# RESEARCH PROTOCOL

**Project Title**:

The CanDo (Canadian Donor Milk) Trial: Pasteurized human donor milk supplementation in the well-baby unit.

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**Confidentiality Statement**

This document contains confidential information that must not be disclosed to anyone outside of the Sponsor, the Investigator Team, the Health Research Authority, the partner organization, and members of the Research Ethics Committee and Regulatory Authorities, unless given explicit permission to do so.

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## Lay Abstract

Human milk is the ideal source of nutrition for all infants. When parent milk is unavailable, pasteurized human donor milk as a supplement versus formula, reduces the risk of necrotizing enterocolitis, a severe bowel emergency in very preterm infants. Donor milk use in well-baby units is a growing practice despite a lack of scientific evidence for its benefit or an understanding of associated cost. In Canada, where lactation support is readily available in most well-baby units, 35-50% of newborns are supplemented with formula prior to hospital discharge. To date, there is no trial to study the impact on breastfeeding and health outcomes following donor milk versus formula supplementation in the well-baby nursery. We aim to conduct a randomized controlled trial to test whether supplementation with donor milk compared to formula for infants during initial hospital stay increases exclusivity and duration of human milk feeding at 4 months, an important determinant of public health. We will also study the impact of type of supplementation on measures of newborn health, growth, behavior, feeding efficacy, parental stress, and use of informally shared milk. The financial implications of each type of supplementation will also be considered. Our findings will provide evidence-based guidelines for feeding Canadian newborns with potential long term implications for child and family health and well-being.

## Abbreviations

BSES-SF, Breastfeeding Self-Efficacy Scale – Short Form; CanDo, Canadian Donor Milk; IFI, Infant Feeding Intentions; NICU, Neonatal Intensive Care Unit; RCT, Randomized Controlled Trial; SGA, Small for Gestational Age; STAI, The State-Trait Anxiety Inventory; VLBW, Very Low Birth Weight; WHO, World Health Organization

## 1.1 Project Summary

**Background and Importance**: Human milk is the ideal nutrition for all infants, and research has demonstrated that human donor milk can provide health benefits for preterm infants. Approximately 35-50% of late preterm and term infants admitted to a well-baby unit after birth require supplementation due to various reasons including hypoglycemia, weight loss, or insufficient availability of parent's milk. However, there is limited research to guide decision making on which supplement to prescribe. Despite this, many hospitals are introducing donor milk in well-baby units in light of the growing recognition of the health protective effects of human milk.

**Goals/ Research Aims**: The primary research aim of this study is to compare the effects of supplementing parent's milk with donor milk versus formula in infants at increased risk for supplementation (infant born to a mother with diabetes, infant with a birth weight smaller than expected for the gestational age (SGA)) on exclusive human milk feeding at 4 months of age. Secondary research aims include: examining the effects of supplementing parent's milk with donor milk on any or exclusive human milk feeding rates at 1, 2 and 3 months, infant health and growth at hospital discharge and at 1, 2, 3, and 4 months, and breast feeding self-efficacy scores. Exploratory outcomes include: infant temperament scores at 1, 2, 3, and 4 months, and parental mental health scores at 2 and 4 months using validated questionaires, milk cortisol concentrations at 2 and 4 months, the use of informally shared milk, and the associated financial costs of a donor milk program versus formula.

**Methods/ Approaches/ Expertise**: This proposed study is a randomized, controlled, single-center trial that will involve assigning participants to two intervention arms (n=56 infants/arm). The intervention will involve providing donor milk or formula in bottles and will last for the duration of the infant's initial hospitalization, followed by monthly phone calls and a virtual or in-person assessment at 4 months. At each time-point of 1, 2, 3, and 4-month, surveys of breast feeding self-efficacy, and health and infant temparement will be conducted, and the infant's anthropometrics will be measured. Additionally, measures of parental depression, anxiety and stress will be assessed with survey-based tools at 2 and 4 months. A coincident human milk sample will be collected at 2 and 4 months and will be analysed for cortisol concentrations. The research team has experience running feeding interventions and donor milk trials in newborns, and includes experts in nursing, medicine, dietetics, and milk banking.

**Expected Outcomes**: This proposed research holds the potential to make a meaningful impact on public health. In Canada, with approximately 350,000 level I nursery admissions each year and high rates of formula supplementation ranging from 35-50%, there is a clear opportunity for improvement. By promoting exclusive human milk feeding, exploring evidence-based practices to inform hospital guidelines, and reducing the reliance on informal milk sharing, this research aims to enhance the health outcomes for both parents and infants. Additionally, it seeks to deepen our understanding of early parent-child interactions, fostering greater awareness and knowledge in this area.

## 1.2 Research Background

For this proposed research, human milk refers to the totality of parent milk and donor milk. Parent milk is a gender inclusive term used for planning future research whereas mother's milk is used where describing reports from the literature where gender is not further specified. Donor milk refers to pasteurized human donor milk from an accredited milk bank.

Newborns are typically fed via parent milk as the primary source of nutrition and the multitude of benefits of mother's milk are summarized in the 2016 Lancet breastfeeding series (1). According to the recent 2023 Lancet breastfeeding report (2), although breastfeeding has been proven to have health benefits for mothers and infants in both high-income and low-income settings, less than 50% of infants worldwide are being breastfed in accordance with the World Health Organization's (WHO) recommendations. This is due to a variety of factors, including societal, political, and economic influences (3, 4).

When a supplement to parent milk is required in the newborn period, it is uncertain whether this should be formula or donor milk as the former is associated with a reduction in exclusive human milk feeding later in infancy while the latter caries elevated financial cost and inequitable distribution within Canada and globally (5, 6). The impact of using donor milk versus formula as a supplement during initial hospitalization on adherence to breastfeeding guidelines post-discharge is unknown. There are more than 350,000 (7) infants born annually in Canada of whom approximately 90% are admitted to well-baby units with many hospitals reporting a typical formula or donor milk supplementation rate of about 35-50%, particularly for high risk infants (6). The reasons for supplemental feeds include hypoglycemia, hyperbilirubinemia, weight loss, insufficient volume of parent's milk, surrogacy, parental preference or for unknown reasons.

Historically, formula has been the predominant supplement when one is required for any infant; however, as human milk banking has grown rapidly, the interest in and the practice of providing donor milk to infants in a well-baby unit has increased (8-10). Health Canada and WHO recommend exclusive breastfeeding for the first 6 months for all infants (11, 12). The WHO goes on to state that if mother's milk is unavailable or insufficient in volume, both preterm and healthy term infants should be fed donor milk. The use of donor milk in populations other than very low birth weight (VLBW) infants, however, has not been systematically investigated. A recent systematic scoping review of donor milk in populations other than preterm infants concluded that current evidence is limited by the lack of randomized trials as well as direct health measures and recommended further research be conducted (13). One retrospective study compared infants admitted to a well-baby unit in Florida before (n=73) and after (n=49) the commencement of a donor milk program (14). Approximately half of the infants' families were called 6 months post hospital discharge and asked about feeding practices. The rate of exclusive breastfeeding to >6 months had increased from 15% to 58% (adjusted odds ratio=5.13, 95% confidence interval 1.37, 19.23, p=0.015).

A recent pilot study in California investigated the feasibility of use of donor milk versus formula in 32 term and late preterm infants admitted to neonatal intensive care unit (NICU). The consent rate was 52%, and breastfeeding attempts increased significantly over time in the donor milk group compared to the formula group. Growth at multiple time points was similar between the two groups (15). The results of the study confirmed that randomly assigning term and late preterm infants into donor milk or formula groups when their mother's milk is not available is feasible, and that donor milk may increase breastfeeding attempts without having a negative effect on growth and appropriate weight gain.

Few data are available regarding the use of donor milk in Canadian well-baby units, although at least 5 hospitals in Ontario are presently doing so (personal communication to the Rogers Hixon Ontario Human Milk Bank). Among 214 American hospitals surveyed in 2017, the prevalence of donor milk use in well-baby units was 17.6% with the majority (85%) of donor milk programs in these hospitals having started in the previous 5 years (10). In 2018, a survey of American hospitals participating in the Better Outcomes through Research for Newborns Network, showed that of 71 hospitals responding, 28% offered donor milk routinely when a supplement was required and 40% had donor milk available in the well-baby unit (9). A 2018 survey of hospitals in Massachusetts served by a single milk bank (n=51 respondents), showed that 43% of hospitals with access to donor milk use it routinely in their well-baby unit (8). A further concern is

the growing use of informally shared milk (donor milk that is sourced other than from a milk bank and is typically not pasteurized) to meet supplementation needs. Both Health Canada and the Canadian Paediatric Society have issued warnings about this practice (16, 17).

***In view of limited high-quality evidence, adequately powered trials that evaluate the impact of donor milk compared to infant formula as a supplement to parent milk in well-baby units are needed***; we thus propose to conduct a randomized controlled trial (RCT) to investigate whether prescribed supplementation with donor milk compared to formula for infants at higher risk of supplementation during initial hospitalization impacts exclusive human milk feeding at 4 months of age. We will also study the impact of types of supplementation on measures of newborn health, growth, and temperament, feeding efficacy, parental stress, use of informally shared milk and the financial costs of donor milk versus formula supplementation.

## 1.3 Goals/ Research Objectives

### 1.3.1 Primary research objective:

To determine whether the provision of donor milk as prescribed as a supplement to parent's milk for infants at higher risk for supplementation and admitted to a well-baby unit improves exclusive human milk feeding at 4 months of age compared to supplementation with formula.

### 1.3.2 Secondary research objectives:

To explore whether the provision of donor milk as a supplement to parent's milk affects any, or exclusive, human milk feeding rates at 1, 2, and 3 months of age, breast feeding self-efficacy scores at 1, 2, 3 and 4 months, infant health and growth at hospital discharge and at 1, 2, 3, and 4 months, and percent weight loss, glucose levels, length of hospital stay at hospital discharge.

### 1.3.3 Exploratory outcomes:

To explore whether the provision of donor milk as a supplement to parent's milk affects infant temperament scores at 1, 2, 3 and 4 months, and parental stress, anxiety and depression scores at 2 and 4 months using validated questionnaires. To also explore milk cortisol levels at 2 and 4 months and the prevalence of informally shared milk after hospital discharge. An economic evaluation of the donor milk program versus formula will also be conducted concurrently.

## 1.4 Rationale and Significance

The proposed trial will be the first randomized controlled trial that will evaluate the type of nutritional supplementation provided in the immediate newborn period to infants during their initial hospitalization in a well-baby unit. Although the goal is for exclusive human milk feedings from birth, up to 50% of all infants in a well-baby unit receive supplementation. The current standard is for this supplement to be a bovine milk formula. However, the practice of using human donor milk for this population is increasingly growing without any research evidence. With approximately 350,000 level I nursery admissions per year in Canada and a supplementation rate of approximately 35-50% in higher risk infants, this proposed research can have far-reaching public health implications. This may include improved health outcomes for parents and infants by improving exclusive human milk feeding and deepening our understanding of early parent-child interactions, while improving evidence-based practices. Further, this study has the potential to result in risk reduction by reducing the occurrence of informal human milk sharing.

## 1.5 The novelty of the approach

To date, the limited published evidence supporting the use of donor milk is in the form of pre-post implementation reports. We propose the first RCT to study the potential health impact of donor milk use in the well-baby unit. Our proposed research takes a holistic approach with the primary outcome (exclusive breastfeeding) being an important determinant of public health. The exploratory measures assess a wide range of parent-child interactions including measures of infant temperament and parent mental health. These outcomes are increasingly being recognized as critical in early infant development and family well-being and will be evaluated as potential outcome variables for future research. Human milk cortisol levels will be analyzed to further study the potential biological underpinnings of the link between parental stress and infant health.

## 1.6 Trial design

The proposed pragmatic RCT will have 2 arms (open label donor milk/formula supplement, n=56 infants/arm); to assess how awareness of donor milk supplementation impacts feeding and other related outcomes. Families will be recruited upon admission to the well-baby unit and their infant registered for the trial only if a feeding supplement is prescribed. The intervention will last for the duration of the hospitalization and the primary outcome, exclusive human milk feeding, will be assessed at 4 months via an in person or virtual visit, according to the family's wishes and to maximize patient retention.

### 1.6.1 Inclusion criteria:

Infants admitted to the well-baby unit at Sinai Health whose parent(s) intend to feed parent milk and who require supplementation:

- Infants of gestational/ type 1/ type 2 diabetic mothers
- Infants who are born small for gestational age (SGA) by using a sex-specific reference population and determining if their birth weight falls below the 10th percentile for gestational age
- Infants with a birth weight less than 2.5 kg

Note: Families will be approached according to the above stated criteria however only registered for trial participation if consent is secured and the infant require supplementation.

### 1.6.2 Exclusion criteria:

Enrollment in any other clinical study affecting nutritional management during the feeding intervention; anticipated change in primary caregiver (person providing the feed) prior to 4 months; refusal to consent to donor milk; supplementation with formula prior to enrollment; any physical condition that may impact growth (ex: skeletal dysplasia).

### 1.6.3 Guidelines for feeding during the intervention phase

The randomization occur once trial registration occurs. The intervention will continue throughout the hospitalization at any time that a supplementation is recommended by the clinical team, and is expected to last from 0 to 72 hours post-birth.

All donor milk will be aliquoted into individualized containers in the Rogers Hixon Ontario Human Milk Bank which is located in Sinai Health. The containers for study groups 1 and 2 will

be open labelled and will be named as “CanDo trial-donor milk” and “CanDo trial-formula”. The decision regarding the timing and duration of the supplementation will be determined by the medical team in the well-baby unit based on the specific needs and circumstances of each mother-infant dyad. As this study follows a pragmatic approach, the clinical team will assess the need for supplementation based on the mother-infant dyad’s condition. General feeding guidelines, including ad lib/ on-demand parent milk feeding as per the parent's plans, will be in place. The management of hypoglycemia will be according to the recommendations of the Canadian Paediatric Society and in accordance with Mount Sinai’s hypoglcemia protocol including routine glucose monitoring for all children born <37 weeks gestation, <10% birth weight for gestation, <2.5 kg or >4.5 kg birth weight, with a supplement provided (dextrose gel followed by supplemental feed) for any glucose <2.6 mmol/L in the first 72 hours after birth (18).

### 1.6.4 Practical arrangements for allocating participants to trial groups

Families will be invited to participate in the trial during their antenatal visits to the diabetes and placenta clinics. If they agree to participate, they will be asked to sign the consent form. After giving birth, upon admission to the well-baby unit they will be approached again for confirmation of their willingness to participate in the trial and to add infant’s name to the consent form. Infants who are prescribed a supplement will be entered into the trial and randomly assigned to either the donor milk or formula group. Prior to the start of the study, a unique randomization schedule will be generated. The computer-generated randomization schedule will be securely stored in a sealed binder, with each participant's allocation placed in separate envelopes. These envelopes will be securely held in a locked procedure room, either within the well-baby unit. Importantly, to maintain blinding, the study coordinator will access the randomization schedule one envelope at a time during participant enrollment and allocation. This approach ensures that the study coordinator cannot see whether a participant will receive donor milk or formula until the point of assignment.The study allocation will be randomly assigned in a 1:1 ratio, using random blocks of 2 or 4. Furthermore, to prevent potential bias and uphold the integrity of the randomization process, the randomization schedule will incorporate stratification factors. These factors include whether the infant was born to a diabetic mother, whether they are small for gestational age, and the infant's sex. Stratification will facilitate the equitable distribution of supplementation rates across these distinct groups, thereby contributing to the robustness of the randomization process.

### 1.6.5. Communication of group allocation with the nursing staff and parent(s):

The responsibility for communicating group allocation lies with the study coordinator. Following the completion of the randomization process, parents and nursing staff will be informed of the group allocation, as the study is open-label, and this information aligns with the study design. During this discussion, parents will receive a clear explanation of their infants' group assignment, including its implications and details regarding the associated supplementation. It will also emphasize the voluntary nature of participation and the option for parents to withdraw from the study at any point.

## 1.7 The outcome measurements at follow up

All outcome measures post hospital discharge may be assessed in person or virtually according to the family's preference. Outcome measures at birth will be assessed during the initial hospitalization whereas outcomes at 1, 2, and 3 months will be assessed by digital survey (RedCap) or telephone call according to the parent(s)' preference with the option of the 4 month visit being by telephone, video call or in person according to the parent(s)' preference.

***Infant Feeding Intentions**:*

At baseline, the Infant Feeding Intentions (IFI) scale (19) will be employed to quantitatively measure the intensity of mothers' intentions to commence breastfeeding and sustain it as the exclusive source of nourishment for their infants for the recommended duration of 6 months, as advocated by public health agencies. The IFI scale is a valid and simple tool comprised of five carefully constructed questions designed to capture the essence of these intentions accurately.

The initial two items of the scale focus specifically on assessing the strength of intention to initiate breastfeeding, offering valuable insights into the mother's commitment from the outset. Subsequently, the remaining items of the scale examine the intensity of intention to exclusively breastfeed at distinct milestones: 1 month, 3 months, and 6 months of age.

By utilizing the IFI scale, our study aims to obtain a precise and quantitative understanding of the degree of intention and dedication displayed by mothers towards initiating and maintaining breastfeeding. This comprehensive evaluation will provide valuable insights into the dynamics of infant feeding practices, enabling the development of effective strategies and interventions to support and encourage breastfeeding.

***Exclusive human milk feeding and informal shared milk feeding***:

As previously mentioned, parent report will be taken at 1, 2, 3, and 4 months. At each interaction, participants will be asked whether the infant is receiving any human milk (yes/ no) and about exclusive human milk feeding. Exclusive human milk feeding will be defined as the provision of only human milk with no additional fluids (including water) or solids except for vitamins/minerals and medicine (including rehydration solutions). Based on previous research, we will explore human milk feeding “at” each time-point (within the previous 2 weeks of the interaction) as well as “for” each time interval (e.g., from hospital discharge to 4 months) (20). We anticipate the intervention will increase both the duration and exclusivity of human milk feeding due to the value given to human milk provision in this study. In order to understand the potential impact of increased informal milk sharing in situations where donor milk is not available after hospital discharge, we will investigate the frequency of this practice. To gather this information, we will include questions in our monthly check-in questionnaire for parents. Specifically, we will ask parents to indicate the number of times per day their infant was fed expressed human milk using a bottle, supplemental nurser, or other methods. Additionally, we will inquire whether the milk used for these feedings was expressed by the parent themselves or by someone else or whether it was a non-human milk based formula. By collecting this data, we aim to gain insights into the prevalence of informal milk sharing and its potential implications for infant feeding practices.

***Length of hospital stay, percent weight loss and glucose concentrations during initial hospitalization****:*

Infant weight is routinely measured at birth and daily during the initial hospital stay at the recruiting site and therefore these values will be abstracted from the patient chart. Donor milk in preterm children has been linked to slower weight gain, thus an important secondary outcome will be to document any difference in percentage weight loss for infants supplemented with donor milk compared to formula while in hospital. When considering the measure of percentage weight loss, it is important to take into account the natural fluid loss that occurs in the early postnatal period. To ensure the measure is meaningful in relation to fluid loss, the aim of the study is to document any excessive and unanticipated weight loss beyond what would be expected in the normal course of fluid regulation. This measure will help determine if there are any notable variations or concerns related to fluid loss that may affect the infants' overall well-being and growth outcomes. In addition, to investigate the effect of donor milk/ formula on

improving hypoglycemia in infants, the glucose concentrations at discharge will be extracted from patient charts and compared between groups.

For infants in the donor milk group, if infant weight loss is >3% per day, the clinical team will be alerted. If this occurs on any two consecutive days, the infant will exit the feeding intervention. Similarly, if the infant's blood glucose remains <2.6 mmol/L for two consecutive checks, the infant will exit the feeding intervention.

***Infant growth***:

Anthropometric measures including weight, length and head circumference will be measured at birth and monthly until study completion (4 months of age) using standardized procedures including length boards and non-stretchable tape measures for in person visits. For virtual visits, parents will be provided with tape measures at the time of birth as well as information from the SickKids "About Kids Health" website on how to measure their infant's weight and length at home (21). This information is available in print format in multiple languages as well as in audio and video files. These instructions have already been successfully implemented in virtual clinics at SickKids Hospital. Additional information will be provided on measuring the head circumference. Study staff will provide further guidance during virtual visits. In the event that there is an observed decline in growth during any of the follow-up visits (at 1, 2, 3, and 4 months old), as indicated by z-score data aligned with WHO growth standards for weight, length, or head circumference, the following procedures will be implemented: If parents or caregivers are responsible for taking these measurements and have expressed concerns regarding their child's growth, we acknowledge that these concerns may stem from imprecise measurements or possible errors. In such instances, it will be requested that parents or caregivers consult their healthcare provider to verify the measurements and address any potential issues. Comprehensive information and guidance will be offered to facilitate this follow-up, ensuring that any concerns are rigorously assessed and appropriately managed by a qualified healthcare professional.

***Infant health and temperament (Infant Behavior Questionnaire)***:

Parents will be asked to report on a variety of health indicators for their infant at each time-point post hospital discharge including rehospitalization, visits to physicians (virtual or in person), emergency rooms, walk-in clinics or allied health professionals (including lactation consultation) and prescription and non-prescription medication use. It is anticipated that all of these health indicators will be ameliorated with the duration of exclusive human milk feeding. Infant

temperament will be assessed at 1-, 2-, 3- and 4-months with the Infant Behavior Questionnaire- Revised, Very Short Form which is a survey of 37 items spanning 3 broad scales including negative emotionality, positive affectivity, and orienting/ regulatory capacity (22). Our hypothesis is that infant temperament can reciprocally impact human milk feeding outcomes (e.g., a baby with colic may be more likely to receive formula supplementation whereas the use of formula may also impact the baby's temperament).

***Breastfeeding self-efficacy (Breastfeeding Self-Efficacy Scale)***:

This will be administered at each time-point post hospital discharge to assess the trajectory of feeding practices. A parent's perception of their ability to breastfeed has been shown to predict both duration and exclusivity of breastfeeding (23). A tool to measure this perception has been developed by Dennis et al. and validated in Canada. This has been updated to a shortened version (Breastfeeding Self-Efficacy Scale – Short Form, BSES-SF) which consists of 14 items scored on a 5-point Likert scale ranging from not at all confident to always confident (24). Understanding parent's feeding self-efficacy provides important context to the duration and exclusivity of human milk feeding as well as infant growth throughout early infancy; it is possible that low breastfeeding self-efficacy may confound exclusive human milk feeding at 4 months of age. Further these outcomes may illustrate areas for intervention to support the duration and exclusivity of human milk feeding and growth among infants admitted to a well-baby unit following birth.

***Parental stress, anxiety and depression****:*

Very little is known about the relationship between donor milk and parental mental health; this has been highlighted as an area requiring further study (25). Two well-established self-reported assessment tools will be employed to measure parental mental health at 2 and 4 months CA. For families with two parents, both will be asked to independently complete the same questionnaires. Anxiety will be measured with State-Trait Anxiety Inventory (STAI) which has been used to assess trait (20 items) and state (20 items) anxiety. This tool has been validated in different populations including postpartum women (26). STAI has been used to differentiate anxiety from depressive syndromes and to generally assess caregiver distress in research (27). The Edinburgh Postnatal Depression Scale (EPDS) has been classically described for screening for postpartum depression in mothers using a 10-point scale. There is evidence for its validity in fathers and therefore all parents will be invited to complete this screening. The measurement of these outcomes will also allow us to explore how they may impact the duration and exclusivity of human milk feeding at 4 months.

If a participant fills out the survey through our online survey platform, REDCap, and their response to the EPDS suggests a high likelihood of depression or self-harm, we have a well-defined protocol in place to prioritize their safety. Within 24 hours of receiving such a response, our study team will be immediately notified. We will then promptly contact the participant, providing them with clear and detailed instructions on how to access the necessary help and support. This may involve connecting them with a healthcare professional or emergency services as needed. If the survey is conducted via a phone conversation or in-person interaction, we will provide specialized guidance right away in the event of similar concerns.

***Milk cortisol****:*

Milk cortisol is known to be associated with maternal distress and may play an important role in programming infant response to parental stress and may be related to infant growth (28, 29). Timed milk samples (morning sample to account for diurnal variation) will be collected at 2 and 4 months of age. These samples will be collected in the morning, within two hours after waking up, to account for the natural rise in cortisol levels during this time. We aim to collect these samples preferably between 6:30 AM and 8:30 AM. This timing consistency aligns with the screening tool for parental stress, anxiety, and depression questionnaires, which will also be administered at similar time points. Cortisol concentrations will be quantified using an enzyme-linked immunosorbent (ELISA) assay or liquid chromatography-tandem mass spectrometry (LC-MS/MS) in Dr. Deborah O'Connnor's laboratory at the University of Toronto In order to facilitate the milk sample collection process for our participants, we will ensure that special containers designed for this purpose are readily available to them. Our commitment to participant convenience and clarity extends to providing comprehensive instructions. These instructions will not only outline the precise methods and timing for collecting milk samples but will also specify the appropriate storage conditions, with an emphasis on keeping the samples stored in a -20°C freezer. Additionally, participants will receive guidance on the logistics of sending these samples to Dr. Deborah O'Connor's laboratory via courier. Furthermore, for those who opt for an in-person 4-month visit, the option to bring the samples with them will be made available, ensuring flexibility and ease in the participation process.

***Economic evaluation***:

In a recent systematic review, research was conducted on seven studies that compared the cost effectiveness of feeding infants with donor milk versus standard feeding in neonatal care. The studies were evaluated based on published full economic evaluations(30). In all 7 studies, the subjects were VLBW or low birth weight and donor milk interventions resulted in cost savings.

Our aim is to present a cost comparison between the two groups: the donor milk group and the formula group. We will collect medical expenses incurred by each infant during their initial hospital stay from the finance departments of the hospital. Direct costs will be evaluated based on the methodology presented by the Ontario Case Costing Initiative. Physician costs will be computed using the Ontario fee schedules. The cost of enteral feeds will be calculated by including the costs of both milk pump (if purchased or rented) and feeding formula or donor milk.

## 1.8 Sample size estimation and power calculations

According to the systematic scoping review of McCune and Perrin published in January 2021, there are no data from RCTs comparing donor milk to formula for supplementation of late preterm or term newborns (13). The only available RCT is a pilot study that assessed the feasibility of using donor milk versus formula for a small group of 32 full-term and late preterm infants admitted to NICU; even though this study did assess certain aspects related to breastfeeding, such as feeding attempts and growth, the authors emphasized the need for larger-scale studies to validate the findings of this pilot study (15). The current exclusive breastfeeding rate in Toronto at 4 months of age is 28% (31). In one retrospective review following donor milk supplementation in late preterm and term newborns (≥35 weeks gestational age), the rate of exclusive breastfeeding at 6 months of age increased from 15% to 58% (14). A doubling of Toronto's exclusive breastfeeding rate to 56% would be clinically significant and plausible given the published data and was thus used for sample size calculation. With 80% power and an alpha level of 0.05, we require 47 infants in each of the two study arms. We anticipate, based on our prior work, a 15% loss to follow-up to 4 months of age (32); hence 56 infants will be recruited for each study arm or 112 infants in total.

## 1.9 Analyses

### 1.9.1 The proposed type of analyses:

Analyses will be carried out using SAS Version 9.4. Descriptive statistics will be calculated for all variables of interest. Unadjusted and adjusted models will be run to compare primary, secondary and exploratory outcomes. The primary outcome of exclusive human milk feeding will be compared between groups using a linear regression model. Secondary and exploratory outcomes will be assessed using linear and logistic regression models based on the nature of

the outcome measure. The models will be adjusted for risk condition (being born to a diabetic mother or SGA) and sex. The primary analysis for this intent-to-treat study will include all infants as randomized, regardless of feeding protocol compliance.

**1.9.2 The frequency of analyses**:

There will be no interim analysis; all data will be analyzed upon completion of all follow-up assessments.

**1.9.3 Planned subgroup analyses**:

Subgroup analysis of primary and secondary outcomes will be according to risk condition (being born to a diabetic mother or SGA) and sex. The p-value for these analyses will be adjusted to account for multiple comparisons (Bonferroni adjustment).

**1.9.4 Any pilot study been carried out using this design**:

The systematic scoping review of McCune et al. (13) reported on 5 observational studies (donor milk n= 696 and control n=385) and a single RCT (donor milk n=30 and control n=30). The observational studies showed improvements in breastfeeding outcomes while the RCT did not, however, the control arm was exclusive breastfeeding not formula supplementation. A very recent pilot RCT that investigated the use of donor milk versus formula in 32 term and late preterm infants admitted to NICU confirmed the feasibility of randomly assigning term and late preterm infants into donor milk or formula groups when their mother's milk is not available, and showed that donor milk may increase breastfeeding attempts without having a negative effect on growth (15).

## 1.10 Rationale for recruitment target

There are more than 6000 admissions to the well-baby unit at Sinai Health annually with approximately 15% being “at higher risk for supplementation” and typical supplementation rates of about 80%. This makes our timeline of recruiting 112 infants in 12 months (approximately 2-3 infants per week) very realistic (Table 1).

Table 1: Annual admissions to Sinai Health's well-baby unit according to risk group for supplementation

| Year: 2020 | Number | Supplemented |
|---|---|---|
| Total | 6326 | 52% |
| Diabetic mother | 501 | 74% |
| < 2.5 kg | 318 | 88% |

## 1.11 Project milestones and timeline

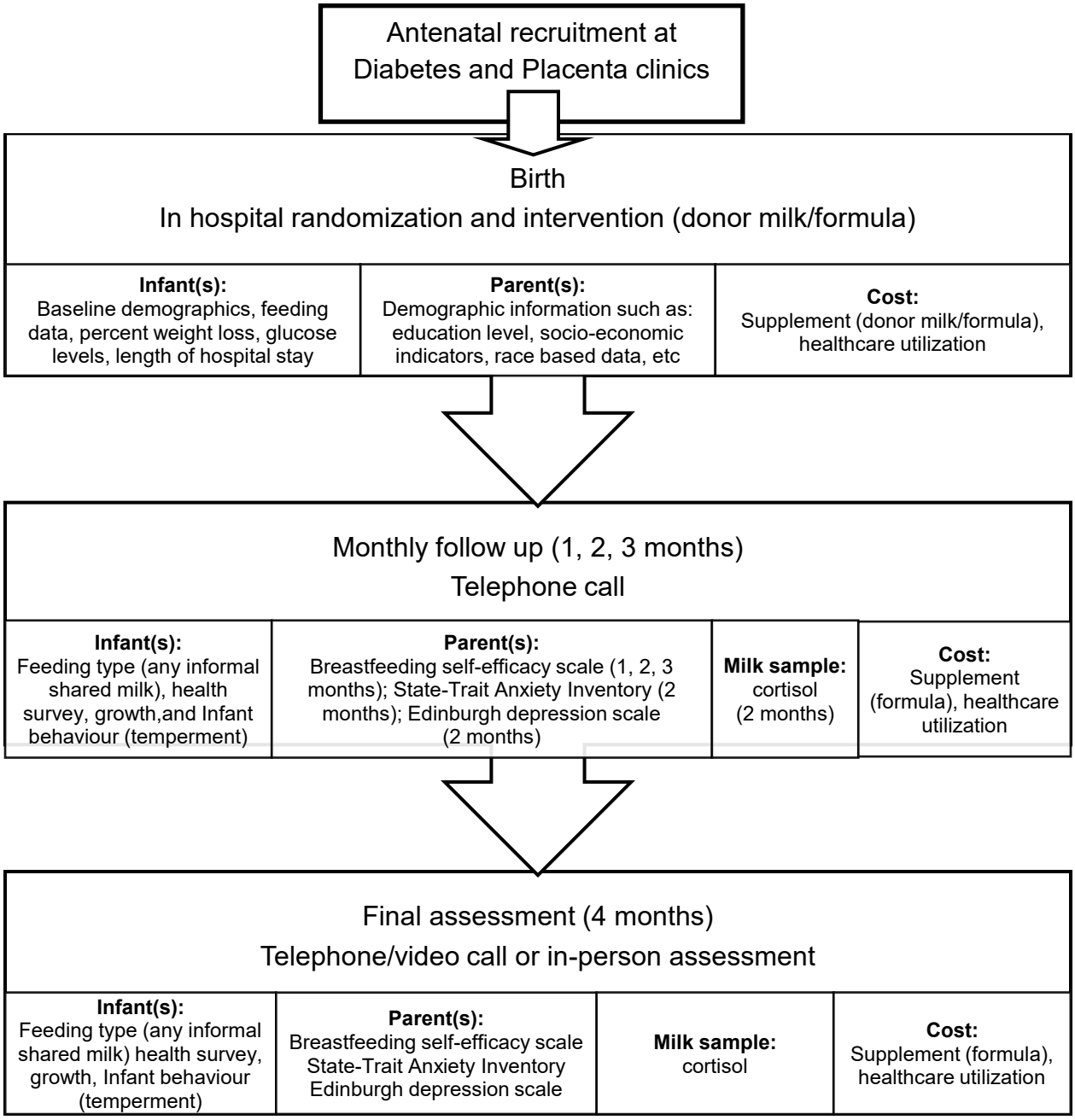
The planned trial will take 6 months of start-up time and 12 months of recruitment (Table 2 and Figure 1). Final patient follow-up assessments, data analysis and end of grant knowledge translation are planned for the final 5 months. The full project will be completed by March 31, 2025.

Table 2**:** Study Timeline

| Task | 2023 Apr-Jun | 2023 Jul-Sep | 2023 Oct-Dec | 2024 Jan-Mar | 2024 Apr-Jun | 2024 Jul-Sep | 2024 Oct-Dec | 2025 Jan-Mar |
|---|---|---|---|---|---|---|---|---|
| Trial preparation<br>Protocol development<br>Trial logistics development<br>REB submission<br>Training on outcome tools<br>Staff in-servicing | | | | | | | | |
| Trial management<br>Patient recruitment<br>Trial intervention<br>Patients follow up<br>Data collection | | | | | | | | |
| Post trial completion<br>Data analysis<br>Manuscript preparation<br>Knowledge translation | | | | | | | | |

Figure 1**:** Summary of study follow up and timing

In the case of multiple gestations (twins), each twin will be assigned to a different group, using a separate randomization plan with blocks of two, allowing for more precise comparisons and analysis. When two parents are available, both parents will be asked to separately complete the questionnaires.



## 1.12 Managing donor milk and formula availability in the well baby unit

### 1.12.1 Human Donor Milk Handling:

The preparation of human donor milk occurs daily at the milk bank in room 4. After thawing, the milk is aliquoted into 12 syringes, each containing 10 ml, as part of the daily routine. Subsequently, study staff transports these aliquoted syringes to the well-baby unit on a daily basis. The storage of these aliquots is carried out within a dedicated fridge, specifically allocated for the CanDo trial, located in the medication room within the well-baby unit. This setup is designed to ensure convenient and immediate access for nurses when administering donor milk as a supplementation to infants.

In addition, it is the responsibility of the study staff to ensure that the donor milk log sheet is completed at each shift by the nurse caring for the infant.

### 1.12.2 Formula Supplementation:

When an infant is randomized to receive formula, the nursing team will utilize the formula resources present within the well baby unit. They will follow established unit procedures to obtain the necessary formula, which includes accessing formula resources in accordance with the standard storage process as required. This aligns with the hospital's typical policy for formula provision and handling.

### 1.12.3 Inventory and Record Keeping:

An inventory and comprehensive record will be maintained to ensure thorough oversight of both human donor milk and formula dispensed to infants. This meticulous record-keeping will be managed through the maintenance of a readily accessible log sheet. This practice safeguards the accuracy of documentation related to the supplements given to infants.

In summary, this comprehensive approach ensures the availability of both human donor milk and formula for supplementation within the well baby unit. The detailed procedures outlined herein, in conjunction with the diligent inventory and record-keeping practices, establish a systematic plan to fulfill the supplementation needs of the study.

## 1.13 Data Storage and Security:

Regarding data security, our study is committed to ensuring the protection of computerized data. We have implemented the following measures to secure all data, including hospital and study visit information: All data, including hospital and study visit information, will be securely stored on the Mount Sinai server. Access to this data will be limited to authorized study team members to ensure confidentiality and data protection. Data security measures, including password protection and access restrictions, will prevent unauthorized access or manipulation of the data.

### 1.13.1 Survey Data Using REDCap:

Data collected during the study will be securely managed using the REDCap platform hosted on the University of Toronto's server. This setup ensures robust security during data collection. After completing data collection, all information will be securely transferred to the Mount Sinai Hospital server for storage, maintaining the highest level of data security and confidentiality.

### 1.13.2 Data Sharing and Collaboration Agreement:

A Data Sharing and Collaboration Agreement has been established between the University of Toronto and Mount Sinai Hospital. This agreement formalizes data sharing procedures, specifying terms and conditions. It ensures compliance with data protection regulations and ethical considerations, providing a framework for secure and responsible data sharing between the two institutions.These measures collectively safeguard the computerized data collected during our study, ensuring its integrity and confidentiality. We prioritize the security of participant information and data throughout the research process.

## 1.14 Health service research issues to be addressed

The use of human donor milk nearly ceased at the time of the HIV epidemic. During the current era of neonatal care and the appreciation of human milk and its health protective effects, donor milk use is growing exponentially. Although there are no statistics from Canadian well-baby units, two surveys from the United States document rapid increases in donor milk programs in well-baby units despite a lack of evidence. It is important to emphasize the timeliness of conducting an RCT to investigate the use of donor milk for this specific group of infants. Simultaneously, studying the associated health economics and health services is equally important.The majority of infants in Canada are admitted to a well-baby unit and many require supplementation while in hospital which is known to be one of the strongest predictors of non-exclusive human milk feeding in the long-term with its consequent negative health impacts. A change in policy of method of supplementation during initial hospitalization must thus be well researched prior to implementation as it may be an important public health initiative, or it may be costly without benefit.

## 1.15 Expected project deliverables

The proposed trial will provide enough data to either support or refute the need for improved access to donor milk in well-baby units. At present, 5 of 52 well-baby units in Ontario offer donor milk supplementation. It is anticipated that positive results from this trial may be utilized to advocate for greater equity in accessing donor milk in all well-baby units. On the other hand, null or negative trial results may call into question the use of donor milk for this population of infants and may suggest redirection of funds to other programs within a well-baby unit. The carefully selected secondary and exploratory outcome variables will provide evidence for the importance of nutrition on early parent-child interactions and subsequent infant health, growth and development. As this early period in child development is believed to have a significant impact on life-long health and well-being, the importance of research in supporting families during this time period cannot be over-stated. Our findings may influence pediatric guidelines and thus the short- and long-term health outcomes in this large target group of newborns. We anticipate data from this study, including a cost analysis, will impact the manner in which donor milk is allocated in Canada and beyond. The existing relationships of the two supervisors for this project, Dr. Unger and Dr. O'Connor, with policy makers at the World Health Organization, Health Canada, the Canadian Pediatric Society and Dietitians of Canada will facilitate rapid and effective

knowledge translation. We will utilize well established social media platforms such as Today's Parent, Life with a Baby and the Roger's Hixon Ontario Human Milk Bank Instagram and Facebook to message parent groups. The primary publication for this trial will be led by Dr. Maryam Razaghi, the postdoctoral research fellow and will be targeted at a high impact open access journal such as the Canadian Nutrition Society's journal, Applied Physiology, Nutrition and Metabolism. Reports will be prepared for presentation at both national and international scientific conferences (for example, the International Society for Research in Human Milk and Lactation annual meeting) and parent webinars (for example, the Canadian Premature Babies Foundation webinar series).

## 1.16 Interactions

This project will be jointly led by the Rogers Hixon Ontario Human Milk Bank at Sinai Health in Toronto and the University of Toronto. The trial will take place in the well-baby unit at Sinai Health which admits >6000 newborns annually. The clinical trial will be under the direct supervision of Dr. Sharon Unger who is a neonatologist at Sinai Health and the medical director for the Rogers Hixon Ontario Human Milk Bank. Dr. Unger is also a member of the Departments of Pediatrics and Nutritional Sciences in the Temerty Faculty of Medicine at the University of Toronto. The Rogers Hixon Ontario Milk Bank, partially funded by the Ontario Ministry of Health and Long Term Care, is the only milk bank in Ontario. The milk bank has the capacity to process approximately 250 L of milk per week with the ability to scale up as required. It is anticipated that the study will require approximately 1 L of donor milk per week to meet the needs of trial participants. All human milk analyses (milk cortisol) will take place in Dr. O'Connor's laboratory at the University of Toronto. In-person follow up visits at 4 months of age will take place at the Nutrition Intervention Centre at the University of Toronto located in the David Naylor Building, just a short walk from Mount Sinai Hospital.

## 1.17 Study Steering Committee

The Steering Committee, consisting of the principal investigator, co-investigators, study coordinator, milk bank representative, and parent advisor/representative, will hold the authority for making decisions concerning the arrangement and execution of the trial. This committee has been entrusted with the responsibility of ensuring that the trial progresses smoothly and according to the set objectives.

To ensure effective collaboration and consistent decision-making, the Steering Committee will convene bi-monthly, twice a month, for the entire planned duration of the study. During these meetings, a wide range of topics will be discussed, including the study's progress, challenges faced, emerging findings, potential adjustments to methodologies, ethical considerations, participant engagement strategies, and more. This regular engagement ensures that the trial remains on track, ethical standards are upheld, and decisions are made collectively to achieve the study's objectives successfully.

## 1.18 References


1. Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. The lancet. 2016;387(10017):475-90.
2. Pérez-Escamilla R, Tomori C, Hernández-Cordero S, Baker P, Barros AJ, Bégin F, et al. Breastfeeding: crucially important, but increasingly challenged in a market-driven world. The Lancet. 2023;401(10375):472-85.
3. Rollins N, Piwoz E, Baker P, Kingston G, Mabaso KM, McCoy D, et al. Marketing of commercial milk formula: a system to capture parents, communities, science, and policy. The Lancet. 2023;401(10375):486-502.
4. Baker P, Smith JP, Garde A, Grummer-Strawn LM, Wood B, Sen G, et al. The political economy of infant and young child feeding: confronting corporate power, overcoming structural barriers, and accelerating progress. The Lancet. 2023;401(10375):503-24.
5. Dennis CL, Brown HK, Chung-Lee L, Abbass-Dick J, Shorey S, Marini F, et al. Prevalence and predictors of exclusive breastfeeding among immigrant and Canadian-born Chinese women. Maternal & child nutrition. 2019;15(2):e12687.
6. Vehling L, Chan D, McGavock J, Becker AB, Subbarao P, Moraes TJ, et al. Exclusive breastfeeding in hospital predicts longer breastfeeding duration in Canada: Implications for health equity. Birth. 2018;45(4):440-9.
7. Statistics Canada. Table 13-10-0415-01 Live births, by month 2021 [14 April 2023]. Available from: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310041501.
8. Brown B, RileyJennifer F, GregoryKatherine E, PhilippBarbara L, ParkerMargaret G. Prevalence and trends in donor milk use in the well-baby nursery: A survey of northeast United States birth hospitals. Breastfeeding Medicine. 2018.
9. Kair LR, Phillipi CA, Lloyd-McLennan AM, Ngo KM, Sipsma HL, King BA, et al. Supplementation practices and donor milk Use in US well-newborn Nurseries. Hospital pediatrics. 2020;10(9):767-73.
10. Parker MG, Burnham LA, Kerr SM, Belfort MB, Perrin MT, Corwin MJ, et al. National prevalence of donor milk use among level 1 nurseries. Hospital Pediatrics. 2020;10(12):1078-86.
11. Health Canada; Canadian Paediatric Society; Dietitians of Canada; Breastfeeding Committee for Canada. Nutrition for healthy term infants: recommendations from birth to six months. Canadian journal of dietetic practice and research. 2012;73(4):204.
12. World Health Organization. Infant and young child feeding 2021 [14 April 2023]. Available from: https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding.
13. McCune S, Perrin MT. Donor human milk use in populations other than the preterm infant: a systematic scoping review. Breastfeeding Medicine. 2021;16(1):8-20.
14. Merjaneh N, Williams P, Inman S, Schumacher M, Ciurte A, Smotherman C, et al. The impact on the exclusive breastfeeding rate at 6 months of life of introducing supplementary donor milk into the level 1 newborn nursery. Journal of Perinatology. 2020;40(7):1109-14.
15. Pithia N, Grogan T, Garg M, Kesavan K, Calkins K. A Pilot Single-Site Randomized Control Trial: Investigating the Use of Donor Milk in the Late Preterm and Term Infant in the Neonatal Intensive Care Unit. 2023.
16. Pound C, Unger S, Blair B. Pasteurized and unpasteurized donor human milk. Paediatrics & Child Health. 2020;25(8):549-.
17. Health Canada. Safety of donor human milk in Canada 2014 [Available from: https://www.canada.ca/en/health-canada/services/canada-food-guide/resources/infant-feeding/safety-donor-human-milk-canada.html.

18. Narvey MR, Marks SD. The screening and management of newborns at risk for low blood glucose. Paediatrics & Child Health. 2019;24(8):536-44.
19. Nommsen-Rivers LA, Dewey KG. Development and validation of the infant feeding intentions scale. Maternal and Child Health Journal. 2009;13:334-42.
20. Dewey KG, Cohen RJ, Brown KH, Rivera LL. Effects of exclusive breastfeeding for four versus six months on maternal nutritional status and infant motor development: results of two randomized trials in Honduras. The Journal of nutrition. 2001;131(2):262-7.
21. SickKids. Virtual care: How to accurately measure your child's height and weight at home 2021 [Available from: https://www.aboutkidshealth.ca/article?contentid=3910&language=english.
22. Putnam SP, Helbig AL, Gartstein MA, Rothbart MK, Leerkes E. Development and assessment of short and very short forms of the Infant Behavior Questionnaire–Revised. Journal of personality assessment. 2014;96(4):445-58.
23. McQueen K, Sieswerda LE, Montelpare W, Dennis CL. Prevalence and factors affecting breastfeeding among Aboriginal women in Northwestern Ontario. Journal of Obstetric, Gynecologic & Neonatal Nursing. 2015;44(1):51-68.
24. Dennis CL. The breastfeeding self-efficacy scale: Psychometric assessment of the short form. Journal of Obstetric, Gynecologic, & Neonatal Nursing. 2003;32(6):734-44.
25. Bramer S, Boyle R, Weaver G, Shenker N. Use of donor human milk in nonhospitalized infants: An infant growth study. Maternal & Child Nutrition. 2021;17(2):e13128.
26. Dennis C-L, Coghlan M, Vigod S. Can we identify mothers at-risk for postpartum anxiety in the immediate postpartum period using the State-Trait Anxiety Inventory? Journal of affective disorders. 2013;150(3):1217-20.
27. Greene J, Cohen D, Siskowski C, Toyinbo P. The relationship between family caregiving and the mental health of emerging young adult caregivers. The journal of behavioral health services & research. 2017;44:551-63.
28. Aparicio M, Browne PD, Hechler C, Beijers R, Rodríguez JM, de Weerth C, et al. Human milk cortisol and immune factors over the first three postnatal months: Relations to maternal psychosocial distress. PLoS One. 2020;15(5):e0233554.
29. Pundir S, Gridneva Z, Pillai A, Thorstensen EB, Wall CR, Geddes DT, et al. Human milk glucocorticoid levels are associated with infant adiposity and head circumference over the first year of life. Frontiers in Nutrition. 2020;7:166.
30. Zanganeh M, Jordan M, Mistry H. A systematic review of economic evaluations for donor human milk versus standard feeding in infants. Maternal & Child Nutrition. 2021;17(2):e13151.
31. Toronto Public Health. Infant feeding surveillance project. Technical report 2018 [14 April 2023]. Available from: https://www.toronto.ca/wp-content/uploads/2018/05/98af-Final-IFSP-Technical-Report-Jan.-19-2018.pdf.
32. O'Connor DL, Gibbins S, Kiss A, Bando N, Brennan-Donnan J, Ng E, et al. Effect of supplemental donor human milk compared with preterm formula on neurodevelopment of very low-birth-weight infants at 18 months: a randomized clinical trial. Jama. 2016;316(18):1897-905.