Association between infant feeding practices and infant growth by maternal HIV and antiretroviral therapy status: A prospective study in Cape Town, South Africa

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PREAMBLE

Declaration

I, **Janice Lorean Rhoda**, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Background

Infants who are HIV-Exposed Uninfected (HEU) compared to HIV Unexposed Uninfected HUU may experience adverse growth outcomes. Breastfeeding (BF) provides infants with nutrients to grow optimally irrespective of maternal HIV status and initiation and duration of BF may be related to infant growth. In a cohort of mother-infant pairs enrolled from the same community in Cape Town, South Africa (SA), growth parameters were compared between infants who were HEU and HUU from birth up to 12 months of age investigating whether these were associated with types of infant feeding practices (BF and complementary feeding) and Household Food Security Status (HFSS) under the current era of universal Antiretroviral therapy (ART) in pregnancy policies in SA.

Methods

Pregnant women who attended antenatal care (ANC) at the Gugulethu Midwife Obstetric Unit (MOU) were included in this secondary analysis of data from a larger cohort study. Maternal demographic data were collected at enrolment. Data on infant feeding, food security and infant anthropometry were collected at postpartum visits: at seven days, 10 weeks, six months and 12 months. To convert infant weight and length at birth to weight-for-age z-scores (WAZ) and length-for-age z-scores (LAZ), Intergrowth-21st software was used, while the World Health Organization (WHO) Anthro survey analyzer tool was used to obtain these and weight-for-length z-scores (WLZ) at 10 weeks, six months and 12 months. Linear mixed effects (LME) models were fit to compare WAZ, LAZ and WLZ between infants who were HEU and HUU. Malnutrition including stunting, underweight, wasting and overweight were compared between the two groups at each timepoint from birth up to 12 months of age.

Results

Overall, 796 mother-infant pairs were included, 400 (50%) were HUU and 396 (50%) were HEU of whom 210 (53%) were exposed to both HIV and ART at conception while 186 (47%) were exposed to ART after conception but during gestation. A high proportion of all infants had ever breastfed in both

groups, however this was lower for infants who were HEU compared to HUU (90% vs 93%; p = 0.118) with continued BF at 10 weeks (67% vs 85%; p < 0.001), six months (41% vs 64%; p < 0.001), and 12 months (23% vs 46%; p < 0.001). Infants who were HEU vs HUU had a significantly shorter median duration of BF [73 days; IQR 12-222 vs 209 days; IQR 72-365 (p < 0.001]). Complementary feeding practices were similar in infants who were HEU compared to HUU: 50% vs 55% (p = 0.41) were introduced to solid, semi-solid or soft foods by six months old; around one-third to sweet beverages 33% vs 35% (p = 0.80); and 9% vs 9% (p = 0.92) consumed fruits and/or vegetables between 6 to 12 months of age.

For LME models, WAZ on average were lower for infants HEU than those who were HUU [β = -0.147; 95% CI: -0.327, 0.033; p = 0.11] keeping age at visit, maternal age, duration of BF, HFSS, employment and marital status constant. On average, LAZ were lower for infants who were HEU than HUU [β = -0.146; 95% CI: -0.339, 0.471; p = 0.14] keeping the same variables constant. Being married/cohabiting compared to not married/not cohabiting was associated with a significantly higher average WAZ and LAZ holding all other variables constant. This association was significant across the BF variables; duration of BF, any BF and exclusive breastfeeding (EBF). Being food secure compared to food insecure was associated with a significantly higher only for average WLZ but across all BF variables. By 12 months of age, 19% of all infants had high proportions of overweight.

Discussion

Infants who were HEU had consistently lower WAZ and LAZ compared to those who were HUU across all LME models. The findings suggest that being married/cohabiting compared to not married/not cohabiting and being food secure compared to food insecure across all BF categories may have a greater impact on infant growth outcomes than being in the population of infants who were HEU. The quality of complementary feeding was poor in both groups, high proportions of sweetened beverages and a low proportion of fruit and/or vegetables at six months. This is coupled with the observation of approximately 20% of infants being overweight by 12 months old.

Conclusion

To ensure optimal growth among infants, public health interventions need to be aimed at strengthening infant feeding practices among infants who are HEU compared to HUU in terms of initiation and duration of BF and improving introduction to complementary feeding practices for all infants. Consistent with previous studies WAZ remained lower up to 12 months old in infants who were HEU, likely associated with socio-economic factors (food insecurity and single parenthood).

Keywords: HIV Exposed Uninfected, HEU, infant growth, feeding practices, weight-for-age, length-for-age, South Africa

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List of abbreviations

AFASS	Acceptable, Feasible, Affordable, Sustainable and Safe
ANC	Antenatal Care
ART	Antiretroviral Therapy
AUDIT	Alcohol Use Disorders Identification Test
β	Beta
BF	Breastfeeding
CI	Confidence Interval
DNA	Deoxyribonucleic
EBF	Exclusive Breastfeeding
GA	Gestational age
HEU	HIV-Exposed Uninfected
HFSS	Household Food Security Status
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
HUU	HIV-Unexposed Uninfected
IQR	Inter-quartile range
IYCF	Infant and Young Child Feeding
LAZ	Length-for-age z-score
LBW	Low Birth Weight
LME	Linear-mixed effects
MOU	Midwife and Obstetric Unit

NDoH	National Department of Health
PCR	Polymerase chain reaction
PEP	Post-exposure Prophylaxis
p	P-value
REDCap	Research Electronic Data Capture
RTHB	Road to Health Booklet
SA	South Africa
SD	Standard Deviation
SES	Socio-economic status
SGA	Small for gestational age
SSA	Sub-Saharan Africa
UCT	University of Cape Town
VTP	Vertical Transmission Prevention
WAZ	Weight-for-age z-score
WHO	World Health Organization
WLHIV	Women living with HIV
WLZ	Weight-for-length z-score

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PART A: PROTOCOL

Background

Infants who are HIV-Exposed Uninfected (HEU) are exposed to maternal Human Immunodeficiency Virus (HIV) infection *in utero* but are not themselves infected with HIV despite maternal infection. Infants who are HIV-Unexposed Uninfected (HUU) are neither exposed to nor infected with HIV because their mothers have an HIV negative status. Infants who are HEU infants comprise an emergent subpopulation in sub-Saharan Africa (SSA) due to advances in vertical transmission prevention (VTP) policies in women living with HIV (WLHIV). In South Africa (SA), the number of children who are HEU was estimated at 3.5 million in 2018, among the five highest countries recorded globally[1]. These infants are subject to various exposures during early development including maternal HIV infection and antiretroviral therapy (ART) exposure *in utero* and via breastmilk; and directly via post-exposure prophylaxis (PEP)[1, 2]. Despite infants who are HEU not living with HIV, the exposures that they come into contact with impacts their health in several ways. Various studies[3-7] have demonstrated that this vulnerable population is at risk of their health and growth being negatively influenced in comparison to their HUU counterparts.

The health outcomes of infants who are HEU differ from that of their HUU counterparts.[4] Infections including common respiratory tract infections and diarrhoea, and developmental delay are more common, placing them at an increased risk of both morbidity and mortality.[8] Although ART prevents VTP of HIV, this additional exposure presents different kinds of challenges and it is unclear how and to what extent either *in utero* and infant HIV and ART exposure influence infant growth. However, both exposures influence the health and well-being of infants. In a prospective cohort study in SA, in univariable linear regression analysis, infants who were HEU had significantly lower mean weight-for-age (WAZ) and length-for-age (LAZ) z-scores than those who were HUU at birth. In multivariable analysis after adjusting for maternal age, gravidity, alcohol use, marital and unemployment status mean WAZ remained significantly lower among infants who were HEU compared to those who were HUU. However mean LAZ was no longer significantly

lower.[5]

In another South African study infants who were HEU were found to have increased infectious-cause hospitalisation between 29 days and 12 months of age in comparison to infants who were HUU.[6] Other studies within the sub-Saharan region demonstrate similar patterns including a prospective study[1] of Zimbabwean children who were breastfed exclusively for six months reported both significantly lower mean WAZ and LAZ for infants who were HEU compared to HUU from birth up to 16 weeks of age. Another prospective study[3] consisting of a Nigerian cohort of infants who were HEU compared to HUU studied from birth to 18 months found both lower WAZ and LAZ when adjusting for maternal education, marital status, breastfeeding status, prematurity, baseline WAZ and maternal weight in a longitudinal linear regression analysis. On the other hand, a cohort study in Kwazulu-Natal reported lower WAZ at nine months among infants who were HEU compared to HUU and this was independent of breastfeeding duration,[9] Breastfeeding however is important to consider in this context because of the benefits that breastmilk provides to infants' growth and development irrespective of HIV exposure.

Previously, before the widespread use of ART during pregnancy and breastfeeding, many WLHIV did not breastfeed because health policy Acceptable, Feasible, Affordable, Sustainable and Safe (AFASS) did not promote breastfeeding unless optimal conditions existed. Additionally, they feared vertical transmission and were provided with formula by public health facilities for their infants. In response to evidence from randomized controlled trials, the World Health Organization (WHO) published recommendations that WLHIV breastfeed exclusively for six months, risk of vertical transmission being reduced by maternal ART and/or infant PEP.[10] However, a recent study conducted in SA found that the proportion of WLHIV who exclusively breastfed their infants was lower compared to those who did not have HIV irrespective of the various timepoints from birth up to six months old. Factors such as mixed messages during infant feeding counselling, social and economic constraints, and fear of HIV transmission play a role in both starting and continuing breastfeeding.[11] Breastfeeding has however been recognised as being important to infant health and development since breastmilk has the ability to provide infants with the necessary nutrients which they need to grow optimally. The benefits of breastfeeding are well known and exclusive breastfeeding up to six months and continued breastfeeding up to two years old is advocated for infants who are HEU and HUU.[12, 13] Studies have

demonstrated numerous benefits of breastfeeding such as improved performance in intelligence tests[14], the protection against diarrhoea related illness[13] and the reduction of the risk of pneumonia[15]. These studies demonstrate how morbidity and mortality can be reduced among infants through breastfeeding and highlight the valuable nature of the benefits of breastfeeding in infants.

The WHO Global strategy for Infant Feeding in 2010 provided guidelines for infant feeding with SA adopting[16] these guidelines in 2011. The South African National Department of Health (NDoH) has amended their policy recommendation for Infant and Young Child Feeding (IYCF) of 2013 to align with the updated WHO/ United Nations Children's Fund 2016 guidelines[17] (Table 1; Appendix 1 Table 4) and WLHIV have since been recommended to continue breastfeeding for 24 months as opposed to 12 months which aligns the recommendations for women living with and without HIV[17].

Table 1. Summary of IYCF indicators for breastfeeding[10]

Breastfeeding guidelines	**Definition**
Ever breastfed	Percentage of children born in the last 24 months
Early start of breastfeeding	Percentage of children born in the last 24 months who breastfed within one hour of birth
Exclusively breastfed only for two days after birth	Percentage of children born in the last 24 months who exclusively breastfed for the first two days after birth
Exclusively breastfed below six months	Percentage of infants aged 0-5 months who exclusively breastfed the day before
Mixed milk feeding below six months	Percentage of infants aged 0-5 months who were formula fed and/or given animal milk in addition to breast milk the day before
Continued breastfeeding from 12-23 months of age	Percentage of children 12-23 months who breastfed the day before

Breastfeeding is not only known for its ability to influence the health of infants but also particularly the duration of breastfeeding is of interest and may be important. A study in Uganda found an association between early cessation of breastfeeding and increased risk of morbidity among infants who were HEU compared to those who were HUU.[18] There is evidence that exclusive breastfeeding up to six months has health benefits for infants. A study in Ethiopia found that premature cessation of exclusive breastfeeding both before four months and between 4-6 months in comparison to six months was associated with at least one infectious morbidity including increased occurrence of diarrhoea, fever and acute respiratory illnesses[19]. This highlights the importance of exclusive breastfeeding up to six months as opposed to four months or less than three months and the implications duration of breastfeeding on infant health.

A recent study[2] suggested that breastfeeding practices should be improved for infants who are HEU to achieve optimal growth. The WHO recommends introducing complementary foods to infants from six months of age[10] (Table 2. Appendix 2 Table 5). Limited data exists that investigates complementary feeding, including differences by maternal HIV status.[20] Breastfeeding and complementary feeding both affect the long-term health of infants.[21] In a recent cross-sectional study[22] among children aged six months in Kwazulu-Natal in SA a big increase was detected in respect of prevalence of obesity between birth and six months old. In addition, 17.6% of children started fluids or food in the first month of their lives.[22] Complementary feeding is thus recognised as a critical time for infants because of its influence on food preferences and its impact on long-term growth.[23]

Table 2. Summary of IYCF indicators for complimentary feeding[10]

Complementary feeding guidelines	**Definition**
Introduction to solid, semi-solid or soft foods	Infants aged 6-8 months who consumed any of these foods.
Minimum dietary diversity	Children aged 6-23 months who consumed food and drinks from a minimum of five out of eight defined food groups
Minimum meal frequency	Children aged 6-23 months who consumed solid, semi-solid or soft foods including milk feeds for non-breastfed children the minimum amount of time or more
Minimum milk feeding frequency for non-breastfed children	Non-breastfed children aged 6-23 months who consumed a minimum of two milk feeding
Minimum acceptable diet	Children age 6-23 months who consumed a minimum acceptable diet
Egg and/or flesh food consumption	Children aged 6-23 months who consumed egg and/or flesh food
Sweet beverage consumption	Children aged 6-23 months who consumed sweet beverage
Unhealthy food consumption	Children aged 6-23 months who consumed certain sentinel unhealthy foods
Other guidelines	
Bottle-feeding	Children aged 0-23 months who were bottle fed with a nipple
Infant feeding area graphs	Infants aged 0–5 months who were fed exclusively with breast milk, breast milk and water only, breast milk and non-milk liquids, breast milk and animal milk/formula, breast milk and complementary foods, and not breastfed
Note each of the above definitions in this particular table includes the appropriate age group of infants/children and the percentage of them who consumed the food and/or drink or other during the previous day	

A recent systematic review[24] suggested that there are insufficient data regarding growth outcomes by maternal HIV and ART status of children who were HEU in relation to their HUU counterparts. Early studies investigating growth in infants who are HEU were often confounded by breastfeeding practice[25,

[26] as international guidelines recommended that WLHIV avoid breastfeeding. More recent studies suggest that growth differences between infants who are HEU and HUU persist up to at least six months old.[27] The impact on longer-term growth is uncertain as is the effect of feeding practices during the first 12 months. In addition, a study[9] suggests that limited data exists for the association between feeding practices and women not living with HIV. The main aim of this study is therefore to identify, describe and compare associations of infant feeding practices with infant growth in infants who are HEU compared to HUU under the era of universal ART in pregnancy. The recognition of the increasing population of infants who are HEU and their health needs is important to understand so that efforts can be made at prevention and intervention strategies to reduce the burden of adverse health outcomes for these children[1].

Study aims and objectives

Aim

The main aim of this study is to identify, describe and compare associations of infant feeding practices and growth in infants who are HEU and HUU at the Gugulethu Midwife and Obstetric Unit (MOU), under the era of universal ART policies.

Objectives

1. To describe infant feeding practices from birth to 12 months old by maternal HIV and ART exposure status with specific reference to:

a. The WHO breastfeeding recommendations:

- breastfed within one hour after birth
- exclusively breastfed for six months
- infants still breastfeeding at 12 months and
- those who received mixed milk feeding with formula and/or animal milk in addition to breast milk under six months

b. Duration of breastfeeding, if any

c. Type of complementary feeding

2. To describe and compare growth WAZ and LAZ between HEU and HUU infants and whether these are associated with types of feeding practices and Household Food Security Status (HFSS)

Methods

Study design

Overview of the proposed study

To address the study aim, we will conduct a secondary analysis of a B-Positive prospective cohort study which enrolled 989 pregnant women at their first antenatal visit. These mother-infant pairs were followed up for up to 12 months from birth. Of the 989 women 796 mother-infant pairs included in the final analysis. Of the 796 women with a singleton livebirth, we will include mother-infant pairs with at least two postnatal visits including 400 infants who are HUU and 396 who are HEU, of whom 210 were exposed to preconception ART and 186 exposed to ART during gestation.

The postnatal visits are of interest to this study in which the B-Positive study evaluated mother-infant pairs by the study teams at various time points; birth, 10 weeks, six months and 12 months. The measures that were administered from the B-Positive study that will be used for this analysis include Infant feeding practices questionnaires for each study visit and mother's and infants' growth anthropometry.

Study setting

The B-Positive study was an observational prospective cohort study which enrolled pregnant women attending antenatal care (ANC) at a primary maternity healthcare facility in Gugulethu, an urban township in Cape Town, SA. The Gugulethu MOU is a public centre primary care obstetric facility. Midwives at the Gugulethu MOU provide ANC, delivery and immediate postnatal services for uncomplicated pregnancies. Women can be referred to the hospital for further care at any stage

according to standard operating procedures. HIV screening is provided regularly throughout pregnancy for women with negative or unknown HIV status.

Characteristics of study population

Participants

The Gugulethu facility serves a population of about 350, 000 with an estimated antenatal HIV prevalence of 30%[28] in 2012. Pregnant women aged 18 years and above, living with and without HIV, were screened and recruited at their first ANC visit. Eligible participants were enrolled into the study between January 2017 to July 2018. Women were eligible to participate in the study if they had a confirmed maternal HIV test and if they planned to reside in Cape Town for at least one year postpartum with their infants. For women not known to be living with HIV a rapid antibody test was made use of to confirm their HIV status as this has sufficient sensitivity and specificity for screening and is the standard of care in routine ANC[29]. Women who tested negative for HIV based on a rapid antibody test were retested up to twice during pregnancy depending on their GA at enrolment. Women who seroconverted during the study were not included in the analysis.

Study procedures

All pregnant women who were eligible and able to provide informed consent were enrolled into the study. Women were followed antenatally for one to three study visits depending on the gestational age (GA) at enrolment. Mother-infant pairs were evaluated by the study teams postnatally at birth, 10 weeks, six months and 12 months. Only mothers who gave birth to a liveborn baby will be included in this secondary analysis. Questionnaires were administered in both isiXhosa and English with participants having access to refer to both these languages on their questionnaire. The questionnaires were administered at each study visit by trained study staff. Questionnaires were designed by several researchers and standardised instruments were used. Pilot studies were done on all the B-Positive questionnaires prior to data collection to establish reliability and validity. The training for study staff included general research issues, ethical conduct, topics on administration of questionnaires. The study clinician conducted regular anthropometric training for all study staff with structured, supervised and

competency assessments. Verbal questionnaires were administered face-to-face by trained interviewers in a closed room to ensure privacy.

Maternal Demographics Clinical History

Information such as maternal demographics, pregnancy history and healthcare information were collected using identical standardized questionnaires administered to all women by trained study interviewers.

Maternal HIV and ART history

The primary exposure for this secondary analysis is HIV exposure *in utero* (infants who are HEU vs HUU). Maternal HIV status was self-reported and confirmation was provided through medical chart review. Women who tested negative for HIV at enrolment based on a rapid antibody test were routinely tested either right after delivery or during newborn visits within 3-7 days after delivery and approximately once every three months during breastfeeding. HIV deoxyribonucleic (DNA) Polymerase Chain Reaction (PCR) testing of infants was taken at birth and 6 to 10 weeks according to national guidelines. Maternal HIV history and other data related to medical conditions were based on medical records.

Infant feeding

Infant feeding practices were assessed for the B-Positive study according to the WHO guidelines. Infant feeding practices questionnaires were administered postnatally to all women by trained study interviewers at up to four study visits. The first infant feeding practices questionnaire will assess breastfeeding behaviour (ever given breastmilk, when mothers put their baby to the breast after birth) and thereafter questionnaires from study visit two up to four will assess feeding behaviour (since last visit, for how long either weeks, days or months). In addition, complementary feeding will be assessed by the first questionnaire (food or drink given to your baby three days after birth) and following from the second up to the fourth questionnaire (the age at which you gave your baby food or drink besides breastmilk). Other questions assessed by each study visit relate to (reasons for stopping breastfeeding, 24-hour recall of breast/formula feeding, medications given to infants and who the baby was left with).

Infant growth

Infant growth is the outcome of this secondary analysis. Birth weight and length were extracted from the South Africa's child Road to Health Booklet (RTHB) to derive anthropometric outcomes, WAZ and LAZ to represent surrogate measures of fetal growth. Birth weight and length were measured at each study visit (seven days, 10 weeks, six months and 12 months) by study staff. During the 6-10 week and 6-month study visit infant weight was assessed with precision to the nearest 10 grams using a calibrated digital infant scale (MTB 20 Baby Scale, Adam Equipment, Milton Keynes, UK), by trained study staff without clothing and diapers. All the infant measurements were taken while the infant was recumbent with the use of an infant length measuring board. Length measurements were recorded to the nearest 0.25 centimetres using a rigid recumbent length board (ADE MZ 1013 Baby Length Measuring Rod, ADE, Hamburg, Germany). Two measurements of infant weight and recumbent length were taken at each visit by study staff and the average was calculated to ensure accuracy and avoid outliers. No big differences were found between the two measures for weight and length. Birth weights and lengths were converted to WAZ and LAZ using Intergrowth-21st software[30] adjusting for age and sex.

Analytic considerations

Sample size considerations

The sample size was determined by the power calculations for the parent study. In this secondary analysis of existing data, we performed a power analysis to determine the statistical power for growth disparities between infants who are HEU and HUU. Based on insights from previous research and past studies conducted in SA, we considered that a sample size of 600 provides adequate power (>80%) to identify absolute differences in growth patterns between infants who were HEU and HUU, with a significance level set at 0.05.

Data analysis

For the B-Positive study, Research Electronic Data Capture (REDCap)[31] a secure, web-based application was used to capture the study data. For this analysis data will be exported to R. Descriptive

statistics will be used to describe infant and maternal characteristics and will be summarised by proportions (%), means and standard deviation for normally distributed data and median and interquartile range (IQR) for non-normally distributed data. Descriptive statistics will also be used to describe the duration and type of feeding practices. Pearson's Chi-squared test or Fisher's Exact test will be used to assess differences in categorical variables. The Kruskal-Wallis test will be used to compare medians for non-parametric data.

WAZ and LAZ at birth will be generated using Intergrowth-21st software, which adjusts for infant GA at birth and infant sex. WAZ and LAZ for 10 weeks, six months and 12 months will be calculated using the WHO Child Growth Standards.[32] The z-score results will be reported as mean and standard deviation at each age. Student t-tests will be used to compare WAZ and LAZ differences between infants who are HEU and HUU. We will use the lme4 package in R to conduct a mixed-effects longitudinal analysis. These models will allow for the inclusion of both fixed and random effects. A random effect for subject will be included in these models since we have repeated measured for each infant on the outcome variables WAZ, LAZ and WLZ. The feeding variables will be included to assess whether they are positively or negatively correlated with these outcome variables and whether the associations are significant.

Ethics

Ethical review

For this secondary analysis, we will request approval from the University of Cape Town's Faculty of Health Sciences Research Ethics Committee (UCT-HREC). The study protocol, informed consent forms, the relevant data collection tools and any other requested information will be submitted to UCT-HREC). In addition, the B-Positive parent study has received approval by UCT-HREC number :749/2015 and approved all questionnaires that will be used for this analysis.

Informed Consent

For the B-Positive cohort study, the informed consent form explained the purpose of the study to participants and what would happen if they decided to take part in the study. There were two informed

consent forms one for the pregnant mother and one for the infant. Written informed consent was obtained for mother-infant pairs who participated in the B-Positive study. Participants were informed that if they refused to participate in the study or later decided to withdraw from the study they could do so at any point during the study and this would not affect the health care that they receive at the Gugulethu MOU or any other health care facility. The informed consent documents are provided below in the Appendices section of this document.

Potential Risks

Minimal potential risk was attributable to mothers and their infants. Mothers were informed that they may feel uncomfortable when asked personal questions. They were however made aware that they were not required to answer any questions that they did not want to. There was the risk of sharing personal and medical information however this information was to be kept as private as possible.

Potential Benefits

Participants were informed that there would be no direct benefits to them for taking part in the B-Positive study but that the information obtained from that study may however be useful to improve the services of pregnant women and their children in SA.

Compensation

Participants were notified that for the B-positive study, they would not receive any costs for participating in the study. They will however at the end of each study visit receive a R100 grocery voucher, R20 for transport and food, and drink while they are at the visit.

Privacy and Confidentiality

For the B-Positive prospective study participants were informed that their information will be kept strictly confidential. This means that their names will not be written on their study forms or be linked to any information or lab samples that were to be collected as part of the study. All staff involved in the data collection and data management process received specific training with respect to confidentiality. Personnel involved in the study process were required to sign a confidentiality agreement form. Hard

copy participant-related information was stored in locked filing cabinets for the B-Positive study and electronic records were made password-protected and files were encrypted. For the secondary analysis, an agreement of confidentiality will be signed when handling the B-Positive data to protect the confidentiality of the participants who participated in the study.

Timeline and Budget

Secondary data will be used for the proposed project. No payment for the student is required.

Table 3. Study Timeline

Activity	**Time in months**				
	2022		**2023**		**2024**
	Mar-Oct	**Nov-Dec**	**Jan-Oct**	**Nov-Dec**	**Jan-Feb**
Protocol submission to Ethics					
Merge and clean data. Data analysis					
Finalise dissertation for marking					

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PART B: MANUSCRIPT

Association between infant feeding practices and infant growth by maternal HIV and antiretroviral therapy status: A prospective study in Cape Town, South Africa

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Abstract

Background

Infants who are HIV-Exposed Uninfected (HEU) may experience adverse growth outcomes compared to those who are HIV Unexposed Uninfected (HUU). Breastfeeding (BF) provides infants with the necessary nutrients to grow optimally irrespective of maternal HIV status. The initiation and duration of BF may also be related to infant growth. We compared growth parameters from birth up to 12 months old in infants who were HEU and HUU, investigating associations with types of infant feeding practices (BF and complementary feeding) and Household Food Security Status (HFSS) under the current era of universal Antiretroviral therapy (ART) in pregnancy policies in South Africa.

Methods

Pregnant women living with and without HIV were enrolled at their first antenatal visit. Feeding data and infant anthropometry were collected at birth, 7 days, 10 weeks, 6 months, and 12 months postpartum. Infant weight and length at birth were converted to weight-for-age (WAZ) and length-for-age (LAZ) using Intergrowth-21st software, and the World Health Organization (WHO)-Anthro survey analyzer tool was used to obtain these and weight-for-length z-scores (WLZ) from 10 weeks old. Linear mixed effects (LME) models were fit to compare WAZ, LAZ and WLZ between infants who were HEU and HUU controlling for *a priori* selected variables.

Results

Overall, 796 mother-infant pairs were included, 400 (50%) were HUU and 396 (50%) were HEU. A high proportion of all infants had ever breastfed, although this was lower in infants who were HEU compared to HUU (90% vs 93%; p = 0.118). Infants who were HEU vs HUU had a significantly shorter median duration of BF [73 days; IQR 12-222 vs 209 days; IQR 72-365 [p < 0.001]). There were no differences between the two groups regarding the types of complementary feeding. By 12 months, both infants who were HEU vs HUU had high proportions of overweight (17% vs 21%; p 0.22). WAZ and LAZ on average were lower in infants HEU than HUU [β = -0.147; 95% CI: -0.327, 0.033] and [β = -

0.146; 95% CI: -0.339, 0.471] keeping age at visit, maternal age, duration of BF, HFSS, employment and marital status constant.

Conclusion

Infants who were HEU had lower WAZ and LAZ compared to those who were HUU after adjusting for covariates. At 12 months, high proportions among both groups were overweight; which may be partly related to sub-optimal complementary feeding practices. Public health interventions need to be aimed at strengthening BF practices among the population of infants who are HEU and improving complementary feeding practices for all infants**.**

Keywords: HIV Exposed Uninfected, HEU, infant growth, feeding practices, weight-for-age, length-for-age, South Africa

Introduction

Infants HIV-Exposed Uninfected (HEU) constitute a sub-population who are exposed to HIV infection *in-utero* but are not infected with HIV. Infants HIV-Unexposed Uninfected (HUU) are born to women without HIV and therefore are neither infected with nor exposed to HIV. Due to the advancement of vertical transmission prevention of HIV (VTP) policies infants who are HEU comprise a growing population in sub-Saharan Africa (SSA). In 2022, approximately 16 million children globally were HEU,[1] including over 90% living in SSA, 3.5 million (23.8%) in South Africa (SA), which accounted for one of the top five countries globally.[2] Infants who are HEU experience various exposures including maternal HIV infection and antiretroviral therapy (ART) exposure *in-utero,* during breastfeeding as well as directly to infant post-exposure prophylaxis (PEP).[2, 3] Studies have shown that infants who are HEU are a vulnerable group who are at risk of adverse health effects and poorer growth.[4-8] They experience more respiratory infections, diarrhoea related illnesses and developmental delays increasing their risk for morbidity and mortality.[2] Studies in SA[5] and SSA[8-10] reported lower weight-for-age z-scores (WAZ) and length-for-age z-scores (LAZ) in infants who are HEU infants compared to their HUU counterparts. Mean birth weight among infants who were HEU were lower than those who were HUU, including WAZ and LAZ up to 12 months.[11] In addition, mean birth WAZ and LAZ was lower among infants HEU compared to HUU and up to 18 months.[8] These findings suggest that lower birth weight may contribute to lower WAZ and LAZ up to 12 and 18 months old respectively among infants HEU. Lower birth weight was found to be the main predictor of undernutrition among infants who were HEU compared to those who were HUU.[12]

The benefits of breastfeeding (BF) are well understood and breastmilk is recognised as a crucial part of infant health and development irrespective of HIV exposure. In 2011 SA adopted recommendations for infant feeding by the World Health Organization (WHO) Global Strategy for Infant Feeding 2010.[13] The South African National Department of Health (NDoH) revised its policy recommendation for Infant and Young Child Feeding (IYCF) in 2017 in line with the WHO/United Nations International Children's Emergency Fund recommendations, with mothers living with HIV advised to continue BF up to 24 months instead of up to 12 months aligning with guidelines for women living without HIV.[14] Exclusive

breastfeeding (EBF) up to six months with continued BF up to 24 months is advocated for in both infants who are HEU and HUU[15] with premature ending of BF both before four and between 4-6 months associated with at least one morbidity including increased occurrence of diarrhoea, fever and acute respiratory illnesses.[16] In addition, lower rates of BF in infants who were HEU vs HUU corresponded with poorer growth outcomes for this population.[11] Numerous benefits of BF exist including improved performance in intelligence tests, school performance and higher income in adulthood.[17] These findings illustrate the value of BF in reducing morbidity and mortality among infants. Improvement of BF practices is vital for infants who are HEU to achieve optimal growth.[3] In addition, addressing maternal nutritional needs can assist infants in receiving the necessary nutrients through breastmilk, as maternal nutrition before conception and at early gestation was reported to positively impact the growth outcomes of infants up to six months of age.[18]

The WHO recommends the introduction to complementary feeding to infants from six months of age.[19] Limited data exist that explores introduction of complementary feeding and the differences by maternal HIV status.[20] Early introduction of complementary feeding is still a common practice in SA[11] and inappropriate types of complementary feeding may result in infant growth faltering.[21] Early introduction to complementary feeding has been found to negatively impact growth, with an increased prevalence of obesity as opposed to underweight.[22] In addition, early infant feeding practices impact the development of obesity as early as 12 weeks old.[23] We compared growth parameters between infants who were HEU and HUU from birth to 12 months of age and investigated whether these were associated with types of infant feeding practices (BF and complementary feeding) and Household Food Security Status (HFSS) under the era of universal ART in pregnancy policies.

Methods

Study setting

The B-Positive study was a prospective cohort study which enrolled pregnant women attending antenatal care (ANC) at a primary maternity healthcare facility in Gugulethu, an urban township in Cape Town, SA and aimed to comprehensively monitor the effectiveness, impact, and risks of the WHO Option B+ prevention of vertical transmission of HIV approach. WHO Option B+ is a prevention of vertical transmission of HIV strategy that recommends life-long ART for all WLHIV irrespective of their CD4 count or clinical status. The Gugulethu Midwife Obstetric Unit (MOU) is a public sector primary care obstetric facility. Midwives provide ANC, delivery, and immediate postnatal services for uncomplicated pregnancies. Women can be referred to the hospital for further care at any stage according to standard operating procedures. HIV screening is provided regularly throughout pregnancy for women with negative or unknown HIV status. The facility serves a population of approximately 350,000 with an antenatal HIV prevalence of 30% in 2015.[24]

Study design and study participants

Pregnant women 18 years and above were recruited at their first antenatal visit at Gugulethu MOU irrespective of their HIV status between January 2017 to July 2018. They were followed up until the 12-month study visit. Eligible participants included pregnant women who were able to provide informed consent, had a confirmed HIV status and planned to reside in Cape Town at least one year postpartum with their infants. Clinic records were used to confirm maternal HIV status. Women with an unknown HIV status were tested using a rapid antibody test to confirm their HIV status. The women attended between one to three antenatal visits prior to delivery depending on the gestational age (GA) at enrolment. GA was determined by an ultrasound specialist specifically assigned to the study and repeated at each antenatal visit. After the birth of the baby, mother-infant pairs attended four postnatal visits at seven days, 10 weeks, six and 12 months. Of the 989 women who enrolled in the B-positive study, a total of 796 mother-infant pairs with live singleton births and birth anthropometry available were included in this analysis (Fig.1). We included those with singleton live births who attended at least two postnatal visits.

Study procedures and data collection

Birth weight and length as recorded in the South African Road to Health Booklet (RTHB) were abstracted to derive birth WAZ and LAZ to represent surrogate measures of fetal growth. Weight and length were measured at each study visit by study staff. Infants with anthropometric data for at two study visits were included in this analysis. During the 6-10 week and 6-month study visit infant weight was assessed with precision to the nearest 10 grams using a calibrated digital infant scale (MTB 20 Baby Scale, Adam Equipment, Milton Keynes, UK), by trained study staff without clothing and diapers. All the infant measurements were taken while the infant was recumbent with the use of an infant length measuring board. Length measurements were recorded to the nearest 0.25 centimetres using a rigid recumbent length board (ADE MZ 1013 Baby Length Measuring Rod, ADE, Hamburg, Germany). Two measurements of infant weight and recumbent length were taken at each visit and the average was calculated to ensure accuracy and avoid outliers. No big differences were found between these two measures for weight and length. Additionally, medical information was collected from antenatal, obstetric, medical and laboratory records.

Data were collected via interviewer-administered questionnaire regarding maternal demographics, the woman's pregnancy history, and healthcare-related information. Maternal HIV status and testing results were self-reported and confirmed through medical chart review. Women who tested negative for HIV based on a rapid antibody test were retested up to two times during pregnancy depending on their GA at enrolment as per prevention of vertical transmission of HIV guidelines.[25] All infants who seroconverted at any time during follow-up were excluded from the analysis to avoid misclassification. Women who seroconverted during the study were not included in the analysis. HIV Polymerase Chain Reaction (PCR) testing of infants was taken at birth and six to 10 weeks according to national guidelines.[25] Infants who were infected with HIV were excluded. Maternal HIV history and other data related to medical conditions were confirmed from medical records. Relevant to this study were the Infant Feeding Practices and Food Security questionnaires as well as infant growth anthropometry. To assess infant feeding practices and introduction to complementary feeding, standardised questionnaires were used based on 24-hour recall. The Alcohol Use Disorders

Identification Test (AUDIT)[26] a tool validated in SA[27] was used to collect alcohol related information to measure alcohol intake during pregnancy. The Six-Item Short Form of the Food Security Survey Module (HFSS) was used to identify households with food insecurity and those with very low food security. It asks questions about the food eaten in the household in the last 12 months, since (current month) of last year and whether mothers were able to afford the food that they needed.[28] This tool was used to measure household food security.

Exposures and outcomes

For this secondary analysis, data collected at the enrolment and postpartum visits were included. The primary exposure was maternal HIV. The primary outcome was infant growth up to 12 months of age, including at birth, 10 weeks, six and 12 months using WAZ and LAZ. These scores at birth were produced using Intergrowth-21st software[29] which adjusted for GA at birth and infant sex. WHO Anthro Survey Analyser tool[30] was used to generate WAZ, LAZ and weight-for-length z-scores (WLZ) at 10 weeks, six months, and 12 months. Prevalence of malnutrition with malnutrition defined as deficits in one's intake of energy and/nutrients and comprises of undernutrition (stunting, wasting, underweight) and overnutrition (overweight).[31] Stunting was defined as LAZ < -2 SD, underweight as WAZ < -2 SD, overweight as WLZ > +2 SD and wasting as WLZ < -2 SD as per WHO guidelines.[30]

Z-scores > 3 or < -3 were reviewed and corrected in the case of data capture errors. Unexplained values > 5 for WAZ and WLZ, > 6 for LAZ or < -6 for WAZ and LAZ and < -5 for WLZ were excluded from the analysis. These scores accounted for < 3.5% of all measurements. Low birth weight (LBW) was defined as infants with a birth weight < 2.5kg. Preterm delivery was defined as infants' GA at delivery < 37 completed weeks. Small for gestational age (SGA) was defined as a weight less than the 10th centile for GA. Infant GA at delivery was extracted using GA determined through the mother's ultrasound taken at enrolment, date of enrolment and the infant's date of birth. To determine socio-economic status (SES) an asset score was calculated (toilet, running water, electricity, refrigerator, landline, and television inside the home measured as binary variables). In addition, level of education and employment status were added to the asset score and three subcategories were created: low, middle and high.

Statistical analysis

Data were exported from REDCap into the R statistical package (version 4.1.1) for analysis. REDCap[32] was used to collect and manage the study data. Variables related to the types of complementary feeding by six months were defined based on the Guidelines of the WHO Global Strategy for Infant Feeding recommendations, introduction of: solid, semi-solid or soft foods; egg and/or flesh flood consumption; sweet beverage consumption; and vegetables and/or fruit consumption.[19] Descriptive statistics were used to describe maternal and infant characteristics, infant feeding practices, age at introduction to complementary feeding, z-scores at each age, and P-values for categorical data were calculated using either Pearson's chi-squared test, Fisher's exact test or Cochran-Armitage test as appropriate. P-values for means were calculated using Student's t-test and for medians using Wilcoxon rank sum test. The lme4 package in R was used to conduct a mixed-effects longitudinal analysis. These models allowed for the inclusion of both fixed and random effects. A random effect for subject was included in these models since we have repeated measured for each infant on the outcome variables WAZ, LAZ and WLZ. The feeding variables were included to assess whether they were positively or negatively correlated with these outcome variables and whether these associations were significant. Linear mixed-effects (LME) models were fit separately for WAZ, LAZ and WLZ entered into the model as continuous dependent variables. Each model included a random effect for each subject and explanatory variables included HIV status, time (study visit), maternal age, employment status, HFSS and marital status. Infant feeding variables including any BF, duration of BF or EBF up to 6 months were entered separately into the models as fixed effects to avoid multicollinearity. All covariates for the LME models were selected *a priori*. A directed acyclic graph was developed which included factors known to be associated with HIV infant exposure status and infant growth outcomes. We conducted a sensitivity analysis for the BF variables including ever BF and EBF and restricted the analysis for LME models to duration of BF.

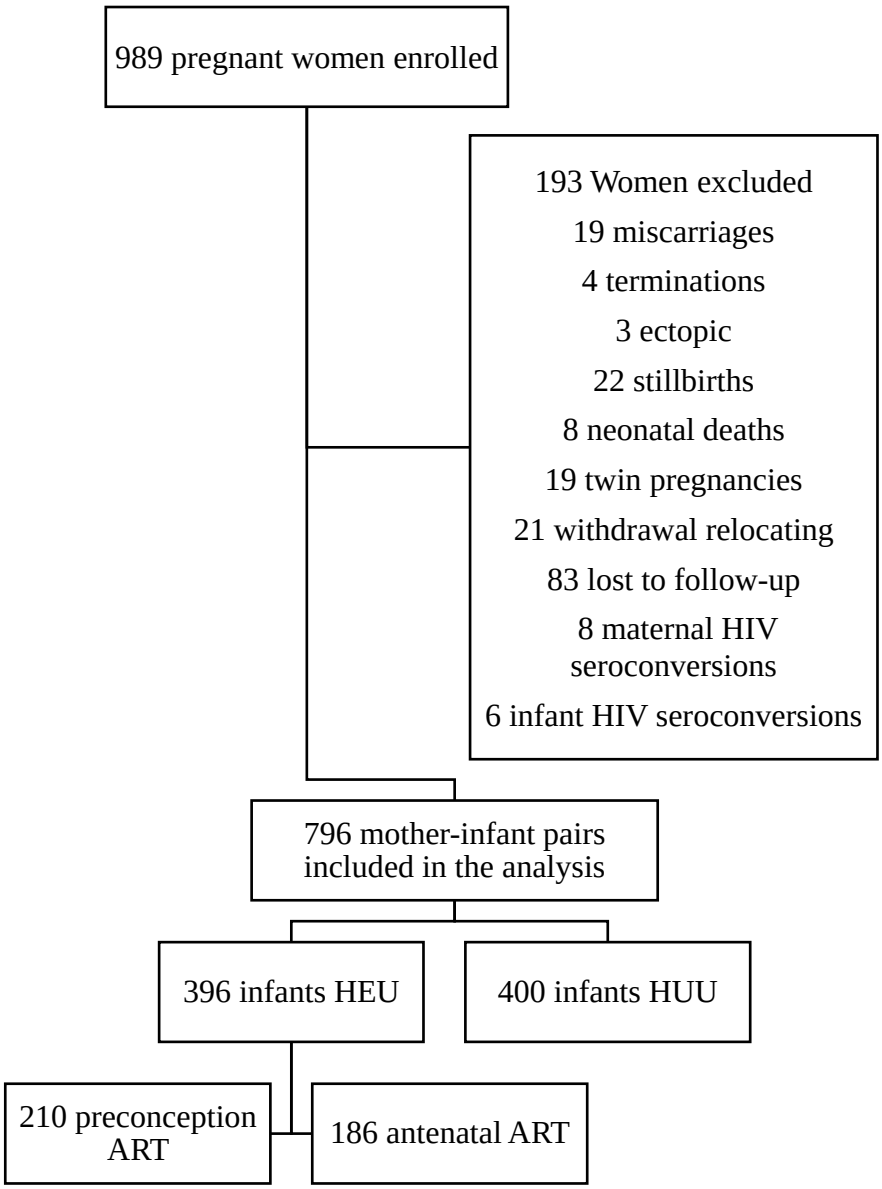
Ethical considerations

This study received ethical approval from the University of Cape Town's Faculty of Health Sciences Research Ethics Committee (UCT-HREC-665/2022 and 749/2015). To participate in the study written informed consent was obtained from women antenatally, and again for themselves and their infants

after birth.

Results

Maternal demographics and birth anthropometry are shown in Table 1. Of the 796 infants, 396 (50%) were HEU and 400 (50%) were HUU by study design. WLHIV were older [31 years; IQR 27-35] than women who were living without HIV [27 years; IQR 23-32 ($p < 0.001$)]; and more likely to be multigravida [median 3 pregnancies; IQR 2-3 versus 2 pregnancies; IQR 1-3 ($p < 0.001$)]. There were no significant differences between WLHIV and women without HIV concerning marital status, employment status, SES, alcohol use during pregnancy and GA at delivery. At birth, female infants who were HEU had significantly lower birth weight, length and head circumference than infants who were HUU. In males the differences did not meet statistical significance. No significant differences were found between WLHIV and women without HIV concerning infant sex, delivery mode, SGA, LBW, birth WAZ and LAZ. In addition, mothers of infants who were HEU experienced similar proportions of household food insecurity in the last 12 months (76% vs 76%; $p = 0.74$) compared to mothers of infants who were HUU. Maternal demographics and birth anthropometry by maternal ART status in WLHIV are shown in (Supplementary Table 6, Additional File 1). Of the 396 infants who were HEU, 210 (53%) were exposed to both HIV and ART from conception, while 186 (47%) were exposed to ART after conception but during gestation. WLHIV on ART from conception were older [33 years; IQR 29-37] than women who were exposed to ART after conception [29 years; IQR 26-33 ($p < 0.001$)]. Proportions with household food insecurity in the last 12 months were similar (76% vs 76%; $p = 0.61$).



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235 Fig.1 Study flow diagram of participants

Table 1. Mother-infant pair characteristics and household characteristics by HIV exposure status

	All women and infants N = 796	**Women living without HIV and infants HUU N = 400 (50%)**	**Women living with HIV and infants HEU N = 396 (50%)**	***p*-value**
Maternal characteristics				
Age (years), median (IQR)	29 (25-34)	27 (23-32)	31 (27-35)	< 0.001
Level of education completed n (%)				
Primary school	30 (4)	11 (3)	19 (5)	0.02
Secondary school	749 (94)	376 (94)	373 (94)	
Tertiary school	16 (2)	12 (3)	4 (1)	
Missing	1 (0.1)	1 (0.3)	0 ()	
Marital status n (%)				
Married/ Cohabiting	352 (44)	175 (44)	177 (45)	0.78
Not married/ Not cohabiting	404 (51)	205 (51)	199 (50)	
Missing	40 (5)	20 (5)	20 (5)	
Employment status n (%)				
Formal employment	267 (33)	121 (30)	146 (37)	0.26
Informal employment	4 (0.5)	3 (1)	1 (0.2)	
Attending school/ College	63 (8)	46 (11)	17 (4)	
Other working/ Studying	2 (0.2)	1 (0.2)	1 (0.2)	
Not employed/ Not studying	460 (58)	229 (57)	231 (58)	
Socio-economic status* n (%)				
Lowest SES	287 (36)	137 (34)	150 (38)	0.93
Moderate SES	263 (33)	135 (34)	128 (32)	
Highest SES	243 (30)	126 (31)	117 (29)	
Missing	3 (0.4)	2 (0.5)	1 (0.2)	
Alcohol use in this pregnancy n (%)				
Yes	81 (10)	40 (10)	41 (10)	0.87
No	715 (90)	360 (90)	355 (90)	
GA at enrolment (weeks) n (%)				
>= 20 weeks	432 (54)	234 (58)	198 (50)	0.02
< 20 weeks	361 (45)	165 (41)	196 (49)	
Missing	3 (0.4)	1 (0.3)	2 (0.5)	
GA at delivery (weeks), median (IQR)	39 (38-40)	40 (38-41)	39 (38-40)	0.15
Gravidity, median (IQR)	3 (2-3)	2 (1-3)	3 (2-3)	< 0.001
Parity, median (IQR)	1 (0-2)	1 (0-2)	2 (1-2)	< 0.001
Household food security status last 12 months n (%)**				
Food secure	65 (8)	34 (8)	31 (8)	0.74
Food insecure	604 (76)	303 (76)	301 (76)	
Missing	127 (16)	63 (16)	64 (16)	
Birth and infant characteristics				
Infant sex n (%)				
Female	407 (51)	199 (50)	208 (53)	0.43
Male	389 (49)	201 (50)	188 (47)	
Delivery mode n (%)				
Vaginal	502 (63)	245 (61)	257 (65)	0.80
Scheduled C-section	108 (14)	55 (14)	53 (14)	
Emergency C-section	166 (21)	89 (22)	77 (19)	
Other	20 (2)	11 (3)	9 (2)	
Preterm delivery (< 37 weeks) n (%)	97 (12)	38 (10)	59 (15)	0.02
Birth weight (kg), median (IQR)				
Female	3.2 (2.9-3.5)	3.2 (3.0-3.5)	3.1 (2.8-3.4)	0.003

Male	3.2 (2.9-3.5)	3.2 (2.9-3.5)	3.1 (2.9-3.5)	0.34
Birth length (cm), median (IQR)				
Female	50 (48-52)	50 (49-53)	50 (48-51)	0.03
Missing	17	6	11	
Male	50 (48-52)	50 (49-52)	50 (48-52)	0.61
Missing	22	12	10	
Head Circumference (cm), median (IQR)				
Female	35 (34-36)	36 (34-37)	35 (33-36)	< 0.001
Missing	59	32	27	
Male	36 (34-37)	36 (35-37)	36 (34-37)	0.56
Missing	64	32	32	
Small for GA < 10th centile n (%)	80 (10)	40 (10)	40 (10)	0.96
Low birthweight < 2.5kg n (%)	85 (11)	37 (9)	48 (12)	0.19
Birth z-score, mean (SD)				
Weight-for-age	-0.06 (1.09)	0.01 (1.09)	-0.12 (1.10)	0.09
Length-for-age	0.64 (1.71)	0.70 (1.74)	0.57 (1.69)	0.29
Missing	39	18	21	
Head circumference	1.31 (1.54)	1.44 (1.58)	1.18 (1.49)	0.03
Missing	123	64	59	

IQR Interquartile range, *SD* Standard deviation, *GA* Gestational age, *HEU* HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected

*SES included; housing type, toilet, water, electricity, refrigerator, landline, television, level of education, employment status. Cut offs used were >= 7.000 high, <= 4.000 low and other medium

**Household food security status measured using U.S. Household Food Security Module: Six-Item Short Form Economic Research Service, USDA September 2012

Infant feeding practices are presented in Table 2. A high proportion of all infants had ever breastfed, however this was lower for infants who were HEU compared to those who were HUU (90% vs 93%; p = 0.118) with continued BF at 10 weeks (67% vs 85%; p < 0.001), six months (41% vs 64%; p < 0.001), and 12 months (23% vs 46%; p < 0.001). Infants who were HEU compared to those who were HUU had a significantly shorter median duration of BF [73 days; IQR 12-222 vs 209 days; IQR 72-365 (p < 0.001]). In Table 3, infants who were HEU compared to HUU had similar proportions of introduction to complementary feeding by 6 months: 50% vs 55%; (p = 0.41) were introduced to solid, semi-solid or soft foods; 33% vs 35%; (p = 0.80) were introduced to sweet beverages from 6 to 12 months; and 9% vs 9%; (p = 0.92) consumed fruits and/or vegetables between 6 to 12 months of age.

Table 2. Infant feeding practices from birth to 12 months

	All women and infants N = 796	**Women living without HIV and infants HUU N = 400 (50%)**	**Women living with HIV and infants HEU N = 396 (50%)**	***p*-value**
Breastfeeding n (%)				
Yes	726 (91)	371 (93)	355 (90)	0.12
No	6 (1)	1 (0.2)	5 (1)	
Missing	64 (8)	28 (7)	36 (9)	
Early initiation of breastfeeding n (%)				
Within 1 hr	604 (83)	314 (85)	290 (82)	0.69
After 1 hour	39 (6)	19 (5)	20 (5)	
Missing	83 (11)	38 (10)	45 (13)	
Duration of breastfeeding (days), median (IQR)				
	181 (48-365)	209 (72-365)	73 (12-222)	< 0.001
Breastfeeding, n (%)				
10 weeks				
Yes	553 (76)	317 (85)	236 (67)	< 0.001
No	114 (16)	28 (8)	86 (24)	
Missing	59 (8)	26 (7)	33 (9)	
6 months				
Yes	379 (52)	236 (64)	143 (41)	< 0.001
No	264 (37)	96 (26)	168 (47)	
Missing	83 (11)	39 (10)	44 (12)	
12 months				
Yes	253 (35)	171 (46)	82 (23)	< 0.001
No	387 (53)	154 (42)	233 (66)	
Missing	86 (12)	46 (12)	40 (11)	
Exclusive breastfeeding 3 days after birth n (%)				
Yes	542 (75)	292 (79)	250 (70)	0.002
No	114 (16)	43 (11)	71 (20)	
Missing	70 (9)	36 (10)	34 (10)	
Exclusive breastfeeding up to 6 months n (%)				
Yes	51 (7)	24 (7)	27 (8)	0.51
No	603 (83)	313 (84)	290 (82)	
Missing	72 (10)	34 (9)	38 (10)	

HEU HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected, IQR Interquartile range, SD Standard deviation

P-values for categorical data were calculated using Pearson's chi-squared or Fisher's exact test

Table 3. Introduction to complementary feeding

	All women and infants N = 796	**Women living without HIV and infants HUU N = 400 (50%)**	**Women living with HIV and infants HEU N = 396 (50%)**	***p*-value**
Solid, semi-solid or soft foods at 6 months n (%)				
Yes	376 (53)	203 (55)	173 (50)	0.41
No	214 (30)	108 (29)	106 (31)	
Missing	126 (18)	60 (16)	66 (19)	
Egg and/or flesh food consumption 6-12 months n (%)				
Yes	501 (70)	261 (70)	240 (70)	0.72
No	115 (16)	62 (17)	53 (15)	
Missing	100 (14)	48 (13)	52 (15)	
Sweet beverage consumption 6-12 months n (%)				
Yes	241 (34)	128 (35)	113 (33)	0.80
No	367 (51)	191 (51)	176 (51)	
Missing	108 (15)	52 (14)	56 (16)	
Vegetables or fruit consumption 6-12 months n (%)				
Yes	62 (9)	32 (9)	30 (9)	0.92
No	566 (79)	296 (80)	270 (78)	
Missing	88 (12)	43 (12)	45 (13)	

HEU HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected

P-values for categorical data were calculated using Pearson's chi-squared or Fisher's exact test

Solid, semi-solid or soft foods include:

- any dairy products e.g., yoghurt, cheese, or cream.
- cereals, porridge, or bread
- eggs
- any fruits or vegetables
- any meat or fish

Egg and/ or flesh consumption include:

- eggs
- any meat or fish

Sweet beverage consumption includes any fruit juice

Vegetables or fruit consumption include fruits and/ or vegetables consumed

Stunting (8% vs 6%; $p = 0.22$) and underweight (4% vs 4%; $p = 0.98$) at birth were not significantly different for infants who were HEU compared to those who were HUU (Table 4). At 10 weeks old, infants who were HEU were significantly more likely to be underweight than those who were HUU (9% vs 5%; $p = 0.02$). From birth up to 12 months the proportions of infants who were stunted remained higher among infants who were HEU in comparison to those who were HUU; at birth (8% vs 6%; $p = 0.22$), 10 weeks (18% vs 15%; $p = 0.25$), six months (15% vs 14%; $p = 0.57$) and 12 months (11% vs 9%; $p = 0.17$). At 12 months old the prevalence of undernutrition in both groups was low, with stunting being more common than underweight and wasting (10%, 2%, 1%) respectively. Simultaneously, 19%

of all infants, including among those who were HEU compared to those who were HUU (17% vs 21%; $p = 0.22$) were overweight.

Table 4. Prevalence of Malnutrition by Infant HIV Status from Birth up to 12 months

Characteristic	Indicator	Total infants N = 796	Infants HUU N = 400 (50%) Frequency (n%)	Infants HEU N = 396 (50%) Frequency (n%)	*p*-value
	birth				
Stunting (n=757)	LAZ < -2	52 (7)	22 (6)	30 (8)	0.22
Missing		39 (5)	18 (4)	21 (5)	
Underweight (n=796)	WAZ < -2	30 (4)	15 (4)	15 (4)	0.98
Missing		0 (0)	0 (0)	0 (0)	
Wasting (n=)	-	-	-	-	-
Missing	-	-	-	-	-
Overweight (n=)	-	-	-	-	-
Missing	-	-	-	-	-
	10 weeks				
Stunting (n = 636)	LAZ < -2	130 (16)	60 (15)	70 (18)	0.25
Missing		160 (20)	78 (20)	82 (21)	
Underweight (n = 645)	WAZ < -2	56 (7)	20 (5)	36 (9)	0.02
Missing		151 (20)	73 (18)	78 (20)	
Wasting (n = 618)	WLZ < -2	13 (2)	7 (2)	6 (2)	0.81
Missing		178 (22)	88 (22)	90 (23)	
Overweight (n = 618)	WLZ > +2	120 (15)	66 (17)	54 (14)	0.27
Missing		178 (22)	88 (22)	90 (23)	
	6 months				
Stunting (n= 613)	LAZ < -2	114 (14)	56 (14)	58 (15)	0.57
Missing		183 (23)	84 (21)	99 (25)	
Underweight (n= 612)	WAZ < -2	24 (3)	13 (3)	11 (3)	0.80

Missing		184 (23)	84 (21)	100 (25)	
Wasting (n= 603)	WLZ < -2	9 (1)	7 (2)	2 (1)	0.18
Missing		193 (24)	88 (22)	105 (26)	
Overweight (n= 603)	WLZ > +2	143 (18)	72 (18)	71 (18)	0.70
Missing		193 (24)	88 (22)	105 (27)	
	12 months				
Stunting (n= 612)	LAZ < -2	79 (10)	35 (9)	44 (11)	0.17
Missing		184 (23)	85 (21)	99 (25)	
Underweight (n=609)	WAZ < -2	17 (2)	8 (2)	9 (2)	0.71
Missing		188 (24)	87 (22)	101 (26)	
Wasting (n= 604)	WLZ < -2	9 (1)	5 (1)	4 (1)	1.00
Missing		192 (24)	90 (23)	102 (26)	
Overweight (n= 604)	WLZ > +2	149 (19)	83 (21)	66 (17)	0.22
Missing		192 (24)	90 (22)	102 (26)	

LAZ Length-for-age z-score, *WAZ* Weight-for-age z-score, *WLZ* Weight-for-length z-score,
HEU HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected
P-values for categorical data were calculated using Pearson's chi-squared or Fisher's exact test

LME models of mean WAZ, LAZ and WLZ between infants who were HEU and HUU for duration of BF are shown in Table 5. On average, infants who were HEU had lower WAZ and LAZ than those who were HUU [β = -0.147; 95% CI: -0.327, 0.033; p = 0.11] and [β = -0.146; 95% CI: -0.339, 0.471; p = 0.14] keeping age at visit, maternal age, duration of BF, HFSS, employment and marital status constant. However, the association between infant HIV exposure status and WAZ and infant HIV exposure status and LAZ were not statistically significant. On average, infant's mothers who were married/cohabiting compared to those who were not had higher WAZ and LAZ [β = 0.286; 95% CI: - 0.112, 0.459; p = 0.001] and [β = 0.295; 95% CI: 0.109, 0.480; p = 0.002] holding the same variables constant. On average, infant's mothers who were food secure had higher WLZ than those who were food insecure [β = 0.342; 95% CI: 0.029, -0.655; p = 0.03] keeping all other variables constant. Associations between visits and WAZ, LAZ and WLZ were significant in all models. There were no statistically significant

associations between duration of BF and WAZ, LAZ or WLZ. Models for mean WAZ, LAZ and WLZ between infants who were HEU and HUU for any BF and EBF are shown in (Supplementary Tables 7 and 8, Additional File 2 and 3) respectively.

Table 5. Linear mixed effects models of WAZ, LAZ and WLZ between infants HEU and HUU for duration of breastfeeding

Model 1: WAZ between infants HEU and HUU for duration of breastfeeding N of observations = 2138			
Effect	**Estimate**	**(95% CI)**	***p*-value**
Visits			
7 days	Ref		
10 weeks	-0.067	-0.166 – 0.033	0.19
6 months	0.363	0.262 – 0.463	< 0.001
12 months	0.621	0.523 – 0.719	< 0.001
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.147	-0.327 – 0.033	0.11
Maternal age	-0.016	-0.031 – -0.000	0.04
Employment status			
Not employed/ Not studying	Ref		
Formal employment	0.178	-0.005 – 0.361	0.06
Informal employment	0.321	-0.827 – 1.470	0.58
Attending school/ College	-0.016	-0.338 – 0.305	0.92
Other, work or studying	0.522	-0.944 – 1.988	0.48
Marital status			
Not married/Not cohabiting	Ref		
Married/ Cohabiting	0.286	0.112 – 0.459	0.001
Duration of breastfeeding	0.000	-0.000 – 0.000	0.78
Household food security status			
Food insecure	Ref		
Food secure	0.175	-0.106 – 0.457	0.22
Model 2: LAZ between infants HEU and HUU for duration of breastfeeding N of observations = 2111			
Effect	**Estimate**	**(95% CI)**	***p*-value**
Visits			
7 days	Ref		
10 weeks	-1.550	-1.698 - -1.400	< 0.001
6 months	-1.380	-1.531 - -1.233	< 0.001
12 months	-1.260	-1.408 - -1.115	< 0.001
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.146	-0.339 – 0.471	0.14
Maternal age	-0.014	-0.031 – 0.002	0.09
Employment status			
Not employed/ Not studying	Ref		
Formal employment	-0.010	-0.206 – 0.186	0.92
Informal employment	0.897	-0.316 – 2.110	0.15
Attending school/ College	0.197	-0.147 – 0.540	0.26
Other, work or studying	0.672	-0.923 – 2.265	0.41
Marital status			
Not married/Not cohabiting	Ref		
Married/ Cohabiting	0.295	0.109 – 0.480	0.002
Duration of breastfeeding	-0.000	-0.000 – 0.000	0.62
Household food security status			
Food insecure	Ref		
Food secure	-0.065	-0.367 – 0.237	0.67
Model 3: WLZ between infants HEU and HUU for duration of breastfeeding N of observations = 1525			
Effect	**Estimate**	**(95% CI)**	***p-value***
Visits			
10 weeks	Ref		
6 months	0.125	-0.022 – 0.272	0.09
12 months	0.174	0.029 – 0.319	0.02
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	0.008	-0.191 – 0.207	0.94
Maternal age	-0.010	-0.027 – 0.007	0.24

Employment status			
Not employed/ Not studying	Ref		
Formal employment	0.173	-0.029 – 0.375	0.09
Informal employment	-0.588	-1.810 – 0.634	0.34
Attending school/ College	-0.094	-0.448 – 0.261	0.60
Other, work or studying	0.553	-1.147 – 2.252	0.52
Marital status			
Not married/ Not cohabiting	Ref		
Married/ Cohabiting	0.134	-0.057 – 0.324	0.17
Duration of breastfeeding	0.000	-0.000 – 0.000	0.33
Household food security status			
Food insecure	Ref		
Food secure	0.342	0.029 – 0.655	0.03

LAZ Length-for-age z-score, *WAZ* Weight-for-age z-score, *WLZ* Weight-for-length z-score, *HEU* HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected

Discussion

In this study, we found that on average, infants who were HEU had lower mean WAZ and LAZ compared to those who were HUU over 12 months when adjusting for all other variables. These differences did not reach statistical significance. In addition, this was irrespective of BF variable (duration of BF, any BF, or EBF up to 6 months) (Table 5; Supplementary Tables 7 and 8, Additional files 2 and 3). Female infants who were HEU were found to have significantly lower birth weight compared to those who were HUU suggesting that there may be sex differences in fetal programming based upon in utero exposures. Infants born to married/cohabiting mothers compared to not married/not cohabiting had an associated higher average WAZ and LAZ across the feeding variables. Being food secure compared to food insecure was associated with a higher average WAZ and WLZ irrespective of feeding variables. At 10 weeks, while a significantly higher occurrence of underweight was found in infants who were HEU compared to HUU the two groups had similar proportions from six months. Stunting was consistently more prevalent among infants who were HEU than HUU from birth up to 12 months. These proportions and similarly in the underweight category however decreased from six months of age. Among both groups, infants experienced high proportions of overweight by 12 months. The most recent national estimates for overweight among children younger than the age of five years in SA is 13%.[33, 34]

The findings of this study in terms of lower WAZ and LAZ among infants who were HEU compared to HUU were consistent with findings in other African populations.[3, 10, 35, 36] In SSA lower WAZ [8, 11] and LAZ[8] among infants who were HEU compared to those who were HUU were found at birth and up to 12 and 18 months old respectively. The findings in this study show that being married/cohabiting and food secure may have a greater impact on infant growth outcomes than being among the population of infants who were HEU. It could mean that married/cohabiting households have a combined income with increased availability of food options. However, more research is needed to investigate what about being married impacts infant growth. A recent study in SA[37] has demonstrated the impact of food security as part of a HIV-related syndemic on the health of infants who were HEU compared to HUU.

Moreover, neither duration of BF, nor any BF or EBF had a significant impact on the growth outcomes of infants. Higher proportions of stunting among infants who were HEU compared to HUU remained by 12 months, while similar proportions of underweight were observed among infants who were HEU compared to those who were HUU from 6 to 12 months. Furthermore, at 12 months stunting remained the most common type of undernutrition among all infants. High proportions of overweight among both groups of infants were more common than all forms of undernutrition at 12 months. Another study in SA[35] reported similar findings of linear growth faltering being the most common form of undernutrition by 12 months of age while at the same time authors observed a high prevalence of overweight among both infants who were HEU and HUU. The findings from this study therefore contributes to the literature that the double burden of undernutrition and overnutrition remains common in SSA.

WHO and South African guidelines recommend the introduction of complementary foods from six months old, while BF continues. Similar proportions among infants who were HEU compared to HUU were introduced to: solid, semi-solid or soft foods; egg and/or flesh food; sweet beverages; and consumption of vegetables and/or fruit by six months of age. A study in SA[11] reported similar results in which both groups did not consume any fruit or vegetables at 6 months, however, the prevalence decreased at 12 months. Appropriate introduction to complementary foods especially increased consumption of fruits and vegetables must be promoted while consumption of fruit juice needs to be discouraged to prevent early obesity among infants. This may explain the reason why overweight was the most common form of malnutrition from six months of age. Obesity in childhood is a predictor of obesity in adulthood making it critical to address this during the early stages of life. Duration of BF among infants who were HEU was significantly shorter than among those who were HUU. Infants who were HEU compared to HUU had significantly lower proportions of any BF from 10 weeks up to 12 months of age. Inappropriate types of complementary foods were prevalent among both infants who were HEU and HUU. Studies in SA[35, 38] and SSA[8, 11] reported similar findings of lower proportions of BF and shorter BF duration among infants who were HEU compared to HUU which highlights the need for longer duration of BF to be promoted among mothers of infants who are HEU.

Several strengths and limitations were identified for this study. Infants who were HEU and HUU were enrolled contemporaneously from the same community which allowed for the comparison of common socio-behavioural and economic characteristics between the two groups as well as temporal comparisons. Robust GA estimates were obtained by antenatal ultrasound. We had data for the timing of ART exposure in WLHIV. However, the findings may not be generalizable to other settings such as rural areas or with less-well implemented VTP programmes. Additionally, timing of ART was not included in the final LME models although all WLHIV were receiving ART. When doing observational research, it is challenging to determine causal effects due to potential unmeasured confounders. Overall, the study and data analytic approach did its best to reduce confounding by consulting the literature to determine which covariates may be associated with both the independent and dependent variables and included these covariates as fixed effects in the LME models.

Conclusion

In this study, we found that on average over 12 months the growth outcomes of infants who were HEU were negatively impacted compared to those who were HUU. These differences did not reach statistical significance. This was regardless of duration of BF/other feeding variables including other covariates adjusted for in the LME models. Being married/cohabiting and household food security appeared to have a greater impact on the growth of infants than HIV exposure. Higher proportions of stunting among infants who were HEU vs HUU remained by 12 months, while similar proportions of underweight were observed among infants who were HEU compared to HUU from 6 to 12 months. Among all infants, at 12 months overweight was the most common form of malnutrition with similar proportions between infants who were HEU and HUU. Infants who were HEU had lower proportions of both any and duration of BF. However, introduction to complementary feeding was similar and suboptimal among all infants which may have contributed to the high proportion of overweight in both groups. While improvements have been made to support BF mothers and their infants in the context of universal ART and BF, public health interventions are needed to strengthen; BF practices among infants who are HEU. Studies may need to investigate the introduction to complementary feeding among infants before the age of six months including the frequency at which these liquids and/or foods

were consumed to target growth faltering and the increasing prevalence of overweight among infants. Furthermore, studies may need to explore the impact of marital status and HFSS on infant growth.

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

JLR, EK, DCN, LH collaborated in the write-up of the manuscript. All authors contributed to; the design, conduct, research aims and objectives, research questions and statistical analyses of the manuscript. The manuscript was reviewed by EK, DCN and LH. The final manuscript was approved by all authors.

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Author's information (optional)

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519

PART C: APPENDICES

Supplementary material: Protocol

Appendix 1: WHO breastfeeding recommendations

Table 4. Breastfeeding guidelines by World Health Organization[10]

Breastfeeding guidelines	**Age group**	**Definition**
Breastfeeding guidelines		
Ever breastfed	Children born in the last 24 months	Percentage children born in the last 24 months who were ever breastfed
Early initiation breastfeeding	Same as above	Percentage children breastfed within one hour after birth
Exclusively breastfed first two days after birth	Same as above	Percentage children breastfed exclusively
Exclusively breastfed below six months	Infants aged 0-5 months	Percentage infants breastfed exclusively with breast milk during previous day
Mixed milk feeding under six months	Same as above	Percentage infants given formula and/or animal milk in addition to breast milk during previous day
Continued breastfeeding	Children 12-23 months	Percentage children breastfed during previous day

Appendix 2: WHO complementary feeding recommendations

Table 5. Complementary feeding guidelines by World Health Organization[10]

Guidelines	Age group	Definition
Complementary feeding guidelines		
Start of solid, semi solid or soft foods	Infants aged 6-8 months	Percentage infants who consumed solid, semi-solid or soft foods during previous day
Minimum dietary diversity	Children aged 6-23 months	Percentage children who consumed foods and drinks from at least five out of eight defined food groups during previous day
Minimum meal frequency	Same as above	Percentage children who consumed soldi, semi solid or soft foods (including milk feeds for non-breastfed children) the minimum amount of times or more during the day before
Minimum milk feeding for non-breastfed	Same as above	Percentage children who consumed at least two milk feeds the day before
Minimum acceptable diet	Same as above	Percentage children who consumed a minimum acceptable diet the day before
Egg and/ or flesh food consumption	Same as above	Percentage children who consumed egg and/ or flesh food the day before
Sweet beverage consumption	Same as above	Percentage children who consumed a sweet beverage during the day before
Unhealthy food consumption	Same as above	Percentage children who consumed selected sentinel unhealthy foods the day before
Zero vegetable or fruit consumption	Same as above	Percentage children who did not consume any vegetables or fruits the day before
Other guidelines		
Bottle feeding	Children aged 0-23 months	Percentage children bottle fed with a nipple the day before
Infant feeding area graphs	Infants aged 0-5 months	Percentage infants fed exclusively with breast milk, breast milk and water only, breast milk and non-milk liquids, breast milk and animal milk/formula, breast milk and complementary foods, and not breastfed the day before

PROTOCOL SYNOPSIS

Background

Infants who are HIV-Exposed Uninfected (HEU) are exposed to maternal HIV infection *in utero* but are not themselves infected with Human Immunodeficiency Virus (HIV) despite maternal infection. These infants and children comprise an emergent subpopulation in sub-Saharan Africa due to advances in the vertical transmission prevention policies in women living with HIV (WLHIV). In South Africa, the number of children who are HEU was estimated at 3.5 million in 2018, among the five highest countries recorded globally. These infants are subject to various exposures during early development including maternal HIV infection and antiretroviral therapy (ART) exposure *in utero* and via breastmilk; and directly via post-exposure prophylaxis (PEP). Their health outcomes differ from those of their HIV- Unexposed Uninfected (HUU) counterparts. Infections, diarrhoea and developmental delay are more common, placing them at an increased risk of both morbidity and mortality. Hospital admissions are increased and there is evidence of lower growth weight-for-age z-scores (WAZ) and length-for-age z-scores (LAZ) in comparison to their HUU counterparts.

By investigating harmful exposures and their impact on the health of infants who are HEU it is important to consider whether various infant feeding practices could impact on the growth of these infants as both exposure to HIV and feedings practices, including patterns and duration of breastfeeding, impact on their growth. It is well understood that breastmilk given to infants both during exclusive breast feeding and throughout continued breast feeding provide them with nutrients to help them grow optimally. However, it is unclear how breastfeeding practices and the addition of complementary feeding impact the growth of infants who are HEU in comparison to those who are HUU. Guidelines for feeding practices have been provided by World Health Organization (WHO) and the South African Department of Health. However, it is unclear what the growth outcomes are for infants who are HEU compared to HUU infants owing to limited research.

Aims and objectives

The overall aim of this study is to identify, describe and compare associations of infant feeding practices and growth in infants who are HEU and HUU at Gugulethu Midwife Obstetric Unit (MOU), in an era of universal ART policies. The first objective is therefore to describe infant feeding practices from birth to 12 months old by maternal HIV and ART exposure status with specific reference to, the WHO breastfeeding recommendations; breastfed within one hour after birth, exclusively breastfed for six months, infants still breastfeeding at 12 months and those who received mixed milk feeding with formula and/or animal milk in addition to breast milk under six months. Infant feeding practices will also be described from birth to 12 months old by maternal HIV and ART exposure status with specific reference to; duration of breastfeeding if any and the type of complementary feeding. Finally, the second objective is to describe and compare infant growth measured by WAZ and LAZ between infants who are HEU and HUU and whether these are associated with types of feeding practices and Household Food Security Status (HFSS).

Methodology

Study design

This study will evaluate the association between infant feeding practices and infant growth over a period of 12 months in Cape Town where limited research has been conducted in this setting pertaining to the aims and objectives. The study is designed as a secondary analysis of the B-Positive cohort study. The B-Positive study enrolled participants at their first antenatal care (ANC) visit at a primary maternity healthcare facility in Gugulethu, an urban township in Cape Town, South Africa. They comprise of pregnant women 18 years and above and their infants who were enrolled between January 2017 to July 2018.

For the B-Positive study eligible participants included women who had a confirmed maternal HIV test and who planned to reside in Cape town at least one year postpartum with their infants. Women who gave birth to a stillborn baby, who had a miscarriage or a termination of pregnancy were not included in this study. A total of 796 mother-infant pairs will be included in this secondary data analysis comprising of 396 infants who are HEU and 400 who are HUU. Of the WLHIV, 210 started ART preconception and 186 during this pregnancy.

Research procedure and data collection

The primary exposure for this secondary analysis is HIV exposure *in utero* (HEU vs HUU). Infant growth is the outcome. Maternal HIV status was self-reported and confirmation was provided through medical chart review. Women who tested negative for HIV at enrolment based on a rapid antibody test were routinely tested either right after delivery or during new born visits within 3-7 days after delivery and approximately once every three months both during breastfeeding. HIV Deoxyribonucleic (DNA) Polymerase Chain Reaction (PCR) testing of infants were taken at birth and 6 to 10 weeks according to national guidelines. Infants with HIV were excluded. Maternal

HIV history and other data related to medical conditions were based on medical records. At each of the four postpartum visits, maternal weight and infant anthropometry were performed and participants completed interviewer-administered standardized questionnaires. Relevant to this study are the Infant Feeding Practices and Food Security questionnaires as well as mothers' and infants' growth anthropometry.

Infant feeding practices were assessed according to the WHO guidelines. Infant feeding questionnaires were administered postnatally to all women by trained study interviewers at four study visits. Birth weight and length were extracted from South Africa's Road to Health Booklet (RTHB) to derive anthropometric outcomes, WAZ and LAZ to represent surrogate measures of fetal growth. Weight and length were measured at each study visit (birth, 10 weeks, 6 months and 12) by study staff. During the 6-10 week and 6-month study visit infant weight was assessed with precision to the nearest 10 grams using a calibrated digital infant scale (MTB 20 Baby Scale, Adam Equipment, Milton Keynes, UK), by trained study staff without clothing and diapers. All the infant measurements were taken while the infant was recumbent with the use of an infant length measuring board. Length measurements were recorded to the nearest 0.25 centimetres using a rigid recumbent length board (ADE MZ 1013 Baby Length Measuring Rod, ADE, Hamburg, Germany). Two measurements of infant weight and recumbent length were taken at each visit by study staff and the average was calculated to ensure accuracy and avoid outliers. No big differences were found between the two measures for weight and length.

Data analysis

Data will be exported from REDCap into the R statistical package for analysis. Descriptive studies will be used to describe infant and maternal characteristics and to describe the duration and type of feeding practices. WAZ and LAZ at birth will be generated using Intergrowth-21 software which accounts for gestational age at birth and sex. WAZ and LAZ for 10 weeks, 6 months and 12 months will be calculated using the WHO Child Growth Standards for age and sex. The z-score results will be reported as mean and standard deviation at each age. Student t-tests will be used to compare WAZ and LAZ differences between infants who are HEU and those who are HUU. We will use the lme4 package in R to conduct a mixed effects longitudinal analysis with a set of independent covariates.

Ethical considerations

The B-Positive cohort study has received ethical approval from the University of Cape Town's Faculty of Health Sciences Research Ethics Committee (UCT-HREC) (HREC REF: 749/2015). Written informed consent was obtained from women in which participants were informed of potential risks. Women consented to participation antenatally, and again for themselves and their infants after birth. The level of risk which was low has remained unchanged throughout the study. For the B-Positive study participants were informed that there were no individual benefits for them but that the data obtained from the study would be useful to improve the services of pregnant women and their children in South Africa. In addition, for the B-Positive study participants received at the end of each study visit a R100 grocery voucher, R20 for transport and food, and drink at their visit. Staff involved in the data collection and management process received specific training with respect to confidentiality. In addition, personnel were required to sign a confidentiality agreement form.

For this secondary analysis, it is required that an agreement of confidentiality be signed when working with the B-Positive data. The risk of harm in this study is also low. The main risk is that of loss of confidentiality and the researcher will have access to health and other personal information. This risk has been minimized by the use of a unique study number allocated to each participant and used on all electronic and paper forms. No identifying information was collected on study forms and the researcher will not have access to this information. Hard copy participant-related information was stored in locked filing cabinets for the B-Positive study and electronic records were made password-protected and files were encrypted. All data will be stored on the researcher's password-protected laptop and deleted after submission of the dissertation and publication of the manuscript (original data remains on the B-positive REDCap database until five years after the closure of this study). Every effort will be made to protect the privacy of the participants.

Supplementary material: Manuscript

Additional file 1

Table 6. Mother-infant pair characteristics and household characteristics in WLHIV by ART exposure status

	All women and infants HEU N = 396	**Preconception ART N = 210 (53%)**	**Antenatal ART N = 186 (47%)**	***p*-value**
Maternal characteristics				
Age (years), median (IQR)	31 (27-35)	33 (29-37)	29 (26-33)	< 0.001
Level of education completed n (%)				
Primary school	19 (5)	10 (5)	9 (5)	0.66
Secondary school	373 (94)	199 (95)	174 (93)	
Tertiary school	4 (1)	1 (0.4)	3 (2)	
Marital status n (%)				
Married/ Cohabiting	177 (45)	101 (48)	76 (41)	0.26
Not married/ Not cohabiting	199 (50)	102 (49)	97 (52)	
Missing	20 (5)	7 (3)	13 (7)	
Employment status n (%)				
Formal employment	146 (37)	80 (38)	66 (35)	0.84
Informal employment	1 (0.2)	1 (0.5)	0 (0)	
Attending school/ College	17 (4)	8 (4)	9 (5)	
Other working/ studying	1 (0.2)	1 (0.5)	0 (0)	
Not employed/ Not studying	231 (58)	120 (57)	111 (60)	
Socio-economic status* n (%)				
Lowest SES	150 (38)	72 (34)	78 (42)	0.38
Moderate SES	128 (32)	68 (32)	60 (32)	
Highest SES	117 (29)	69 (33)	48 (26)	
Missing	1 (0.2)	1 (0.5)	0 (0)	
Alcohol use in this pregnancy n (%)				
Yes	41 (10)	16 (8)	25 (13)	0.06
No	355 (90)	194 (92)	161 (87)	
GA at enrolment (weeks) n (%)				
>= 20 weeks	198 (50)	97 (46)	101 (54)	0.11
< 20 weeks	196 (49)	112 (53)	84 (45)	
Missing	2 (0.5)	1 (0.5)	1 (0.5)	
GA at delivery (weeks), median (IQR)	39 (38-40)	39 (39-40)	39 (38-40)	0.37
Gravidity, median (IQR)	3 (2-3)	3 (2-4)	3 (2-3)	0.03
Parity, median (IQR)	2 (1-2)	2 (1-2)	1 (1-2)	0.06
Household food security status last 12 months n (%)**				
Food secure	31 (8)	15 (7)	16 (9)	0.61
Food insecure	301 (76)	160 (76)	141 (76)	
Missing	64 (16)	35 (17)	29 (15)	
Birth and infant characteristics				
Infant sex n (%)				
Female	208 (53)	109 (52)	99 (53)	0.79
Male	188 (47)	101 (48)	87 (47)	
Delivery mode n (%)				
Vaginal	257 (65)	139 (66)	118 (63)	0.65
Scheduled C-section	53 (14)	27 (13)	26 (14)	
Emergency C-section	77 (19)	37 (18)	40 (22)	
Other	9 (2)	7 (3)	2 (1)	
Preterm delivery (< 37 weeks) n (%)	59 (15)	22 (10)	37 (20)	0.009
Birth weight, median (IQR)				

Female	3.12 (2.80-3.38)	3.16 (2.87-3.42)	3.11 (2.68-3.36)	0.33
Male	3.15 (2.86-3.47)	3.22 (2.97-3.48)	3.13 (2.80-3.43)	0.13
Birth length, median (IQR)				
Female	50 (48-51)	50 (49-52)	50 (47-51)	0.08
Missing	11	5	6	
Male	50 (48-52)	50 (48-52)	50 (48-52)	0.62
Missing	10	5	5	
Head Circumference, median (IQR)				
Female	35 (33-36)	35 (33-36)	35 (34-36)	0.86
Missing	27	14	13	
Male	36 (34-37)	36 (35-37)	36 (34-37)	0.97
Missing	32	16	16	
Small for GA < 10th centile n (%)	40 (10)	20 (10)	20 (11)	0.69
Low birthweight < 2.5kg n (%)	48 (12)	21 (10)	27 (15)	0.17
Birth z-score, mean (SD)				
Weight-for-age	-0.12 (1.10)	-0.12 (1.05)	-0.13 (1.16)	0.94
Length-for-age	0.57 (1.69)	0.59 (1.72)	0.55 (1.65)	0.82
Missing	21	10	11	
Head circumference	1.18 (1.49)	1.10 (1.44)	1.27 (1.54)	0.30
Missing	59	30	29	

IQR Interquartile range, *SD* Standard deviation, *GA* Gestational age, *HEU* HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected, *ART* Antiretroviral therapy

*SES included; housing type, toilet, water, electricity, refrigerator, landline, television, level of education, employment status. Cut offs used were >= 7.000 high, <= 4.000 low and other medium

**Household food security status measured using U.S. Household Food Security Module: Six-Item Short Form Economic Research Service, USDA September 2012

Additional file 2

Table 7. Linear mixed effects models of WAZ, LAZ and WLZ between infants HEU and HUU for any breastfeeding

Model 1: WAZ between infants HEU and HUU for any breastfeeding N of observations = 2138			
Effect	**Estimate**	**(95% CI)**	***p*-value**
Visits			
7 days	Ref		
10 weeks	-0.067	-0.166 – 0.033	0.19
6 months	0.363	0.262 – 0.463	< 0.001
12 months	0.621	0.523 – 0.719	< 0.001
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.152	-0.325 – 0.022	0.09
Maternal age	-0.015	-0.031 – 0.000	0.05
Employment status			
Not employed/ Not studying	Ref		
Formal employment	0.176	-0.007 – 0.358	0.06
Informal employment	0.324	-0.823 – 1.470	0.58
Attending school/ College	-0.020	-0.340 – 0.301	0.90
Other, work or studying	0.542	-0.917 – 2.001	0.47
Marital status			
Not married/ Not cohabiting	Ref		
Married/ Cohabiting	0.285	0.112 – 0.458	0.001
Any breastfeeding			
No	Ref		
Yes	0.304	-0.586 – 1.194	0.50
Household food security status			
Food insecure	Ref		
Food secure	0.177	-0.104 – 0.459	0.22
Model 2: LAZ between infants HEU and HUU for any breastfeeding N of observations = 2111			
Effect	**Estimate**	**(95% CI)**	***p*-value**
Visits			
7 days	Ref		
10 weeks	-1.550	-1.698 - -1.400	< 0.001
6 months	-1.380	-1.531 – -1.233	< 0.001
12 months	-1.260	-1.408 - -1.116	< 0.001
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.132	-0.317 – 0.054	0.16
Maternal age	-0.014	-0.031 – 0.002	0.09
Employment status			
Not employed/ Not studying	Ref		
Formal employment	-0.006	-0.201 – 0.189	0.95
Informal employment	0.878	-0.334 – 2.090	0.15
Attending school/ College	0.202	-0.141- 0.545	0.25
Other, work or studying	0.634	-0.953 – 2.221	0.43
Marital status			
Not married/ Not cohabiting	Ref		
Married/ Cohabiting	0.291	0.105 – 0.476	0.002
Any breastfeeding			
No	Ref		
Yes	0.108	-0.832 – 1.048	0.82
Household food security status			
Food insecure	Ref		
Food secure	-0.061	-0.363 – 0.241	0.69
Model 3: WLZ between infants HEU and HUU for any breastfeeding N of observations = 1525			
Effect	**Estimate**	**(95% CI)**	***p*-value**
Visits			
10 weeks	Ref		

6 months	0.126	-0.021 – 0.273	0.09
12 months	0.177	0.032 – 0.321	0.02
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.018	-0.209 – 0.172	0.85
Maternal age	-0.009	-0.026 – 0.008	0.30
Employment status			
Not employed/ Not studying	Ref		
Formal employment	0.163	-0.039 – 0.364	0.11
Informal employment	-0.563	-1.783 – 0.658	0.37
Attending school/ College	-0.106	-0.460 – 0.248	0.55
Other, work or studying	0.623	-1.071 – 2.318	0.47
Marital status			
Not married/ Not cohabiting			
Married/ Cohabiting	0.135	-0.055 – 0.325	0.16
Any breastfeeding			
No	Ref		
Yes	0.440	-0.523 – 1.402	0.37
Household food security status			
Food insecure	Ref		
Food secure	0.340	0.027 – 0.653	0.03

LAZ Length-for-age z-score, *WAZ* Weight-for-age z-score, *WLZ* Weight-for-length z-score, *HEU* HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected

Additional file 3

Table 8. Linear mixed effects models of WAZ, LAZ and WLZ between infants HEU and HUU for exclusive breastfeeding up to 6 months

Model 1: WAZ between infants HEU and HUU for exclusive breastfeeding up to 6 months N of observations = 2042

Effect	Estimate	(95% CI)	*p*-value
Visits			
7 days	Ref		
10 weeks	-0.060	-0.162 – 0.041	0.24
6 months	0.366	0.265 – 0.467	< 0.001
12 months	0.626	0.525 – 0.727	< 0.001
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.141	-0.323 – 0.042	0.13
Maternal age	-0.016	-0.032 – 0.000	0.06
Employment status			
Not employed/ Not studying	Ref		
Formal employment	0.180	-0.014 – 0.373	0.07
Informal employment	0.352	-0.816 – 1.520	0.55
Attending school/ College	-0.001	-0.336 – 0.334	0.99
Other, work or studying	-0.089	-2.113 – 1.936	0.93
Marital status			
Not married/ Not cohabiting	Ref		
Married/ Cohabiting	0.297	0.114 – 0.479	0.001
Exclusive breastfeeding up to 6 months			
No	Ref		
Yes	-0.087	-0.405 – 0.231	0.59
Household food security status			
Food insecure	Ref		
Food secure	0.249	-0.048 – 0.547	0.10

Model 2: LAZ between infants HEU and HUU for exclusive breastfeeding up to 6 months N of observations = 2016

Effect	Estimate	(95% CI)	*p*-value
Visits			
7 days	Ref		
10 weeks	-1.510	-1.660 - -1.355	< 0.001
6 months	-1.360	-1.516 - -1.212	< 0.001
12 months	-1.220	-1.369 - -1.066	< 0.001
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.135	-0.327 – 0.058	0.17
Maternal age	-0.011	-0.028 – 0.006	0.21
Employment status			
Not employed/ Not studying	Ref		
Formal employment	-0.005	-0.208 – 0.199	0.96
Informal employment	0.924	-0.295 – 2.143	0.14
Attending school/ College	0.201	-0.152 – 0.554	0.26
Other, work or studying	-0.455	-2.568 – 1.658	0.67
Marital status			
Not married/ Not cohabiting	Ref		
Married/ Cohabiting	0.286	0.094 – 0.478	0.003
Exclusive breastfeeding up to 6 months			
No	Ref		
Yes	-0.252	-0.586 – 0.082	0.14
Household food security			
Food insecure	Ref		
Food secure	0.051	-0.264 – 0.365	0.75

Model 3: WLZ between infants HEU and HUU for exclusive breastfeeding up to 6 months N of observations = 1472			
Effect	**Estimate**	**(95% CI)**	***p*-value**
Visits			
10 weeks	Ref		
6 months	0.136	-0.012 – 0.285	0.07
12 months	0.177	0.029 – 0.325	0.02
Infant HIV exposure status			
Infants HUU	Ref		
Infants HEU	-0.002	-0.199 – 0.194	0.98
Maternal age	-0.012	-0.030 – 0.005	0.18
Employment status			
Not employed/ Not studying	Ref		
Formal employment	0.178	-0.030 – 0.386	0.09
Informal employment	-0.576	-1.803 – 0.651	0.36
Attending school/ College	-0.128	-0.490 – 0.235	0.49
Other, work or studying	0.108	-2.020 – 2.236	0.92
Marital status			
Not married/ Not cohabiting	Ref		
Married/ Cohabiting	0.164	-0.032 – 0.360	0.10
Exclusive breastfeeding up to 6 months			
No	Ref		
Yes	0.157	-0.179 – 0.494	0.36
Household food security status			
Food insecure	Ref		
Food secure	0.366	0.043 – 0.689	0.03

LAZ Length-for-age z-score, *WAZ* Weight-for-age z-score, *WLZ* Weight-for-length z-score, *HEU* HIV-exposed-uninfected, *HUU* HIV-unexposed uninfected

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

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26 October 2022

HREC REF: 665/2022

Dr E Kalk
Centre for Infectious Disease Epidemiology
& Research
Public Health & Family Medicine
Email: Emma.kalk@uct.ac.za
Student: rhdjan003@myuct.ac.za

Dear Dr Kalk

PROJECT TITLE: ASSOCIATION BETWEEN INFANT FEEDING PRACTICES AND INFANT GROWTH BY MATERNAL HIV AND ANTIRETROVIRAL THERAPY STATUS: A PROSPECTIVE STUDY IN CAPE TOWN, SOUTH AFRICA-SUB-STUDY LINKED TO 749/2015- (MASTERS CANDIDATE-MS JANICE RHODA)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 October 2023.

Please submit a progress form, using the standardised Annual Report Form (FHS016) if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Ms Janice Rhoda will also be involved in this study.

Please quote the HREC REF 665/2022 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Consent for publication

Informed consent for mothers

B POSITIVE SENTINEL COHORT – PREGNANT WOMEN INFORMATION AND INFORMED CONSENT

WHAT IS THE PURPOSE OF THIS STUDY?

We are researchers from the University of Cape Town (UCT) and we are asking you to participate in a study we are conducting at the Gugulethu Midwife Obstetric Unit (MOU) and its referral centres. The purpose of this study is to understand and monitor the health of pregnant women and how they are cared for at health facilities during pregnancy and after they deliver their baby. It includes women's mental and physical health and any medicines and remedies that are taken during pregnancy.

We know that it is important for their own health as well as that of their baby, that pregnant women receive all Type text here the care and treatment they need both during pregnancy and after delivery. Information learned in this study will help us to improve health services for pregnant women.

You are being asked to take part in this study because you are pregnant and you are getting your pregnancy care here at the Gugulethu MOU. The purpose of this consent form is to give you information to help you decide if you want to take part in this study.

WHAT DO I HAVE TO DO IF I AGREE TO TAKE PART?

If you agree to take part, you will come in for **up to 7 study visits**. The first visit will take place today while you are in the clinic, and then up to two more before delivery depending on how far your pregnancy is. These visits will occur after your routine ANC clinic visit. After you have given birth, the next visit will occur within one week of delivering your baby and again when your baby is about 6 weeks, 6 months and 12 months old. These study visits are separate from the usual clinic visits that you will have for your pregnancy or your baby's health but we can time the study visits so that they take place on the same days as your usual pregnancy and baby checkups. The first visit today will take about 90 minutes and each of the subsequent visits will take about 60 minutes.

At the visits that are conducted *while you are pregnant,* you will do the following:

- Answer questions about your pregnancy and related healthcare issues/problems
- Answer questions about any other health concerns or ailments
- Discuss any chronic health conditions (if present)
- Discuss any medicines and remedies you are taking
- Answer questions to assess whether you are stressed or unhappy (depressed)
- Discuss some aspects of your personal relationships
- Answer questions about the care you are receiving

If we feel there are any serious problems, we will refer you to the relevant specialized care.

One-week after delivery

One week after you give birth to your baby, you will come to the clinic for a visit that will include the following:

- A repeat of some of the questions discussed previously (health, medicines, stress)
- Questions about your delivery, your baby's health and feeding.

At the other visits *after your baby is born* (6 weeks, 6 months and 12 months old) you will do the following:

- Answer questions about your recent health and any health concerns
- Answer questions about your baby's health, feeding practices and immunizations

- Discuss any problems with taking chronic medications
- Answer questions to assess whether you are stressed or unhappy (depressed)
- Discuss some aspects of your personal relationships
- Answer questions about the care you are receiving

Review of medical records
As part of this study, we will also be looking at and taking information from your antenatal, obstetric, medical, and laboratory records. We will also be looking at and taking information from the health care records of your baby after she or he is born. From these records, we are interested in learning about the pregnancy care you received, medicines you took as well as information about your delivery and your baby's health. All data that we review and abstract is confidential and no participant names are recorded on study documents.

Follow-up of missed visits
You will be asked to provide contact information so that we may get in touch with you during the study. Study staff will talk with you about the best way to contact you. In the event that you miss one of the scheduled study visits, a member of the study staff will contact you in order to find another day and time to complete your visit. If you repeatedly miss study visits or the staff is unable to contact you using the information that you provide, it may be necessary to visit you at home in order to reschedule the missed study visit.

Contact for future study
After the completion of the visit one week after delivery, we will speak to you again to make sure you are happy to be involved in further research.

WHAT ARE THE POTENTIAL RISKS?
You may feel uncomfortable about some of the personal questions you are asked. You may refuse to answer any question that you do not want to answer. There is some risk in sharing personal and medical information. We will be careful to keep all your information as private as possible.

WHAT ARE THE POTENTIAL BENEFITS?
There is no direct benefit to you if you take part in this study. The information gained in this study may help to improve healthcare services for pregnant women and their babies in Cape Town, the Western Cape Province, and across South Africa.

WHAT ARE THE ALTERNATIVES TO TAKING PART?
The alternative to taking part in this study is to continue with your usual health care. If you decide not to participate in this study, your usual health care will not be changed in any way.

WHAT ABOUT CONFIDENTIALITY?
If you agree to take part, all information collected during the study will be kept strictly confidential. Your name will not be written on the study forms and will not be used in connection with any information or lab samples that are collected as part of the study.

All study materials will be stored in locked filing cabinets. Only study staff and personnel involved in routine audits will have access to these materials. All staff involved in data collection and management will get specific training in confidentiality.

Even with these procedures in place if the study staff learns that you are at risk of hurting yourself or someone else or of possible child abuse and/or neglect, study staff will tell the proper authorities.

ARE THERE ANY COSTS?
There is no cost for being in this study.

CAN I LEAVE THE STUDY?
You have the right to decide not to not take part in the study, to refuse to answer any questions, or to withdraw from the study at any time without any penalty. It will have no effect on the care that you receive at the Gugulethu MOU or any other health facility.

WILL I BE GIVEN ANYTHING FOR TAKING PART?
There is no payment for participation. At the end of each visit, you will be given a R100 grocery voucher, R20 for transport, and food and drink while you are at the visit.

If you are HIV infected, you will be asked to provide a dried blood spot (DBS) sample at each visit. This involves a prick on the tip of your finger with a sterile lancet, and the drops of blood are used to fill a maximum of five spots on a filter paper.

The DBS sample will be stored and used to check on your immune system (the part of your body that fights infections like HIV) and ARV levels at a later time. Results from these tests are purely for research and will not be available to you, the clinic, or the study staff. If the health care providers at the clinic need to check your blood, they will take a separate blood sample. When it is stored, your blood and test results will not have your name or any other way of identifying you attached to it.

Please initial below to indicate whether or not you give permission for us to take DBS sample from you. You may still remain in the study, even if you choose not to give a sample.

_____ (initial) I agree to have DBS sample as part of this research.

_____ (initial) I do **NOT** agree to have a DBS sample as part of this research.

FUTURE USE OF SAMPLES:
If you are HIV-infected and agree, some of the blood drawn from you (DBS sample) as part of the study may be used for future HIV-related research. At this time, we cannot provide details of when this testing may be conducted, or exactly what tests we would like to do. However, additional testing will not be done using these stored samples without the approval of the appropriate research ethics committees involved in this research.

If you agree to let us keep your stored sample from you for future research, they may be kept in a locked freezer for up to 5 years. If we do use these samples in the future, the names of other identifiers will not be included with this information (as with the rest of the information we collect for this study).

Please initial below to indicate whether or not you give permission for these samples to be used for future research. You may still remain in the study, no matter which you choose.

_____ (initial) I agree to have my blood stored for future research.

_____ (initial) I do **NOT** agree to have my blood stored for future research.

DO YOU HAVE ANY QUESTIONS?

If there is anything that is unclear or if you need further information, please ask us and we will provide it.

Do you have any questions?

FOR ADDITIONAL INFORMATION:

If you have any questions or have any problems while taking part in this research study, you should contact:

Professor Landon Myer
School of Public Health and Family Medicine
Faculty of Health Sciences
University of Cape Town
Tel: 021 406-6661
Email: Landon.Myer@uct.ac.za

Professor Andrew Boulle
School of Public Health and Family Medicine
Faculty of Health Sciences
University of Cape Town
Tel 021 406-6715
Email: Andrew.Boulle@uct.ac.za

If you have any questions about your rights as a research participant, you may contact the following member of the ethics committee:

Prof Marc Blockman
Chair, Human Research Ethics Committee
Faculty of Health Sciences, University of Cape Town
Tel: 021 406-6338

SIGNATURE PAGE

For participant to complete (please tick):

☐ I have read the information in this document (or it has been read to me). I have been offered a copy of this consent form. I was encouraged and given time to ask questions and all my questions about the study and my participation in it have been answered. I freely consent to be in this research study and agree to participate and know that I may withdraw at any time. My being in the study is voluntary. I understand that whether or not I participate will not affect my health care services received today, or at any time in the future.

☐ I agree that the study team can access my medical records at this hospital or another hospital if necessary for this study. My information will be kept confidential.

☐ I agree to provide contact information for myself which will be kept confidential by the study team.

☐ I agree to be called on my telephone during the course of the study

Participant Name (Please print)	Participant Signature	Date/Time

Interviewer Name (Please print)	Interviewer Signature	Date/Time

If this consent form is read to the participant because the participant is unable to read the form or if the participant must use a thumbprint to sign his/her name, an impartial witness not affiliated with the research or investigator must be present for the consent and sign the following statement:

I confirm that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the participant. The participant freely consented to be in the research study.

Witness Name (Please print)	Witness Signature	Date/Time

Thank you!

Informed consent for infants

B-POSITIVE SENTINEL COHORT – INFANT DIAGNOSTIC COHORT INFORMATION AND INFORMED CONSENT

YOU ARE CURRENTLY ENROLLED IN THE B-POSITIVE COHORT STUDY. NOW THAT YOU HAVE DELIVERED YOUR BABY WE WOULD LIKE TO INVITE YOU AND YOUR BABY TO CONTINUE TO PARTICIPATE IN THE STUDY. THIS INFORMATION AND CONSENT FORM CONCERNS YOUR BABY'S ENROLMENT IN THE STUDY.

WHAT IS THE PURPOSE OF THIS STUDY?

We are researchers from the University of Cape Town (UCT) and we are asking you to participate in a study we are conducting at the Gugulethu Midwife Obstetric Unit (MOU) and its referral centres. The purpose of this study is to understand and monitor the health of pregnant women and how they are cared for at health facilities during pregnancy and after they deliver their baby. It includes women's mental and physical health and any medicines and remedies that are taken during pregnancy.

We know that it is important for their own health as well as that of their baby, that pregnant women receive all the care and treatment they need both during pregnancy and after delivery. Information learned in this study will help us to improve health services for pregnant women and their infants.

You are being asked to take part in this study because were enrolled in the earlier parts of this study and you have recently given birth. The purpose of this consent form is to give you information to help you decide if you and your baby want to continue in the next part of the study.

WHAT DO I HAVE TO DO IF I AGREE TO TAKE PART?

If you agree to take part, you will come in for at least **4 study visits**. These visits will take place today while you are in the clinic (after 7 days' post delivery), and then when your baby is about 6 weeks, 6 months and 12 months old. All of these study visits are separate from the usual clinic visits that you will have for your baby's health. Study visits can be arranged for dates and times that suit you. Each visit will take about 1 hour: 30 minutes. We may also speak to you over the phone in between these visits.

At these visits you will do the following:

- Answer questions about the use of any medicines and remedies
- Answer questions about your baby's health, feeding practices and infant health care
- Answer questions about any other health concerns
- Answer questions to assess whether you are stressed or unhappy (depressed)
- Discuss some aspects of your personal relationships
- Answer questions about the care you and your baby are receiving
- Have your baby's weight and height measured

Review of medical records

As part of this study, we will also be looking at and taking information from your medical, and laboratory records. We will also be looking at and taking information from the health care records of your baby (such as the Road to Health Card and details of any hospital visits). From these records, we are interested in learning

about the health care you receive as well as information about your baby's health. All data that we review and abstract is confidential and no participant names are recorded on study documents.

Follow-up of missed visits
You will be asked to provide contact information so that we may get in touch with you during the study. Study staff will talk with you about the best way to contact you. In the event that you miss one of the scheduled study visits, a member of the study staff will contact you in order to find another day and time to complete your visit. If you repeatedly miss study visits or the staff is unable to contact you using the information that you provide, it may be necessary to visit you at home in order to reschedule the missed study visit.

Other contact
As part of participating, we may ring you on your cellphone or send you reminder SMS's regarding your appointments. If we do this, we will not mention any aspect of your health or health care. We will simply refer to the "Appointment for you and your baby at the Gugulethu CHC".

Contact for future study
After the completion of the last study visit, we will speak to you again to see if you are happy to be involved in further research.

WHAT ARE THE POTENTIAL RISKS?
You may feel uncomfortable about some of the personal questions you are asked. You may refuse to answer any question that you do not want to answer. There is some risk in sharing personal and medical information. We will be careful to keep all your information as private as possible.

WHAT ARE THE POTENTIAL BENEFITS?
There is no direct benefit to you if you take part in this study. The information gained in this study may help to improve services of pregnant women and their babies in Cape Town, the Western Cape Province, and across South Africa.

WHAT ARE THE ALTERNATIVES TO TAKING PART?
The alternative to taking part in this study is to continue with your usual health care. If you decide not to participate in this study, your usual health care will not be changed in any way.

WHAT ABOUT CONFIDENTIALITY?
If you agree to take part, all information collected during the study will be kept strictly confidential. Your name will not be written on the study forms and will not be used in connection with any information or lab samples that are collected as part of the study.

All study materials will be stored in locked filing cabinets. Only study staff and personnel involved in routine audits will have access to these materials. All staff involved in data collection and management will get specific training in confidentiality.

Even with these procedures in place if the study staff learns that you are at risk of hurting yourself or someone else or of possible child abuse and/or neglect, study staff will tell the proper authorities.

WILL I BE GIVEN ANYTHING FOR TAKING PART?

There is no payment for participation. At the end of each visit, you will be given a R100 grocery voucher, R20 for transport and food, and drink while you are at the visit.

ARE THERE ANY COSTS?

There is no cost for being in this study.

CAN I LEAVE THE STUDY?

You have the right to decide not to take part in the study, to refuse to answer any questions, or to withdraw from the study at any time without any penalty. It will have no effect on the care that you or your baby receives at this or any other health facility.

DO YOU HAVE ANY QUESTIONS?

If there is anything that is unclear or if you need further information, please ask us and we will provide it.

Do you have any questions?

FOR ADDITIONAL INFORMATION:

If you have any questions or have any problems while taking part in this research study, you should contact:

Professor Landon Myer
School of Public Health and Family Medicine
Faculty of Health Sciences
University of Cape Town
Tel: 021 406-6661
Email: Landon.Myer@uct.ac.za

Associate Professor Andrew Boulle
School of Public Health and Family Medicine
Faculty of Health Sciences
University of Cape Town
Tel 021 406-6715
Email: Andrew.Boulle@uct.ac.za

If you have any questions about your rights as a research participant, you may contact the following member of the ethics committee:

Prof Marc Blockman
Chair, Human Research Ethics Committee
Faculty of Health Sciences
University of Cape Town
Tel: 021 406-6338

SIGNATURE PAGE

For participant to complete (please tick):

☐ I have read the information in this document (or it has been read to me). I have been offered a copy of this consent form. I was encouraged and given time to ask questions and all my questions about the study and my participation in it have been answered. I freely consent to my baby's participation in this research study and know that I may withdraw at any time. My and my baby's being in the study is voluntary. I understand that whether or not I participate will not affect my health care services received today, or at any time in the future.

☐ I agree that the study team can access my and my baby's medical records at this hospital or another hospital if necessary for this study. All information will be kept confidential.

☐ I agree to provide contact information for myself which will be kept confidential by the study team.

☐ I agree to be called on my telephone to be interviewed.

Participant Name (Please print)	Participant Signature	Date/Time

Interviewer Name (Please print)	Interviewer Signature	Date/Time

If this consent form is read to the participant because the participant is unable to read the form or if the participant must use a thumbprint to sign his/her name, an impartial witness not affiliated with the research or investigator must be present for the consent and sign the following statement:

I confirm that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the participant. The participant freely consented to be in the research study.

Witness Name (Please print)	Witness Signature	Date/Time

Thank you!

PWID: _ _ _ _ _ _ - _ _

Infant feeding practices questionnaire

Study Visit 1

INFANT FEEDING PRACTICES

This CRF applies to ALL enrolled BPOS Infants
Complete during <7 Days Postpartum Study Visit

Visit Date								
D	D	M	M	M	Y	Y	Y	Y

Visit Code	
P	1

Ngoko sizokubuza malunga nendlela omtyisa ngayo umntwana wakho
We are going to ask you some questions about how you are feeding your baby

INFANT #: _____ Initials: __ __

A: BREASTFEEDING INITIATION

1. Wakhe waluncancisa ibele usana lwakho? *Have you ever given breast milk to your baby?*	☐ Ewe *Yes* ☐ Hayi *No* → **Gqithela ku Q4**
2. Ulubeke nini usana lwakho ebeleni emva kokuba ubelekile? *When did you put your baby to the breast after birth?*	☐ Kwiyure yokuqala *Within the first hour* ☐ Emva kweyure yokuqala ukuya kwiyure eziyi-12 *After the first hour and up to12 hours* ☐ Emva kweyure eziyi-12 ukuya kwiyure eziyi-24 *After 12 hours and up to 24 hours* ☐ Emva kweyure eziyi-24 ukuya kwiyure eziyi-48 (usuku lwesibini) *After 24 hours and up to 48 hours (2nd day)* ☐ Emva kweyure eziyi-48 ukuya kwiyure eziyi 72(usuku lwesithathu) *After 48 hours and up to 72 hours (3rd day)* ☐ Emva kweyure eziyi-72 (emva kosuku lwesithathu) *After 72 hours (After the 3rd day)*
3. Ingaba ubisi lokuqala walinika umntwana wakho okanye ingaba walikhama waze walilahla? *Did you give the first milk (colostrum) to your baby or did you express and discard it?*	☐ Ndamnika ubisi lokuqala *Gave the first milk* ☐ Ndalikhama ndaze ndalilahla ubisi lokuqala *Expressed and discarded the first milk* ☐ Zombini ndamnika kwaye ndamkhamela *Both gave and expressed it* ☐ Enye, cacisa:_____ *Other, specify*

4. Kwintsuku zokuqala ezintathu emva kokuba ubelekile, ingaba umntwana wakho wayinikwa nantoni na eselwayo okanye engcamlwayo (nokuba ngamaqabaza ambalwa ento okanye into encinci engonogcwala nomlomo) *Within the first three days after birth, was your baby given anything to drink or taste (even a few drops of something or less than a mouth full) other than breastmilk?*	☐ Ewe *Yes* ☐ Hayi *No* → **Gqithela ku Q6** ☐ Andazi *Don't know* → **Gqithela ku Q6**

5. Kwintsuku ezintathu emva kokuba ubelekileyo yintoni eyanikwa umntwana wakho ukuba ayisele okanye ayingcamle?
What was your baby given to drink or taste within the first 3 days after birth?

Funda zonke,kheta **ZONKE** ezifanelekileyo
*Read all, select **ALL** that apply*

☐ Amanzi *Water*	☐ Amanzi aneswekile *Water with sugar (or glucose)*
☐ Amanzi anetyuwa *Water with salt*	☐ Nayiphi na into engemanzi njengelenxalenye yesiko *Any liquid as part of a ritual.*
☐ Ubisi lwenkomo oluxutyiweyo *Diluted cow's milk*	☐ Ubisi lwenkomo olungaxutywanga *Not diluted cow's milk*
☐ Ubisi lwabantwana olungumgubo *Infant formula*	☐ Naluphi na ubisi olungumgubo *Any other powdered milk*
☐ Nayiphi na ipapa *Any porridge*	☐ Nayiphi na isuphu *Any soup*
☐ Ubusi *Honey*	☐ Utywala *Alcohol*
☐ Amayeza akhutshwe ngugqirha *Any prescribed medicine* Cacisa *specify:* _____	☐ Amayeza angakhutshwanga ngu gqirha *Non-prescribed medicine* Cacisa *specify:* _____
☐ Iyeza lesintu *Traditional medicine*	☐ Amanye *Other* Cacisa *specify:* _____

B: INFANT FEEDING RECALL

6. Ingaba uyamncancisa na ngoku umntwana wakho? *Are you currently breastfeeding your baby*	☐ Ewe *Yes* → **Gqithela ku Q9** ☐ Hayi *No*
7. Wamncancisa ixesha elingakanani umntwana wakho? *For how long did you breastfeed your child?*	Iintsuku: _____ *Days*

	☐ Zange ancance *Never breastfed* ☐ Andazi *Don't know*

8. Zeziphi izizathu ezakwenza uyeke ukumncancisa/okanye ungamncancisi umntwana wakho?
What were your reasons for stopping to breastfeed/not breastfeed your child?

Funda zonke, khetha **ZONKE** ezifanelekileyo
*Read all, select **ALL** that apply*

☐ Umsebenzi okanye Imfundo *Work or Education*	☐ Ndandingafuni ukumosulela ngentsholongwane ka gawulayo umntwana wam. *Did not want to give my baby HIV infection*
☐ Ukugula,ngaphandle kwengxaki zokuncancisa *Illness, other than lactation problems*	☐ Ndandinengxaki yokuncancisa *Lactation problems*
☐ Mastitis – breast	☐ *Ndandingenabisi lwaneleyo lebele* *Not enough breast milk*
☐ Umtwana wayengakhuli kakuhle *Child not grow well*	☐ Umntwana wayekhala kakhulu *Child crying a lot*
☐ Iingcebiso/ukunyanzeliswa ngabanye *Advice/pressure from others*	☐ Ezinye *Other* Cacisa *specify:* _____

C: DIETARY 24hr RECALL

Ngoku sizokubuza imibuzo malunga nendlela omtyisa ngayo umntwana wakho ukusukela izolo
We will now ask you some questions about your baby's feeding since yesterday morning.

9. Ngexesha obuvuka ngalo izolo ukuyokuma ngexesha obuvuka ngalo ngalentsasa ingaba uye wamncancisa na umntwana wakho? *From the time you woke up yesterday morning till you woke up this morning did you breastfeed your baby?*	☐ Ewe *Yes* ☐ Hayi *No*
10. Ngexesha obuvuka ngalo izolo ukuyokuma ngexesha obuvuka ngalo ngalentsasa ingaba uye wancancisa ubisi olungumgubo na umntwana wakho? *From the time you woke up yesterday morning till you woke up this morning did you feed your baby formula milk?*	☐ Ewe *Yes* ☐ Hayi *No*

11. Ngexesha obuvuka ngalo izolo ukuyokuma ngexesha obuvuka ngalo ngalentsasa: Ingaba ubukhe wamnika nanye na yezi zinto zilandelayo umntwana wakho?
From the time you woke up yesterday morning till you woke up this morning: Did you give any of the following items to your baby?

Funda zonke, khetha **ZONKE** *ezifanelekileyo*
*Read all, select **ALL** that apply*

☐ Nawaphi na amanzi *Any water*	☐ Nawaphi na amanzi aneswekile *Any water with sugar or glucose*
☐ Naziphi na ingcambu emanzini *Any herbs in water*	☐ Amanzi erayisi *Rice water*
☐ Nayiphi na iti enobisi *Any tea with milk*	☐ Nayiphi na iti engenabisi *Any tea without milk*

☐ Ubisi lwenkomo olungaxutywanga *Non diluted cow's milk*	☐ Ubisi lwenkomo oluxutyiweyo *Diluted cow's milk*
☐ Ubisi lwebhokhwe *Goat's milk*	☐ Olunye ubisi olungumgubo *Other powdered milk*
☐ Ijusi yeziqhamo *Any fruit juice*	☐ iGripe water *Gripe water*
☐ Nabuphi na utywala njenge bhiya, umqombothi *Any alcohol like beer or brew*	☐ Naziphi na ezinye izinto njenge yogathi, itshizi okanye ikhrim *Any other dairy product like yoghurt, cheese or cream*
☐ Ipapa, isidudu okanye isonka *Cereals, porridge or bread*	☐ Amaqanda *Eggs*
☐ Naziphi na iziqhamo/imifuno *Any fruits/vegetables*	☐ Nayiphi na inyama okanye intlanzi *Any meat or fish*
☐ Nawaphi na amayeza abhalwe ngugqirha *Any prescribed medicine*	☐ Nawaphi na amayeza angabhalwanga ngugqirha *Any non-prescribed medicine*
☐ Ezinye *Other*, Cacisa, *Specify:*_____	☐ Akukho nanye kwezi zikhankanywe ngentla *None of the Above*

12. Ngawaphi kulamayeza afunyanwa ngumntwana wakho:
Which of these medicines is your baby currently receiving?
Khetha onke afanenelekileyo
Select all that apply

☐ Multivitamins (eg Kiddievite)	☐ Iron drops
☐ Zinc syrup	☐ Nevirapine
☐ Iantibiotic yokukhusela ulwasuleleko lwesifuba *Co-trimoxazole, Bactrim, Trimethoprim-Sulphamethoxazole, Resmed* ***These are antibiotics to prevent chest infection!!!***	☐ Ezinye Ii-antibiotics *Other Antibiotics* Cacisa *specify:*_____
☐ TB drugs	☐ Ezinye *Other* Cacisa *Specify:*_____

Initials of interviewer: ____

13. Ingaba umntwana wakho ufumana nayiphi na okanye amanye amayeza angakhutshwanga ngu gqirha okanye unesi?
Is your baby receiving any other medication that has not been prescribed by a doctor or nurse?

Khetha **ONKE** afanelekileyo
*Select **ALL** that apply*

☐ Iyeza lesintu *Traditional medicine*	☐ Amayeza angakhutshwanga ngu gqirha *Non-prescribed medicine* Cacisa *specify:* _____
☐ Amanye *Other* Cacisa *specify:* _____	☐ Akukho nanye kwezi zikhankanywe ngentla *None of the Above*

LEAVING YOUR CHILD

14. Sewukhe wohlukana nosana lwakho oko walubeleka kwenzeka ancanciswe ngomnye umntu? Ingaba wawukhe wohlukana na nomntwana wakho ukusukela egqibozalwa ukuze omnye omntu amtyise? *Have you ever been separated from your baby since childbirth so that someone else has fed him/her?*	☐ Ewe *Yes* ☐ Hayi *No* → **Gqithela ku END** ***Skip to END***

15. Bamtyisa ntoni umntwana wakho ngelaxesha wawungekho?
What did they feed your baby the last time you were away?

Khetha **ZONKE** ezifanelekileyo
*Select **ALL** that apply*

☐ Umxube wamanzi *Water based liquids*	☐ Umxube wobisi/ukutya okuthambileyo *Milk based liquids/semi-solid feeds*
☐ Ubisi lwam lwebele endandilikhamile *My own expressed breast milk*	☐ Umntwana wancanciswa ngomnye umdlezana *Baby "wet nursed" (breastfed by another woman)*
☐ Ubisi olungumgubo *Formula milk*	☐ Ukutya endandikuhlafunile komntwana *Food that I chewed for the baby*
☐ Ezinye *Other* Cacisa *specify:* _____	☐ Andazi *Do not know*

Signed Interviewer completing CRF: _____ Date: _____ / _____ / _____
DD MMM YYYY

Signed QC Officer: _____ Date: _____ / _____ / _____
DD MMM YYYY

Signed Study Coordinator: _____ Date: _____ / _____ / _____
DD MMM YYYY

Initials of interviewer: ____

PCID: __ __ __ __ __ __ - __ __

Study Visit 2 to 4

INFANT FEEDING PRACTICES

This CRF applies to ALL enrolled BPOS Infants
Complete during 10 weeks Postpartum Study Visit

Visit Date								
D	D	M	M	M	Y	Y	Y	Y

Visit Code	
P	**2**

Siza kubuza imibuzo malunga nendlela olutyisa ngalo usana lwakho
We are going to ask you some questions about how you are feeding your baby

INFANT #: _____ Initials: __ __

INFANT FEEDING RECALL
Ukukhumbula indlela zokondla usana

1. Uye waqhubekeka ukuncancisa ibele usana lwakho oko ugqibele ukuza kuthi *Since your last visit, did you breastfeed your baby?*	☐ Ewe *Yes* → **Gqithela ku Q4** ☐ Hayi *No*
2. Uluncancise ibele usana lwakho ixesha elingakanani *For how long did you breastfeed your child?*	Intsuku/iveki/inyanga:_____ *Days/weeks/months* ☐ Zange lube sebeleni *Never breastfed* ☐ Andazi *Don't know*

3. Zeziphi izizathu ezibangele uyeke ukuluncancisa usana/okanye ungaluncancisi
What were your reasons for stopping to breastfeed/not breastfeed your child?

Khetha ZONKE izizathu
*Read all, select **ALL** that apply*

☐ Umsebenzi okanye Imfundo *Work or Education*	☐ Andifuni ukumosulela ngentsholongwane *Did not want to give my baby HIV infection*
☐ Ukugula,ngaphandle kwengxaki zokuncancisa *Illness, other than lactation problems*	☐ Ingxaki zokuncancisa *Lactation problems*
☐ Mastitis – breast problems	☐ Ubisi lwebele alwanelanga *Not enough breast milk*
☐ Usana alukhuli kakuhle *Child not grow well*	☐ Usana lukhala kakhulu *Child crying a lot*
☐ Iingcebiso/ukunyanzeliswa ngabanye *Advice/pressure from others*	☐ Ezinye *Other* Cacisa *specify:*_____

4. Ebengakanani umntwana ukuqala kwakho ukumnika ezinye izinto ngaphandla kwebisi lebele okanye amayeza? Ngamany'amazwi, ebengakanani yena xa wayeqala ukufumana amanzi okanye ukutya okanye ubisi lomgubo wabantwana? *How old was your baby when you FIRST gave him/her anything other than breast-milk or medicine? In other words, how old when he/she first had any water or food or formula milk?*	☐ Zange ndamnika enye into umntwana ngaphandle kwebisi lwebele *Have only given baby breast-milk and medicine since birth* # weeks/months old _____ ☐ Andiqinisekanga *Unsure*

DIETARY 24hr RECALL

We will now ask you some questions about your baby's feeding since yesterday morning

5. Ukuvuka kwakho izolo ekuseni kude ibe kukuvuka kwakho namhlanje ekuseni uye waluncancisa usana lwakho? *From the time you woke up yesterday morning till you woke up this morning did you breastfeed your baby?*	☐ Ewe *Yes* ☐ Hayi *No*
6. Ukuvuka kwakho izolo ekuseni kude ibe kukuvuka kwakho namhlanje ekuseni uye waluseliswa ubisi usana lwakho? *From the time you woke up yesterday morning till you woke up this morning did you feed your baby formula milk?*	☐ Ewe *Yes* ☐ Hayi *No*

7. Ukuvuka kwakho izolo ekuseni kude kube kukuvuka kwkho namhlanje ekuseni: Ulunikile usana lwakho ezinye zezi zinto.

From the time you woke up yesterday morning till you woke up this morning: Did you give any of the following items to your baby?

Kheta ZONKE omnike zona.

*Read all, select **ALL** that apply*

☐ Amanzi *Any water*	☐ Amanzi aneswekile *Any water with sugar or glucose*
☐ Ingcambu emanzini *Any herbs in water*	☐ Amanzi erayisi *Rice water*
☐ Iti enobisi *Any tea with milk*	☐ Iti engenabisi *Any tea without milk*
☐ Ubisi lwenkomo olungaxutywanga *Non diluted cow's milk*	☐ Ubisi lwenkomo oluxutyiweyo *Diluted cow's milk*
☐ Ubisi lwebhokhwe *Goat's milk*	☐ Olungolunye ubisi olungumgubo *Other powdered milk*
☐ Ijusi yeziqhamo *Any fruit juice*	☐ iGripe water *Gripe water*
☐ Into ebutywalara njenge bhiya, umqombothi *Any alcohol like beer or brew*	☐ Ezinye izinto njenge yogati, itshizi, ikhrim *Any other dairy product like yoghurt, cheese or cream*
☐ Ipapa yabantwana, ipapa, okanye isonka *Cereals, porridge or bread*	☐ Amaqanda *Eggs*
☐ Iziqhamo/vegi *Any fruits/vegetables*	☐ Inyama, intlanzi *Any meat or fish*
☐ Amayeza abhalwe ngugqira *Any prescribed medicine*	☐ Amayeza angabhalwanga ngugqira *Any non-prescribed medicine*
☐ Ezinye, cacisa*:* _____	☐ Nanye kwezi zikhankanyewe ngenthla *None of the Above*

8. Ngawaphi kulamayeza atyiwa lusana lwakho:

Which of these medicines is your baby currently receiving:

Kheta ZONKE ezenziweyo

Select all that apply

☐ Multivitamins (eg Kiddievite)	☐ Iron drops
☐ Zinc syrup	☐ Nevirapine
☐ Iantibiotic yokukhusela ulwasuleleko lwesifuba *Co-trimoxazole, Bactrim, Trimethoprim-Sulphamethoxazole, Resmed* **These are antibiotics to prevent chest infection**	☐ Ezinye Ii-antibiotics *Other Antibiotics* Cacisa *specify:*_____
☐ TB drugs	☐ Ezinye *Other* Cacisa *Specify:*_____
☐ Nanye kwezi zikhankanyewe ngenthla *None of the Above*	

PCID: _ _ _ _ _ _ _ - _ _

9. Ingaba umntwana wakho ukhe waseleliswa amayeza angawafumenanga kugqirha okanye kumongikazi?
Is your baby receiving any other medication that has not been prescribed by a doctor or nurse?

Kheta ZONKE ezenziweyo
Select all that apply

☐ Iyeza lesintu *Traditional medicine*	☐ Amayeza angekhoyo kuluhlu *Non-prescribed medicine* Cacisa *specify:*_____
☐ Ezinye *Other* Cacisa *specify:*_____	☐ Nanye kwezi zikhankanyewe ngenthla *None of the Above*

LEAVING YOUR CHILD

10. Sewukhe wohlukana nosana lwakho oko walubeleka kwenzeka ancanciswe ngomnye umntu? *Have you ever been separated from your baby since childbirth so that someone else has fed him/her?*	☐ Ewe *Yes* ☐ Hayi *No* → **Gqithela ku END**

11. Luye lwancanciswa ntoni usana oku kokugqibela ungekho?
What did they feed your baby the last time you were away?

Kheta ZONKE ezenziweyo
*Select **ALL** that apply*

☐ Umxube wamanzi *Water based liquids*	☐ Umxube wobisi/ukutya okuthambileyo *Milk based liquids/semi-solid feeds*
☐ Ubisi lwam lwebele ebendilukhamile *My own expressed breast milk*	☐ Usana beluncanciswe ibele ngomnye umdlezana *Baby "wet nursed" (breastfed by another woman)*
☐ Ubisi lomgubo *Formula milk*	☐ Ukutya ebendikuhlafunile kosana *Food that I chewed for the baby*
☐ Ezinye *Other* Cacisa *specify:*_____	☐ Andazi *Do not know*

Signed Interviewer completing CRF: _____ Date: _____ / _____ / _____
DD MMM YYYY

Signed QC Officer: _____ Date: _____ / _____ / _____
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Signed Study Coordinator: _____ Date: _____ / _____ / _____
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Doe, J: Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt (1999). Accessed 12 Nov 1999.

Organization site

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See our editorial policies for author guidance on good citation practice.

Please check the submission guidelines for the relevant journal and article type.

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Preparing tables

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- Tables should be numbered and cited in the text in sequence using Arabic numerals (i.e. Table 1, Table 2 etc.).
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- Tables larger than one A4 or Letter page in length can be placed at the end of the document text file. Please cite and indicate where the table should appear at the relevant location in the text file so that the table can be added in the correct place during production.
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- Tables should not be embedded as figures or spreadsheet files, but should be formatted using 'Table object' function in your word processing program.
- Color and shading may not be used. Parts of the table can be highlighted using superscript, numbering, lettering, symbols or bold text, the meaning of which should be explained in a table legend.
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Preparing additional files

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All Additional files will be published along with the accepted article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files, if requested, should be sent by email to the journal's editorial email address, quoting the manuscript reference number. Please do not send completed patient consent forms unless requested.

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Additional files

Guidelines

This page provides general information for authors creating additional files to maximize the quality of those files. It includes tips on handling files like these as well as supported formats.

How to use additional files

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- Title of data.
- Description of data.

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Additional files should be numbered in the order they are first mentioned in the text, and uploaded in this order.

File size and resolution

The maximum file size for additional files is 20 MB each and files will be virus-scanned on submission.

Data over 20 MB should be deposited in a suitable permanent repository for that type of data, where one exists (e.g. GEO for microarray data). Please see our list of recommended repositories for guidance.

Additional files can be submitted in any format.

Instructions for commonly submitted file types are available within guidelines for specific types of additional files.

Frequently asked questions

What are Additional files?

A. Additional files are files containing additional information that supports or expands upon items referred to in the main manuscript. All additional files must be referenced in the main manuscript.

What is the difference between an additional file and a figure file?

A. A figure file is an image file that will appear in the final published manuscript. On the other hand, additional files will be made available alongside the published manuscript, but will not be visible within it. Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article.

Can I upload my figures files as additional files?

A. The only time you should do this is when the figure image must be larger than A4 in order to be legible.

Why is it important to include additional files?

A. Additional information is considered integral to articles published by BMC. You are encouraged to provide data sets, tables, movie files, software or other information as additional information. Results that would otherwise be indicated as "data not shown" can and should be included as additional files.

How large can additional files be?

A. You should aim for all additional files to be as low a file size as possible. The maximum file size for additional files is 20 MB each.

Is there a limit to how many additional files I can include?

A. No; you can include as many relevant additional files as necessary.

Can I use image manipulation software to increase the clarity of images within additional files?

A. Enhancement of digital images using image-editing software is acceptable practice if carried out responsibly. However, it is crucial that artefacts are not introduced and the original data is not misrepresented.

Within my manuscript, can I make references to individual items within my additional files?

A. Yes, items within additional files can be referenced in the main manuscript. However, please use the format set out in the following example: “See Supplementary Table 1, Additional File 1”.

Can I submit earlier versions of the final manuscript as additional files?

A. No.

Can I cite references in additional files?

A. You can cite references in additional files, as long as you list them in a separate section within the additional file; any references that are only cited in the additional files should not be listed in the main manuscript reference list.

Additional files formats

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This section provides information on which formats you should use for specific types of additional files.

Additional documentation and Algorithms

Additional documentation and algorithms can be provided in a number of formats, including PDF (Adobe Acrobat), DOC (Microsoft Word), TXT, RTF, EPS, HTML and PPT.

Animations and Movies

Animations can be provided in SWF (Shockwave Flash) format (or converted in a video format).

Movies in mp4, mpeg, mov, avi, swf and animated GIF formats will be embedded in the additional files page. The first frame of the movie will be used as 'poster frame' and will be shown before the user starts movie playback. Other formats will be available for download.

MOV is a common multimedia format, which is often used for saving movies and other video files. This format uses a proprietary compression algorithm developed by Apple Computer; compatible with both Macintosh and Windows platforms. MPG is a common digital video format, which typically incorporates MPEG-1 or MPEG-2 audio and video compression. AVI files can contain both audio and video data in a file container that allows synchronous audio-with-video playback.

Audio

Audio files can be uploaded in a number of formats, including: WAV, MP3, FLAC, AIFF and AU. WAV is a Microsoft and IBM audio file format standard for storing audio bitstreams. It is the main format used on Windows systems for raw and typically uncompressed audio.

Chemical Structures

CDX (ChemDraw) is the file format for saving chemical reaction schemes prepared using ChemDraw. Suggested ChemDraw settings are:

- Chain Angle 120°
- Bond spacing 18%
- Fixed length 0.406 cm (11.5 pt)
- Bold width 0.056 cm (1.6 pt)
- Line width 0.018 cm (0.5 pt)
- Margin width 0.046 cm (1.3 pt)
- Hash spacing 0.071 cm (2 pt)

TGF (ISIS/Draw) is the file format for saving chemical reaction schemes prepared using ISIS/Draw.

Computational models

We encourage authors to prepare models of biochemical reaction networks using the Systems Biology Markup Language (SBML).

Generic Data Files

DAT (Data file) files are generic data files created by specific applications. They are not an ideal format to use, as they can typically only be accessed by the application that created the file. However, text within DAT files can sometimes be viewed using a text reader.

Geospatial Data

KML (Keyhole Markup Language) is an XML-based language schema for expressing geographic data in two or three-dimensions. KML can be used for geospatial biomedical data suited to 3D spatial visualisation. Google Earth will be used as a viewing application for KML data.

Genomic sequences

Genomic sequences should be formatted according to the Genomic Standards Consortium (GSC) and follow the minimum information about a genome sequence (MIGS) specification. The sequence should be deposited to the International Nucleotide Sequence Database Collaboration (INSDC) prior to submission and the accession numbers provided in the text of the manuscript.

Metabolic networks

Networks can be defined as a collection of interactions between different pairs of nodes. Frequently used formats include KML, BioPax, SBML , PSI-MI, SIF, XML and KGML.

Microarray data

The Minimum Information About a Microarray Experiment (MIAME) or Minimum Information about a high-throughput SeQuencing Experiment (MINSEQE) guidelines should be adhered to when reporting microarray data and we recommend using the spreadsheet-based MAGE-TAB format. We also recommend that you include a copy of the appropriate MIAME checklist.

Mini-websites

Small self-contained websites can be submitted as additional files, in such a way that they will be browsable from within the full text HTML version of the article. In order to do this, please follow these instructions:

1. Create a folder containing a starting file called index.html (or index.htm) in the root
2. Put all files necessary for viewing the mini-website within the folder, or sub-folders
3. Ensure that all links are relative (ie "images/picture.jpg" rather than "/images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters
4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems; it is ideal to check this on a different machine
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article

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Tabular data can be provided in formats including: DAT, TXT, XLS, CSV, XML and TSV.