculate the follow-up time for the transmission analyses. If no previous samples existed, the ages of the two positive samples were used. To avoid distinguishing between infants who were infected and those who were uninfected, sample follow-up time was the average between the two ages, regardless of infection status. Follow-up time was defined as the minimum of sample follow-up time and age of cessation of breastfeeding. There were 898 infants with known HIV status. Data from 445 children whose mothers were in the no vitamin A arm was used for this analysis as this arm was not associated with increased risk of HIV transmission. Breastfeeding transmission was defined as infection after 6 weeks of age among infants who were uninfected at 6 weeks. The Kaplan-Meier method of analysis was used to calculate the probability of breastfeeding transmission. The data were severely interval-censored because not many PCR or ELISA tests were conducted for each participant. The Turnbull method was use to incorporate the interval censored nature of the data. The two survival curves were similar; therefore only Kaplan-Meier methods were presented.
Participants	**Sample size:** The total number of women randomly assigned to the interventions was 1078. There were 445 infants in the no-vitamin A arm who were tested for HIV infection. There were 312 infants who were HIV-uninfected at 6 weeks and who contributed to the estimate of risk of breastfeeding transmission after 6 weeks. **Eligibility criteria:** HIV-1 infected pregnant women between 12-27 weeks gestation who attended antenatal clinics, were residents of Dar es Salaam, and who intended to live in the city post delivery and for one year thereafter were enrolled in the study. **Baseline maternal and infant characteristics** were similar between the groups. Mean duration of breastfeeding among infants breastfed for ≥ 24 months: 20.3 months (SD = 4.4 months; median 20.5 months) Children alive and breastfeeding, %: 3 months: 96% 6 months: 95% 12 months: 92% 18 months: 79% 24 months: 25%
Interventions	The women were randomly assigned to receive a daily oral dose of one of four regimens - vitamin A alone; multivitamins excluding vitamin A (vitamins B_1, B_2, B_6, niacin, B_{12}, C, E, and folic acid); multivitamins including vitamin A; placebo.

	All women were counselled about the benefits and risks associated with the different infant feeding regimes according to guidelines from the World Health Organisation and the Tanzanian Ministry of Health. Mothers then made the decision on their choice of infant feeding based on social circumstances. At each monthly visit, women were questioned on infants being breastfed, and the date at of cessation of breastfeeding if relevant. The period of breastfeeding was taken as the mean of the age at which the child was last reported as breastfeeding and the age at which the child was reported as not breastfeeding for at least two consecutive monthly sessions.
Outcomes	**Cumulative incidence HIV transmission through breastfeeding in infants uninfected at 6 weeks (N=312)** 6 months: 4.6 (13/312) (95% CI, 2.1 – 7.0) 12 months: 7.8 (21/312) (95% CI, 4.6 – 11.1) 15 months: 11.3 (29/312) (95% CI, 7.4 – 15.2) 18 months: 13.3 (33/312) (95% CI, 9.1 – 17.5) 21 months: 15.4 (36/312) (95% CI, 10.6 – 20.2) 24 months: 17.9(37/312) (95% CI, 11.2 – 24.5) The test for trend over time was not significant (P = 0.40) There were 37 infections during the 4372 child-months of follow-up evaluation, or 10.2 cases per 100 child-years.
Notes	

Risk of bias table

Item	Judgement	Description
Adequate sequence generation?	No	No applicable to this review
Allocation concealment?	Yes	"Active tablets and placebo were identical in size and colour." However, allocation concealment of the vitamin A status is not relevant to this review.
Blinding?	No	"Breastfeeding was almost universally adopted in this population"
Incomplete outcome data addressed?	Yes	"Turnbull method was used to incorporate the interval censored nature of the data"
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

OBSERVATIONAL TRIALS – PROSPECTIVE COHORTS

VTS – Vertical Transmission Study

Coovadia 2007 (29)

Methods	**Study design:** Non-randomized intervention cohort **Duration of enrolment:** 29 Oct 2001 to 16 April 2005 **Setting:** antenatal clinics in KwaZulu-Natal, South Africa (7 rural, 1 semi-urban, 1 urban) **Blinding**: Patient: not blinded Assessor: field monitors and feeding counsellors were unaware of mothers' HIV status **Definition of feeding practice:** Exclusive breastfeeding was defined according to WHO guidelines. The protocol allowed the inclusion of 3 days of water or formula milk, either on separate or continuous days, without exclusion from the exclusive breastfeeding group. The authors of the study also allowed paracetamol administration for up to 7 days and oral rehydration solution for treatment of diarrhoeal disease up to 72 hours. Replacement feeding was defined as feeding with any non-human milk, and the exclusion of all breastmilk, with or without other liquids or solids. Mixed breastfeeding was defined according to WHO, UNICEF and UNAIDS guidelines as breastfeeding and providing non-human milk, other liquids, or solids. **Follow-up: (censored at ±6 months)** Planned length of follow-up: 26 weeks Observed length of follow-up: 26 weeks Lost to follow-up: • At birth: 14/1372 (1.0%) • Birth to 4 weeks: 32/1276 (2.5%) • 4-14 weeks: 60/1183 (5.1%) • 14-26 weeks: 106/1077 (4.4%)
Participants	**Sample size**: Number of women Number of infants: 1276 infants with complete data on infant feeding modality Inclusion criteria: HIV-infected and uninfected pregnant women were enrolled in the study if they were ≥ 16 years, planned to reside in the study area for at least 3

	months following delivery, and provided written informed consent. **Exclusions:** **Baseline characteristics** in the overall group (n=1372) Median maternal age: 25.1 (16.1 – 45.8) Enrolment clinic: urban 257 (19%), semi-urban 514 (37%), rural 601 (44%) Some primary to 10 years education: 1087 (79.2%) House type: 761 (55%) concrete, 423 (31%) board/mud/sticks/stones Toilet type: 249 (18%) flush/chemical, 792 (58%) pit latrine/bucket, 289 (21%) none Water type: 536 (39%) piped indoors/yard, 384 (28%) piped public tap, 297 (22%) river Maternal CD4 count (per µl): 453 (2 - 1835) Gender of infants: 676 (49%) male Birth weight of infants (g): 3100 (1150-4600) Feeding practice: exclusively breastfed from birth 1132 (83%) replacement from birth109 (8%) mixed breastfed from birth 35 (3%) feeding information unavailable 96 (7%)
Interventions	Intervention: exclusive breastfeeding Median duration of exclusive breastfeeding: 159 days (first quartile to third quartile 122 – 174 days) for women who initiated exclusive breastfeeding and for infants who had HIV-1 test results (n=1034) Women counselled: in pregnancy about infant feeding modality
Outcomes	**Probability of HIV-free survival** 1 month: 0.8484 exclusively breastfed (797/1034), 0.8263 replacement fed (54/101), 0.8156 mixed fed (5/32) 3 months: 0.7901 exclusively breastfed (615/1034), 0.7582 replacement fed (30/101) 6 months: 0.7541 exclusively breastfed (410/1034), 0.7582 (17/101) replacement fed **Cumulative mortality** 3 months: 6.1% (95% CI 4.74 – 7.92) in exclusively breastfed infants, 15.1% (95% CI 7.63-28.73) in replacement fed infants (HR 2.06; 95% CI, 1.00-4.27, P=0.51)
Notes	This study is the VTS study and presents findings for the first 6 months of life. Rollins et al 2008 is also a VTS study extending the follow-up to 18 months

Item	Judgement	Description
Adequate sequence generation?	No	Not a randomized controlled trial
Allocation concealment?	No	Not a randomized controlled trial
Blinding?	No	The study was not blinded
Incomplete outcome data addressed? All outcomes	Yes	"Data for nevirapine uptake and ingestion were inconsistent and are not included in the analysis"
Free of selective reporting?	Yes	No apparent problems
Free of other bias?	Yes	No apparent problems

Rollins 2008 (30)

Methods	**Study design** Non-randomized intervention cohort Duration of enrolment: October 2001 to mid-April 2005 Setting: rural and semi-urban antenatal clinics in KwaZulu-Natal, South Africa Blinding: Not applicable **Follow-up:** Planned length of follow-up: 18 months Observed length of follow-up: 18 months Lost to follow-up : • 82/1400 immediately after birth • 56/1193 by 3 months of age • 45/952 between 3 and 6 months of age **Definition of feeding practices**: WHO definition for exclusive breastfeeding, mixed breastfeeding and replacement feeding **Analysis** The analysis was based on a database created in April 2007 and consisted of live-born children of HIV-infected women. Kaplan-Meier methods of analysis were used to assess cumulative HIV-free survival probabilities in the first 18 months of life. Feeding categories were ascertained from the analytical database by applying algorithms that first classified infants according to feeding practices on each day of life and then determined the cumulative pattern from birth. The 18-month HIV-free survival was calculated firstly, according to feeding practices initiated at birth, and secondly on cumulative feeding recall histories. Exclusively breastfed infants that were given additional fluid or milk even on one occasion were immediately reclassified as mixed fed, regardless of subsequent feeding practices.

	Censoring rules regarding HIV rate and survival: • Infants with missing data were censored at time the infant was last seen. • Infants with no feeding or test data were excluded from the analyses
Participants	**Sample size**: Number of women:1460 Number of infants: 1193 **Inclusion criteria:** Pregnant women attending antenatal clinics with confirmed diagnosis of HIV-1 **Exclusions:** multiple births, stillbirths, infants not tested for HIV infection **Baseline characteristics** between women who breastfed < 6 months (n=136) compared to women who breastfed >6 months (n=688) • Unemployed: 19.9% versus 11.5% (P=0.001) • Education level: similar in both groups • Access to toilets: similar in both groups • Mean CD4 count (cells/mm ^{3}): 487 versus 514 cells/µl (P=0.24) • Proportion with CD4 < 200cells/µl: 10.8% versus 8.1% (P=0.31) **Feeding practice**: • exclusively breastfed from birth 937/1193 (78.5%) • exclusively replacement fed from birth 118/1193 (9.9%) • mixed fed from birth 105/1193 (8.8%) ▪ breast milk and water based milk or solids 75 (71.4%) ▪ breast milk and formula milk 30 (28.6%) ▪ infants excluded from analysis as received water only or intravenous feeds or missing feeding data 33/1193 (2.8%)
Interventions	Intervention: Breastfeeding or replacement feeding Duration of exclusive breastfeeding: from birth to 3 months 533 (56%) from birth to 6 months 278 (31%) Duration of mixed feeding: from birth to 3 months 259 (27%) from birth to 6 months 410 (46%) Duration of exclusive replacement feeding: from birth to 3 months 78 (8%) from birth to 6 months 69 (8%) Women counselled: during pregnancy about infant feeding options **Maternal and infant ARV:** single-dose nevirapine were provided from 28 weeks gestation to all women and their infants

Outcomes	Exclusive breastfeeding n=937 Mixed feeding n= 105 Replacement feeding n =118 **Probability of HIV free survival (n=1160)** 6 months (n=841): exclusive breastfeeding 0.79 (95% CI, 0.77 – 0.82), mixed feeding 0.81 (95% CI, 0.71 – 0.87) and exclusively replacement feeding 0.82 (95% CI, 0.74 – 0.88) 12 months (n=703): exclusive breastfeeding 0.76 (95% CI, 0.74 – 0.79), mixed feeding 0.81 (95% CI, 0.71 – 0.87) and exclusively replacement feeding 0.80 (95% CI, 0.72 – 0.87) 18 months (n=592): exclusive breastfeeding 0.75 (95% CI, 0.72 – 0.78), mixed feeding 0.79 (95% CI, 0.70 – 0.86) and exclusively replacement feeding 0.80 (95% CI, 0.72 – 0.87) **Probability of survival (n=1160)** 6 months (n=841): exclusive breastfeeding 0.93 (95% CI, 0.91 – 0.94), mixed feeding 0.97 (95% CI, 0.91 – 0.99) and exclusively replacement feeding 0.91 (95% CI, 0.84 – 0.95) 12 months (n=703): exclusive breastfeeding 0.89 (95% CI, 0.86 – 0.90), mixed feeding 0.94 (95% CI, 0.86 – 0.97) and exclusively replacement feeding 0.89 (95% CI, 0.81 – 0.93) 18 months (n=592): overall probability of survival 0.76 (95% CI, 0.73-0.78) exclusive breastfeeding 0.86 (95% CI, 0.83 – 0.88), mixed feeding 0.87 (95% CI, 0.78 – 0.93) and exclusively replacement feeding 0.87 (95% CI, 0.78 – 0.92) **HIV transmission** 18 months: overall risk of transmission for breastfeeding was 9.1 cases per 100 child-years (95% CI, 5.8-12.5) **Mortality** 18 month probability of death HIV infected 0.53 (95% CI, 0.46-0.60) HIV uninfected 0.04 (95% CI, 0.03-0.06)
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	Not applicable
Allocation concealment?	No	Not applicable
Blinding?	No	Not a randomized controlled trial
Incomplete outcome data addressed?	No	"An independent group of field monitors visited mothers at home every week from birth until the infant was 9 months of age and documented all feeds and morbidity episodes for each day of the preceding week"
Free of selective reporting?	Unclear	No problems apparent
Free of other bias?	Yes	No problems apparent

DITRAME PLUS

Becquet 2007 (31)

Methods	**Study design:** open-label cohort **Duration of enrolment:** March 2001 and July 2003 **Setting:** six community-run health facilities in Abidjan, Côte d'Ivoire **Definition of feeding practice:** Feeding practices were defined according to WHO guidelines. Exclusive breastfeeding: children received no other food or drink, including no water, in addition to breastfeeding with the exception of medicines, vitamin drops or syrups, and mineral supplements Predominant breastfeeding: children who were breastfed and also given small amounts of water or water-based drinks. Food based fluid, solid foods or non-human milk was not allowed under this definition Mixed feeding: children who were breastfed and also given non-human milk or solid foods Artificial feeding: children who were fed artificial feeds (including non-human milk such as infant formula and powdered animal milk) and not breastfed at all. **HIV testing** Blood samples were taken at day 2 then 4, 6, and 12 weeks of age and every 3 months thereafter until 2 months after breastfeeding was completely stopped. HIV infection was determined by positive HIV-1 RNA PCR taken at any age or by reactive HIV serology if ≥ 18 months. Peripartum transmission was defined as HIV infection at 4 weeks of age. Late postnatal transmission was defined as HIV infections occurring beyond 4 weeks of age

	assessed on negative RNA PRC from a sample obtained at ≥ 30 days of age. **Follow-up:** Mother-infant pairs were followed up at birth, 48 hours after delivery, weekly until 6 weeks of age, monthly until 9 months, then 3 monthly until 2 years of age. Planned length of follow-up: 24 months Observed length of follow-up: 18 months Lost to follow-up There were 44/808 (5.4%) women lost to follow-up at delivery. Before 18 months of age, 43 (14%) children in the formula feeding group and 65 (20%) children in the breastfeeding group (P=0.006) were lost to follow-up. Definitive HIV status was ascertained for all infants in the formula feeding group and 38 of 65 (69%) infants lost to follow-up in the breastfeeding group had a definitive HIV test 2 months after complete cessation of breastfeeding. Therefore, definitive HIV status was possible for 69% of infants lost to follow-up in the breastfeeding group. **Analysis** Timing of HIV transmission was estimated to be at the midpoint of the date of the last negative HIV test and the first positive HIV test. The intervals between the HIV tests were short (less than 3 months) hence Kaplan-Meier methods were appropriate as opposed to Turnbull's extension of Kaplan-Meier methods. Kaplan-Meier methods of analysis were used to calculate the cumulative probability of transmission at 18 months of age among infants in the peripartum period. Cox regression analyses were used to adjust for maternal and infant variables. Some women did not express their choice of infant feeding antenatally therefore allocation to feeding group was based on mode of feeding 2 days after delivery as reported by the mother
Participants	**Sample size**: There were 730 of 808 eligible women enrolled in the study. Number of infants: 622 infants uninfected during the peripartum period were included in this analysis – 298 in the formula feeding group and 324 in the breastfeeding group **Eligibility:** HIV-1 or HIV-2 infected pregnant women ≥ 18 years diagnosed within one of the six facilities, at < 36 weeks gestation, and haemoglobin ≥ 7g/dl were included in the study. **Exclusions:** second or third born infants of multiple pregnancies, stillborns, neonates not tested for HIV infection , infants infected in the peripartum period, and infants with unknown timing of transmission **Baseline characteristics** were similar between the breastfeeding group (n=324) and the formula feeding group (n=298) Median maternal age (interquartile range [IQR]): breastfeeding group 26.7 (5.4); formula feeding 27.2 (5.1) (P=0.22) Median maternal CD4 count (cells/mm ^{3}): breastfeeding group 400; formula feeding 405 (P=0.21)

	No maternal education: breastfeeding 141 (43.5%); formula feeding 73 (24.5%) (P<0.001) Maternal employment in formal sector: breastfeeding 172 (53.1); formula feeding 150 (50.3%) (P=0.49) Access to water with tap inside home: breastfeeding 84 (25.9); formula feeding 124 (41.6) (P<0.001) Individual housing: breastfeeding 96 (29.6); formula feeding 128 (43.0) (P<0.001) Peripartum scZDV + sdNVP: breastfeeding 150 (46.3); formula feeding 179 (60.1) (P<0.001) Peripartum scZDV+sc3TC+sdNVP: breastfeeding 174 (53.7); formula feeding 119 (39.9) Female infant: breastfeeding 159 (49.1); formula feeding 145 (48.7) (P=0.92) Birth weight of infants < 2.5kg: 87 (26.9); 69 (23.1) (P=0.29) Feeding practice Median duration of breastfeeding: 124 days (interquartile range, 97 -199) Breastfeeding duration ≥ 6 months: n= 92 Breastfeeding duration < 6 months: n=232 Exclusive breastfeeding during the first month of life: yes (n=75); no (n=249) Exposure to mixed feed during the first month of life: yes (n=27), no (n=297)
Interventions	**Maternal ARV 2001 – 2002** Mothers received oral zidovudine (ZDV) daily from 36 weeks of gestation and single dose nevirapine (sd NVP) at the onset of labour. Infants received directly observed sd-NVP on day 2 and 1 week of zidovudine syrup. **2002 – 2003** The maternal regimen was provided from 32 weeks gestation with the addition of lamivudine (3TC). ZDV+3TC were provided to the mothers for 3 days postpartum. The neonatal regimen was not altered. **Feeding practice** Women were counselled individually regarding the benefits and risk associated with breastfeeding and the safe preparation of formula. They were then offered two alternative option to prolonged breastfeeding; either exclusively formula feeding from birth (with a drug inhibiting lactation), or exclusive shortened breastfeeding with early cessation within the 4th month. All women could express their choice antenatally and were supported in their choice of feeding. Both options were provided free of charge with the provision of free infant formula milk and utensils needed from birth or the date of weaning until 9 months of age.
Outcomes	18 months: 15 children acquired HIV-infection postnatally, 13 in the breastfeeding group and 2 in the formula feeding group. Overall estimated risk of late postnatal transmission 8.7 per 100 child years of breastfeeding (95% CI, 4.4 – 13.0)

Probability of remaining free from postnatal HIV infection

6 months: formula feeding 0.99 (95% CI, 0.98 – 1.00); breastfeeding ≤ 6 month 0.98 (95% CI, 0.96 – 0.99); breastfeeding > 6 months 0.97 (95% CI, 0.90 – 0.99)

12 months: formula feeding 0.99 (95% CI, 0.97 – 1.00); breastfeeding ≤ 6 month 0.98 (95% CI, 0.96 – 0.99); breastfeeding > 6 months 0.92 (95% CI, 0.94 – 0.96)

18 months: formula feeding 0.99 (95% CI, 0.97 – 1.00); breastfeeding ≤ 6 month 0.98 (95% CI, 0.96 – 0.99) (log rank test, P<0.001); breastfeeding > 6 months 0.90 (95% CI, 0.81 – 0.95)

In multivariate analysis, breastfeeding beyond 6 months and mixed feeding were associated with 7.5 (P=0.003) and 6.3 (P=0.04) times greater risk of postnatal transmission respectively.

24 months

Leroy 2008(32)

Study population: 808 women included in the ANRS 1201/1202 Ditrame-Plus cohorts with enrolment of 711 first live-born infants. Of these, 375 received ZDV + sd-NVP and 336 were given ZDV+3TC+sdNVP. From September 1995 to 1998, 249 first live-born infants whose mothers received ZDV only were included in the historical cohort in the same health facilities. There were 241 children exposed to ZDV and breastfeeding long-term (reference group).

HIV transmission according to infant feeding modality

	DITRAME ZDV (1994 – 1999)	**Ditrame-Plus ZDV+sdNVP (2001-2002)**		**Ditrame-Plus ZDV+3TC+sdNVP (2002 – 2003)**	
	Long term breastfed (n=238)	**Formula-fed (n=195)**	**Shortened breastfed (n=169)**	**Formula fed (n=126)**	**Shortened breastfed (n=198)**
HIV-1 infected (%)	**47 (19.8)**	**18 (9.2)**	**22 (13.0)**	**7 (5.6)**	**13 (6.6)**
Peri-partum infection (<4 weeks) (%)	**28 (60)**	**16 (89)**	**9 (41)**	**7 (100)**	**10 (77)**
Postnatal infection (≥ 4 weeks) (%)	**15 (32)**	**2 (11)**	**11 (50)**	**0 (0)**	**2 (15)**
Timing of	**4 (8)**	**0(0)**	**2 (9)**	**0(0)**	**1 (8)**

Notes	Becquet 2009(33) and Becquet 2008 (34) not included in this table but data will be extracted to populate grade profiles for recommendations

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	Yes	
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

MITRA

Kilewo 2008 (35)

Methods	**Study design:** open-label nonrandomized prospective cohort study investigating prevention of mother to child transmission of HIV through breastfeeding by treating infants prophylactically with Lamivudine **Duration of enrolment:** August 2001 to August 2003 **Setting:** Three primary care antenatal clinics, one each from the 3 municipal districts of Dar es Salaam and from the antenatal clinic at the Muhumbili National Hospital in Dar es Saalam, Tanzania (same setting as Petra trial). **Definition of feeding practice:** WHO definitions of feeding practice are followed by the study **HIV testing** **Maternal** • HIV antibody screening carried out using Capillus rapid simple assay (Trinity Biotech, Bray, Ireland) • Reactive samples were tested on the Determine rapid simple assay (Abbott Laboratories, Tokyo, Japan) • Confirmatory tests were carried out using 2 anti-HIV enzyme-linked immunosorbent assays (ELISAs) • Maternal HIV infection was defined as two positive ELISA tests.

	• Women with discordant results were tested using Western blot assay; if positive the women was classified as HIV-1 infected **Infant** • HIV tests performed at 6 weeks, 3 and 6 months of age • HIV-1 DNA version 1.5 qualitative polymerase chain reaction (PCR) assay (Roche Diagnostics, Branchburg, NJ) was used to detect HIV infection • Positive PCR test was confirmed by repeat PCR at the next visit • HIV-infected was defined as two positive HIV test results • Children who died or were lost to follow-up after one positive test result were considered HIV-1 positive in the analyses **Follow-up:** Mother-infant pairs were followed up 1, 3, and 6 weeks and thereafter at 3, 6, 9, 12, 15, 18, 21 and 24 months of age. Planned length of follow-up: 24 months Observed length of follow-up: 6 months Lost to follow-up • 13 of 468 (2.8%) mothers enrolled lost to follow-up before delivery • 31 of 455 (6.8%) infants lost to follow-up without an HIV test **Analysis** Kaplan-Meier methods were used to estimate HIV-1 transmission, mortality, and the composite outcome “HIV infection or death”, and breastfeeding. Timing of HIV-1 infection was defined as the midpoint between the date of the last negative and the first positive test. A direct comparison was conducted between the Mitra study and the Petra study Arm A, facilitated by individual data provision by the data management centre at the International Antiviral Therapy Evaluation Centre. Censoring rules regarding HIV rate and survival: Uninfected infants (considered at risk only while breastfeeding continued) were censored on the day of breastfeeding cessation or the date of the last visit to the clinic if the child was still breastfed at that time. HIV infections up to 6 months following delivery were considered as events. In the analysis of HIV infection or death, censoring at cessation of breastfeeding was not done.
Participants	**Sample size**: Number of women: 468 of 1029 eligible women enrolled in the study Number of infants: 470 infants delivered including twins. There were 398 infants included in the transmission analysis. **Eligibility criteria:** HIV-1 seropositivity , intention to breastfeed, haemoglobin >7g/dL, age ≥ 18 years, willingness to take drugs or administer drugs to the newborn

	as prescribed, resident of Dar es Salaam, deliver at the study site, and follow-up with the study for 18 months **Exclusions:** second born twins **Baseline characteristics** were similar between the Mitra (n=398) Median maternal age (IQR): 26 (23 – 30) Median Hb, g/dL (IQR): 9.6 (8.6 – 10.6) Median CD4 count (cells/µL)(IQR): 411 (269 – 611) CD4 count < 200 cells/µl: 15.4% Caesarean section: 18.6% Maternal education level: not reported Female infants: 53% Birth weight of infants <2.5kg: 14.6% Feeding practice from birth: 6 week: 95% 12 week: 86% 16 week: 61% 20 week: 44% 24 week: 30% 26 week: 18% 28 week: 15%
Interventions	Pregnant women were randomized into the study at 36 weeks gestation. **Maternal ARV:** antiretroviral (ARV) treatment according to Petra Arm A regimen, which was 300mg zidovudine (ZDV), lamivudine (3TC) 150mg 12 hourly from 36 weeks of gestation, intrapartum, and for 1 week postpartum. **Infant ARV:** ZDV 4mg/kg 12 hourly and 3TC 2mg/kg 12 hourly from birth to 1 week of age (Petra Arm A) and thereafter with 3TC alone2mg/kg 12 hourly from 2 to 4 weeks and 4mg/kg administered 12 hourly after 4 weeks during breastfeeding (for a maximum of 6 months) and 2 weeks after cessation of breastfeeding. Women counselled: in pregnancy to exclusively breastfeed with weaning initiated between 5 and 6 months. The study did not provide replacement feeding for infants, except in case of failure to thrive after cessation of breastfeeding.
Outcomes	**Kaplan-Meier cumulative risk of transmission of HIV-1** Overall at 6 months (n=380): 4.9% (95% CI, 2.7 – 7.1%)

	Kaplan-Meier Cumulative risk of HIV-1 infection in infants uninfected at 6 weeks 6 months: 1.2% (4/380) (95% CI, 0.0 – 2.4%) **Kaplan-Meier estimated HIV-1 infection or death rate** 6 months: 8.5% (95% CI, 5.7 – 11.4%) **Kaplan Meier estimated mortality** 6 months: 3.7% (95% CI, 1.9 – 5.6%)
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	Yes	Infants who missed 2 consecutive study visits were traced to their homes. Information about participants who had moved away from the study site was obtained from friends or relatives. The sample size was estimate at 450 mothers to allow for loss to follow-up.
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	No	Although the study follows the WHO definitions of infant feeding, no further data was collected from mothers about their feeding practices, for instance mixed feeding.

MITRA PLUS

Kilewo 2009 (36)

Methods	**Study design:** Open-label nonrandomized prospective cohort study of mothers on HAART (zidovudine [ZDV], lamivudine [3TC], and nevirapine [NVP]) while breastfeeding **Duration of enrolment:** April 2004 to June 2006 **Setting:** Three primary care antenatal clinics, one each from the 3 municipal districts of Dar es Salaam and from the antenatal clinic at the Muhumbili National Hospital in Dar es Saalam, Tanzania (same setting as Petra trial and Mitra study).

	Definition of feeding practice: WHO definitions of feeding practice were followed by the study but no data collected on mixed feeding and exclusive replacement feeding **HIV testing** **Maternal** • HIV antibody screening carried out using Capillus rapid simple assay (Trinity Biotech, Bray, Ireland) • Reactive samples were tested on the Determine rapid simple assay (Abbott Laboratories, Tokyo, Japan) • Confirmatory tests were carried out using 2 anti-HIV enzyme-linked immunosorbent assays (ELISAs) • Maternal HIV infection was defined as two positive ELISA tests. • Women with discordant results were tested using Western blot assay; if positive the women was classified as HIV-1 infected **Infant** • Infants were tested at 6 weeks and at 3, 6, and 9 months by the Amplicor HIV-1 DNA version 1.5 qualitative polymerase chain reaction (PCR) assay (Roche Diagnostics, Randburg, South Africa) • At 12, 15 and 18 months children were tested by Enzyngost anti-HIV 1+2 plus ELISA. Reactive samples were tested with a second ELISA test (Vironostika HIV uniform II antigen/ antibody ELISA). • Amplicor HIV-1 RNA Monitor version 1.5 assay were used to test samples that were positive by ELISA at 12 and 15 months (diagnostic threshold 10 000 copies/ml). • HIV infection in infants was defined as having 2 consecutive positive tests **Follow-up:** Mother-infant pairs were followed up 1, 3, and 6 weeks and thereafter at 3, 6, 9, 12, 15, 18, 21 and 24 months of age. Planned length of follow-up: 24 months Observed length of follow-up: 18 months Lost to follow-up • There were 9 of 501 (1.8%) women lost to follow-up before delivery • There were 14 of 491 (2.9%) of infants lost to follow-up with unknown HIV status **Analysis**

	Kaplan-Meier methods were used to estimate HIV-1 transmission, mortality, and the composite outcome "HIV infection or death", and breastfeeding. Timing of HIV-1 infection was defined as the midpoint between the date of the last negative and the first positive test.
Participants	**Sample size** Number of women: there were 501 of 1508 eligible women enrolled in the study Number of infants: there were 491 live born infants, of whom 441 mother-infant pairs were included in the transmission analysis **Eligibility criteria:** HIV-1 seropositivity , intention to breastfeed, haemoglobin >7g/dL, age ≥ 18 years, willingness to take drugs or administer drugs to the newborn as prescribed, resident of Dar es Salaam, deliver at the study site, and follow-up with the study for 18 months **Exclusions:** second born twins **Baseline characteristics** were similar between the two groups Median maternal age (IQR): 26 (24 – 30) Median CD4 count ,cells/mm ^{3} (IQR): 415 (265 – 577) When CD4 count was obtained: not reported Median plasma viral load (IQR): 14621 (2954 – 59 738) Maternal education level: not reported Female infants: 49% Median birth weight of infants, kg: 2.9 (2.5 – 3.2) Birth weight < 2.5kg: 16.8% Kaplan Meier estimates of feeding practice from delivery: 6 week: 97% 12 week: 90% 16 week: 80% 20 week: 74% 24 week: 51% 26 week: 17% 28 week: 13%
Interventions	Women were enrolled into the study was at 34 weeks gestation. Women with symptomatic HIV infection (WHO clinical stage 3 or 4) or with CD4 count < 200 cells/µl HAART was started earlier than 34 weeks if possible. **Maternal ART/ARV** • ZDV 12 hourly + 3TC 150mg 12 hourly + NVP 200mg lead dose for 2

	weeks; increased to 400mg per day given in 2 doses the rest of the study period • Same regimen continued intrapartum and postnatally for 6 months, then stopped • ZDV + 3TC was continued for 1 week after stopping NVP • Women who had adverse reaction to NVP were administered nelfinavir (NFV) • From 1 October 2005, women with CD4 > 200 cells/µl were given NFV instead of NVP because of NVP-related side effects in women with CD4 > 250 cells/µl **Infant ARV** • ZDV 4mg/kg 12 hourly + 3TC 2mg/kg 12 hourly from birth to 1 week of age (same as Petra Arm A) Women counselled: in pregnancy to exclusively breastfeed with weaning initiated between 5 and 6 months. The study did not provide replacement feeding for infants, except in case of failure to thrive after cessation of breastfeeding.
Outcomes	**Kaplan-Meier estimated HIV-1 infection or death in children negative at 6 weeks** 6 months: 8.6% (39/400) (95% CI, 6.0 -11.2) 9 months: 11.2% (50/388) (95% CI, 8.2 – 14.2) 12 months: 12.8% (57/369) (95% CI, 9.6 – 16.0) 18 months: 13.6% (60/334) (95% CI, 10.3 – 16.9) **Kaplan-Meier estimated transmission of HIV-1** 8 infants were PCR uninfected at 6 weeks but became infected for the first time at 3 months (n=1), 6 months (n = 3), 9 months (n = 1), 12 months (n = 2) or 15 months (n = 1) 6 months: 5.0% (22/397)(95% CI, 2.9 – 7.1) 9 months: 5.3% (23/387) 95% CI, 3.2 – 7.4) 12 months: 5.8% (25/368) (95% CI, 3.6 – 8.0) 18 months: 6.0% (26/333) (95% CI, 3.7 – 8.3) **Kaplan-Meier estimated mortality** 6 months: 4.3% (19/417) (95% CI, 2.4 – 6.2) 9 months: 7.1% (27/407) (95% CI, 4.7 – 9.5)

	12 months: 8.5% (37/386)(95% CI, 5.8 – 11.2) 18 months: 9.2% (40/350) (95% CI, 6.4 – 12.0)
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	Yes	Home tracing on children who missed two consecutive study visits. Information was collected from friends or relatives if participants moved. A minimum of 450 mothers were required to allow for deaths and loss to follow-up.
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	No	Data was not collected on feeding practices although the study supported exclusive breastfeeding.

KIBS

Lando 2009 (37) 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention (abstract)

Methods	**Study design:** Phase IIb open-label one arm trial assessing maternal HAART. **Duration of enrolment:** July 2003 to November 2006 **Setting:** Kisumu, Kenya **Definition of feeding practice:** Women were advised to exclusively breastfeed for 6 months with rapid weaning. No definition of exclusive breastfeeding is provided in the abstract. **Follow-up:** Planned length of follow-up: 24 months Observed length of follow-up: 18 months Lost to follow-up was not reported in the abstract. **Analysis**

	Kaplan-Meier methods were used to estimate rates of HIV infection overall, by infant sex, by maternal enrolment CD4 cell count (≤ 250, or >250 cells/mm^3), and by initial regimen (nevirapine or nelfinavir) for mothers with CD4 cell counts > 250 cells/ mm^3. The abstract does not report censoring rules with regards to breastfeeding cessation.
Participants	**Sample size**: There were 601 women screened, of which 522 were enrolled. Number of infant: 502 infants born alive. 497 infants were included in the analysis of HIV infection **Eligibility:** HIV infected pregnant women with CD 4 >250 cells/mm^3 who chose to breastfeed following counselling **Baseline characteristics** were not reported in the abstract. Median age in years: 23 Median CD4 count, cells/mm^3: 392 Median plasma viral load, $\log_{10}$: 4.5
Interventions	**Maternal ART/ARV:** women received Combivir and Nevirapine (NVP) initiated at 34 weeks gestation through 6 months postpartum. The regimen was later changed to Nelfinavir for maternal CD > 250 cells/mm^3 (because of possible hepatotoxicity with NVP in women with CD4 over 250). **Infant ARV:** single dose NVP at birth
Outcomes	**HIV transmission** **Overall** 6 months: 5.0% (95% CI, 3.4 – 7.3) 12 months: 5.9% (95% CI, 4.0 – 8.5) 18 months: 6.7% (n=29) No differences were found in HIV transmission rates by maternal CD4 count or HAART. **Mortality** 12 months: 44/502 (87.6 per 1000 live births) The 3 most common causes of mortality were gastroenteritis [17 (39%)], pneumonia [7 (16%)], and respiratory distress [5 (11%)]. Five of the 44 deaths were in HIV-infected infants, of which 2 deaths were due to gastroenteritis, 2 due to pneumonia and 1 death due to tuberculosis. Fifteen (34%) deaths were due to gastroenteritis around the weaning period.
Notes	

Item	Judgement	Description

Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	
Free of selective reporting?	Unclear	Abstract reviewed
Free of other bias?	Unclear	Abstract reviewed

AMATA

Peltiera 2009 (38)

Methods	**Study design:** Non-randomized intervention cohort study **Duration of enrolment:** May 2005 to January 2007 **Setting:** four public sector health facilities in Rwanda of which 1 was rural, 2 were urban and 1 was semi-rural **Definition of feeding practice:** Mixed feeding was defined according to WHO definitions as a breast fed infant who received any solids or liquids (with the exception of drugs) even once. **HIV testing** All infants were tested at birth, 6 weeks, and 3, 7, and 9months of age by DNA PCR (Amplicor technique version 1.5; Roche Molecular Systems, Branchburg, New Jersey, USA). *In utero* transmission was defined as infants with HIV-1 DNA PCR reactive within 48 hours of life and confirmed by a second test. Peripartum transmission was defined as a non-reactive result at birth followed by a positive result at 6 weeks of age. Breastmilk transmission was defined as infants who had a non-reactive PCR test from birth and day 15 and were then subsequently tested positive with DNA PCR. All positive HIV tests were confirmed by a second test. **Follow-up:** Mother-infant pairs were followed up at birth (within 48 hours), 15 days, 6 weeks and at 3, 6, 7, and 9 months following delivery. Adherence to HAART and feeding method were assessed by maternal interview and clinical examination. Planned length of follow-up: 9 months Observed length of follow-up: 9 months Lost to follow-up:

	11/562 (2.0%) women lost to follow-up in the prepartum period 18/532 (3.4%) infants lost to follow-up after delivery **Analysis** The allocation to a feeding group was on the basis of feeding modality chosen before delivery. Kaplan-Meier methods of analysis were used to calculate the 9 month cumulative probabilities of events. Comparisons were made using the log-rank test. Infants were censored at the date of last available HIV test if lost to follow-up. Lost to follow-up was defined as having missed planned appointments after having been actively sought at home. The timing of HIV transmission was estimated at the midpoint between the last negative test and the first positive test. Cox model was used to determine factors for HIV-free survival.
Participants	**Sample size**: There were 562 women enrolled in the study - 240 chose breastfeeding with HAART and 322 chose formula feeding. There were 532 mother-infant pairs included in this analysis **Eligibility:** HIV-infected pregnant women from 28 weeks gestation entering the PMTCT programmes in the 4 centres in Rwanda **Exclusions:** second born twins **Baseline characteristics** were similar between the two groups Median maternal age, years: formula 29; breastfeeding + HAART 28 (P=0.000) Mean CD4 count (/µl): formula 434; breastfeeding + HAART 498 (P=0.005) When CD4 count was obtained: at delivery (within 48 hours) and 6 months postpartum Delivery plasma viral load: formula 247; breastfeeding + HAART 184 Maternal education level: None 461 (89.0%); secondary/university 57 (11.0%) Water source (n=518): 363 (70.0%) public tap; 61 (11.7%); 85 (16.4%) Had electricity at home: 117/520 (22.5%) Female infants: 262/532 (49.2%) Gestational age (mean weeks): formula 39.5; breastfeeding + HAART 39.8 (P=0.032) Median weight of infants, kg: 3.1 in both groups (P=0.281) Feeding practice: exclusively breastfed 94.2% (number not reported) mixed fed 13 women
Interventions	**Maternal ART/ARV** According to Rwandan national protocol the following were prescribed in 2005: Lifelong HAART regimen (stavudine [D4T], lamivudine [3TC], and nevirapine [NVP])

	to pregnant women with CD4 cell count < 350 cells/µl or WHO clinical stage 4, or both; or Prophylactic HAART regimen (zidovudine [ZDV], 3TC, and efavirenz [EFV]) to pregnant women with CD4 count > 350 cells/ µl or WHO clinical stage 1, 2, 3. After delivery prophylactic HAART was terminated for women who opted to formula feed unless they became eligible for HAART. In breastfeeding women prophylactic HAART was continued to 7 months (1 month after weaning) to reduce the risk of postnatal transmission associated with mixed feeding if abrupt weaning was not carried out. In each case a backbone of ZDV and 3TC was administered for 1 week after stopping NVP/EFV, to decrease the risk of resistance. **Infant ARV** All infants were administered NVP syrup 2mg/kg at birth and ZDV 4mg/kg 12 hourly for 1 week. Women counselled: in pregnancy. Women who chose to formula feed were educated about safe preparation of formula and were provided with free formula. Women who chose to breastfeed were counselled about exclusive breastfeeding for 6 months followed by rapid weaning. Women were provided with nutritional supplement of "sosoma" (mixture of soya, sorghum, and maize) during the weaning period for 1 month and also to the formula feeding group. All food supplements was seased at 7 months unless ever malnutrition was diagnosed (weight-for0age <5th percentile) after excluding underlying medical conditions
Outcomes	One child became infected with HIV-1 between 3 and 7 months (cumulative risk of transmission 0.5% at 9 months of age. Seven infants died by 9 months of age in the breastfeeding group (3.1%). No child in the formula feeding group acquired HIV-1 infection between birth and 9 months. There were 17 deaths (5.6%) in the formula feeding group by 9 months of age. **Probability HIV-free survival** Overall: 1 month: 0.985 (n=524) (95% CI, 0.980 – 0.990) 6 months: 0.962 (n=512) (95% CI, 0.954 – 0.970) 7 months: 0.955 (n=508) (95% CI, 0.946 – 0.964) 8 months: 0.949 (n=505) (95% CI, 0.939- 0.959) 9 months: 0.945 (n = 503) (95% CI, 0.935 – 0.955) **1 month**:

	formula feeding 0.986 (n=301) (95% CI, 0.979 – 0.993); BF + ART 0.982 (n=223)(95% CI, 0.973 – 0.991) **6 months**: formula feeding 0.960 (n=293) (95% CI, 0.949 – 0.977); BF + ART 0.964 (n=219)(95% CI, 0.952 – 0.976) **7 months**: formula feeding 0.950 (n=290) (95% CI, 0.938 – 0.962); BF + ART 0.960 (n=218)(95% CI, 0.947 – 0.973) **8 months**: formula feeding 0.944 (n=288) (95% CI, 0.931-0.957); BF + ART 0.955 (n=217)(95% CI, 0.941 – 0.969) **9 months**: formula feeding 0.940 (n=287) (95% CI, 0.927 – 0.953); BF + ART 0.951 (n=216)(95% CI, 0.937 – 0.965) (log rank test, P=0.66) **Cumulative probability of HIV transmission** 6 weeks: formula feeding 1% (95% CI, 0.3 – 3.0); BF + ART 1.3% (95% CI, 0.4 – 4.1%) 9 months: formula feeding 1% (95% CI, 0.3 – 3.0); BF + ART 1.8% (95% CI, 0.7 – 4.8%) (log rank test, P=0.43) **Cumulative probability of death** 9 months: formula feeding 5.7% (n=17) (95% CI, 3.6 – 9.2); BF + ART 3.3% (n=7)(95% CI,1.6 – 6.9)(log rank test; P=0.20) The following was reported "overall 7 children were infected with HIV-1 of which six *in utero* (3 in each infant feeding group). Only one child in the breastfeeding group infected between months 3 and 7, and no child acquired HIV infection between birth and 9 months in the formula feeding group...overall, by 9 months of age 7 children died in the breastfeeding group and 17 in the formula feeding group". **However, the reported events of death and HIV infection do not correlate with figure 2 in the test (n=524 at 1 month and 503 at 9 months)**
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No r	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	Yes	"Allowing for 10% LTFU, we decided to include at least 225 pregnant women in each group".
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

DREAM

Palombi 2007 (39)

Methods	**Study design:** Comparative analysis of two prospective cohort studies enrolled in the DREAM Drug Resource Enhancement against AIDS and Malnutrition) programme **Duration of enrolment:** Cohort 1 patients were enrolled from January 2004 to December 2006. Cohort 2 were enrolled from August 2005 to July 2006 **Setting:** Cohort 1 was enrolled in Mozambique, Malawi and Tanzania. The setting for the cohort 2 was Mozambique **Definition of feeding practice:** Infants were exclusively breastfed or formula-fed. The study does not define exclusively breastfeeding and formula feeding **Follow-up:** Observed length of follow-up: 6 months Lost to follow-up: Cohort 1: 4% of women lost to follow-up during pregnancy. No infants lost to follow-up between 1 to 6 months. Of 879 infants, 40 infants were excluded because maternal HAART was administered for less than 1 month and 30 (3.4%) infants died without a definitive HIV-1 diagnosis and were excluded from the analysis. Cohort 2: 2.5% of women lost to follow-up during pregnancy. 21/341 (6.2%) infants lost to follow-up between 1 and 6 months. **Analysis** Confidence intervals, exact test, Student's *t* test, Levine's *F* test, chi-squared, odds

	ratio and Mantel-Haentzel adjustment for odds ratio were calculated.
Participants	**Sample size**: Cohort 1: There were 914 women were enrolled in the study. 809 of 879 infants enrolled in the study were included in the study analysis. Cohort 2: 341 infants were born to women in the cohort. The infants in this cohort are still being followed up with 251 of 341 infants having data in the first month of life completing follow-up for this analysis at 6 months. **Eligibility:** HIV-infected pregnant women from 25 weeks of gestation on HAART. All women in the DREAM programme receive HAART for PMTCT purposes free of charge irrespective of their clinical and immunological stage of disease. **Exclusions:** twins, stillbirths, and miscarriages **Maternal ART:** All women in the DREAM programme receive HAART free of charge irrespective of their clinical and immunological stage of disease. **Infant ARV:** infants receive post-exposure prophylaxis **Baseline characteristics** between the groups were not reported in the study. Feeding practice: Number breastfed from birth: cohort 2 - 341 Number formula fed from birth: cohort 1 - 809
Interventions	Cohort 1 was provided supplementary formula and water filters for use during the first six months of life Cohort 2 was given the choice of taking HAART during the first 6 months postpartum and to breastfeed exclusively.
Outcomes	**Cumulative Incidence of mother-to-child transmission of HIV-1 over time** Overall: formula fed 2.7 %(95% CI, 1.7 – 4.1); breastfed 2.0% (95% CI, 0.6 – 3.8) ($\times^2$ = 0.27, P = 0.60 1 month: formula fed (7/809) 0.9% (95% CI, 0.3 – 1.8); breastfed (4/341) 1.2% (95% CI, 0.3 – 3.0) 6 months: formula fed (15/809) 1.8% (95% CI, 1.0 – 3.0); breastfed (2/251) 1.8%(95% CI, 0.1 – 2.8) ($\times^2$ = 0.77, P = 0.38 [NS]) 12 months (Palombi 2009): HIV infection rate 1.4% **HIV-free survival rate** 1 month: formula fed (802/809) 99.2%, breastfed (337/341) 99.4% 6 months: formula fed (794/809) 98.2%; breastfed (249/251) 99.2% 12 month (Palombi 2009): breastfed 94%

	Mortality rate (no details presented on calculation of the mortality rate) 6 months: formula fed 27 per 1000 person-years, breastfed 28.5 per 1000 person-years, lower than 100 per 1000 person years in Mozambique. **Morbidity** Haemoglobin < 8g/dl: formula fed infants 40/809 (4.9%), breastfed infants 17/251 breastfed infants (6.8%) (x^2 = 0.92, P = 0.33[NS]) **(Palombi 2009 – released at the 2009 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention)(40)** Mothers on HAART were counselled to exclusively breastfeed their infants for 4 months, initiate weaning at 17 weeks and stop breastfeeding at 6 months after which mothers were no longer provided with HAART. Weaning foods included rice, corn flour and no formula. Records were analysed for July 2007 to January 2009 from 2452 infants enrolled in Mozambique (n=1618) and Malawi (n=834). Weight and length were measured monthly. Weight-for- age, height-for-age and weight-for-height Z scores (WAZ, HAZ, and WHZ) were calculated at various time points. 1month: WAZ 0.39±0.9; HAZ -1.03± 1.2; WHZ 0.39 ± 0.9 3 months: WAZ -0.08 ± 0.98; HAZ -1.08± 1; WHZ +1 ± 1.1 6 months: WAZ -0.53 ± 1.0; HAZ -1.25 ± 1.1; WHZ 0.56 ± 1.1 12 months: WAZ -1.45± 1.1; HAZ -1.57 ± 1.1; WHZ -0.40 ± 1.0 18 months: WAZ -1.73 ± 1.2; HAZ -1.73 ± 1.2; WHZ 0.46 ± 0.9
Notes	The study does not report compliance within each cohort to formula feeding or breastfeeding but comments on probable mixed feeding in cohort 1 “HIV-1 infection rates were greater among formula-fed infants. The plausible explanation for this finding is that many women in the formula arm were probably delivering a mixed feeding regimen consisting partly of breast milk and partly of formula.” In addition, duration of formula feeding and breastfeeding is not specified.

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial

Incomplete outcome data addressed?	No	Loss to follow-up is small (6.2%) in the breastfed group.
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	No	Cohort 1 and 2 were followed up in different countries. The characteristics of the mother-infant pairs were not reported in the study. Cohort 2 is comprised of a smaller group of participants with follow-up not yet complete at the time the study was published. Some selection bias with 14% of eligible women refusing to participate in the formula group and 11% of women refusing to participate in the study in the breastfed group.

Homsy 2009 (41)

Methods	**Study design**: prospective cohort study of participants enrolled in a randomized controlled trial to assess HIV transmission and infant survival **Duration of enrolment:** 1 March 2003 to 1 January 2007 **Setting:** Tororo and Busia districts in rural Uganda **Definition of feeding practice:** Exclusive breastfeeding was defined as infants' receiving only breast milk. The duration of exclusive breastfeeding stopped the instant infants received any amount of additional liquid or solid food with the exception of medicines. Data on breastfeeding status, type and date of breastfeeding cessation were collected at the time of regular home visits. **HIV testing** **Infant** • Infant HIV testing was performed at 6 weeks, then quarterly until at or after more than 6 weeks of breastfeeding cessation • Qualitative HIV-1 DNA PCR testing (Amplicor HIV-1 DNA PCR test, version 1.5; Roche; Branchburg) was performed on dried blood spots • Positive PCR tests were confirmed by repeat tests • HIV enzyme-linked immunosorbent assay (ELISA) antibody testing was performed in parallel on 2 plasma specimens for infants ≥ 18 months of age **Follow-up:** Median follow-up duration, interquartile range (IQR): 18.1 (9.3 – 26.0) Lost to follow-up: not reported **Analysis**

	Kaplan-Meier methods were used to calculate infant survival in relation to breastfeeding status. Child-time at risk was defined by whether a child was still breastfeeding, had stopped breastfeeding, or had never been breastfed at the time of death. Duration of total breastfeeding referred to the combined duration of exclusive breastfeeding and mixed feeding for all infants, and was equivalent to the age at which infants were weaned. Infants who died who were unable to breastfeed because of their illness were not treated as "weaned" unless the mothers reported that they had taken definitive action to wean the infants before, and independent of the infants' illness. No censoring rules reported by the study.
Participants	**Sample size**: Number of women on HAART during the study: 102 Number of infants: 118 infants born to 102 women on HAART; 80 of 95 (84%) children who were alive at the end of the follow-up were followed for ≥ 12 months **Eligibility:** HIV-1 infected pregnant women between 18-49 years receiving HAART **Baseline characteristics** by children alive (n=118) and deceased (n=23) Median maternal age: not reported Median CD4 count close to delivery (cells/ml)(IQR): alive 309 (253 – 409); deceased 369 (237 – 614) (P=0.33) Mothers who started HAART during pregnancy: alive 3.8 (2.8 – 6.3); deceased 5.5 (5.3 – 5.5) (P=0.25) Median time on HAART by time of delivery (IQR) (mo): alive n=14; deceased n=5 Mothers with detectable viral load close to delivery (cps/ml) alive 6 (6.3%); deceased 3 (13%) (P=0.37) Socio- demographics: not reported Male infants: alive 46 (48%); deceased 12 (52%) Gestational age at birth: not reported Median age at death/end of follow-up: alive 20.8; deceased 3.7 (P<0.001) Median weight of infants: not reported Feeding practice: Number breastfed from birth (%): alive 89 (94); deceased 20 (87) (P=0.37) Median duration of exclusive breastfeeding (mo): alive 4.0 (3.0 – 6.0); deceased 3.0 (1.5 – 0.0) (P=0.001) Median duration of total breastfeeding (mo): alive 5.5 (3.0 – 7.1); deceased 3.0 (1.5 – 4.4) (P<0.001)

	Feeding status at 6 mo, n (%) Exclusive breastfeeding: alive 28 (30); deceased 1 (4) Mixed feeding: alive 19 (20); deceased 4 (17) Weaned: alive 42 (44); deceased 15 (65) Never breastfed: alive 6 (6); deceased 3 (13)
Interventions	**Maternal ART/ARV** Mothers were eligible for HAART in the RCT if they had a CD4 count ≤ 250 cells/µl or had WHO clinical stage III or IV. The HAART regimen consisted of lamivudine (3TC), stavudine (D4T) and nevirapine (NVP) **Infant ARV** Single dose NVP syrup was administered to the infant at 2mg/kg within 72 hours of delivery. After September 2005, NVP was supplemented with zidovudine (ZDV) 4mg/kg twice daily for 1 week post delivery (or for 28 days if the mother had started HAART < 1 month before her expected due date) Women counselled: in pregnancy to exclusively breastfeed for 3-6 months followed by rapid weaning and safe replacement feeding according to Ugandan Ministry of Health guidelines. Women in the study were not provided with any food supplements.
Outcomes	There were 23 of 118 (19%) infants who died during follow-up. **Feeding status at death and causes of death** Never breastfed: 3/23 (13.0%) – 1 death from fever and 2 deaths from gastroenteritis Exclusively breastfed: 5/23 (21.7%) – 3 deaths from gastroenteritis, 1 death from respiratory illness and 1 sudden death Weaned: 13/23 (56.5%) – 8 deaths due to gastroenteritis, 3 deaths from respiratory ill, 1 sudden death and 1 death related to fever Mixed fed: 2/23 (8.7%): 2 deaths from gastroenteritis (1 with gastroenteritis and respiratory illness) PCR results on infants who were deceased were not definitive as 9 infants were still breastfeeding and 3 infants were weaned for < 6 weeks at time of testing. No HIV infection was identified among the 19 infants tested before their death. However, 12 of the 19 did not have a definitive PCR positive test and 1 ELISA antibody negative could not be taken as final. In the Cox proportional hazards analysis, there was a 6 times increased risk of death among infants breastfed for less than 6 months (after adjusting for maternal CD4

	count, maternal marital status or maternal death) (adjusted HR 6.19, 95% CI, 1.41 – 27.0, P=0.015)
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not randomized controlled trial
Allocation concealment?	No	The study is not randomized controlled trial
Blinding?	No	The study is not randomized controlled trial
Incomplete outcome data addressed?	No	The study does not detail loss to follow-up
Free of selective reporting?	Yes	
Free of other bias?	No	Lack of data on socio-demographics of the participants and infant birth weights (not adjusted for in the Cox proportional models). Feeding data was collected retrospectively from the some mothers which may have led to an underestimate of mixed feeding (social desirability bias)

Tonwe-Gold 2007 (42)

Methods	**Study design:** prospective cohort study – 2 tiered design **Duration of enrolment:** August 2003 to December 2004 **Setting**: community-based antenatal clinics in Abidjan, Côte d'Ivoire. The cohort included women enrolled in the MTCT-Plus program which is a multi-centre comprehensive HIV care and treatment program offering prophylactic and treatment services to pregnant women and their families. The MTCT-Plus was built on existing PMTCT services. **Blinding**: No **Definition of feeding practice:** The study followed WHO/ UNAIDS/UNICEF guidelines on infant feeding. Exclusive breastfeeding was encouraged for 6 months with initiation of early weaning at 4 months **Follow-up:** Infants were followed up at 4 weeks and 12 months Planned length of follow-up: 12 months Observed length of follow-up: 12 months Lost to follow-up:

	2/261 women lost to follow-up at start of the study 21 children lost to follow-up after 4 weeks **Analysis** In the case of multiple births, the first live-born infant was included in the analysis. Two survival analyses were used for two different outcome measures in infants: HIV infection or HIV-free survival (ending of occurrence of HIV infection or death, which came first). The Turnbull method and Kaplan-Meier estimates were used to determine cumulative transmission risk of HIV and (HIV or death). Kaplan-Meier estimates were retained as results for both methods were similar. Timing of transmission was defined as the time of the first positive test for peripartum cases and the midpoint date between the last negative test and the first positive test for postnatal transmission. Postnatal transmission was defined as infants who were HIV-1 negative at 4 weeks of age.
Participants	**Sample size**: There were 261 women enrolled; 250 women received antiretroviral antenatally and/or during delivery. Number of infants: 231/246 infants who were live born and singleton. **Eligibility criteria:** HIV-infected pregnant women attending two community-based antenatal clinics in two low-income, urban areas in Abidjan were eligible for the MTCT-Plus program. **Baseline characteristics** were similar between the two groups Median maternal age: 27 years (interquartile range [IQR] 24-31 years) Median CD4 count (cells/mm ^{3}): 338 (IQR 206-488) WHO staging: stage 1 – 105/250 (42.0%), Stage 2 101/250 (40.4%), Stage 3 41/250 (16.4%), Stage 4 3/250 (1.2%) Eligible for HAART: 105/250 (42%) Treatment HAART: 107 Treatment short course antiretroviral: 143 Median gestational age (weeks): 30 weeks (IQR 25-33 weeks) Feeding practice: breastfed from birth 172/241 (71.3%) – 62 in the HAART group and 110 in the short course antiretroviral for PMTCT group Median duration of HAART in breastfeeding women: 14.9 mo (IQR, 14.5 – 16.2 mo)
Interventions	**Maternal ART/ARV:** Women were divided into two cohorts. The first cohort included all HIV-infected pregnant women eligible for HAART (highly active antiretroviral treatment) based on WHO criteria (high risk of transmission). The second cohort included HIV-infected pregnant women not eligible for HAART (low risk transmission) who were prescribed short course-PMTCT.

	HAART cohort: treatment started at 24 weeks gestation with zidovudine (ZDV), lamivudine (3TC), and nevirapine (NVP) and was continued during labour and postnatally. sc-PMTCT cohort: validated sc-ARV prophylactic regimens Most women received sc(ZDV+3TC) from 32 weeks gestation until 3 days postpartum and sdNVP in labour, or scZDV from 28 weeks, or sdNVP alone, or both scZDV and sdNVP. **Infant ARV:** all infants received ZDV syrup for 7 days and sdNVP syrup on the third day regardless of maternal ARV regimen. Median duration of breastfeeding: overall 5.4 mo (IQR 4.0 – 6.8); HAART (4.6 mo), scARV for PMTCT (5.8 mo) (P = 0.010) Women counselled: in pregnancy/after delivery
Outcomes	**Probability of HIV-infection (Overall)** 1 month: 2.2 (95% CI, 0.3-4.2) (5/241) 3 months: 3.7 (95% CI, 1.2 – 6.2) (8/197) 6 months: 5.2 (95% CI, 2.2 – 8.2) (11/192) 12 months: 5.7 (95% CI, 2.5 – 9.0) (12/188) There were no statistically significant difference found according to infant feeding practice (P=0.48) **Transmission of HIV according to feeding status** Breastfed infants (n=138): HAART 1.9% (1/52) (95% CI, 0.04 – 10.2%); short course ARV 3.5% (3/86) (95% CI, 0.7 – 9.9%) Formula fed infants (n=53): no cases of postnatal transmission **Probability of HIV-infection or death (overall)** 1 month: 4.3% (95% CI, 1.7 – 7.0) (10/218) 3 months: 7.1% (95% CI, 3.7 – 10.4) (16/203) 6 month: 10.8% (95% CI, 6.7 – 14.8) (24/194) 12 month: 11.7% (95% CI, 7.5 – 15.9) (26/189) **Probability of HIV infection, HAART** 1 month: 1.0% (95% CI, 0.0 – 3.1) 1/95 3 month: 2.2% (95% CI, 0.0 – 5.1) 2/88 6 month: 3.3% (95% CI, 0.0 – 6.9) 3/86

	12 month: 3.3% (95% CI, 0.0 – 6.9) 3/86 (P = 0.18) **Probability of HIV infection, sc ARV** 1 month: 3.1% (95% CI, 0.1 – 6.1) 4/122 3 month: 4.9% (95% CI, 1.1 – 8.7) 6/109 6 month: 6.6% (95% CI, 2.2 – 11.1) 8/106 12 month: 7.5% (95% CI, 2.8 – 12.3) 9/102 (P = 0.90) **There were no significant differences between the two groups.**
Notes	The study is a description of two cohorts with one group receiving HAART and the other receiving scARVs for PMTCT. The study is not comparative. A limitation is the small sample size in each cohort.

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	Nor	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	The study had a high level of retention with 2% of infants lost to follow-up and final HIV status available in 86% of infants
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

Miotti 1999(43)

Methods	**Study design** prospective cohort study **Duration of enrolment:** 1994 to 1997 **Setting:** Postnatal clinic of tertiary care hospital, Blantyre, Malawi **Definition of feeding practice:** "In accordance with World Health Organization and government of Malawi recommendations at the time of this study, HIV-infected women, who were told their HIV-infection status, were not discouraged from breastfeeding." **HIV testing** Maternal status was determined by repeated reactive results from enzyme-linked immunosorbent assay (ELISA) performed on umbilical cord blood. Indeterminate

	HIV ELISA tests were confirmed by immunoblotting. Infants' HIV status was determined with DNA polymerase chain reaction (PCR) using heel stick blood. In infants' ≥ 15 months, HIV ELISA was used to confirm HIV infection status. All new, repeated positive results were confirmed by immunoblotting. **Timing of HIV infection status** The timing of HIV infection status of the infant was estimated at the midpoint between the last negative and the first positive PCR test. The date of the last negative PCR test was used as the date at which the infant was still uninfected for infants who did not seroconvert. Censoring occurred at the date of cessation of breastfeeding, determined by the mother's report. If breastfeeding was ceased during the infection interval, the first positive HIV result was moved backward in time to the date of last breastfeeding. The midpoint between the date of the last known breastfeeding and the date when the infant was known to have been weaned was used for infants who were known to have been weaned but for whom the date of weaning was uncertain. Observations were terminated at 24 months as follow-up of infants after 2 years was minimal. **Follow-up:** Mother-infant pairs were followed up at 6 weeks and at 3, 6, 9, 12, 15, 18, and 24 months. Planned length of follow-up: 24 months Observed length of follow-up: 24 months follow-up uninfected infants while breastfeeding 7155 person-months (596 person-years) Lost to follow-up There were 786 of 2157 (36.4%) infants with no postnatal follow-up visit at 6 weeks 6 weeks and 24 months: 329/1371 (24.0%) infants lost to follow-up. **Analysis** Risk of HIV and analysis of the time of breastfeeding cessation was determined using Kaplan-Meier methods of analysis. Infants were censored when breastfeeding was ceased or when follow-up ended.
Participants	**Sample size**: Two thousand and ninety four HIV-infected women were enrolled in the study. Number of infants: There were 2157 live births, of which 1012 infants had breastfeeding data available. There were 672 infants included in this analysis. **Eligibility:** HIV-infected pregnant women who had previously participated in a clinical trial of birth canal cleansing. Infants included in the study had to be HIV-

	uninfected at the first postnatal visit, have breastfeeding data, and have a second follow-up visit. Twins and triplet births were included in the analysis. **Exclusions:** HIV-positive infants at the 6 week visit, **Maternal ART/ARV:** none of the mothers in the study received antiretrovirals antenatally, intrapartum, or postnatally. **Infant ARV:** no antiretrovirals were provided to the infants. **Baseline characteristics** of the mother-infant pairs were not reported. Median infant age at first visit (uninfected infants): 1.7 months (interquartile range [IQR] 1.4 – 2.1; range 0.7 – 16.8 months) Median duration of follow-up while breastfeeding, months: 11.5 (IQR 4.5 – 22.0)
Interventions	No intervention was delivered in this prospective study.
Outcomes	Forty-seven of 672 infants with at least 2 follow-up visits were HIV-infected while breastfeeding. No infant became infected after breastfeeding was stopped (268 person-months of follow-up). **Cumulative risk of HIV transmission** 5 months: 3.5% 11 months: 7.0% 17 months: 8.9% 23 months: 10.3% **Incidence rate of HIV-infection per person-month** 6-11 months: 0.6% 12-17 months: 0.3% 18-23 months: 0.2% (P=0.01 for trend)
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	The study does not report the baseline characteristics of mother-infant pairs that did not return for a postnatal follow-up visit. Loss to follow-up substantial.

Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	No problems apparent

Coutsoudis 2005b (44)

Methods	**Study design** prospective cohort study **Duration of enrolment:** January 2000 to December 2003 **Setting:** primary health clinic in Cato Manor, Durban, South Africa **Definition of feeding practice:** Exclusive breastfeeding for 6 months as defined by the WHO guidelines. The Safer Breastfeeding Programme was implemented by HIV counsellors who attended a 10 day WHO/United Nations Children's Fund breastfeeding course and a 3 day WHO HIV infant feeding course. A breastfeeding promotion campaign was also conducted for all breastfeeding women in the clinic and the community so that all women would be comfortable practicing exclusive breastfeeding for the first 6 months of life. **HIV testing** Infant blood was collected at 6 weeks, 9, 12 and 15 months for testing with polymerase chain reaction (PCR). ELISA and p24 antigen testing was carried out at 9, 12, and 15 months. Furthermore, a blood spot was collected at 9 months, dried and stored. If the test results from the ELISA and p24 antigen were inconclusive at 9 months, the blood spot was tested for HIV by PCR. **Follow-up:** Infants were followed up at 6, 10, 14 weeks and then at 9, 12, and 15 months. Observed length of follow-up: 15 months Lost to follow-up: 9 months: 40 of 233 (17.2%) infants
Participants	**Sample size**: 315 women were enrolled in the study Number of infants: 233 infants of 275 infants who had an HIV test were negative at 6 weeks. 188 of 193 breastfed infants were included in this analysis. **Eligibility:** HIV-infected pregnant women enrolled into the Prevention of Mother to Child Transmission programme. **Exclusions:** infants who were never breastfed **Baseline characteristics** The study population consisted of women from an informal settlement with approximately 50% having no running water, sanitation or water. An estimated 80% of women were unemployed.

	Feeding practice: Number breastfed from birth: 188 Number formula fed from birth: 5
Interventions	**Maternal ART/ARV:** 200mg single dose nevirapine (NVP) (72% of mother and/or their infants received single dose NVP. **Infant ARV:** 2mg/kg single dose nevirapine syrup Data was available on feeding practices of 148 infants between 6 and 9 months.56 of 148 (38%) were breastfed only, 57 (38.5%) were formula fed only, and 26 (17.6%) were breastfed and formula fed. 9 (6%) received heat treated expressed breast milk. Women counselled: in pregnancy to exclusively breastfeed for the first 6 months. After women had chosen their modality of feeding, they were given further counselling on feeding practices in the first week of life including early initiation of breastfeeding, correct positioning of the infant, frequent feeding, heat treatment of expressed breast milk, shorter duration for 6 months, and exclusivity of breastfeeding.
Outcomes	Postnatal HIV transmission: any transmission after 6 weeks of age in a previously negative infant was attributed to breastfeeding. At 9 months 4 of 188 (2.6%) infants were infected with HIV. The risk of postnatal transmission was assumed to be constant over time (7.5 months of breastfeeding) and approximately 0.35% per month of breastfeeding.
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No r	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	The study does address loss to follow-up of infants
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	Yes	Loss to follow-up with 17.2% of infants not returning at 9 months

Olayinka 2000 (45)

Methods	**Study design** prospective cohort study **Duration of enrolment:** 1992 to 1995 **Setting:** antenatal clinic in a tertiary care teaching hospital in Harare, Zimbabwe **Definition of feeding practice:** Breastfeeding only not defined Mixed feeding was defined as breastfeeding with formula supplementation Formula feeding only not defined **HIV testing** Maternal HIV status was determined using enzyme-linked immunosorbent assay (ELISA) and confirmed if reactive by western blot (WB). Infant HIV status was determined using DNA polymerase chain reaction (PCR) at 3, 6, 9 and 12 months and by ELISA at 18 and 24 months of age. Confirmation of infant HIV infection was determined by WB. All infants who tested HIV positive as a result of reactive ELISA tests and HIV negative as a result of PCR before 15 months of age were considered to still have HIV-1 maternal antibodies. **Analysis** The HIV infection status of infants at delivery was excluded as only a small proportion of infants were tested shortly after delivery (5/326; 2.1%). The overall mother to child transmission (MTCT) rate was calculated by dividing the total number of HIV-1 infected infants by the total number of HIV-1 positive mothers. Incidence of HIV-1 at each time point of follow-up was defined as the number of new HIV-1 cases per 100 child months of follow-up. Kaplan-Meier methods of analysis was used to ascertain if there were statistically significant differences between infant feeding practices and the risk of MTCT of HIV-1 by time of follow-up (log rank X^2 test). Cox Proportional Hazards regression method was used to calculate the cumulative risk of MTCT among infants who were breast fed and mixed fed compared to formula fed infants. Hazard ratios were used unadjusted and adjusted estimates of relative risks in reporting the outcomes of Cox proportional hazards regression.
Participants	**Sample size**: Number of mother-infant pairs analysed for the study 236 **Eligibility:** HIV-infected pregnant women >28 weeks, live-born singleton infants **Baseline characteristics** The majority of the women were married 94.1% (n=222); with 37.3% aged between 21-25 years (n=88), and 66.5% educated to secondary level (n=157) Feeding practice: breastfed exclusively from birth to 3 months 120/203 (59.1%) formula fed only from birth to 3 months 2/203 (1.0%) mixed fed from birth to 3 months 81/203 (39.9%)

Interventions	Study is a prospective cohort study with no intervention
Outcomes	**HIV transmission risk** Overall 95/236 (40.3%) infants were HIV-infected **Incidence of HIV-1 per 100 child months of follow-up** 3 months: 8.33 (30/120) in breastfed, 8.64 (21/81) in mixed fed, and 0.0 (0/2) in the formula fed group 6 months: 0.0 (0/7) in the breastfed, 1.43 (13/152) in mixed fed, 3.33 (1/5) in the formula fed group 12 months: 0.0 (0/1) in the breastfed, 0.20 (2/126) in the mixed fed, 0.44 (1/19) in the formula fed group 18 months: 0.26 (3/63) mixed fed, 0.64 (9/78) in the formula fed group 24: 0.25 (8/131) in the formula fed group
Notes	Small numbers in each age category and results were therefore not statistically significant.

Item	Judgement	Description
Adequate sequence generation?	No	Not a randomized controlled trial
Allocation concealment?	No	Not a randomized controlled trial
Blinding?	No	Not a randomized controlled trial
Incomplete outcome data addressed?	No	Loss to follow-up data not reported in the study
Free of selective reporting?	No	Loss to follow-up not mentioned
Free of other bias?	No	Study conducted in an antenatal clinic in a tertiary teaching hospital; hence population selected may be systematically different from the rest of antenatal clinic attendees. Methods of estimation total MTCT rates were problematic.

Good Start Study

Jackson 2007 (46)

Methods	**Study design** prospective cohort study
	Duration of enrolment: October 2002 to November 2004
	Setting: 3 sites in South Africa – Rietvlei (rural) in Eastern Cape, Paarl (peri-urban/rural) in Western Cape and Umlazi (peri-urban) in KwaZulu-Natal.
	Definition of feeding practice:
	"Ever breastfed" was defined as reported exposure to any breastmilk feeding between birth and 36 weeks. The variable was included to reflect postnatal exposure to HIV and to reflect any specific infant feeding modality
	Follow-up:
	Planned length of follow-up: 36 weeks
	Observed length of follow-up: 36 weeks
	Lost to follow-up:
	140/665 (21%) mother-infant pairs at 36 weeks
	Follow-up:
	Mother-infant pairs followed up at 3,5,7,9,12,16,20,24,28,32,and 26 weeks
	Observed length of follow-up: 24 months
	Lost to follow-up: not reported
	HIV testing:
	Maternal: HIV status was ascertained from routine Prevention of Mother- to- Child Transmission (PMTCT) records. Mothers recorded as HIV positive with undetectable viral loads had a repeat laboratory enzyme-linked immunosorbent assay carried out. 12 mothers were confirmed negative after testing (1.9%) and were excluded from the analysis.
	Infants:
	HIV infection in infants determined at 3, 24 and 36 weeks of age by heel/finger prick. HIV detection was by means of viral load using quantitative HIV-1 RNA NASBA (Nuclisens ECL; bioMerieux, Marcy l'Etoile, France) and/or positive test using quantitative HIV-1 DNA polymerase chain reaction assay (Amplicor version 1.5; Roche Molecular Systems, Branchburg, New Jersey, USA)
	Analysis
	HIV-free survival was the primary outcome. Cox's proportional hazards regression models were fitted to examine the risk factors for HIV transmission. The midpoint between the last negative HIV test and the first positive test was used to estimate time of infection and Efron's method was used to adjust for tied survival times. Infant

	mortality rates were calculated using Kaplan-Meier estimates.
Participants	**Sample size**: 665 mother-infant pairs recruited– 149 from Paarl, 192 from Rietvlei and 324 from Umlazi. 525 mother-infant pairs were included in this analysis. **Eligibility:** HIV-1 infected pregnant women were recruited from the local hospital or clinic offering PMTCT services. Refusal rates for both participation in the PMTCT programme and the study were 19%. **Baseline characteristics** Women in the Rietvlei group were in the lowest socio-economic group. Birth weight was similar across the 3 groups (P<0.001). The mean maternal age was lower in Rietvlei (24.1) compared to Umlazi (26.1) and Paarl (25.8) (P<0.01). Feeding practice: "Ever breastfed "– 51 (36.4%) from Paarl 224 (80.9%) from Umlazi 110 (65.5%) from Rietvlei
Interventions	Study is a prospective cohort with no intervention
Outcomes	**HIV-free survival rates** Paarl 84% Rietvlei 64% Umlazi 73% (*P = 0.0003) **Cumulative overall HIV transmission** **(Calculated as the number of infants who were shown to be HIV infected by any assay at 3, 24, or 36 weeks of age divided by the total number of infants HIV infected at any visit, those HIV uninfected at 36 weeks and those uninfected at the 24 week visit with no 36 week visit and no breastfeeding recorded from 20 week visit forward)** Birth to 36 weeks Paarl: n=21 (15.3%) Rietvlei: n= 42 (25.6%) Umlazi: n= 59 (22.4%) (*P=0.07) *P values indicate differences between sites "Ever breastfed" increased the risk of infection with HIV and/or death, although this was not statistically significant. (HR 1.38, 95% CI, 0.91 – 2.08, P= 0.13) **Infant mortality rate** Birth to 36 weeks Paarl: n=7 (5.6%) Rietvlei: n = 34 (18.6%)

	Umlazi: n= 26 (11.4%) (P=0.0005) **Goga 2009 (47) (2009 4th South African AIDS conference)** Sample: 665 HIV-infected women and 218 HIV-uninfected women in 3 routine PMTCT sites in South Africa Data collection: data on infant feeding was collected at 3, 5, 7, 9, 12, 16, 20, 24, 28, 32, and 36 weeks post-delivery. **Goga 2009 (48) (2009 4th South African AIDS conference)** Data was collected on complete breastfeeding cessation (CBC) and "not breastfeeding" (NBF) for four days prior to the last follow-up visit at or before 24 weeks. Data was collected at 3, 5, 7, 9, 12, 16, 20, and 24 weeks during home visits. Univariate analyses, logistic regression, Kaplan-Meier survival analysis and Cox regression were conducted. 88 (43.6%) women reported CBC. The probability of NBF at 5,7,9,12,16,20, and 24 weeks was 2.8% (95% CI, 1.8 – 3.8), 4.3% (95% CI, 3.0 – 5.6), 5.9% (95% CI, 4.4 - 7.4%), 9.8% (95% CI, 7.9 – 11.7%), 16.1% (95% CI, 13.8 – 18.4%), 23.1% (95% CI, 20.5 – 25.7%) and 37.6% (34.6 – 40.6%) respectively. NBF was not protective against 9 month infant HIV or death in univariate and multivariable analysis.
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	The study does not report how incomplete data was dealt with
Free of selective reporting?	Yes	No problems apparent
Free of other bias?	No	Possible loss to follow-up bias – loss to follow-up was not reported.

Magoni 2005 (49)

Methods	**Study design** prospective cohort study **Duration of enrolment:** 30 September 2000 to 30 October 2002 **Setting:** St Francis Hospital, Nsambya, Kampala, Uganda **Blinding**: no **Definition of feeding practice:** For the analyses women were initially classified according to the choice of feeding at delivery, either breastfeeding (BF) or formula feeding (FF). For the secondary analyses, mother-infant pairs were reclassified as exclusive breastfeeding (EBF), exclusive formula feeding (EFF) and mixed feeding (MF) according to data collected on feeding practices during the follow-up. Children who were weaned early and children who ceased to breastfeed before 4 months and were switched to formula feeding were included as part of the EBF group. The EBF group was defined as children who received breast milk only with no concomitant fluid or food. The EFF group was defined as infants who were formula fed only and who were never breastfed. The MF group was defined as infants receiving breast milk as well as other foods or fluids, or formula feeding and breastfeeding. **Follow-up:** Mother-infant pairs were followed up at 1, 6, 10, 14 weeks and 4, 5, and 6 months. Observed length of follow-up: 6 months Lost to follow-up: 28/277 (10.1%) infants HIV-uninfected at week 6 lost to follow-up at 6 months (17 in the BF group and 11 in the FF group). **HIV test** Children were tested for HIV RNA or DNA measured by polymerase chain reaction (Amplicor version 1.5, Roche Molecular Systems, Branchburg, New Jersey, USA) HIV infection in children was defined as one positive HIV RNA or DNA result. **Analysis** HIV transmission rates were calculated by dividing the number of HIV infected infants by number of mother-infant pairs. Univariate analysis used Fisher's exact test to compare rates of HIV transmission. Kaplan-Meier's life tables and multivariate Cox's proportional hazards model were used to calculate relative risks of transmission in the secondary analyses. All tests were two sided and P values < 0.05 were considered significant.

Participants	**Sample size**: Number of women eligible and enrolled not reported Number of infants included in the analyses: 306 – 181 BF, 125 FF **Eligibility:** HIV-infected pregnant women attending the Prevention of Mother to Child Transmission Programme (PMTCT) in St Francis Hospital were asked to enrol in the study. **Antiretrovirals administered:** Yes – short course zidovudine or nevirapine. Adequate compliance was based on self-report from the participant of taking drugs as prescribed, and for zidovudine for at least 1 week before delivery. **Baseline characteristics** were similar between the two groups Mean maternal age was 27.2 (SD 4.8). Overall, 47.1% of women attended either secondary school or university, 19.3% were housewives, 31.2% were classified as other employment, and 52.1% had access to either a protected spring or piped water. 71.8% of women received zidovudine for a mean duration of 28 days. Feeding practice: BF group - 83% breastfed at 6 weeks 37% breastfed at 3 months 9% breastfeeding at 6 months 45 (25%) mixed fed FF group – 13 (11%) received breast milk at least once 8 mixed med
Interventions	Prospective cohort study with mother-infant pairs analysed according to infant feeding practice. Pregnant women in the PMTCT programme received either short course zidovudine or nevirapine according to Ugandan PMTCT guidelines. Women counselled: in pregnancy. If participants opted to breastfeed, they were counselled to breastfeed exclusively for 6 months, followed by abrupt weaning and avoidance of mixed feeds. Mothers who chose not to breastfeed were provided with formula feeds free of charge in powder form and given education on safe preparation. The home environment was assessed for feasibility of formula feeding.
0	**Relative risk HIV infection in the BF and FF group** 6 weeks: Overall 27/304 (8.9%) BF 23/179 (12.8%), FF 4/125 (3.3%) (95% CI, 1.4 – 11.3; P = 0.0036) 6 months: Overall 33/276 (12.0%) BF 27/162 (16.7%), FF 6/114 (5.3%) (95% CI, 1.3 – 7.4; P = 0.0043) **HIV transmission rates** 6 weeks:

	EFF 4/117 (3.4%), EBF 17/152 (11.2%), MF 6/35 (17.1%) 6months: EFF 4/108 (3.7%), EBF 19/119 (16.0%), MF 10/49 (20.4%) **Cumulative probability of HIV infection (figure 1)** ~ 30 days: never breastfed ~ 0.03; EBF ~ 0.04, MF ~0.15 100 days: never breastfed ~0.03, EBF ~ 0.11, MF ~0.18 150 days: never breastfed ~0.03, EBF ~0.12, MF ~0.18 200 days: never breastfed ~0.03, EBF ~0.15, MF ~ 0.24 EBF (HR, 4.4, 95% CI, 1.5 – 13.1; P = 0.007) and MF (HR 6.6, 95% CI, 2.0 – 22.1; P = 0.002) were significantly associated with higher risk of HIV infection **Mortality** Before 6 months: Overall 5/306 deaths (1.6%) (EFF 1/304, EBF 3/304, 1/304 MF) of which 2 were HIV uninfected (0.65%) and 3 (0.98%) were HIV infected **Morbidity** Respiratory tract infections (%): BF 16.5%, FF 12.6% (P<0.05) Acute diarrhoea/vomiting (%): BF 5.0%, FF 4.6% Multivariate Cox's model
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	Incomplete outcome data not addressed in the study
Free of selective reporting?	No	The study does not address the issue of reclassification of infants weaned early and started on formula feeding as EBF
Free of other bias?	No	Loss to follow-up bias Selection bias as HIV-1infected pregnant women chosen from a hospital setting and were well educated. Their feeding practices may be systematically different resulting in bias in the study.

Noel 2008 (50)

Methods	**Study design** prospective cohort study **Duration of enrolment:** 1995 to 2005 **Setting:** the *Groupe Häitien d' Études du Sarcome de Kaposi et des Infections Opportunistes* (GHESKO), a voluntary counselling and testing centre in Port – au - Prince, Haiti **Definition of feeding practice:** The study reports mixed feeding, formula feeding and breastfeeding, but does not define these practices. **HIV testing** Maternal HIV testing was carried out with rapid whole blood antibody testing using Determine HIV1/2 (Abbott Laboratories Park IL) and Capillus 1 and 2 (trinity Biotech, Bray, Ireland). Maternal HIV infection was defined as two positive results. Indeterminate results were verified with Western Blot. Infant HIV status was determined with nucleic acid sequence-based assay (NASBA) (BioMerieux Boxtel, The Netherlands) for the first 3 years of the study. Thereafter, polymerase chain reaction (PCR) testing for HIV-1 RNA was carried out on infants at birth, 2-3 months, and 6 months of age. HIV antibody testing was performed every 3 months until seroreversion confirmed that maternal antibodies had cleared. An infant was defined as HIV infected based on serology and nucleic acid detection. *In utero* and peripartum transmission was defined. *In utero* transmission was defined as a positive test within 72 hours after delivery and peripartum transmission was defined as a negative test at birth and a subsequent positive test. Postnatal transmission was not defined. **Follow-up:** Mother-infant pairs were followed up twice per month for 6 months, thereafter once a month until 24 months of age. Observed length of follow-up: 15 months Lost to follow-up defined as children who did not follow-up at the clinic for 3 months and could not be located by the field worker. There were 84 of 551 infants lost to follow-up before an HIV status could be determined. **Analysis** Mortality rate was defined as the number of deaths per year of follow-up. Follow-up was censored at 15 months. The association of maternal antiretroviral regimen and Infant death < 15 months was determined with Pearson's chi squared test.

Participants	**Sample size**: Number of women enrolled 508 Number of infants: 467 of 551 infants were included in this analysis **Eligibility:** HIV-1 infected pregnant women in the Prevention of Mother to Child Transmission programme (PMTCT) at GHESKIO. **Baseline characteristics** were similar between the two groups Median maternal age, interquartile range [IQR]: 27 years Mean parity: pre-HAART 2.5; post-HAART 1.3 (P<0.001) Maternal stage of disease – asymptomatic: pre-HAART 103 (49.5%); post-HAART 232 (73%) (P<0.001) Clinical stage AIDS: asymptomatic: pre-HAART 85 (41%); post-HAART 48 (15%) (P<0.001) Median CD4 count (cells/µl): pre-HAART 460; post-HAART 462 (P=0.30) When CD4 count was obtained: not reported Median plasma viral load: not reported Maternal education level: not reported Male infants: pre-HAART 100 (46%); post-HAART 167 (50%) Gestational age (weeks): not reported Birth weight of infants > 2.5kg: pre-HAART 161 (83%); post-HAART 234 (79%) (P=0.34) Feeding practice: ((P< 0.001) Formula feeding: pre-HAART 183 (85%); post HAART 286 (86%) Breastfeeding: pre-HAART 9 (4%); post-HAART 37 (11%) Mixed: pre-HAART 24 (11%); post-HAART 12 (4%)
Interventions	Intervention: no intervention was provided. The study was an evaluation of a PMTCT programme before and after the initiation of HAART in pregnant women. **Maternal ART/ARV:** the standard of care from 1999 until 2003 was short course zidovudine (scZDV) to mothers and infants. From 2003 HAART became available, after which the PMTCT programme shifted to a two-tiered approach recommended by WHO (based on CD4 count and clinical stage of HIV). Pregnant women with advance disease were given HAART and women who did fit the criteria for HAART were prescribed scZDV. 1999: scZDV (300mg 12 hourly) ≥ 36 weeks gestation and during labour (300mg every 3 hours until delivery) administered to all HIV-infected pregnant women Women who presented close to labour were given 200mg sdNVP and instructed to

	self administer the NVP at the onset of labour. 2003: CD4 < 350cells/dL were given ZDV, NVP and lamivudine (3TC) or if the mother was anaemic stavudine (d4T), 3TC and NVP. Women who did qualify for HAART received scZDV or sdNVP. Duration of breastfeeding: not reported Women counselled about feeding practices before delivery and were given a choice of formula feeding or breastfeeding. Formula was provided free of charge for the first nine months and women were educated about safe preparation of formula.
Outcomes	**Mortality rate** Overall: 15.2 per 100 live births (95% CI, 12.8 – 18.8) **HIV Transmission** No ARVs: 13% (8/62) (95% CI, 5.7 – 23.9) scNVP/sdNVP: 10.8% (38/353) (95% CI, 7.7 – 14.5) HAART: 1.9% (1/52) (95% CI, 0.05 – 10.3) **Infant feeding related to HIV transmission and mortality** The study reports "while infant feeding practice was not associated with a change in HIV-1 transmission or neonatal and post-neonatal mortality (data not shown) we were severely limited in power to make any meaningful conclusions as 85% of women chose to formula feed".
Notes	

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	Lost to follow-up not addressed.
Free of selective reporting?		
Free of other bias?	No	Loss to follow-up bias. Misclassification bias with regards to feeding practices as feeding modality was not defined.

Kagaayi 2008 (51)

Methods	**Study design** prospective cohort study of mother-infant pairs participating in the ARV-Related Maternal-Infant Study (ARMIS) **Duration of enrolment:** from 2005 (end date not reported) **Setting:** Rakai, Uganda **Definition of feeding practice:** Exclusively breastfeed was defined as breastfeeding with no added supplements. Mixed feeding was defined as breastfeeding with the addition of supplements (excluding medications). Exclusive replacement or formula feeding not defined. Structured questionnaires were used to determine infant feeding practices. Midwife counsellors followed the mothers up at home visits. **HIV testing** Infant HIV status is determined by HIV-1 PCR (Roche Amplicor 1.5) test on heel prick blood sample collected from one month. HIV testing is not carried out for infants at birth. No confirmatory test was reported by the study. **Follow-up:** Infants were followed up at 1, 6 and 12 months. Observed length of follow-up: 12 months Lost to follow-up Eight of 182 (2.2%) infants lost to follow-up at 1 month Five of 168 (3.0%) infants lost to follow-up at 6 months One of 97 (1.0%) infants lost to follow-up at 12 months **Analysis** Kaplan Meier methods of analysis used to calculate infant mortality, and the composite outcome of HIV infection or death (complement of HIV-free survival). Censoring for mortality occurred due to the loss to follow-up at the visit this was first noted. For composite outcome HIV infection or death censoring occurred due to loss to follow-up or absence of an HIV result. All mother-infant pairs with HIV transmission at one month were censored to exclude *in utero*/peripartum and early breastmilk transmission.
Participants	**Sample size**: Number of eligible and enrolled mothers not reported Number of infants: 187 infants were liveborn of which 182 were included in this analysis (107 infants were breastfed and 75 infants were formula fed) **Eligibility:** HIV-1 infected pregnant mothers identified through the Rakai Community Cohort Study **Exclusions:** first born in twin pregnancy

	Baseline characteristics Mean maternal age (SD): formula fed 29.3 (5.3); breastfed 26.1 (5.4) (P<0.01) Married: formula fed 53 (70.7); breastfed 77 (72.0) (P=0.87) Post-primary education: formula fed 40 (53.3); breastfed 61 (57.0) (P=0.65) Mean CD4 count (cells/µl): formula fed 412 (76); breastfed 606 (254) (P<0.01) Birth weight of infants (SD): : formula fed 3.1 (0.5); breast fed 3.1 (0.6) (P=0.92) Infant NVP/ZDV syrup for PMTCT: : formula fed 72 (96.0); breast fed 90 (84.1) (P<0.01)
Interventions	Intervention: no intervention but women were provided with ARVs as part of a routine Prevention of Mother to Child Transmission (PMTCT) programme. **Maternal ART/ARV:** single dose Nevirapine (NVP) at the onset of labour. After September 2007 prophylaxis was changed to a combination of zidovudine from 28 weeks gestation, single dose NVP and 3TC given at the onset of labour, and 1 week tail of 3TC and ZDV with ZDV syrup for the infant. Mothers with WHO clinical stage 4 disease or CD4≤ 250 cells/µl were provided with free antiretroviral treatment (ART) through a community based programme. Women counselled: in pregnancy about HIV transmission through breast milk and were allowed to decide their mode of feeding and were supported in their choice. Free formula was provided by the PMTCT programme. Mothers who chose to breastfeed were counselled to breastfeed exclusively for 6 months and then wean their infants. Mothers who chose to formula feed were counselled about safe preparation of feeds and provided with free utensils and a thermos flask to store feeds at night.
Outcomes	**Cumulative probability of infant HIV infection or death** 12 months: Formula feeding 14% (2/57) (95% CI, 8-25%); Breastfeeding 8 %(1/66) (95% CI, 4-16%) (adjusted HR = 2.8 [95% CI 0.67 -11.7]) **HIV-free survival** 12 months: formula feeding 86%; breastfeeding 92% (P=0.16) (reported at 96% in the study) **Cumulative probability of death** 12 months: Formula feeding 18% (3/61) (95% CI, 11-29%);

	Breastfeeding 3 %(0/87) (95% CI, 1-9%) (unadjusted HR = 6.1 [95% CI, 1.7 – 21.4; P<0.01])
Notes	Although the study defines exclusive breastfeeding the study is analysed according to formula feeding and breastfeeding groups (which includes infants that were mixed fed). The small numbers limit statistical power.

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	No	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	
Free of selective reporting?	No	Limitations of the study are not acknowledged. Number of mothers eligible and enrolled are not detailed and feeding practices were not well defined
Free of other bias?	No	Recall bias – feeding practice detailed at each visit (1 month, 6 month, 12 months). Baseline characteristics between the formula and breastfeeding group differed. An estimated 26.7% of women in the formula group were on maternal ART before delivery compared to 0.9% in the breastfeeding group.

Gupta 2007 (52)

Methods	**Study design** prospective cohort study of women enrolled in a phase III randomized controlled trial (the details of the randomized controlled trial were not reported or referenced in the text). **Duration of enrolment:** 16 August 2002 to 8 July 2004 **Setting:** antenatal clinic and postpartum ward at Sassoon Hospital, an urban public hospital of Byramji Jeejeebhoy Medical College (BJMC) in Pune, Maharashtra, India **Blinding**: not reported for the randomized controlled trial **Definition of feeding practice:** Exclusive formula fed and mixed fed practices reported but not defined in the study **HIV testing** HIV-1 DNA polymerase chain reaction (PCR) was used for testing infants at 9 follow-up visits (excluding week 3 and 5). The infants was designated as HIV-infected if the PCR positive test was confirmed with HIV-1 quantitative viral load using the Amplicor Monitor Standard Assay, version 1.5 (Roche Molecular Systems, Alameda, CA)

	Follow-up: Mother-infant pairs followed up at weeks 1, 2, 3, 4, 5, 6, 10,14 and months 6,9, and 12 Planned length of follow-up: 12 months Observed length of follow-up: 12 months Lost to follow-up: 16% lost to follow-up at 12 months **Analysis** Kaplan-Meier estimates of distribution of time-to-HIV infection in infants (also probability of HIV transmission)
Participants	**Sample size**: 41 of 82 women enrolled participated in the study. Number of infants: 41/42 live born (1 twin delivery). **Eligibility criteria:** HIV-infected pregnant women >32 weeks gestation who planned not to breastfeed and women who planned to breastfeed but declined enrolment in the randomized trial, >18 years, haemoglobin =7.5gm/dl, creatinine <1.2mg/dl, liver function tests <3 times the upper limit of normal, lack of serious pregnancy complications, and written informed consent. **Exclusions:** twin delivery **Baseline characteristics** were similar between the two groups Median maternal age: 22 years Median CD4 count (cells/mm ^{3}) at delivery: 469 (range 106 – 1916) Marital status: 32 (93%) married Median plasma viral load: 28 611 copies/ml (range <400 – 285 121) No formal education: 32 (13%) Median gestational age (weeks) at delivery: 38 (range 32 – 40) Median weight of infants (g): 2426 Feeding practice: Exclusively formula fed *32 (76%) Mixed fed *10 (24%) (*as presented but the sample size then becomes 42 not 41)
Interventions	**Maternal ART/ARV:** Twenty-seven of 41 (66%) women received some form of perinatal prophylaxis. Of the 14 women who did not receive prophylaxis, 6 were in advance labour, 3 were unaware of their HIV status at the time of delivery, 3 had a clinical contraindication and no cause was identified in 2 women Women received either short course zidovudine (AZT) 300mg 12 hourly orally from 36 weeks until onset of labour (n=3)(11%), or single dose nevirapine (NVP) 200mg

	(n=19) (70%), or both AZT and NVP (n=5) (19%). The study does not detail how choice of antiretrovirals was decided. Reasons for not taking AZT were as follows: 13 women opted not to take AZT and preferred the option of single dose NVP, 6 women were unaware of their HIV status at the time of early labour, 4 women were in advanced labour at time of presentation to the hospital and could not be offered AZT, 2 had severe pregnancy induced hypertension and could not be prescribed AZT, and no specific reason was found in 8 women. **Infant ARV:** all infants (n=42) received single dose (NVP (2mg/kg) within 72 hours of delivery
Outcomes	**Probability of HIV transmission** Overall: 8.0% (95% CI, 3.2-22.1%) at 14 weeks Formula fed group (n=31): 3.0% at 14 weeks (1/31) (95% CI, 0.5-22.0%) **Infant mortality** 4/41 (9%) infants died – 2/4 HIV-infected died at 2 and 7 months and 2/38 (5.2%) HIV uninfected died at 2 and 4 months.
Notes	Selection bias in the study of a small cohort of HIV-infected women in a largely formula-fed population in a hospital setting with only 50% of eligible women participating in the study. Furthermore, no information was provided on the collection of infant feeding data. Misclassification of infant HIV status is also likely as confirmatory test is by HIV-1 quantitative viral load.

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized clinical trial
Allocation concealment?	No	The study is not a randomized clinical trial
Blinding?	No	The study is not a randomized clinical trial
Incomplete outcome data addressed?	No	The study does not report handling of loss to follow-up.
Free of selective reporting?	No	Limitation of the study is not well described
Free of other bias?	No	There is likely to be a loss to follow-up bias

CHARACTERISTICS OF EXCLUDED STUDIES

Makhoka 2002 (53)

Methods	**Study design** prospective cohort study **Duration of enrolment:** June to November 1998 **Setting:** 7 centres from 4 districts in Western Kenya (Matayos, Khunyangu, Siaya, Usigu, Kombewa, Chulaimbo, and Nyahera) **Definition of feeding practice:** All mothers were allowed to breastfeed their infants. The study does not further define breastfeeding. **HIV testing** Infants were tested for HIV-infection using HIV-1 DNA polymerase chain reaction (PCR) on samples taken at 1,2,3,4,6 and 9 months and confirmed by serology at 18 months. Maternal and infant antibody status was determined by testing serum/plasma with Particle Agglutination (PA test, Fujirebio, Japan) and anti-HIV-1/2 antibody ELISA (Enzygnost^{R} anti-HIV-1/2 Plus ELISA, Behring-Marburg, Germany). Infant samples were tested for antibodies to HIV at 18 months of age. Positive samples were confirmed by Western Blot assay (HIV Blot, Genelab, and Diagnostics, USA). **Follow-up:** Maternal follow-up: antenatally at 6 and 8 months of pregnancy, postpartum at 2 and 6 months Infant follow-up: within 2 weeks of birth, then 1,2,3 and 4 months, then every 3 months until 18 months of age Observed length of follow-up: 18 months Lost to follow-up 7/107 (6.5%) mothers lost to follow-up.
Participants	**Sample size**: Out of 107 eligible women, only 59 (55%) completed the follow-up and were included in the analysis. Eight women died, 15 women had a miscarriage and declined further participation in the study, 18 women refused to have blood drawn and 7 women were lost to follow-up. **Eligibility:** HIV-infected pregnant women participating in a Mother-to-Child-Transmission study in 4 districts in Western Kenya (Busia, Siaya, Bondo, and Kisumu). **Baseline characteristics** were similar between the women who transmitted HIV infection to their infants and those who did not. Almost half (52.5%) of the women in the cohort were between 19-29 years of age. The mean parity overall was 2

	pregnancies. Maternal absolute CD4 count was greater than 500µl in 35.6% of women and between 200-499 µl in an estimated 30.5% of women. Absolute CD4 counts were not taken for 28.8% of women participating in the study. Almost all women had a vaginal delivery (91.5%) Most infants (76.9%) were born after 37 weeks gestation. Median CD4 count (cells/mm ^{3}): When CD4 count was obtained: Feeding practice: Number breastfed from birth 59
Interventions	**Maternal ARV:** Zidovudine (AZT) 300mg 12 hourly at 36 weeks of gestation until the onset of labour, 300mg at the start of labour, and 300mg 8 hourly during labour until delivery. Duration of breastfeeding: not reported in the study Women counselled in pregnancy on the risk of transmission associated with HIV.
Outcomes	**HIV transmission** 18 months: 12/59 infants were HIV-infected (20.3%)
Notes	The limitations of the study are its small sample size and selection bias.

Item	Judgement	Description
Adequate sequence generation?	No	The study is not a randomized controlled trial
Allocation concealment?	Unclear	The study is not a randomized controlled trial
Blinding?	No	The study is not a randomized controlled trial
Incomplete outcome data addressed?	No	
Free of selective reporting?	No	
Free of other bias?	No	Small sample size. "Out of 107 mothers who tested HIV seropositive and were thus eligible for the present study, only 59 (55%) successfully completed the study."

Chung 2005 (54)

Reason for exclusion	No outcomes of relevance to this review

Coutsoudis 1999 (55)

Reason for exclusion	Data from this study included in the review in Coutsoudis 2001

Coutsoudis 2000 (56)

Reason for exclusion	Data from this study included in the review in Coutsoudis 2001

Coutsoudis 2005a (57)

Reason for exclusion	Outcomes of this study reported in Coutsoudis 2003

Coutsoudis 2004 (58)

Reason for exclusion	Individual studies in the meta-analysis included in this review

Embree 2000 (59)

Reason for exclusion	Outcomes not relevant to this review. Primary study published in 1997 and does not meet inclusion criteria for the review

Fawzi 2000a (60)

Reason for exclusion	No outcomes of relevance to this review

Fawzi 2002b (61)

Reason for exclusion	The outcomes are not relevant to this review. Data from this trial relevant to this review presented in Fawzi 2002

Fox 2008 (62)

Reason for exclusion	Outcomes of this study not relevant for this review

Fox 2009 (63)

Reason for exclusion	Outcomes of this study presented in Kuhn 2009

Ngatiri 2009 (64)

Reason for exclusion	Outcomes of this study not relevant to this review

Mofenson 2009 (65)

Reason for exclusion	Outcome not relevant to this review

Obimbo 2004(66)

Reason for exclusion	Small cohort of patients consists of HIV-infected infants only

Simpore 2006 (67)

Reason for exclusion	The study included patients with HIV-2 (not part of this systematic review)

Chisenga 2005 (68)

Reason for exclusion	No outcomes of relevance to this review

References

1. Kuhn L, Aldrovandi GM, Sinkala M, Kankasa C, Semrau K, Mwiya M, et al. Effects of early, abrupt weaning on HIV-free survival of children in Zambia. N Engl J Med. 2008 Jul 10;359(2):130-41.
2. Kuhn L, Sinkala M, Kankasa C, Semrau K, Kasonde P, Scott N, et al. High uptake of exclusive breastfeeding and reduced early post-natal HIV transmission. PLoS One. 2007; 2(12):e1363.
3. Fawzy A, Arpadi S, Aldrovandi G, Kankasa C, Sinkala M, Mwiya M, et al., editors. Diarrhoea morbidity and mortality increases with weaning prior to 6 months among uninfected infants born to HIV-infected mothers in Zambia. 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009; Cape Town, South Africa.
4. Kuhn L, Aldrovandi GM, Sinkala M, Kankasa C, Semrau K, Kasonde P, et al. Differential effects of early weaning for HIV-free survival of children born to HIV-infected mothers by severity of maternal disease. PLoS ONE. 2009; 4(6)(e6059).
5. Thior I, Lockman S, Smeaton LM, Shapiro RL, Wester C, Heymann SJ, et al. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. JAMA. 2006 Aug 16; 296(7):794-805.
6. Shapiro RL, Smeaton L, Lockman S, Thior I, Rossenkhan R, Wester C, et al. Risk factors for early and late transmission of HIV via breast-feeding among infants born to HIV-infected women in a randomized clinical trial in Botswana. J Infect Dis. 2009 Feb 1; 199(3):414-8.
7. Nduati R, John G, Mbori-Ngacha D, Richardson B, Overbaugh J, Mwatha A, et al. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. JAMA. 2000 Mar 1; 283(9):1167-74.
8. Mbori-Ngacha D, Nduati R, John G, et al. Morbidity and mortality in breastfed and formula-fed infants of HIV-1 infected women: a randomized clinical trial. JAMA. 2001; 286(19):2413-20.
9. de Vincenzi I, Kesho Bora Study Group. Triple-antiretroviral (ARV) prophylaxis during pregnancy and breastfeeding compared to short-ARV prophylaxis to prevent mother-to-child transmission of HIV-1 (MTCT): the Kesho Bora randomized controlled clinical trial in five sites in Burkina Faso, Kenya and

South Africa. 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009; Cape Town, South Africa. 2009.

10. Chasela C, Hudgens M, Jamieson D, Kayira D, Hosseinipour M, Ahmed Y, et al., editors. Both maternal HAART and daily infant nevirapine (NVP) are effective in reducing HIV-1 transmission during breastfeeding in a randomized trial in Malawi: 28 week results of the Breastfeeding, Antiretroviral and Nutrition (BAN) Study 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009 Jul 19-22; Cape Town, South Africa.
11. Shapiro R, Hughes M, Ogwu A, Kitch D, Lockman S, Moffat C, et al., editors. A randomized trial comparing highly active antiretroviral therapy regimens for virologic efficacy and the prevention of mother-to-child HIV transmission among breastfeeding women in Botswana (The Mma Bana Study) 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009 Jul 19-22; Cape Town, South Africa.
12. Kumwenda NI, Hoover DR, Mofenson LM, Thigpen MC, Kafulafula G, Li Q, et al. Extended antiretroviral prophylaxis to reduce breast-milk HIV-1 transmission. N Engl J Med. 2008 Jul 10; 359(2):119-29.
13. Taha T, Kumwenda J, Cole S, Hoover D, Kafulafula G, Li Q, et al., editors. Effect of Maternal HAART on Postnatal HIV-1 Transmission after Cessation of Extended Infant Antiretroviral Prophylaxis. 16th Conference on Retroviruses and Opportunistic Infections; 2009; Montreal, Canada.
14. Kafulafula G, Hoover, Taha T, Thigpen M, Li Q, Fowler MG, et al. Frequency of Gastroenteritis and Gastroenteritis-Associated Mortality with Early Weaning in HIV-1-Uninfected Children Born to HIV-Infected Women in Malawi. JAIDS. 2009.
15. Bedri A, Gudetta B, Isehak A, Kumbi S, Lulseged S, Mengistu Y, et al. Extended-dose nevirapine to 6 weeks of age for infants to prevent HIV transmission via breastfeeding in Ethiopia, India, and Uganda: an analysis of three randomized controlled trials. Lancet. 2008 Jul 26; 372(9635):300-13.
16. Gupte N, Bollinger R, Gupta A, SWEN India Study Team editors. High infant mortality rate among in-utero HIV-infected infants compared to postnatal mother-to-child HIV transmissions (MTCT) - survival benefit if early HIV transmission can be prevented 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009 Jul 19-22; Cap Town, South Africa.
17. Dabis F, Msellati P, Meda N, Welffens-Ekra C, You B, Manigart O, et al. 6-month efficacy, tolerance, and acceptability of a short regimen of oral zidovudine to reduce vertical transmission of HIV in breastfed children in Cote d'Ivoire and Burkina Faso: a double-blind placebo-controlled multicentre trial. DITRAME Study Group. DIminution de la Transmission Mere-Enfant. Lancet. 1999 Mar 6; 353(9155):786-92.
18. 15-month efficacy of maternal oral zidovudine to decrease vertical transmission of HIV-1 in breastfed African children. DITRAME ANRS 049 Study Group. Lancet. 1999 Dec 11; 354(9195):2050-1.
19. Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa, and Uganda (Petra study): a randomized, double-blind, placebo-controlled trial. Lancet. 2002 Apr 6; 359(9313):1178-86.
20. Coutsoudis A, Pillay K, Kuhn L, Spooner E, Tsai WY, Coovadia HM. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa. AIDS. 2001 Feb 16; 15(3):379-87.
21. Coutsoudis A, Pillay K, Spooner E, Coovadia HM, Pembrey L, Newell ML. Morbidity in children born to women infected with human immunodeficiency virus in South Africa: does mode of feeding matter? Acta Paediatr. 2003 Aug; 92(8):890-5.

22. Gray GE, Urban M, Chersich MF, Bolton C, van Niekerk R, Violari A, et al. A randomized trial of two postexposure prophylaxis regimens to reduce mother-to-child HIV-1 transmission in infants of untreated mothers. AIDS. 2005 Aug 12; 19(12):1289-97.
23. Chasela C, Chen YQ, Fiscus S, Hoffman I, Young A, Valentine M, et al. Risk factors for late postnatal transmission of human immunodeficiency virus type 1 in sub-Saharan Africa. Pediatr Infect Dis J. 2008 Mar; 27(3):251-6.
24. Jackson JB, Musoke, Fleming T, Guay LA, Bagenda D, Allen M, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomized trial. Lancet. 2003 13 September 2003; 362:859-68.
25. Onyango-Makumbi C, Bagenda D, Mwatha A, Omer SB, Musoke P, Mmiro F, et al. Early Weaning of HIV-Exposed Uninfected Infants and Risk of Serious Gastroenteritis: Findings from Two Perinatal HIV Prevention Trials in Kampala, Uganda. JAIDS. 2009.
26. Taha TE, Hoover DR, Kumwenda NI, Fiscus SA, Kafulafula G, Nkhoma C, et al. Late postnatal transmission of HIV-1 and associated factors. J Infect Dis. 2007 Jul 1; 196(1):10-4.
27. Iliff PJ, Piwoz EG, Tavengwa NV, Zunguza CD, Marinda ET, Nathoo KJ, et al. Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. AIDS. 2005 Apr 29; 19(7):699-708.
28. Fawzi W, Msamanga G, Spiegelman D, Renjifo B, Bang H, Kapiga S, et al. Transmission of HIV-1 through breastfeeding among women in Dar es Salaam, Tanzania. J Acquir Immune Defic Syndr. 2002 Nov 1; 31(3):331-8.
29. Coovadia HM, Rollins NC, Bland RM, Little K, Coutsoudis A, Bennish ML, et al. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. Lancet. 2007 Mar 31; 369(9567):1107-16.
30. Rollins NC, Becquet R, Bland RM, Coutsoudis A, Coovadia HM, Newell ML. Infant feeding, HIV transmission and mortality at 18 months: the need for appropriate choices by mothers and prioritization within programmes. AIDS. 2008 Nov 12; 22(17):2349-57.
31. Becquet R, Bequet L, Ekouevi DK, Viho I, Sakarovitch C, Fassinou P, et al. Two-year morbidity-mortality and alternatives to prolonged breast-feeding among children born to HIV-infected mothers in Cote d'Ivoire. PLoS Med. 2007 Jan; 4(1):e17.
32. Leroy V, Ekouevi DK, Becquet R, Viho I, Dequae-Merchadou L, Tonwe-Gold B, et al. 18-month effectiveness of short-course antiretroviral regimens combined with alternatives to breastfeeding to prevent HIV mother-to-child transmission. PLoS One. 2008; 3(2):e1645.
33. Becquet R, Bland R, Leroy V, Rollins N, Ekouevi DK, Coutsoudis A, et al. Duration, pattern of breastfeeding and postnatal transmission of HIV: pooled analysis of individual data from West and South African cohorts. PLoS ONE. 2009.
34. Becquet R, Ekouevi DK, Menan H, Amani-Bosse C, Bequet L, Viho I, et al. Early mixed feeding and breastfeeding beyond 6 months increase the risk of postnatal HIV transmission: ANRS 1201/1202 Ditrame Plus, Abidjan, Cote d'Ivoire. Prev Med. 2008 Jul; 47(1):27-33.
35. Kilewo C, Karlsson K, Massawe A, Lyamuya E, Swai A, Mhalu F, et al. Prevention of mother-to-child transmission of HIV-1 through breast-feeding by treating infants prophylactically with lamivudine in Dar es Salaam, Tanzania: the Mitra Study. J Acquir Immune Defic Syndr. 2008 Jul 1; 48(3):315-23.
36. Kilewo C KK, Ngarina M, Massawe A, Lyamuya E, Swai A, Lipyoga R, Mhalu F, Biberfeld G; for the Mitra Plus Study Team,. Prevention of Mother-to-Child Transmission of HIV-1 Through Breastfeeding by

Treating Mothers With Triple Antiretroviral Therapy in Dar es Salaam, Tanzania: The Mitra Plus Study. J Acquir Immune Defic Syndr. 2009.

37. Lando R, Angira F, Masaba R, Ndivo R, Nyangau I, Okanda J, et al., editors. Causes of death among children in a prevention of mother-to-child transmission (PMTCT) trial, Kisumu, Nyanza province, Kenya 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009; Cape Town, South Africa.
38. Peltiera CA, Ndayisaba GF, Lepage P, van Griensven J, Leroy V, Pharm CO, et al. Breastfeeding with maternal antiretroviral therapy or formula feeding to prevent HIV postnatal mother-to-child transmission in Rwanda. AIDS. 2009.
39. Palombi L, Marazzi MC, Voetberg A, Magid NA. Treatment acceleration program and the experience of the DREAM program in prevention of mother-to-child transmission of HIV. AIDS. 2007 Jul; 21 Suppl 4:S65-71.
40. Palombi L, Buonomo E, Scarcella P, Doro Altan AM, Nielsen-Saines K, Tembo D, et al., editors. Provision of highly active antiretroviral therapy (HAART) to mothers while breastfeeding and nutritional supplementation during the weaning process favorably impacts infant growth and survival in African HIV-exposed infants 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009 Jul 19-22; Cape Town, South Africa.
41. Homsy J, Moore D, Barasa A, Were W, Likicho C, Waiswa B, et al. Breastfeeding, Mother-to-Child HIV Transmission, and Mortality Among Infants Born to HIV-Infected Women on Highly Active Antiretroviral Therapy in Rural Uganda. JAIDS. 2009.
42. Tonwe-Gold B, Ekouevi DK, Viho I, Amani-Bosse C, Toure S, Coffie PA, et al. Antiretroviral treatment and prevention of peripartum and postnatal HIV transmission in West Africa: evaluation of a two-tiered approach. PLoS Med. 2007 Aug; 4(8):e257.
43. Miotti PG, Taha TE, Kumwenda NI, Broadhead R, Mtimavalye LA, Van der Hoeven L, et al. HIV transmission through breastfeeding: a study in Malawi. JAMA. 1999 Aug 25; 282(8):744-9.
44. Coutsoudis A. Infant feeding dilemmas created by HIV: South African experiences. J Nutr. 2005 Apr; 135(4):956-9.
45. Olayinka B, Oni AO, Mbajiorgu FE. Impact of infant feeding practices on the risk of mother to child transmission of HIV-1 in Zimbabwe. J Paediatr Child Health. 2000 Aug; 36(4):313-7.
46. Jackson DJ, Chopra M, Doherty TM, Colvin MS, Levin JB, Willumsen JF, et al. Operational effectiveness and 36 week HIV-free survival in the South African programme to prevent mother-to-child transmission of HIV-1. AIDS. 2007 Feb 19; 21(4):509-16.
47. Goga A, Colvin M, Doherty T, Jackson D, Sanders D, Chopra M, et al. Infant feeding practices at routine South African PMTCT sites
4th South African AIDS Conference; 2009; Durban, South Africa. 2009.
48. Goga A, van Wyk B, Jackson D, Doherty T, Colvin M, Chopra M. Operational effectiveness of guidelines on complete breastfeeding cessation to reduce mother-to-child transmission of HIV: results from a prospective observational cohort study at routine PMTCT sites, South Africa
4th South African AIDS Conference; 2009; Durban, South Africa. 2009.
49. Magoni M, Bassani L, Okong P, Kituuka P, Germinario EP, Giuliano M, et al. Mode of infant feeding and HIV infection in children in a program for prevention of mother-to-child transmission in Uganda. AIDS. 2005 Mar 4; 19(4):433-7.
50. Noel F, Mehta S, Zhu Y, De Matteis Rouzier P, Marcelin A, Shi JR, et al. Improving outcomes in infants of HIV-infected women in a developing country setting. PLoS ONE. 2008; 3(11)(e3723).

51. Kagaayi J, Gray RH, Brahmbhatt H, Kigozi G, Nalugoda F, Wabwire-Mangen F, et al. Survival of infants born to HIV-positive mothers, by feeding modality, in Rakai, Uganda. PLoS One. 2008; 3(12):e3877.
52. Gupta A, Gupte N, Sastry J, Bharucha KE, Bhosale R, Kulkarni P, et al. Mother-to-child transmission of HIV among women who chose not to exclusively breastfeed their infants in Pune, India. Indian J Med Res. 2007 Aug; 126(2):131-4.
53. Makokha EP, Songok EM, Orago AA, Koech DK, Chemtai AK, Kobayashi N, et al. Maternal immune responses and risk of infant infection with HIV-1 after a short course Zidovudine in a cohort of HIV-1 infected pregnant women in rural Kenya. East Afr Med J. 2002 Nov; 79(11):567-73.
54. Chung MH, Kiarie JN, Richardson BA, Lehman DA, Overbaugh J, John-Stewart GC. Breast milk HIV-1 suppression and decreased transmission: A randomized trial comparing HIVNET 012 nevirapine versus short-course zidovudine. Aids. 2005 02; 19(13):1415-22.
55. Coutsoudis A, Pillay K, Spooner E, Kuhn L, Coovadia HM. Influence of infant-feeding patterns on early mother-to-child transmission of HIV-1 in Durban, South Africa: a prospective cohort study. South African Vitamin A Study Group. Lancet. 1999 Aug 7; 354(9177):471-6.
56. Coutsoudis A. Influence of infant feeding patterns on early mother-to-child transmission of HIV-1 in Durban, South Africa. Ann N Y Acad Sci. 2000 Nov; 918:136-44.
57. Coutsoudis A, Pillay K, Spooner E, Coovadia HM, Pembrey L, Newell ML. Routinely available cotrimoxazole prophylaxis and occurrence of respiratory and diarrhoeal morbidity in infants born to HIV-infected mothers in South Africa. S Afr Med J. 2005 May; 95(5):339-45.
58. Coutsoudis A, Dabis F, Fawzi W, Gaillard P, Haverkamp G, Harris DR, et al. Late postnatal transmission of HIV-1 in breast-fed children: an individual patient data meta-analysis. J Infect Dis. 2004 Jun 15; 189(12):2154-66.
59. Embree JE, Njenga S, Datta P, Nagelkerke NJ, Ndinya-Achola JO, Mohammed Z, et al. Risk factors for postnatal mother-child transmission of HIV-1. AIDS. 2000 Nov 10; 14(16):2535-41.
60. Fawzi WW, Msamanga G, Hunter D, Urassa E, Renjifo B, Mwakagile D, et al. Randomized trial of vitamin supplements in relation to vertical transmission of HIV-1 in Tanzania. J Acquir Immune Defic Syndr. 2000 Mar 1; 23(3):246-54.
61. Fawzi WW, Msamanga GI, Hunter D, Renjifo B, Antelman G, Bang H, et al. Randomized trial of vitamin supplements in relation to transmission of HIV-1 through breastfeeding and early child mortality. AIDS. 2002 Sep 27; 16(14):1935-44.
62. Fox MP, z, Kuhn L, Aldrovandi G, Sinkala M, Kankasa C, et al. Reduced mortality associated with breast-feeding-acquired HIV infection and breast-feeding among HIV-infected children in Zambia. J Acquir Immune Defic Syndr. 2008 May 1; 48(1):90-6.
63. Fox MP, Brooks DR, Kuhn L, Aldrovandi G, Sinkala M, Kankasa C, et al. Role of breastfeeding cessation in mediating the relationship between maternal HIV disease stage and increased child mortality among HIV-exposed uninfected children. Int J Epidemiol. 2009 Apr; 38(2):569-76.
64. Ngatiri G, Mwangi R, Feced VT, Kimiru S, Bedell R, Macharia G, et al., editors. HIV free infant survival under routine program conditions in resource limited settings: the experience of the Karatina prevention of mother to child transmission of HIV (PMTCT) program implemented by the Ministry of Health in Kenya 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009; Cape Town, South Africa.
65. Mofenson LM, Taha TE, LI Q, Kumwenda J, Kafulafula G, Fowler MG, et al., editors. Infant extended antiretroviral (ARV) prophylaxis is effective in preventing postnatal mother-to-child HIV transmission

(MTCT) at all maternal CD4 counts 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2009; Cape Town, South Africa.

66. Obimbo EM, Mbori-Ngacha DA, Ochieng JO, Richardson BA, Otieno PA, Bosire R, et al. Predictors of early mortality in a cohort of human immunodeficiency virus type 1-infected African children. Pediatr Infect Dis J. 2004 Jun; 23(6):536-43.

67. Simpore J, Pietra V, Savadogo A, Pignatelli S, Nikiema JB, Nadembega WM, et al. Reduction of mother-to-child transmission of HIV at Saint Camille Medical Centre in Burkina Faso. J Med Virol. 2006 Feb; 78(2):148-52.

68. Chisenga M, Kasonka L, Makasa M, Sinkala M, Chintu C, Kaseba C, et al. Factors affecting the duration of exclusive breastfeeding among HIV-infected and -uninfected women in Lusaka, Zambia. J Hum Lact. 2005 Aug; 21(3):266-75.