"TO ANALYSE THE MICRONUTRIENT COMPOSITION OF PASTEURIZED POOLED DONOR HUMAN MILK (DHM) - A ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY"

BY

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LIST OF ABBREVIATIONS

MOM – Mother's own milk

PDHM – Pasteurized donor human milk

HM – Human milk

WHO – World health organization

UNICEF – United Nations International Children's Emergency Fund

NFHS – National Family Health Survey

LBW – Low birth weight

VLBW – Very low birth weight

BPD – Bronchopulmonary dysplasia

ROP – Retinopathy of prematurity

NEC – Necrotizing enterocolitis

LOS – Late onset sepsis

EBF – Exclusive breast feeding

HMO – Human milk oligosaccharides

EGF – Epidermal growth factor

HBEGF – Heparin binding epidermal growth factor

BDNF – Brain derived neurotrophic factor

GDNF – Glial cell line derived neurotrophic factor

CNTF – Ciliary neurotrophic factor

IGF – Insulin like growth factor

VEGF – Vascular endothelial growth factor

IL – Interleukin

INF – Interferon

TNF – Tumor necrosis factor

GCSF – Granulocyte colony stimulating factor

MIF – Macrophage migratory inhibitory factor

BMI – Body mass index

DHA – Docosahexaenoic acid

AAP – American Academy of Pediatrics

NICU – Neonatal intensive care unit

CLMC – Comprehensive Lactation Management Centre

LTLT – Low temperature, long time

HTST – Hight temperature, short time

HMBANA – Human milk banking association of North America

PT – Preterm formula

CGA – Corrected gestational age

RCT – Randomized controlled trial

HC – Head circumference

CC – Chest circumference

PMA – Post menstrual age

SGA – Small for gestational age

GA – Gestational age

ARR – Attributable risk ratio

EHM – Exclusive human milk

LCPUFA – Long chain polyunsaturated fatty acids

AAS – Atomic absorption spectroscopy

MIR – Mid infrared spectroscopy

NG – Nasogastric

OG – Orogastric

USG – Ultrasonography

TLC – Total leucocyte count

ANC – Absolute neutrophil count

ESR – Erythrocyte sedimentation rate

CRP – C Reactive protein

IUGR – Intrauterine growth retardation

LSCS – Lower segment caesarian section

VD – Vaginal delivery

NNH – Neonatal hyperbilirubinemia

RT – Ryles tube

SF – Spoon feeds

DBF – Direct breast feeding

PHC – primary health center

PROM – Premature rupture of membranes

PIH – Pregnancy induced hypertension

ICP AES – Inductively coupled plasma atomic emission spectroscopy

HMA – Human milk analyzer

HPLC – High performance liquid chromatography

SD – Standard deviation

HoP – Holder's pasteurization

p – probability

vs – versus

cm – centimeter

cumm – cubic millimeter

kg – weight

gm – grams

IgA – immunoglobulin A

Min – minutes

ml – millilitre

L – litre

n – total number

i.e – that is

% - percentage

ABSTRACT

Background: Studies on nutrient composition of pasteurized donor human milk limited in India. Analysis of the micronutrient and macronutrient content of PDHM used to assess the growth outcomes by establishing a milk flow to trace the PDHM from the set of donor mothers to the recipient infants and to their growth outcomes.

Research aim: To analyze the micronutrient and macronutrient composition of PDHM and assess the effect on growth of infants, incidence of necrotizing enterocolitis/sepsis, duration of hospital stay and the method of feeding at discharge.

Methods: Observational longitudinal study. Micronutrient content - calcium, zinc and iron analyzed by atomic absorption spectroscopy and macronutrient content of PDHM- energy, carbohydrates, proteins and fats analyzed by mid infrared human milk analyzer. Donor mother characteristics obtained and PDHM dispensed to neonates and followed up in hospital till discharge and at 6 weeks for anthropometry.

Results: 13 batches of pooled PDHM analyzed for micronutrients (mean calcium- 213.68 ± 57.7 mg/dL, zinc- 0.42 +/- 0.15 mg/dl, iron- 0.1 ± 0.03 mcg/dl) and macronutrients (mean energy- 61.09 ± 3.05 kcal/dL, carbohydrates- 7.47 ± 0.21 g/dl, proteins- 1.69 ± 0.03 g/dl, fats- 1.94 ± 0.13 g/dl). PDHM traced to 146 neonates and observed significant increase ($p<0.005$) in all anthropometric parameters over 6 weeks. Negative correlation observed between mean donor gestational age and protein content (p value = 0.023) and between postnatal day of milk donation and zinc, energy, protein and fat content (p value = 0.028, 0.032, 0.021, 0.049). Positive correlation between protein content and average gain in all anthropometric

parameters, with statistical significance ($p<0.05$) reported. Gestational age at birth had positive effect on weight gain while maternal age had a significant negative effect.

Conclusion: The nutrient composition of PDHM in normal expected range of human milk reference values. Positive impact on short term growth of infants at 6 weeks. Lower donor gestational age and early postnatal day – high protein content- greater increase in anthropometry. Other factors affecting growth- gestational age at birth and maternal age. Decreased incidence of NEC/Sepsis, shorter duration of hospital stay and increased breastfeeding rates at discharge.

Keywords: Pasteurized donor human milk, micronutrient, macronutrient, growth outcomes

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INTRODUCTION

Human milk is critical for the optimal growth and development of the newborn infants because of its various benefits in term of immunological, gastrointestinal and neurodevelopmental functions (1). Human milk (MOM and/or PDHM) protects against necrotizing enterocolitis and sepsis due to its essential immunologic components with anti-infective properties especially in preterm infants. The human milk microbiome comprising of commensal and probiotic bacteria plays an important role in infant gut colonization. (2). WHO and UNICEF recommend exclusive human milk feeding for the first 6 months of life, with continued breastfeeding for 2 years of life or longer (3).

Global prevalence of exclusive breastfeeding in infants less than six months has increased significantly from 34.0 % in 2012 to 48 % in 2023 (4). In India, according to the National Family Health Survey – 5 (NFHS 5 2019-2021) conducted by the Ministry of Health and Family Welfare (MoHFW), 63.7% of the infants below the age of 6 months are exclusively breastfed (5). The World Health Assembly set a global target of increasing exclusive breastfeeding for infants under 6 months to at least 50% by year 2025 (4,6).

The World Health Organization recommends use of pasteurized donor human milk (PDHM) as the next best alternative feeding option when mother's own milk is not available (7–11). This recommendation is supported by guidelines published by the American Association of Paediatrics and the European Society for Paediatric Hepatology, Gastroenterology and Nutrition (8). Pasteurized donor human milk (PDHM) is distributed through human milk bank where systematic collection, pasteurization, storage and distribution of human milk is done. (10). Several systematic reviews and meta-analysis have demonstrated the benefits of PDHM

especially in low birth weight (LBW) infants. PDHM is reported to decrease feeding intolerance, improves growth and neurodevelopment and decreases the incidence of necrotising enterocolitis, late-onset sepsis, bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP). PDHM has also been associated with reduced duration of hospital stay and improved maternal breastfeeding rates (12).

PDHM is reported to be associated with poor growth compared to other alternative feeding options in preterm infants. (13–24). Several studies have reported that the preterm infants fed with PDHM have no significant difference in gain in weight, length and head circumference at discharge when compared to preterm formula fed infants (19,21,22,25–30). These observations raise concerns about the long term outcomes of the babies fed with PDHM. The poorer growth may be related to the nutrient composition of donor human milk.

Several studies have reported the micronutrient and macronutrient composition of human milk (3,8,31–34) but the studies on nutrient composition of donor human milk are limited especially in India. The macronutrient composition of PDHM has been more frequently studied and reported as compared to micronutrient composition (35). The composition of PDHM is variable due to wide variability of donors, milk banking and storage process. Donor characteristics such as lactational stage, gestational age and maternal diet result in variations in the PDHM composition. Other factors which affect the PDHM composition are problems in the methods of milk collection such as incomplete expression of breast milk leading to loss of fat and processes involved in human milk bank such as pooling, mixing, multiple container transfers, pasteurization and storage. The variations in the protein content can be justified by the fact that preterm delivery and the stage of lactation significantly affect the levels of protein in human milk (36).

A milk flow process can be established to trace the preterm infants receiving a pooled PDHM sample collected from a set of donor mothers. Analysis of the macronutrient and micronutrient content of this pooled PDHM sample can be used to assess the growth outcomes of the infants receiving this sample of PDHM (36,37). Information regarding the donor mother characteristics is a strong predictor of nutrient composition and in turn can define the growth outcomes.

Knowledge about the nutrient composition of PDHM will help to improve preterm feeding practices including implementation of various fortification strategies (38). It also helps in developing new strategies and initiatives to improve the quality of milk banking processes. Since studies on nutrient composition of donor human milk is limited especially in India, the present study was planned to analyse the micronutrient and macronutrient composition of pasteurized.

AIMS & OBJECTIVES

Primary Objective:

To analyse the micronutrient composition of pasteurized pooled donor human milk (DHM)

Secondary Objectives:

1) To analyse the macronutrient composition of pasteurized pooled donor human milk (DHM)

2) To assess the effect of consumption of pasteurized donor human milk on:

- Growth of infants at discharge and 6 weeks of life
- Incidence of Necrotizing Enterocolitis and Sepsis
- Duration of NICU stay
- Method of feeding of neonates at discharge

REVIEW OF LITERATURE

Human milk, which is optimum for survival and development of the newborns lowers the incidence of necrotizing enterocolitis, retinopathy of prematurity, and late-onset sepsis in preterm infants (3). Additionally, it improves neurodevelopmental outcomes and reduces the number of rehospitalizations in the 1st year of life. Premature babies that are fed human milk are known to have decreased incidences of insulin resistance and metabolic syndrome when they get to adolescence (39). Given the importance of human milk for a child's survival as well as healthy growth and development, the World Health Organisation (WHO) and UNICEF recommend starting breastfeeding within an hour of delivery, exclusive breastfeeding (EBF) during the 1st 6 months, and continuing with breast feeds till at least 2 years of age (40).

Globally, the percentage of infants receiving only breast milk during the first six months of life has risen to 48% in 2023, nearly reaching WHO's objective of 50% by 2025. The Global Collective of Breastfeeding established a 2030 goal of reaching 70%. According to survey data gathered between 2016 and 22, 46% of newborns were initiated with breast feeding within an hour of delivery, compared to the objective of 70%. By the time a child turns one year old, 71% of them are breastfed and by 2 years of age, 45% are breastfed. Exclusive breastfeeding rates increased in 70 of the 100 nations whose Global Breastfeeding Scorecard data had been updated. 22 countries showed a rise of over ten percentage points (4).

According to NFHS 5, the percentage of Indian infants under 6 months of age who were breastfed exclusively increased to 63.7 % from 57% as per NFHS 4. Compared to rural areas (62.9%), it was higher in urban areas (66.2%). Chhattisgarh (71%), Haryana (69.5%), and Jharkhand (64.7%) had higher percentages of exclusive

breastfeeding than the national average of 63.7%. It was lower in Meghalaya (23%), Manipur (24.5%), West Bengal (25.4%) and Uttarakhand (25.5%) (41). According to NFHS-5 (2019-2021), the prevalence of exclusive breast feeding in the first 6 months of life in the state of Karnataka was 61%, compared to 54% as per NFHS-4 (42). In a descriptive study on infant and young child feeding practices in the city of Belgaum by Khanna et al., it was noted that the prevalence of early initiation of breast feeding was 64.89 % and the prevalence of exclusive breast feeding in the first 6 months of life was 65.95% (43).

COMPOSITION OF HUMAN BREAST MILK (44)

Growth and neurodevelopment in neonates is promoted by the ideal composition of human breast milk.

Human breast milk contains-

Water: 87-88%

Carbohydrates: 7% (60–70 g/L)

Protein: 1% (8–10 g/L)

Lipids: 4% (35–40 g/L)

MACRONUTRIENTS:

1) Carbohydrates

Carbohydrates constitute the predominant macronutrient within human breast milk, playing a vital role in nutrition and supporting the intestinal microbiota. Lactose, the major carbohydrate, undergoes digestion facilitated by lactase located on the brush border of the small intestine. Following lactose, the next abundant carbohydrates are human milk oligosaccharides (HMOs). They impede the attachment of microorganisms to the intestinal mucosa, thereby conferring protection against gastrointestinal infections. (45)

2) Proteins

Casein and Whey are the primary proteins found in human breast milk. Casein is present in a micellar structure in the stomach as clots or curd while whey exists in a liquid state and undergoes easy digestion. Mature milk typically maintains a whey/casein ratio of 60:40. The proliferation of potentially pathogenic bacteria is inhibited by lactoferrin and lysozyme, while immunoglobulin A (IgA) shields the intestinal mucosa and leads to bacterial destruction (8,32,46,47).

3) Lipids

Fat is the second abundant macronutrient and plays a very important role in the development of the central nervous system and retina. Its major component is triglyceride (95%–98%) and also contains 2 essential fatty acids, linoleic acid and alpha-linolenic acid (8,32,46).

GROWTH FACTORS:

1) Epidermal growth factor(EGF)

It is essential for the maturation and healing of the intestinal mucosa. It stimulates the enterocyte to increase DNA synthesis, cell division, water and glucose absorption and protein synthesis. Heparin-binding growth factor (HB-EGF), a member of the EGF family, causes resolution of damage following hypoxia, ischemia-reperfusion injury, hemorrhagic shock / resuscitation injury, and necrotizing enterocolitis (3).

2) Brain-derived neurotrophic factor (BDNF) and Glial cell-line derived neurotrophic factor (GDNF)

There is impairment of peristalsis in the preterm gut which is enhanced by these growth factors. These growth factors along with Ciliary neurotrophic factor (CNTF) are present in human milk upto 90 days after birth (3).

3) IGF-I and IGF-II

These along with IGF binding proteins and IGF-specific proteases are found in human milk, their levels being the highest in colostrum. Following intestinal damage due to oxidative stress, they help in survival of enterocytes (3).

4) Vascular endothelial growth factor (VEGF)

Angiogenesis is regulated primarily by the relative expression of VEGF and its antagonists. Pulmonary immaturity, supplemental oxygen and negative regulation of VEGF lead to dysregulated vascularization of the retina and hence, human breast milk is known to provide protection against Retinopathy of Prematurity. Its levels are highest in colostrum (3).

IMMUNOLOGICAL FACTORS (3) :

1) Cells like Macrophages and stem cells – They help in protection against infection, regeneration and repair.
2) Immunoglobulins like IgA, sIgA, IgG and IgM
3) Cytokines like IL6, IL7, IL8, IL10 , INF-gamma and TNF-alpha
4) Chemokines like G-CSF and MIF (Macrophage Migratory Inhibitory Factor)
5) Antimicrobials like Lactoferrin and Lactadherin

HORMONES (3):

1) Erythropoietin - Erythropoiesis and intestinal development
2) Calcitonin - Development of enteric neurons
3) Leptin and ghrelin – Regulates the appetite and BMI of infants.
4) Somatostatin - Regulation of gastric epithelial growth
5) Adiponectin – Decreases inflammation and BMI of infant.

MICROBIOTA:

Breast milk microbiota contains a large number of specific bacterial species with antimicrobial properties and health benefits which include beneficial, commensal and probiotic bacteria. The major bacterial species belong to Lactobacillus and Bifidobacterium (32,33,48,49).

BENEFITS OF HUMAN BREAST MILK

BENEFITS FOR HEALTHY NEWBORNS (50,51):

- Provides optimum nutrition for growth and development and acts like the first immunization for the baby.
- Provides protection against common infective childhood morbidities. Exclusively breastfed infants are 14.2 times less likely to have diarrhoea, 3.6 times less likely to die from respiratory diseases and 2.5 times less likely to die from other infections compared to non- breastfed infants.
- Immunological benefits as it contains microbiota that helps in development of immunity which protects and promotes gut, lung and brain health. It also protects against allergic disorders like asthma and atopic dermatitis.
- Results in optimum neurodevelopment as it contains galactose, taurine and docosahexaenoic acid (DHA)
- Long term protection from cardiovascular and metabolic diseases.

BENEFITS FOR PRETERM AND SICK NEWBORNS (39,50,52,53)

- Decreased incidence of LOS
- Prevents NEC
- Reduced incidence of ROP
- Reduced incidence of hospital readmissions and duration of hospital stay
- Better neurodevelopmental outcomes
- Decreased feed intolerance
- Earlier achievement of full enteral feeds

DONOR HUMAN MILK

According to the World Health Organization, donor human milk is the first alternative when mothers own milk (MoM) is unavailable for infant feeding especially low birth weight infants (8–11,54). This recommendation is also supported by the American Association of Paediatrics and the European Society for Paediatric Hepatology, Gastroenterology and Nutrition. Donor human milk is expressed by a mother and processed by a human milk bank so that it can be used by a recipient that is not the mother's own baby (8).

Origin of the concept of donor human milk was from the early practice of wet nursing, where infants were breastfed by friends, relatives, or strangers. The choice of the nurse was thought to be important as it was believed that infants inherited the physical, mental and emotional traits of their wet nurse. Theodor Escherich established the first human milk bank in 1909 in Vienna. The second one opened in the next year in Boston, followed by more throughout the United States and Europe during the 20th century (55).

The first human milk bank in India was started in the year 1989 by Armida Fernandez at Sion hospital, Mumbai after which their numbers increased throughout the country (9,54).

Donation of breast milk should be done freely and voluntarily. It should be understood that this milk would be utilized for other babies admitted in NICU without monetary benefits to the donor (10).

THE HUMAN MILK BANK

A significant number of newborns, especially premature newborns, are not able to receive sufficient quantity of their mothers' milk due to different reasons. Human milk banks collect, screen, pasteurize, and distribute donated breast milk to in-hospital as well as outpatient recipients. Usually the collection, storage, and processing in a human milk bank follows established protocols. (10)

The main function of milk banks is to function as a storehouse of donated milk so that it can be accessed when needed. Milk banks receive milk from donors, process it, and store it until utilization. Most commonly milk from multiple donors is pooled, although some banks pool milk only of individual donors (single-donor banks). Usually, milk provided by milk banks has undergone pasteurization. Once pasteurized, milk is placed in small (100-150 mL) containers and is stored in a frozen state for up to 1 year depending on local guidelines (10).

CLMC provides all the hospitalized mothers with complete lactation support. (10). The requirement are as follows:

1) INFRASTRUCTURE (10)

i. **LOCATION**- PDHM provision for high-risk infants in the neonatal unit is the main objective of human milk bank. It is ideal to locate the milk bank near or preferably within the neonatal unit.

ii. **SPACE** - The guidelines specify that a minimum of 250 square feet of partitioned space is required. Ideally, the following areas should be included:

- Administrative area: for enrolment and screening of donors.
- Counseling area: for registration and counseling sessions with lactation management counsellors.
- Milk expression area: equipped with stations for mothers to express milk privately, with electric breast pumps.
- Cleaning/autoclave room: located adjacent to the milk expression area.
- Milk processing/storage area: Furnished with necessary equipments, including a water bath, a 190L refrigerator an electronic sealing machine, a 400L deep freezer as well as laminar airflow.
- Microbiological laboratory: necessary for conducting cultures and tests to ensure the safety of processed milk.
- Milk storage area: has a 400-litre vertical and 300-litre horizontal deep freezer for storage of milk after pasteurization.

These facilities are essential to guarantee safety and ensure good quality of milk as well as the efficient running of human milk banks.

2) EQUIPMENTS REQUIRED (10)

- Pasteurizer/Shaker Water Bath
- Deep Freezer
- Hot Oven /Autoclave
- Containers
- Refrigerator

- Breast Milk Pump

3) POWER SUPPLY (UN-INTERUPPTED) / GENERATOR (10)

   Each milk bank must have a reliable backup power supply system, such as a generator or inverter, to ensure uninterrupted operation.

4) ADDITIONAL PREFERRED EQUIPMENT - This category encompasses an infrared spectroscopy-based milk analyser. (10)

5) STAFF AND ADMINISTRATION - A group of experts should be assembled by human milk banks to supervise general development and operations, comprising:

   - Director
   - Milk Bank Officer/Chief Operating Officer
   - Lactation Management Nurses
   - Milk Bank Attendant
   - Microbiologist
   - Receptionist/Record Keeper

6) FUNDS - The overall cost approximation of setting up and running a milk bank can be summed up to 18 lakh +/- 3 lakh per annum including salaries of the staff (10).

7) HEALTHY DONORS - Healthy lactating mothers who would be willing to donate (10).

FUNCTIONS OF CLMC (10) :

STEP 1: SCREENING OF DONOR MOTHERS

- The mother must meet the donor eligibility criteria.
- Breast milk donation must be voluntary without any incentives provided.
- History taking and detailed examination of the breasts should be conducted to check for skin or breast lesions.

- Informed written consent should be obtained.
- Negative results in serological testing for HIV, syphilis and hepatitis need to be obtained.

STEP 2: MILK EXPRESSION AND COLLECTION

Breast milk can be expressed using one of two methods:

1) Manual: This technique improves the oxytocin response and milk ejection reflex and is more gentle than using a breast pump. It is the duty of lactation counsellors or nurses to instruct mothers in the art of hand expression.
2) Electric breast pump: Hospital-grade electric breast pumps employ a funnel shaped attachment that covers the areola and nipple, and they work on the basis of vacuum.

Key Guidelines:

- All expressed milk needs to be properly labelled with the necessary details, such as identity of the lactation counsellor or nurse, the mother's specific identification number, the donation and collection date, and the location.
- Logging of Donor Human Milk (DHM) should be conducted, which involves recording the volume of milk and identifying potential sources of contamination.

STEP 3: MILK PROCESSING

1. Storage prior to pasteurization

- DHM needs to be stored right away in a deep freezer at -20 ºC.
- MoM that is meant to be fed within a day should be kept in a refrigerator with a temperature control between +2 and +4 degrees Celsius.

- If MoM is not going to be used right away, it should be moved to a different deep freezer and kept at -20 degrees Celsius within 24 hours.

2. Thawing of milk

- DHM that is frozen needs to be refrigerated for at least 24 hours, or overnight, until the milk reaches 4 degrees Celsius.
- Alternatively, it can be thawed in a large container of warm water as long as the water temperature stays above 37 ºC and stays away from the lid to avoid contamination when spills occur.
- Microwave ovens should never be used for thawing as they can destroy immunoglobulin A and other antibacterial properties.
- Proper documentation of the thawing date and time is required, and thawed milk should never be refrozen.

3. Pooling and Aliquoting of Milk

- Raw DHM bottles are taken from the refrigerator to the processing room for pooling and subsequent pasteurization.
- Following stringent aseptic measures, milk after pooling is separated into clean containers in specified volumes based on bottle sizes in the presence of laminar airflow.
- DHM undergoes microbial contamination testing to determine its suitability for consumption, with tests conducted both prior to pasteurization and following it.

STEP 4: PASTEURIZATION

- Pasteurization removes bacteria while maintaining most of the beneficial components of milk. This process helps retain Vitamin C levels, prevents lipid oxidation, and deactivates CMV and HIV, as well as eliminating most harmful bacteria in breast milk.
- There are two methods of pasteurization: Low Temperature, Long Time (LTLT) and High Temperature, Short Time (HTST).
- It is recommended to utilise the Holder's pasteurisation procedure, also known as the LTLT heat treatment, for CLMC. In order to do this, DHM must be heated to 62.5°C (±0.5°C) and maintained there for 30 minutes. The milk is then quickly cooled to reduce heat-induced nutrition loss and to create a thermal shock that speeds up the death of microbes and stops the formation of spores.

STEP 5: TESTING OF DHM AFTER PASTEURIZATION

STEP 6: DHM STORAGE AFTER PASTEURIZATION

- After pasteurisation, the milk needs to be kept at -20°C in a deep freezer.
- Preservation of the milk can be done up to 6 months.

STEP 7: DISPENSING OF PROCESSED MILK

It is advised to thaw DHM by placing it in a refrigerator for a maximum of 24 hours at +4°C. Prior to using it in the wards, it needs to be brought to room temperature.

COMPOSITION OF DONOR HUMAN MILK

MACRONUTRIENTS

Valentine et al. combined milk from three to four donors per pool from five different HMBANA milk banks to create pools of DHM. They discovered that while there were some minor differences in the fatty acid and amino acid profiles between pools, the stage of lactation had an impact (37). Pasteurisation majorly lowers the macronutrient composition of DHM, with proteins and fats being the significantly affected components and lactose showing a minor reduction. Thus, the fall in lipid content is the main cause of the decrease in energy content.

Several studies indicate that pasteurization significantly reduces the macronutrient content of DHM, with lipids and proteins being the most affected components, while lactose shows a slight reduction. Therefore, the decrease in energy content primarily stems from the reduction in lipid content (36). According to Vieira et al., the mean fat and protein contents in 57 raw human milk samples notably decreased (5.5% and 3.9%, respectively) after pasteurization. Furthermore, it was observed that different feeding methods such as one-hour infusion (resulting in 40% more loss of lipids and 10% more protein loss) and gravity bolus feeding (resulting in an additional 5% loss of lipids and 3% loss of proteins) resulted in significant nutrient losses (35). A study by Castro et al. on PDHM feedings using 5 Fr Ryle's tubes found that longer infusion times resulted in greater nutrient losses. There was no loss of nutrients in milk fed via gravity bolus method, whereas major fat loss and consequent energy loss with a fall of 8%, 11% and 29% was seen with milk given over a period of 30 minutes, 1 hour and 4 hours respectively. (56). Garcia Lara et al. in his study on frozen breast milk (34 samples) observed that lactose and nitrogen content mostly stayed intact following pasteurization, although there was a noticeable decrease in

lipid (3.5%) and calorie content (2.8%) (57,58). It was also noted that Holder's pasteurization did not significantly affect carbohydrates, as confirmed by various analytical techniques (30). The antioxidant capacity can be reduced by freezing, thawing as well as pasteurization processes in milk (58). Total fat in PDHM was reduced by 6.3% due to the additional effect of pasteurization and 90 days of frozen storage. Compared to carbohydrates and proteins (4 kcal/g), fat has a higher caloric density at 9kcal/g. Therefore, the loss of fat significantly impacts the energy content. (57,58). Czank et al. found that maintaining a specific temperature during pasteurization was more critical than the duration of heat exposure for protein bioactivity preservation in milk. They observed that even a minimal temperature rise from 58°C to 59°C had a more pronounced negative impact on protein activity retention compared to similar variations in the holding time. Pasteurization primarily targets bacterial inactivation through heat but the broader thermal effects also induce protein denaturation. Freezing can affect the raw milk constituents by disrupting the integrity of the membrane of fat globules, leading to cream separation. Additionally, freezing can affect protein characteristics such as the stability of casein micelles and the whey protein's quaternary structure (59).

BIOACTIVE COMPONENTS

Ahrabi et al. in their study demonstrated that lactoferrin and secretory IgA levels were not affected by storage in freezer for up to 9 months. The study evaluated the levels of these components in samples of raw milk that had never been pasteurised using the enzyme-linked immunosorbent test (ELISA) (60).

The levels of bioactive constituents in milk are altered after pasteurisation; their concentrations are typically higher in colostrum than in mature milk (38,61–63). It mainly lowers immunoglobulins, lipoprotein lipase, bile salt-stimulated lipase, and

amylase levels. Pasteurisation has no effect on the levels of glycosaminoglycans or oligosaccharides (54,57,59).

MICRONUTRIENTS

A limited number of studies have reported regarding a few of the micronutrients and there is lack of literature about the vitamin and mineral concentrations in PDHM. Therefore, this is a crucial field for more research (36).

Human milk has a constantly changing composition during a meal, during the day, and when a mother is lactating. For these reasons, the banks usually combine milk from several donors in order to minimise excessive variance. Studies have shown that pooling milk from multiple donors can help distribute the components of the milk evenly throughout the aliquot. Less variation was found in the concentrations of insulin and IgA in milk including more than two pooled donors, according to Young et al. who examined the consequence of milk bank pooling methods on the levels of bioactive compounds in milk. The findings showed that insulin and total IgA levels were lower in single-donor pools than in those with two or more donors ($p < 0.05$). As a result, bioactive components in donated milk were optimised, and compositional variability in created DHM pools was decreased (54,64). Another study by Young et al. determined DHM pools to have lower protein and calorie concentrations than is typically assumed. These relatively low concentrations must be taken into consideration while supplying DHM to the infants in NICU. The study looked at how milk donation timing and pooling procedures affected the energy, zinc, and macronutrient concentrations in the resulting DHM pools (64,65).

EFFECT OF DHM ON INFANT GROWTH

Infants fed the donor milk supplement had a trend towards slower weight gain than those administered a formula supplement, according to a study by Colaizy et al. The reduced protein content of donor milk compared to what VLBW neonates require could be one reason for the slower growth rates observed in these newborns. Intake of protein has a linear relationship with preterm infant growth. VLBW babies fed human milk have a protein deficit in the first few months of life, even though women who give birth prematurely and frequently use human milk fortifiers have milk with a greater protein content (65). Arsanoglu et al. calculated this deficiency to be between 0.5 and 0.8 g/kg/day based on a comparison between expected and actual protein consumption for VLBW babies (66). If diets are adjusted to ensure enough protein intake and this relative protein shortage is identified, growth can be optimised in donor milk specifically. Diets using PDHM may be linked to more growth difficulties than diets including mother's milk. Extra care is needed to fortify foods with protein and calories so that the advantages of a human milk diet can be obtained without compromising growth (65).

In a retrospective analysis study, R Chowning et al. looked at how human donor milk affected 550 VLBW infants' postnatal growth and prevented NEC. The study discovered that the use of DHM led to reduced weight and head circumference growth rates at discharge (14). Lloyd M. et al. observed similar results in 2019 when they compared mother's milk to PDHM. They discovered that the group supplemented with mother's milk gained more weight faster at 34+1 weeks postmenstrual age than the group supplemented with PDHM but the group supplemented with PDHM showed clear catch-up growth by discharge. There was no noticeable variation at an age of 12 months between the two groups (25).

Lucas et al. conducted a study to determine the impact of feeding style on growth not only during the neonatal stage, but also during late infancy and youth. During the neonatal stage, these neonates were randomly assigned to receive either formula feeds or DHM feeds as a supplement or as their only diet. Follow-ups were done at nine months, eighteen months, and between the ages of 7.5yrs and 8 yrs. It was discovered that groups of infants receiving DHM had a slow gain of all the anthropometric in the early newborn phase than groups receiving preterm formula. However, there was no difference in the measures of anthropometry at nine or eighteen months or between 7 and a half and 8 yrs in either groups (26).

Brownell et al. also investigated the association between PDHM, one's own mother's milk, and preterm formula feeds on growth rates. The data show that every ten percent increment in PDHM consumption reduced the mean weight gain velocity by 0.17 grams/kg each day. Furthermore, as PDHM consumption rose, the mean variability in the z-scores decreased, but improved as the percentage of formula intake increased. Finally, it was observed that PDHM consumption and the average rate of rise of HC had a negative correlation (15).

In a setting of VLBW infants, a retrospective case control study carried out in 2012 by Giulliani F et al. evaluated the short-term benefits of MOM as the only diet compared to PDHM individually in terms of anti-infectious properties, gain in anthropometric parameters, tolerance of feeds, prevention of prematurity complications. The study interpreted no major difference in clinical outcomes between the 2 groups. There happened to be only a small, non-statistically significant variation in growth seen favouring MOM (67).

An observational study did a comparison between fortified MOM and fortified DHM in newborns with < 32 weeks of gestational age. This included a

period of exclusively feeding with human milk from the end of parenteral nutrition to the transition to formula. Infants that received at least 80% mother's milk gained weight faster than those who got less than 20% mother's milk, and weight increase was related to the amount of mother's milk intake (68).

In a study of 244 neonates with gestational age < 30 weeks, Schanler and Richard et al. compared neonates who were fed only MOM to infants who were fed MOM plus PDHM from mothers who had preterm births or MOM plus PT. A human milk fortifier was introduced at a daily milk intake of 100 mL/kg. In the PDHM group, 17.7 % of infants were moved to PT due to inadequate growth, whereas none were switched in MOM group. Additionally, it was discovered that while head circumference gains were the same in both the DHM and preterm formula (PF) groups, the weight gain velocity differed and was greater in PF group (68,69).

In comparison to VLBW neonates in whom <50% of the total volume was MOM, a study by Piemontese et al. revealed that a greater amount of lean mass deposition at term corrected gestational age was observed in VLBW neonates fed MOM at > 50% of the volume of total milk (70).

Research has shown that preterm newborns supplemented with fortified donor milk gained less weight than infants fed only on fortified mother's milk. According to a report, babies with birth weights under 1000 g gained weight more slowly in the initial 1st month and had a lesser score in tests on cognition in the 1st 2 years of life if they were fed >50% PDHM supplements in comparison to neonates given only MOM (68).

An observational study comparing MOM vs. PDHM consumption in 90 extreme premature neonates delivered in Gothenburg, Sweden between 2013 and

2015 found that all the anthropometric z scores at 28 days of life showed a positive correlation with unpasteurized mother's milk (71).

In their study, Mol et al. examined the composition of infants both preterm as well as term and the effects of MOM and preterm formula. They found that, at term CGA, the neonates who were fed MOM had a body mass composition identical to the term infants (70,72).

Jacopa Cerasani et al. published a review in 2020 that looked at the association between MOM and preterm newborn growth and composition. The analysis covered 91 articles over a 11 year period from 2009 to 2020 and found that the category of neonates who consumed > 75% MOM had a higher drop in z scores for weight starting from the time of birth till discharge than the category that consumed less than 75%. When the kind of milk (MOM, PDHM, or mixed) was examined, a tendency developed towards greater incidences of growth retardation in newborns at the time of discharge among those getting >75% PDHM in comparison with those getting >75% MOM or a combination of the two. In late preterm newborns on a pure human milk diet, there was a significant effect of MOM consumption on the adipose deposition in the body. This correlation increased as the neonate achieved a term CGA. (72).

In Li et al.'s RCT, mostly formula-fed infants weighed more than the exclusively human milk-fed group at term adjusted age, and their higher rate of variation in z scores for weight during the study was associated with higher non-adipose tissue mass deposition. There was no link established between consumption of PF feeds and increased adiposity at term age (72,73).

Beliaeva et al. investigated growth outcomes and composition of the body in premature newborns, categorising them into three groups based on feeding type. The

authors found that body weight, length, HC and CC at discharge were lesser in purely MOM fed premature infants than in formula-fed infants. Similar variations were found in premature infants who only received MOM versus newborns who received both MOM and PF. However, there was a favourable effect on lean body mass and growth outcomes in MOM fed neonates. In contrast, body composition analysis revealed that formula-fed premature newborns had a larger amount of lipids in body mass compared to infants who were exclusively MOM fed (74).

Another retrospective study, conducted by Kim L Chung in 2017 reviewing 132 infants of birthweights (BWs)<1500g and gestational ages < 32 wks, studying the impact of feeding DHM exclusively on growth outcomes and morbidity of premature neonates during hospital stay reported, lower values of anthropometric measures in PDHM group as compared to PF group on achieving full enteral feeding of 130ml/kg, but showed similar growth results at 36 weeks PMA (17).

Quigley et al in 2019 conducted a meta-analysis review comparing formula v/s PDHM for premature neonate feeding reported that PF fed neonates were found to regain all anthropometric measures quicker and also have greater rates of rise in the anthropometric measures than the neonates who were fed PDHM but resulted in higher incidence of complications like NEC subsequently (18).

Dempsey et al in 2019 conducted a systemic review of randomized and quasi randomized trails comparing preterm DHM v/s term DHM to study the resulting growth and development seen in VLBW infants and reported no literature evidence to demonstrate the effect of pooled preterm PDHM on growth outcomes in preterm infants. Lack of preterm milk secretion and collection in milk banks along with routine practice of pooling term and preterm milk in milk banks along with processes

like pasteurization altering the milk composition before distribution were major obstacles identified (75).

Some studies showed a positive impact of PDHM on the growth outcomes. Verd et al. investigated the eligibility of a combination of MOM and any quantity of PDHM, as opposed to formula feeds. Starting from birth till discharge, no major changes in the anthropometric measures of neonates consuming DHM as compared to those consuming formula were noted (76). Visuthranukul et al. compared the growth following discharge, fat mass, and metabolic outcomes of AGA neonates as compared to SGA neonates given a purely human milk diet at 12-15 months (visit 1) and 22 months (visit 2) of CGA. The study revealed that the SGA group gained the same amount of weight as the AGA group from discharge till visit 1, and that the BMI z-score increased considerably from visit 1 to visit 2, even after accounting for GA and diet at discharge. They concluded that, at two years of age, SGA preterm infants who only received human milk displayed greater compensatory growth with the adipose tissue content being constant (72,77).

Sisk et al. conducted a similar study to evaluate the chances of NEC and growth in premature newborns provided predominantly MOM, DHM, or PF, and reported that MOM and DHM feedings, consumed till a CGA of 34 weeks were related to decreased NEC rates without disturbing the growth. There were no variations detected in the growth parameters starting from birth till discharge (27). Connor et al. and Morley et al. found that a combination of MOM and fortified DHM outperformed formula in terms of rise in weight and HC (61). Adhisivam et al. conducted a RCT in the Southern India in 2018 to determine the effect of PDHM on 80 healthy premature neonates randomised into 2 groups - fortified and unfortified PDHM. The study reported similar growth outcomes in both the group on following

them up till discharge or for 28 days, whichever came sooner (78). Fang L et al analysed the impact of preterm PDHM on tolerance of feeds, growth parameters, and major morbid conditions in LBW newborns. No difference in increase in weight per day or increase in HC per week was observed in both PF and PDHM group proving preterm DHM does not alter the outcome of growth. However, the hospital stay duration and duration of achievement of complete enteral feeding was lower in PDHM group (28). Similar findings were reported in researches by Costa et al. and Canizo Vazquez D et al. (19,79). Hoban et al. used multiple level models of increase in length to analyse the variations in z scores of growth both short and long term depending on amount of PDHM or formula consumed in the 1st 4 weeks of life. Weight and length Z-scores declined during the admitted period in hospital, however all indicators increased after discharge. Short-term growth correlated positively with duration of stay in hospital and GA at birth. A greater amount of PF and not PDHM resulted in lesser fall in the trajectories of growth but the type of feeding did not impact development in the long run, hence concluding that PDHM did not affect growth - both short term and long term (20). During the NICU hospitalisation, the length and weight z-scores of VLBW newborns decreased, with no association observed with the volume of PDHM consumed but with directly proportional relation between the volume of formula consumed and z scores of anthropometry. It was observed that the growth parameters showed an abrupt variation to rising trend of z scores irrespective of the type of feeding in hospital. Hence, use of PDHM has been promoted to ensure long term growth and prevent the complications associated with the use of formula. It is believed that larger number of studies are required to confidently state the long term effect of feeding VLBW newborns with PDHM (20).

The AAP promotes the use of PDHM over PF when MOM is not adequately available and a supplement is required. Bramer et al.'s study presents strong evidence in favour of this proposal, demonstrating that DHM did not have a deleterious effect on newborn growth velocity. The growth of non-hospitalized newborns fed only PDHM or PDHM additional to MOM was observed to be significant and the PDHM was also tolerated properly (80). Recent studies have shown excellent growth results, which could be attributed to both increased consumption of PDHM with improved nutritional composition and the use of fortification strategies (81).

When standardised fortification was used, the protein content variations were not taken into consideration due to which few studies demonstrated a lower growth rate in PDHM fed VLBW infants. It was noticed that infants who were fed MOM or PDHM both attained the expected increase in weight of 18 to 20 g/kg everyday when PDHM had been supplemented using targeted fortification. Hence, when PDHM is utilized with individual fortification, it results in adequate growth in the VLBW newborns (82).

NECROTIZING ENTEROCOLITIS

Necrotizing enterocolitis (NEC), a potentially fatal condition primarily affects the infants. The death rate in NEC might reach 50%. The pathophysiology of NEC involves intestinal inflammation that gives rise to bacterial invasion, cellular destruction, and necrosis of the intestine. As NEC worsens, there may be an intestinal perforation that results in sepsis, peritonitis, and even death. Prematurity, formula feeding, and bacterial dysbiosis are all recognised risk factors. Infants with NEC had an average postmenstrual age of 30-32 weeks. NEC often appears in the second or third week of life, though in VLBW infants, it can appear as late as three months.

Necrotizing enterocolitis presents with a wide range of nonspecific, mild signs and symptoms.

Parents frequently report gastrointestinal symptoms in the infants including nausea, vomiting, diarrhoea, and an increase in abdominal circumference. Additionally, infants may have blood in their faeces. As the illness worsens, the patient may exhibit systemic symptoms including cyanosis and non-responsiveness that are linked to circulatory collapse and respiratory failure. Abdominal distention, abdominal pain to palpation, visible intestinal loops, reduced bowel sounds, abdominal wall erythema and palpable abdominal mass are some of the physical examination findings. Reduced peripheral perfusion, circulatory collapse, and respiratory failure are the important systemic findings. (83). The most crucial test needed to diagnose the condition is an X ray abdomen that includes left lateral decubitus and anterior-posterior views. Diagnosis for necrotizing enterocolitis includes findings of dilated bowel loops, pneumatosis intestinalis, and the presence of portal venous air. Pneumatosis intestinalis is a pathognomonic condition for necrotizing enterocolitis, characterised by the visualisation of air within the intestinal wall. Air in the portal vein is not always present, but when seen it is a poor prognostic marker. When there is a perforation, the abdomen may show free air (84). The results of blood investigations reveal three biochemical abnormalities of metabolic acidosis, hyponatremia, and thrombocytopenia (83).

Management (85) :

1. Suspected NEC (Bell's stage 1) – supportive care is needed.
2. Proven NEC (Bell's stage 2) - total parenteral nutrition, nasogastric decompression, and antimicrobial therapy. In cases that do not respond, surgical management is indicated.
3. Advanced NEC (Bell's stage 3) - may require inotropes to be used in the medical treatment. Surgical intervention is necessary for neonates who have intestinal perforations, suspected gut necrosis, or non-response to medical line of management.

It has been observed that 27–52% of newborns with weight less than 1000 grams need surgery (86).

EFFECT OF DHM ON NEC AND SEPSIS

Because human milk contains immune-modulating and bactericidal bioactive compounds, it is protective against both NEC and sepsis when compared to formula feeds, according to several observational studies and RCTs (87).

The idea that human milk possesses antioxidant qualities and protects against oxidative stress has been supported by Shoji et al. in their study (13,88). In a retrospective study conducted in 2016 by Kantorowska et al. with 10823 newborns from 22 hospitals weighing less than 1500 grams, it was found that when DHM was compared to formula, the incidence of NEC decreased from 5.7% to 2.9% (P =.0006) (89).

Every 10% rise in MoM and PDHM intake was linked to around 8 and 12% decrease in the risk of newborn infection/NEC respectively, according to a study by Lapidaire et al. There was a 12% increase in the likelihood of infection/NEC for every

10% rise in term formula (TF) (90). In comparison to newborns fed preterm formula, Cristofalo et al. discovered that preterm infants fed PDHM enhanced with a fortifier derived from human milk required less time on parenteral feeding. One of the secondary findings was a decrease in NEC, which was linked to a diet consisting solely of human milk. In the preterm formula group, the incidence of NEC was 21%, which is significantly higher than in the majority of NICUs of developed countries (16,29).

According to Schanler et al., donor milk had no impact on the incidence of NEC or late-onset sepsis. They included only cases of sepsis or NEC that developed after 16 to 18 days, when a substantial amount of feeds (50 mL/kg daily) was tolerated (29,91). A retrospective cohort research by Chowning et al. in 2015 with 550 neonates weighing less than 1500 grams at a gestational age of less than 35 weeks revealed a relationship between PDHM and a lower incidence of NEC (13.5% vs. 3.4%; P<.001) (87).

With a 2% reduction in severe NEC and a 4% ARR in any NEC, a larger proportion of human milk was more beneficial than lesser quantities. In situations where mothers are unable to provide milk sufficient for all of their infant's needs, the policy suggests a switch to 100% human milk for NEC protection. There does not seem to be a dosage effect, however an exclusive HM diet was linked to 4% decrease in sepsis. The data about the impact of exclusive or any HM on the prevalence of BPD or ROP is conflicting, with the probable exception of a potential effect of EHM on the 8% fall in severe ROP when compared to formula (92).

EFFECT OF PDHM ON DURATION OF HOSPITALIZATION

Hospital stay duration is a complicated measure with numerous interfering variables. Other than dietary consumption, a number of variables have been proven to be complicating factors in the development of infants and may even show symptoms prior to birth. A 100 gram birth weight difference or one week gestational age difference can have a major effect on perinatal as well as postnatal problems for VLBW babies. These issues affect the duration of hospital stay for these babies either directly or indirectly, and they are expected to confound effects of PDHM (93).

PDHM through the following mechanisms, affects the duration of hospitalization as follows:

(a) Enhancing growth and nutrition of newborns (94)

(b) Lowering the chances of LOS in VLBW newborns (95,96)

(c) by means of essential nutrients including long-chain polyunsaturated fatty acids (LCPUFA) and other neurotrophic components found in human milk (94).

MATERIALS AND METHODS

The study was conducted from January 2023 to December 2023 in the Neonatal Intensive Care Unit (NICU) of Department of Paediatrics, KLES Dr. Prabhakar Kore hospital and Medical Research Centre, attached to Jawaharlal Nehru Medical College, Belagavi.

Study Design:

Observational Longitudinal study

Study Duration:

One year

Study Place:

The study was conducted in Neonatal Intensive Care Unit of Paediatrics, KLES Dr. Prabhakar Kore hospital and Medical Research Centre, attached to Jawaharlal Nehru Medical College, Belagavi.

Source Of Data:

Infants requiring PDHM and admitted in NICU of Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, Karnataka.

Sample Size:

The sample size was calculated from the formula as mentioned below by taking power as 90% and an effect size as 0.38.

$$n_{pairs} = \frac{(Z_{1-\alpha} + Z_{1-\beta})^2 + Z^2_{1-\frac{\alpha}{2}}}{\Delta^2}$$

$$\text{Where } \Delta = \frac{X_2 - X_1}{SD} \quad , \quad SD = \frac{S_1 + S_2}{2}$$

Where

X_1 and X_2 = Means of pre and post test

S1 and S2 are SD of pre and post test

△ (Delta) = Effect size

Standard deviation in the 1st group S1 = 4.2

Standard deviation in the 2nd group S2 = 3.4

Mean difference between 1st and 2nd sample = 1.45

Effect size = 0.381578947368421

Alpha error (%) = 5

Power (%) = 90

Sided = 2

Number needed (n) = 146 babies

The calculated sample size was 146 for the study.

Selection criteria:

Inclusion criteria:

1) Infants getting admitted in NICU requiring pasteurized donor human milk (PDHM).

2) Infants whose mothers are willing to feed pasteurized donor human milk (PDHM).

Exclusion criteria:

Infants with

1) Birth asphyxia

2) Congenital anomalies

3) Not on enteral feeds

4) Surgical problems of gastrointestinal tract

5) Early onset sepsis

6) Death of the neonate

Institutional ethical clearance: The ethical clearance was obtained prior to the conduct of the study, from Institutional Ethics and Research Committee, KAHER's Jawaharlal Nehru Medical College, Belagavi

C.T.R.I registration: The study was registered with Clinical Trial Registry of India prior to sample collection and clearance was granted with registration number CTRI/2023/05/052641.

Method Of data collection:

The study was conducted after obtaining institutional ethical clearance. Informed consent was obtained from the parents /caregivers of the eligible neonates. The neonates fulfilling the selection criteria were enrolled for the study. At enrolment, socio-demographic data viz name, age, gender, occupation, educational status of the parents and baseline maternal and infant parameters viz, maternal history, maternal age, gravida, antenatal care, antenatal risk factors ,birth history like birth weight, gestational age, mode of delivery, age, anthropometry and indication for NICU admission and indication for starting PDHM were recorded on a proforma which was previously prepared and tested.

PDHM was obtained from human milk bank "AMRUTHA" attached to the Level III NICU of KAHER Dr. Prabhakar Kore charitable hospital, Belagavi which was established in 2018. Human milk collected from five donor mothers after screening and obtaining consent was pooled and pasteurised using the Sterifeed pasteurizer based on the Holder's method which involves heating of milk to a controlled temperature of 62.5º celsius for thirty minutes and then swiftly cooling it to 10º celsius. Processing, storage and dispensing of the pooled sample of DHM was done as per the National CLMC guidelines (97). The pooled and pasteurized donor human milk was given a particular batch number. A sample of 10ml from each batch of

PDHM was sent to the food and micronutrient analysis lab for the analysis of macronutrients and micronutrients in each batch of PDHM.

The analysed PDHM was then dispensed to the neonates who were eligible and enrolled in the study. A milk flow process can be established to trace the infants receiving a specific batch of a pooled PDHM sample.

Follow up:

All the enrolled neonates were followed up in the hospital till discharge and at 6 weeks of life. During hospital stay, the infants were observed for complications (necrotizing enterocolitis/ sepsis), feeding type, method and amount of PDHM consumed by the infant per day.

At discharge and at 6 weeks, the infants were assessed for anthropometry (weight, height, head circumference and mid arm circumference), feeding pattern (type and method) and total duration of hospital stay.

Outcomes:

Primary outcome:

Micronutrient analysis:

The micronutrients analysed in every batch of pooled pasteurised donor human milk were calcium, iron and zinc.

Method: Atomic absorption spectroscopy

Atomic absorption spectroscopy (AAS) is a method used to measure the concentration of metallic elements in different materials. As an analytical technique, it uses electromagnetic wavelengths, coming from a light source. Specific elements absorb these specific wavelengths differently, giving a picture of concentrations of a specific element in the material being tested.

Secondary outcomes:

1. Macronutrient analysis

The macronutrients analysed in every batch of pooled pasteurised donor human milk were energy, carbohydrates, proteins and fats.

Method: Mid infrared spectroscopy (MIR)

Infrared chromatography based MIR uses the different wavelengths of infrared energy absorption of the different macronutrients present in PDHM including protein, fat, carbohydrates and energy content.

2. Growth of infants

Parameters assessed were:

- **Weight** - The infant's weight was measured on Essae-BS-250 electronic weighing scale with precision of 0.001 kg. Infant was weighed naked with no clothing or diaper after making sure that scale was placed on flat, hard, even surface with proper calibration. Three readings were noted and mean of the three readings was taken as final value.
- **Length** - The infant's length was measured on an infantometer board after placing it on a horizontal and level surface. Three measurements for each baby were taken and mean of the three values was taken as final value after measuring it to nearest 0.1cm.
- **Head circumference** – Infant's head circumference was measured with Schorr tape with precision of 1 mm by placing it over the occipital protuberance at the back and just over the supraorbital ridge and the glabella in front, once being positioned correctly it was pull tight to compress the hair and the skin, but not too tight causing injury to the baby. Three measurements were taken for each baby and mean of the three values was taken as final value.

- **Mid upper arm circumference** – Infant's mid upper arm circumference was measured with Schorr's tape at the mid-point between the tip of the shoulder and the tip of the elbow (olecranon process and the acromion). Three measurements were taken for each baby and mean of the three values was taken as final value.

3. Type and method of feeding

A) Type Of Feeding–

- Exclusive Breastfeeding: "Exclusive breastfeeding" is defined by WHO as giving no other food or drink, not even water, except breast milk.
- Pasteurized Donor Human Milk: Breast milk expressed by a mother that is then processed by a donor milk bank for use by a recipient that is not the mothers own baby".
- Formula Feeds: "An artificial substitute for breast milk intended for feeding infants, using cow's milk as a base, supplemented with vitamins and minerals".
- Mixed Feeding: Feeding of both PDHM and MOM.

B) Method of Feeding-

- Direct Breastfeeding: WHO defines 'direct breastfeeding' as the provision of human breast milk to the infant by direct feeding at the breast.
- Nasogastric feeding: "A feeding tube is a small, soft, plastic tube placed through the nose (NG) or mouth (OG) into stomach to provide feeds and medicines to the babies after measuring the distance from either the nostril or the mouth (depending on insertion site) to the tragus (lobe of the ear) to the half way point between the xiphi-sternum and the umbilicus"
- Spoon/Paladai Feeding: "The paladai is a cup-like utensil with a narrow tip has been used traditionally to feed preterm neonates who has not developed coordinated Suck-Swallow Reflex"

C) Duration of Feeding: Total number of days receiving MOM, PDHM or Mixed feeding.

D) Amount of Feeding: Amount in (ml) Of PDHM, MOM or Mixed feeding.

4. Duration Of Hospital Stay: Total number of days of NICU stay

5. Incidence of Necrotizing Enterocolitis and Sepsis

Necrotizing Enterocolitis is defined based on:

- Blood and serum studies for hyponatremia, metabolic acidosis and thrombocytopenia
- Radiograph or USG
- Stool for occult blood

Sepsis Screen criteria:

- Leucopenia (TLC < 5000 cubic mm)
- Neutropenia (ANC < 1800/ cubic mm)
- Immature neutrophils to total neutrophil (I/T ratio) > 0.2
- Micro ESR (> 15mm)
- CRP +ve

Statistical Analysis

Data obtained was coded, collected and stored in Microsoft Excel spreadsheet. Data was analyzed with the use of statistical software R version 4.4.0. and Microsoft Excel. The variables which were categorical were expressed by percentages and frequencies. Continuous variables were represented in mean ± SD/ median (min, max) form. The normality of variables was checked by Shapiro Wilk test and QQ plot. Parametric tests were applied when data was adhered to a normal distribution, whereas nonparametric tests were utilized for data that did not follow the rule of normal distribution. Kruskal Wallis test was utilized to analyse the distribution of growth parameters over amount of PDHM received. Friedman's test was utilized to correlate the distribution of variables over different time points. Pairwise Wilcoxon test was used as post hoc analysis. Spearman's rank correlation test was used to check the correlation of variables. A probability value (P-value) of less than or equal to 0.05 at 95 % confidence interval was considered statistically significant.

OBSERVATION & RESULTS

The study was conducted from January 2023 to October 2023 in the Neonatal Intensive Care Unit (NICU) of Department of Paediatrics, KLES Dr. Prabhakar Kore hospital and Medical Research Centre, attached to Jawaharlal Nehru Medical College, Belagavi.

A total of 13 batches of pooled PDHM obtained from the human milk bank - AMRUTHA were analysed for micronutrient and macronutrient composition. 10ml of PDHM from each batch was sent for the analysis. The PDHM from each batch was dispensed among the infants eligible and enrolled in the study. Hence, a milk flow was established for each batch of PDHM which was traced to the number of infants it was provided and growth was assessed in these infants. (Figure 1)

A total of 688 infants were admitted in NICU during the study period of which 402 infants were given PDHM. Of the 402 infants who received PDHM, the first 200 infants were screened. 20 infants were excluded from the study in view of congenital malformations, birth asphyxia and early onset sepsis. A total of 180 infants were considered eligible and were enrolled and analysed in the study. Of the 180 infants enrolled, 8 deaths were noted during the study period. Of the 172 survivors, 26 infants were lost to follow up after discharge. Hence, 146 infants were followed up till 6 weeks of life and analysed. (Figure 2)

Figure 1: Batch wise distribution of PDHM

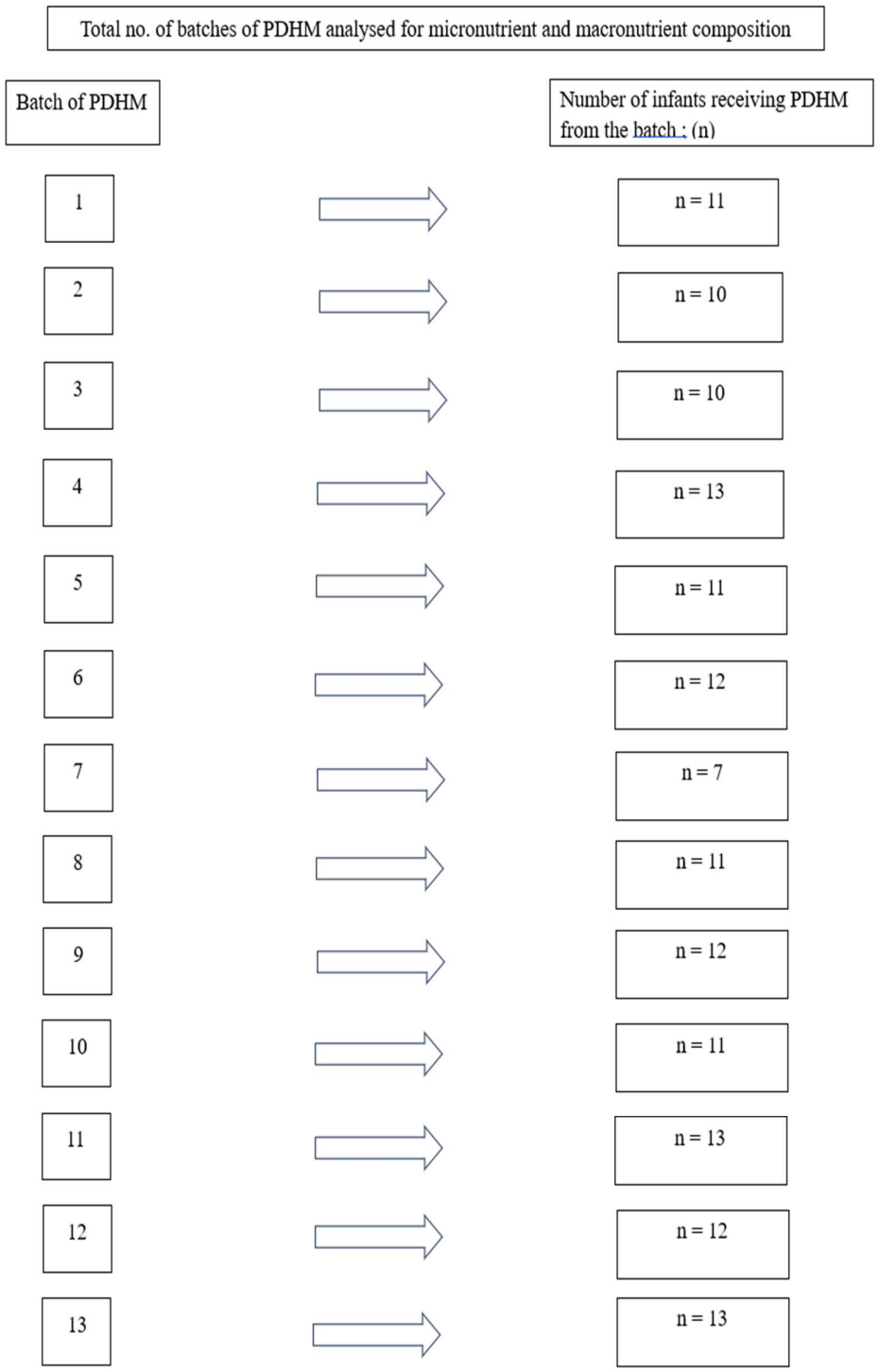
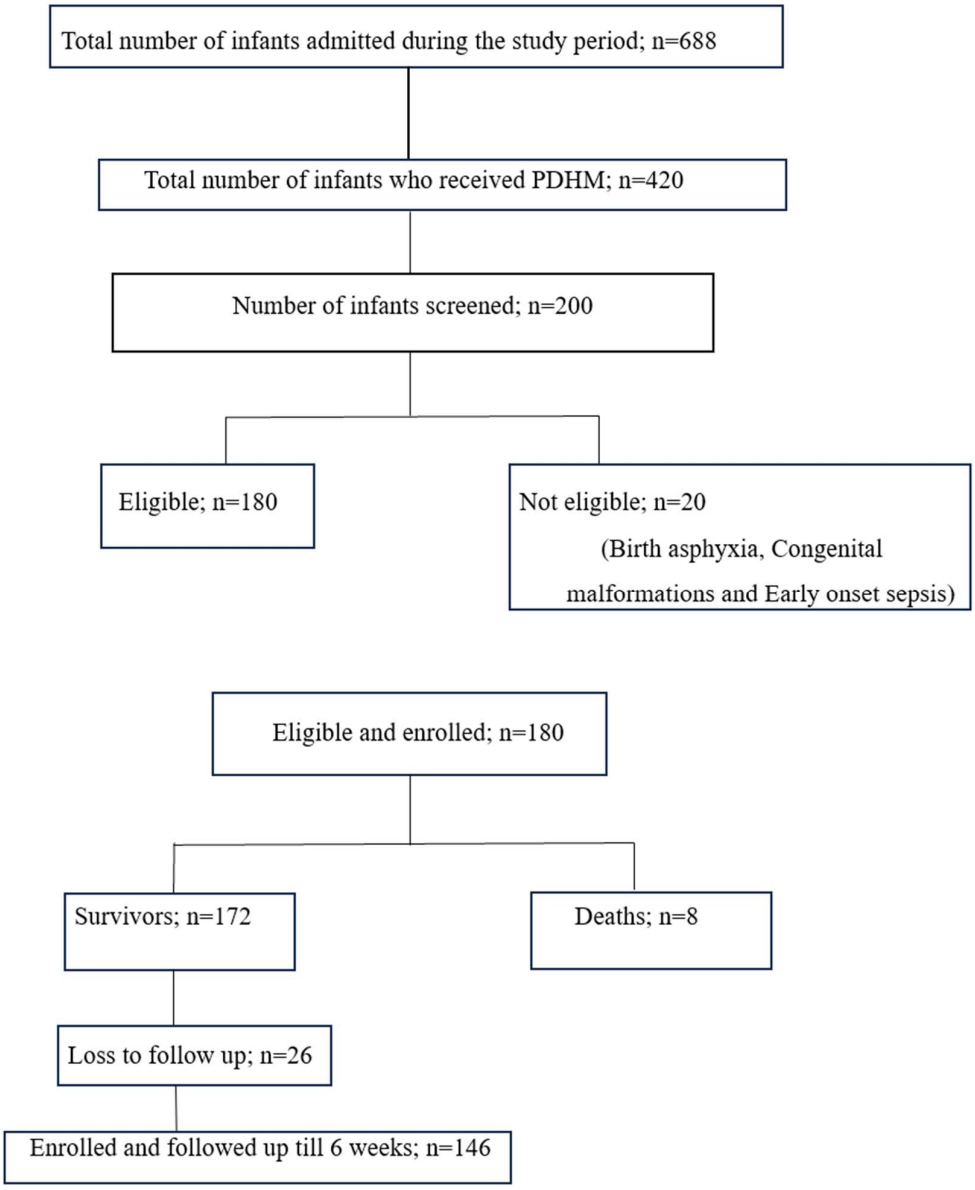


Figure 2: CONSORT diagram for screening and enrolment of infants



1. SOCIO-DEMOGRAPHIC PROFILE

- The following table gives the distribution of infants according to socio-demographic details at enrolment.

Table 1: Socio-demographic profile

Variables	Sub Category	Number of subjects (%)
Age (Days)	Mean ± SD Median (Min, Max)	1.86 ± 1.1 2 (1, 7)
Gender	Female	70 (47.95%)
	Male	76 (52.05%)
Religion	Hindu	125 (85.62%)
	Muslim	20 (13.7%)
	Others	1 (0.68%)
Mother's Education	Primary	23 (15.75%)
	Secondary	85 (58.22%)
	Graduate	38 (26.03%)
Mother's Occupation	Employed	7 (4.79%)
	Homemaker	139 (95.21%)
Father's Education	Primary	7 (4.79%)
	Secondary	40 (27.4%)
	Graduate	97 (66.44%)
	Post Graduate	2 (1.37%)
Socio Economic Status	Upper class	17 (11.64%)
	Upper middle class	85 (58.22%)
	Middle class	37 (25.34%)
	Lower middle class	7 (4.79%)
Consanguineous marriage in parents	No	128 (87.67%)
	Yes	18 (12.33%)

The mean age of the infants at enrolment was 1.86 days (±1.1). Majority of the infants were males (52.05%) and belonged to the Hindu religion (85.62%). Majority of the mothers were homemakers (95.21%) with secondary education (58.22%). A vast majority of the families belonged to upper middle class of the socioeconomic status

(58.22%). Only a few families reported consanguineous marriages among parents (12.33%), while the majority did not (87.67%).

2. MATERNAL PROFILE

- The following table gives the distribution of mothers according to maternal history

Table 2: Maternal profile

Variables	Sub Category	Number of subjects (%)
Mother's age (years)	Mean ± SD	25.7 ± 3.73
	Median (Min, Max)	26 (18, 39)
Gravida	Multigravida	73 (50%)
	Primigravida	73 (50%)
Antenatal Visits and Scans	Yes	146 (100%)
Antenatal Risk Factors	Elderly primigravida > 35 years	1 (0.68%)
	Short statured < 145 cm	2 (1.37%)
	Preeclampsia and eclampsia	35 (23.97%)
	Anemia	8 (5.48%)
	Gestational diabetes mellitus	15 (10.27%)
	Previous still birth, intra uterine death	3 (2.05%)
	Previous caesarean section	28 (19.18%)
	Grand multipara	4 (2.74%)
	Preterm premature rupture of membranes	11 (7.53%)
	IUGR	42 (28.77%)
	Twins/ Triplets	48 (32.88%)
	Placenta previa/placenta abruption	4 (2.74%)
	Rh isoimmunization	9 (6.16%)
	None	26 (17.81%)

The mean age of the mothers was 25.7 years and the study included an equal number of multigravida (50%) and primigravida (50%) mothers. All mothers (100%) had antenatal visits and scans during their pregnancies. Various antenatal risk factors were identified within the cohort: twin/triplet pregnancy being the highest (32.88%) followed by intrauterine growth restriction (IUGR) (28.77%) and preeclampsia and eclampsia (23.97%).

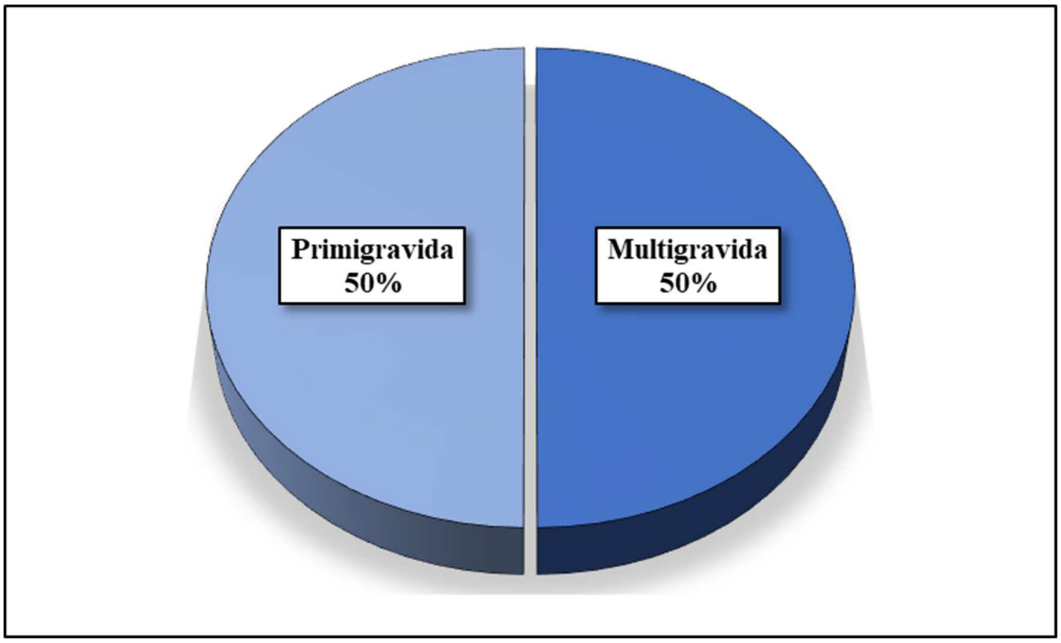


Figure 3: Distribution of mothers according to gravida.

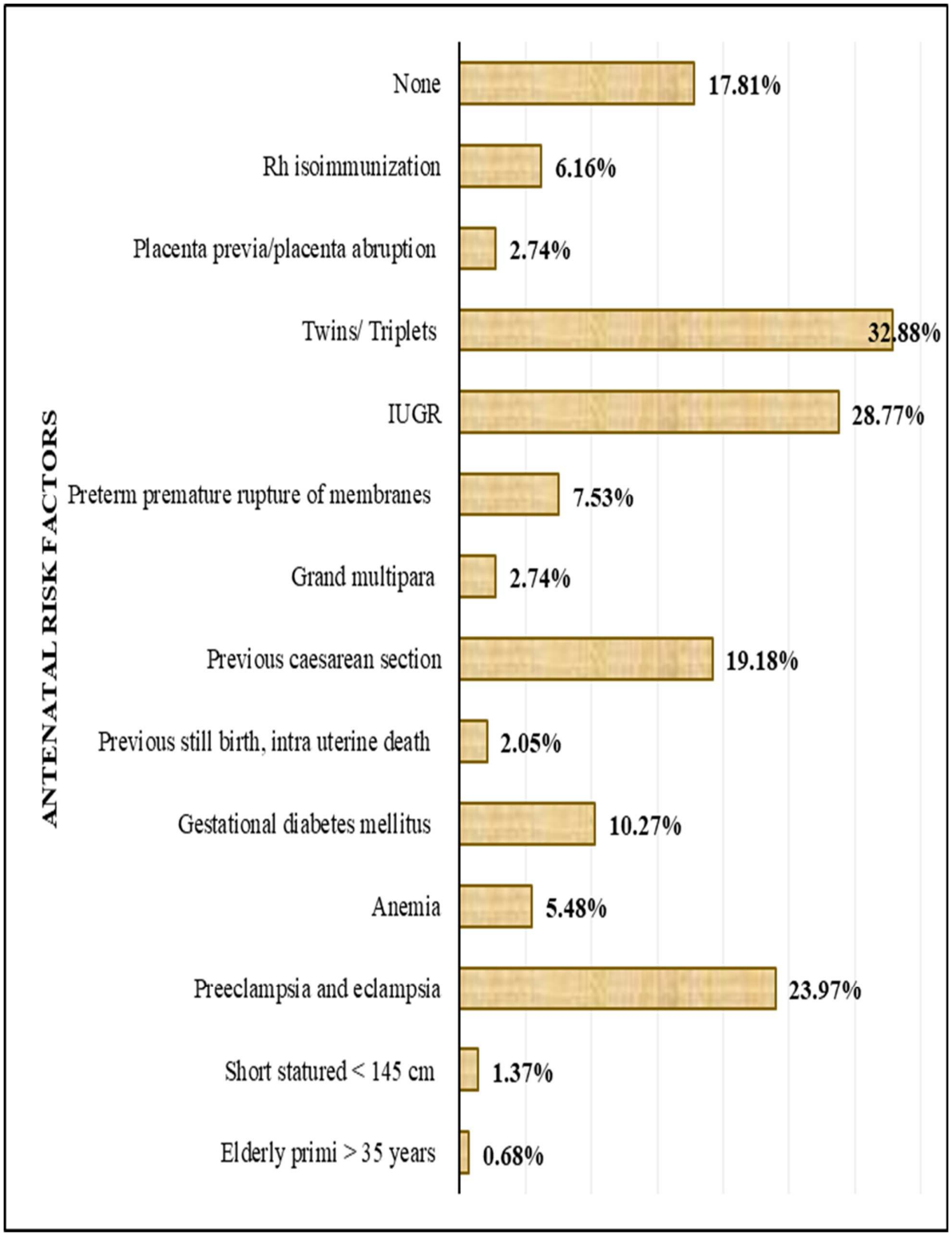


Figure 4: Distribution of mothers according to antenatal risk factors

3. BIRTH PROFILE

- The following table gives the distribution of infants according to birth history

Table 3: Birth profile

Variables	Sub Category	Number of subjects (%)
Mode of Delivery	LSCS	115 (78.77%)
	VD	31 (21.23%)
Gestational age (weeks)	Mean ± SD	35.91 ± 2.34
	Median (Min, Max)	36.29 (30, 40.43)
Birth weight (g)	Mean ± SD	2008.15 ± 436.15
	Median (Min, Max)	1980 (1200, 3300)
Indication for NICU Admission	Low birth weight	125 (85.62%)
	Respiratory distress syndrome	18 (12.33%)
	Meconium aspiration syndrome	2 (1.37%)
	NNH	29 (19.86%)
	Feeding difficulty	0
	Observation	5 (3.42%)
	Hypoglycemia	1 (0.68%)
	Others	6 (4.11%)

The majority of deliveries were conducted via LSCS (78.77%), while vaginal deliveries constituted 21.23%. The mean gestational age at birth was 35.91 weeks (± 2.34) with a mean birth weight of 2008.15 grams (±436.15). The most common indication for NICU admission was low birth weight (85.62%) followed by neonatal hyperbilirubinemia (19.86%) and respiratory distress syndrome (12.33%).

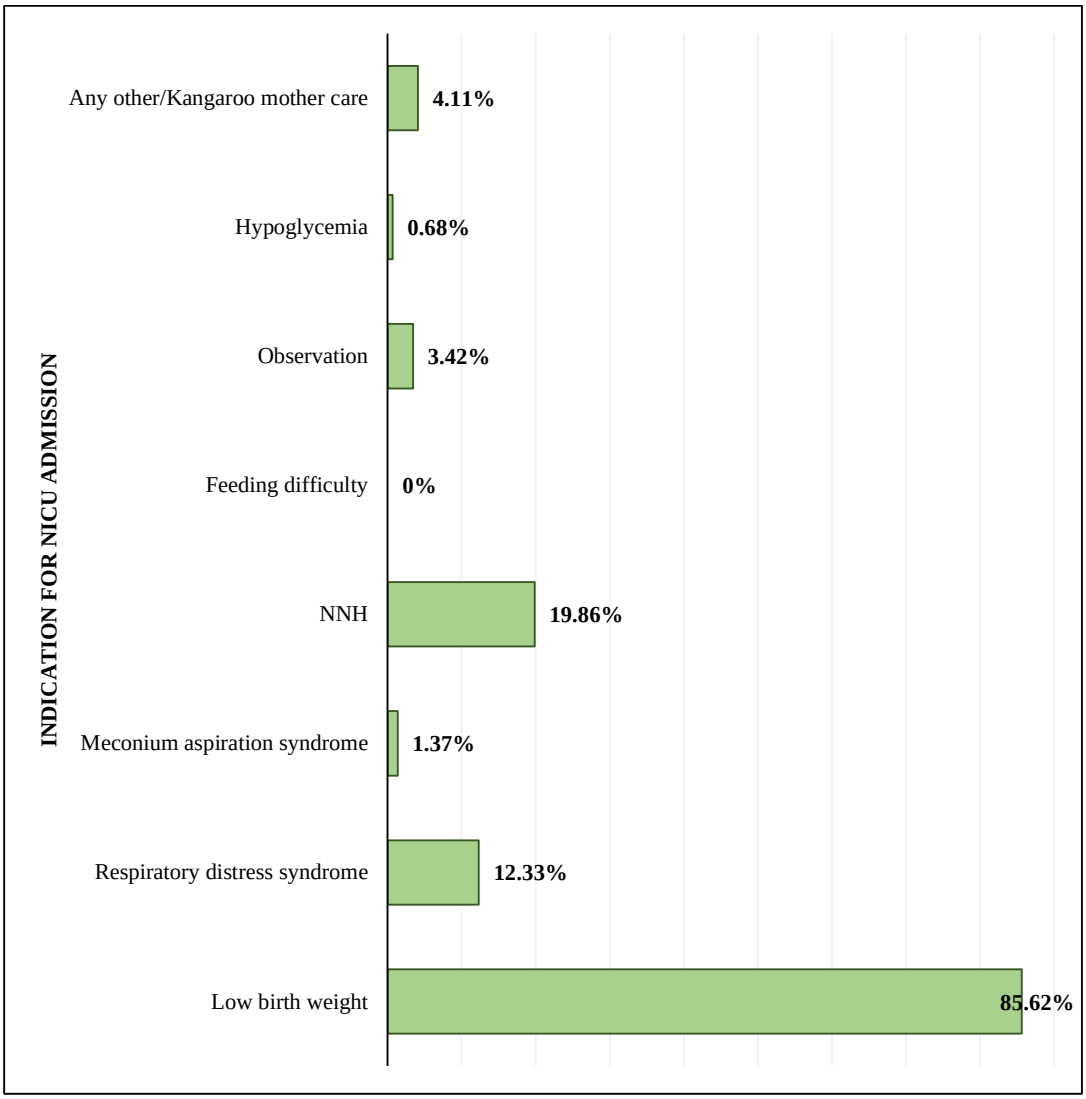


Figure 5: Distribution of infants according to indication for NICU admission

4. FEEDING PROFILE

- The following table gives the distribution of infants according to indication for starting PDHM

Table 4: Indications of starting PDHM

Indication For Starting PDHM	Number of subjects (%)
Mother not healthy to feed	8 (5.48%)
Mother not having enough secretions	133 (91.1%)
Mother is not healthy to feed and does not have enough secretions	5 (3.42%)

The majority of infants (91.1%) were given PDHM because the mother had insufficient milk secretion. A smaller proportion (5.48%) received PDHM due to the mother not being healthy to breastfeed.

- The following table gives the distribution of infants according to the batch numbers of pooled PDHM.

Table 5: Distribution of PDHM batches

Batch Number	Number of subjects (%)
1	11 (7.53%)
2	10 (6.85%)
3	10 (6.85%)
4	13 (8.9%)
5	11 (7.53%)
6	12 (8.22%)
7	7 (4.79%)
8	11 (7.53%)
9	12 (8.22%)
10	11 (7.53%)
11	13 (8.9%)
12	12 (8.22%)
13	13 (8.9%)

Batch no. 4, 11 and 13 of PDHM were dispensed to 13 infants each, batch no. 6, 9 and 12 to 12 infants each, batch no. 1, 5, 8 and 10 to 11 infants each, batch no. 2 and 3 to 10 infants each and batch no. 7 to 7 infants respectively.

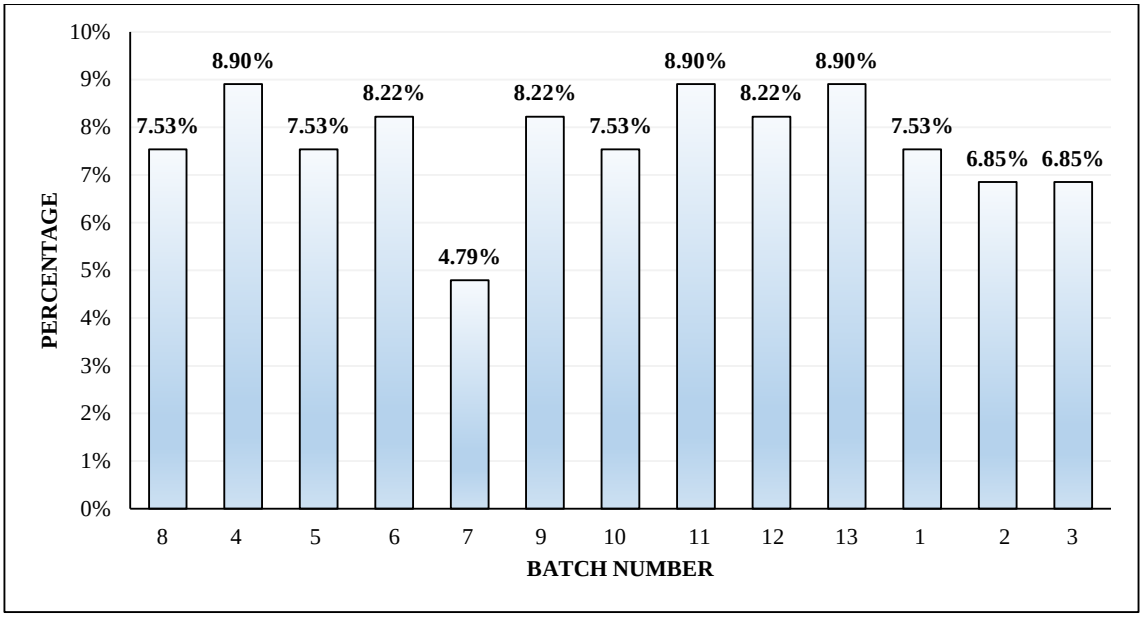


Figure 6: Distribution of infants according to PDHM batch number

- The following table gives the distribution of total volume of PDHM and total duration of PDHM.

Table 6: Total volume and duration of PDHM.

Variables	Sub Category	Number of subjects (%)
Total volume of PDHM (ml)	<500	115 (78.77%)
	500-1000	26 (17.81%)
	>1000	5 (3.42%)
	Mean ± SD Median (Min, Max)	382.26 ± 302.91 300 (70, 2320)
Total duration of PDHM (days)	Mean ± SD Median (Min, Max)	4.03 ± 1.75 3 (2, 10)

The majority of infants (78.77%) received less than 500 ml of PDHM, with only a small percentage receiving larger volumes (17.81% received between 500-1000 ml, and 3.42% received over 1000 ml). The mean total volume of PDHM consumed by the subjects was 382.26 ml (±302.9) with a mean total duration of 4.03 days (±1.75).

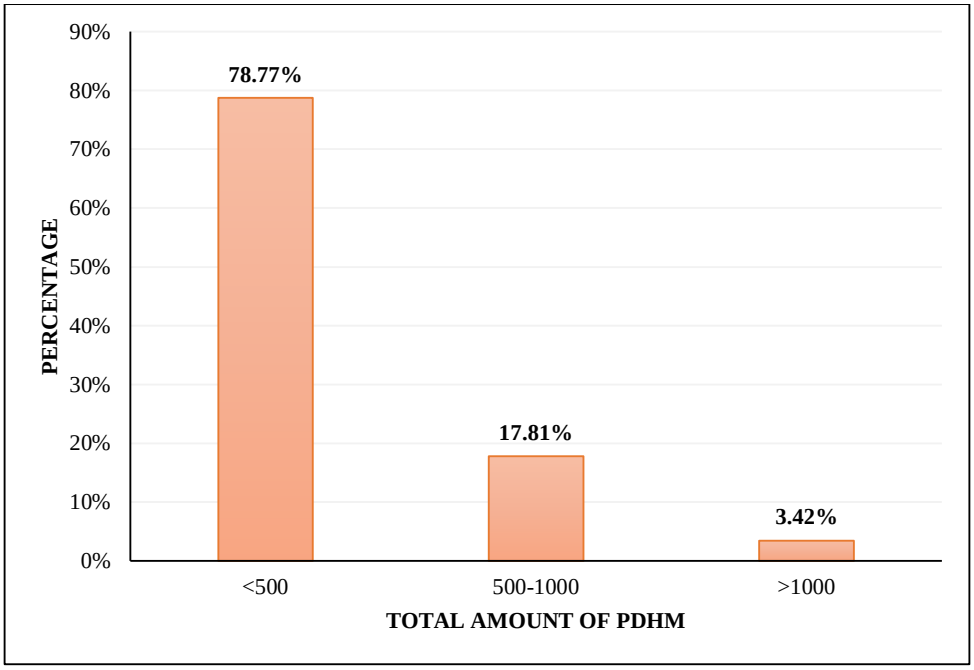


Figure 7: Distribution of infants according to total volume of PDHM.

5. DONOR MOTHER PROFILE

- The following table shows the mean values of donor mother characteristics

Table 7: Donor mother profile

Donor mother characteristics	Mean
Gestational age (weeks)	38.47 ±1.34
Maternal age (years)	23.69 ±2.9
Post natal day (days)	4.61 ±1.6

The mean GA of the donor mothers - was 38.47 (± 1.34) weeks and the mean maternal age was 23.69 (± 2.9) years. The post-natal day at which human milk was donated was a mean of 4.61 days.

6. PRIMARY OUTCOME

MICRONUTRIENT ANALYSIS OF PDHM:

- The following table gives the mean values of micronutrients in PDHM

Table 8: Mean micronutrient composition

Variables	Mean ± SD	Median (Min, Max)
Calcium	213.68 ± 57.7	201.52 (107.5, 320)
Zinc	0.42 ± 0.15	0.4 (0.15, 0.67)
Iron	0.1 ± 0.03	0.1 (0.01, 0.14)

The mean calcium content in PDHM was 213.68 mg ± 57.7 mg, while the mean zinc content was 0.42 mg ± 0.15 mg and mean iron content was 0.1 mg ± 0.03 mg.

- The following table shows the micronutrient composition of PDHM batch wise

Table 9: Batchwise micronutrient composition

Batch no.	Calcium (mg/dl)	Zinc (mg/dl)	Iron (mg/dl)
1	160	0.38	0.08
2	107.5	0.23	0.01
3	158.48	0.67	0.12
4	167.36	0.36	0.14
5	195.36	0.64	0.14
6	240.6	0.28	0.09
7	201.52	0.44	0.08
8	244.36	0.15	0.08
9	209.5	0.44	0.1
10	299.86	0.4	0.12
11	320	0.37	0.08
12	186	0.41	0.12
13	251	0.65	0.14

❖ **Correlation between micronutrient composition of PDHM and donor mother profile:**

➢ The following tables show the correlation between the donor mother characteristics and each of the micronutrients:

Table 10: Correlation between calcium and donor parameters

Pairs	Pearson Correlation (r)	P-value
Calcium (mg/dl) vs mean donor gestational age (weeks)	-0.413	0.161
Calcium (mg/dl) vs mean donor maternal age (years)	0.363	0.223
Calcium (mg/dl) vs mean post-natal day (days)	-0.082	0.789

No significant correlation between the calcium levels in PDHM and the donor mother characteristics was observed.

Table 11: Correlation between zinc and donor parameters

Pairs	Pearson Correlation (r)	P-value
Zinc (mg/dl) vs Mean donor gestational age (weeks)	0.059	0.848
Zinc (mg/dl) vs Mean donor maternal age (years)	0.250	0.411
Zinc (mg/dl) vs Mean post-natal day (days)	-0.690	**0.028**

There is a moderate to strong negative correlation between the zinc content of PDHM and the mean post-natal day of donation, with statistical significance (p=0.028), indicating that increase in postnatal day of milk donation is associated with decrease in the zinc content of PDHM.

Table 12: Correlation between iron and donor parameters

Pairs	Pearson Correlation (r)	P-value
Iron (mg/dl) vs Mean donor gestational age (weeks)	-0.237	0.435
Iron (mg/dl) vs Mean donor maternal age (years)	0.169	0.580
Iron (mg/dl) vs Mean post-natal day (days)	0.273	0.366

No significant correlation between the calcium levels in PDHM and the donor mother characteristics was observed.

7. SECONDARY OUTCOMES

A) MACRONUTRIENT ANALYSIS OF PDHM

- The following table gives the mean values of macronutrients in PDHM

Table 13: Mean macronutrient composition

Variables	Mean ± SD	Median (Min, Max)
Energy	61.09 ± 3.05	60.62 (56.97, 70.04)
Carbohydrates	7.47 ± 0.21	7.51 (6.84, 7.68)
Protein	1.69 ± 0.03	1.68 (1.64, 1.76)
Fat	1.94 ± 0.13	1.92 (1.77, 2.26)

In terms of energy, PDHM provided a mean value of 61.09 ± 3.05 kcal. The mean carbohydrate content was 7.47 ± 0.21 g/dl and mean protein content was 1.69 ± 0.03 g/dl while mean fat content was 1.94 ± 0.13 g/dl.

- The following table shows the macronutrient composition of PDHM batch

Table 14: Batchwise macronutrient composition

Batch no.	Energy (kcal/100gm)	Carbohydrates (g/dl)	Protein (g/dl)	Fat (g/dl)
1	58.79	7.42	1.72	1.8
2	70.04	6.84	1.76	2.26
3	62.61	7.19	1.7	1.92
4	59.7	7.51	1.69	1.93
5	60.8	7.54	1.68	1.85
6	56.97	7.68	1.69	1.77
7	60.15	7.5	1.68	1.9
8	62.34	7.41	1.76	1.97
9	64.09	7.56	1.64	2.12
10	60.45	7.6	1.67	2
11	60.62	7.66	1.68	2.05
12	60.78	7.44	1.65	1.84
13	58.37	7.57	1.67	1.83

❖ **Correlation between macronutrient composition of PDHM and donor mother profile:**

➢ The following tables show the correlation between the donor mother characteristics and each of the micronutrients:

Table 15: Correlation between energy and donor parameters

Pairs	Pearson Correlation (r)	P-value
Energy (kcal/100gm) vs Mean donor gestational age (weeks)	0.460	0.113
Energy (kcal/100gm) vs Mean donor maternal age (years)	-0.347	0.246
Energy (kcal/100gm) vs Mean post-natal day (days)	-0.593	**0.032**

There is a moderate to strong negative correlation between the energy content of PDHM and the mean post-natal day of donation, with statistical significance (p=0.032), indicating that increase in postnatal day of milk donation is associated with decrease in the energy content of PDHM.

Table 16: Correlation between carbohydrates and donor parameters

Pairs	Pearson Correlation (r)	P-value
Carbohydrates (g/dl) vs mean donor gestational age (weeks)	-0.379	0.202
Carbohydrates (g/dl) vs mean donor maternal age (years)	0.312	0.299
Carbohydrates (g/dl) vs mean post-natal day (days)	0.400	0.176

No significant correlation between the carbohydrate levels in PDHM and the donor mother characteristics was observed.

Table 17: Correlation between protein and donor parameters

Pairs	Pearson Correlation (r)	P-value
Protein (g/dl) vs mean donor gestational age (weeks)	-0.626	**0.023**
Protein (g/dl) vs mean donor maternal age (years)	-0.055	0.158
Protein (g/dl) vs mean post-natal day (days)	-0.670	**0.021**

There is a strong negative correlation between the protein content of PDHM and the mean donor gestational age as well as the mean post-natal day of milk donation, with statistical significance ($p<0.021$), indicating that higher gestational age and increase in postnatal day of milk donation are associated with decrease in the protein content of PDHM.

Table 18: Correlation between fat and donor parameters

Pairs	Pearson Correlation (r)	P-value
Fat (g/dl) vs mean donor gestational age (weeks)	0.472	0.104
Fat (g/dl) vs mean donor maternal age (years)	-0.216	0.478
Fat (g/dl) vs mean post-natal day (days)	-0.612	**0.049**

There is a strong negative correlation between the fat content of PDHM and the mean post-natal day of donation, with statistical significance ($p<0.049$), indicating that increase in postnatal day of milk donation is associated with decrease in the fat content of PDHM.

B) GROWTH

- The following table gives the comparison of anthropometric parameters across different time points

Table 19: Comparison of Anthropometric parameters

Time points	Weight (g)	Length (cm)	Head circumference (cm)	Mid arm circumference (cm)
At enrollment	1981.37 ± 425.21 1955 (1200, 3280)	46.2 ± 2.89 46 (38, 54)	31.61 ± 1.89 32 (27, 35.5)	7.86 ± 0.86 8 (5.5, 10)
At Discharge	1974.11 ± 432.36 1925 (1190, 3250)	46.62 ± 2.76 46.4 (38, 55)	31.99 ± 1.73 32 (28, 36)	8.04 ± 0.77 8 (6, 10.5)
Follow-up at 2weeks	2038.58 ± 509.26 2020 (2.32, 3420)	46.87 ± 2.86 46.7 (38, 55)	32.22 ± 1.86 32.3 (28, 36)	8.12 ± 0.85 8 (5.5, 10.5)
Follow-up at 6weeks	2850.51 ± 501.48 2845 (1420, 4700)	49.13 ± 2.88 48.9 (40, 58)	34.33 ± 1.76 34.5 (30, 39)	9.04 ± 0.85 9 (6.5, 11.5)
p-value	**< 0.001^{F}***	**< 0.001^{F}***	**< 0.001^{F}***	**< 0.001^{F}***
At Discharge – At enrollment	-7.26 ± 97.87 -20 (-240, 350)	0.43 ± 0.48 0.4 (0, 2)	0.38 ± 0.46 0 (-1, 1.5)	0.17 ± 0.25 0 (0, 1)
At 2weeks– At enrollment	57.21 ± 213.64 80 (-2197.68, 350)	0.67 ± 0.34 0.5 (0, 2)	0.61 ± 0.34 0.5 (0, 1)	0.26 ± 0.25 0.4 (0, 0.5)
At 6weeks– At enrollment	869.14 ± 209.73 865 (140, 1810)	2.93 ± 0.86 3 (1.5, 6.5)	2.72 ± 0.6 3 (1.5, 4)	1.17 ± 0.33 1 (0.5, 2)

At enrolment, the mean weight was 1981.37 grams (±425.21), length was 46.2 cm (±2.89), head circumference was 31.61 cm (±1.89), and mid arm circumference was 7.86 cm (±0.86). At discharge, there was a decrease in weight to 1974.11 grams (±432.36), while other parameters showed marginal increase. On follow-up at 2 weeks and 6 weeks of life, there was an increase in all anthropometric parameters,

indicating significant growth over time. At the 6 weeks follow-up, the mean weight increased significantly to 2850.51 grams (±501.48), length to 49.13 cm (±2.88), head circumference to 34.33 cm (±1.76), and mid arm circumference to 9.04 cm (±0.85). The Friedman's test indicates statistical significance (p-values < 0.001) for all parameters over time, demonstrating significant changes in anthropometric measures from enrolment till follow up at 6 weeks. Additionally, post hoc analysis reveals significant differences in the distribution of weight, length, head circumference, and mid arm circumference across all time point pairs (p-value < 0.05 for all comparisons).

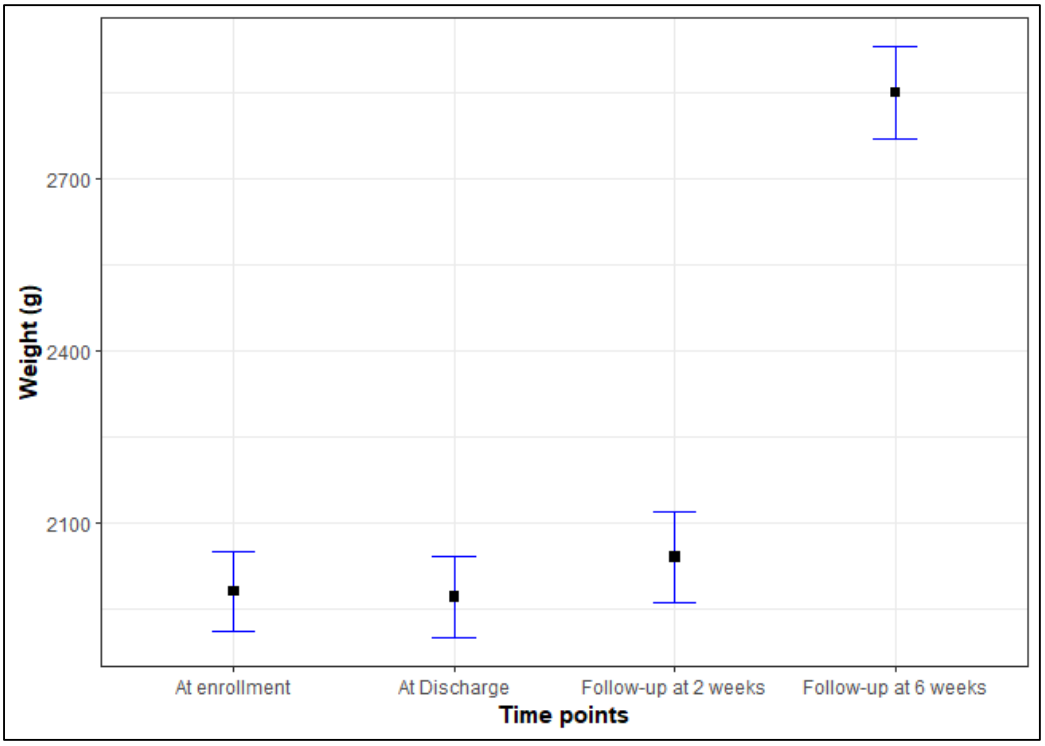


Figure 8: Mean plot of weight over time points.

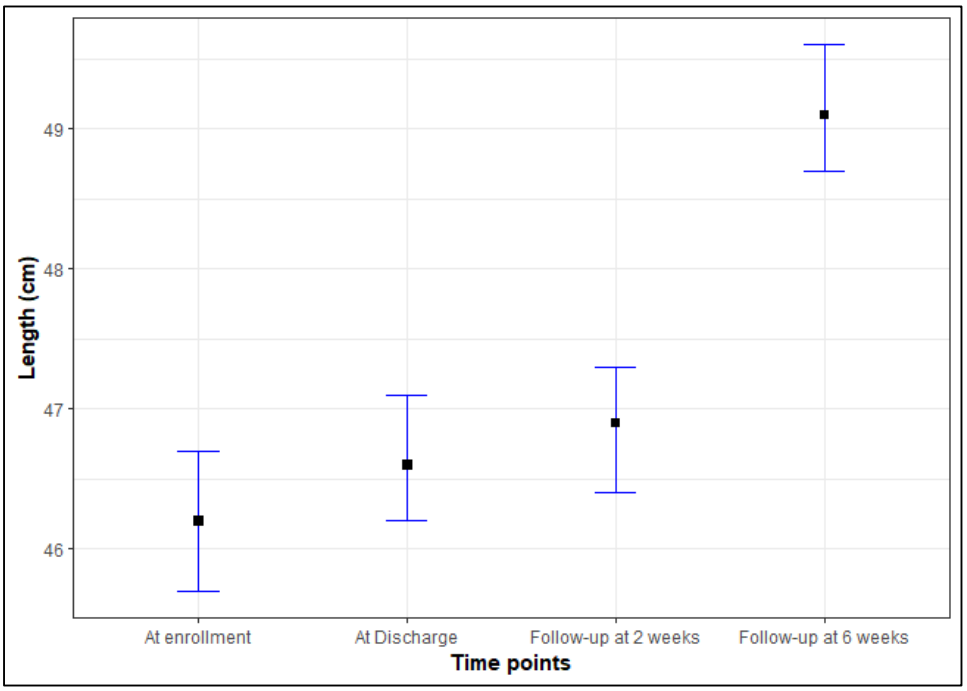


Figure 9: Mean plot of length over time points

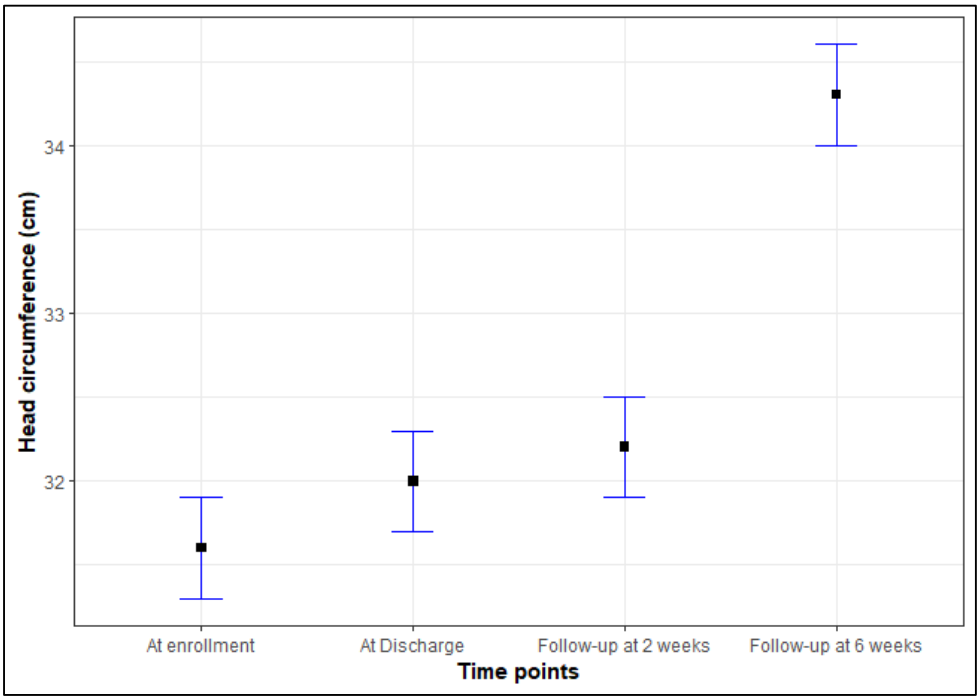


Figure 10: Mean plot of head circumference over time points

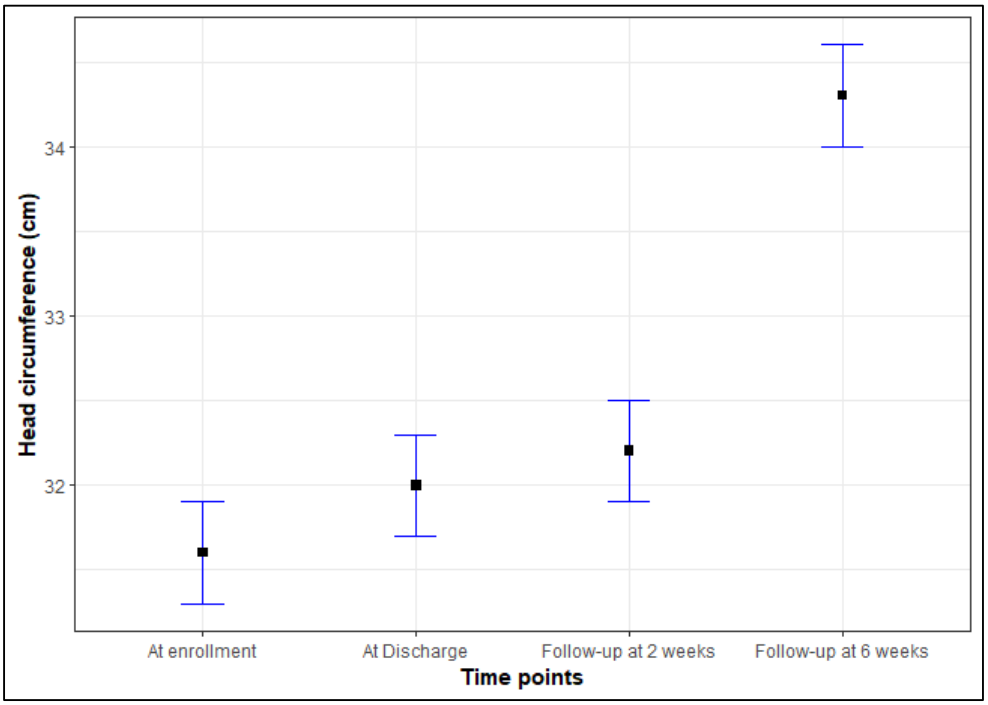


Figure 11: Mean plot of mid arm circumference over time points

C) CORRELATION BETWEEN MICRONUTRIENT AND MACRONUTRIENT COMPOSITION AND GROWTH OUTCOME

- The following table shows the average increase in anthropometric parameters according to batch of PDHM:

Table 20: Batch-wise increase in anthropometric parameters

Batch no.	Average increase in weight (gm)	Average increase in length (cm)	Average increase in HC (cm)	Average increase in MAC (cm)
1	945.45	4.35	3.32	0.955
2	925	3.3	3.15	1.2
3	986	3.65	3.1	1.4
4	964.62	3.31	3.00	1.35
5	913.64	3.00	2.73	1.32
6	860.83	2.96	2.83	1.04
7	731.43	3.07	3.14	1.50
8	960.00	2.90	2.75	1.36
9	842.50	2.48	2.62	1.00
10	756.36	2.78	2.43	1.03
11	743.08	2.42	2.42	0.97
12	633.33	2.11	2.25	1.09
13	706.54	2.19	2.02	1.23

It was noted that average increase in weight was maximum in infants receiving PDHM from batch no. 3 and minimum in those receiving PDHM from batch no. 13. It was also observed that the average increase in length was maximum in infants receiving PDHM from batch no. 1 and minimum in those receiving PDHM from batch no. 12 while the average increase in HC was maximum in infants receiving batch no. 1 and minimum in those receiving batch no. 13 of PDHM. Infants receiving PDHM from batch no. 7 showed maximum average increase in HC and those receiving it from batch no. 1 showed minimum increase.

MICRONUTRIENTS

1. Calcium:

- The following table shows the correlation between calcium content of PDHM and the growth outcomes-

Table 21: Correlation between calcium and growth parameters

Pairs	Pearson Correlation (r)	P-value
Calcium (mg/dl) vs average birth weight gain (grams)	-0.492	0.088
Calcium (mg/dl) vs average length gain (cm)	-0.559	0.067
Calcium (mg/dl) vs average head circumference gain (cm)	-0.652	0.072
Calcium (mg/dl) vs average mid arm circumference gain (cm)	-0.338	0.259

2. Zinc:

- The following table shows the correlation between zinc content of PDHM and the growth outcomes-

Table 22: Correlation between zinc and growth parameters

Pairs	Pearson Correlation (r)	P-value
Zinc (mg/dl) vs average birth weight gain (grams)	-0.155	0.612
Zinc (mg/dl) vs average length gain (cm)	-0.075	0.806
Zinc (mg/dl) vs average head circumference gain (cm)	-0.239	0.432
Zinc (mg/dl) vs average mid arm circumference gain (cm)	0.226	0.457

3. Iron:

- The following table shows the correlation between iron content of PDHM and the growth outcomes-

Table 23: Correlation between iron and growth parameters

Pairs	Pearson Correlation (r)	P-value
Iron (mg/dl) vs average birth weight gain (grams)	-0.168	0.583
Iron (mg/dl) vs average length gain (cm)	-0.264	0.383
Iron (mg/dl) vs average head circumference gain (cm)	-0.462	0.112
Iron (mg/dl) vs average mid arm circumference gain (cm)	0.149	0.627

No significant correlation was observed between the calcium, zinc and iron content of PDHM and the average gain in all the anthropometric parameters.

MACRONUTRIENTS

1. Energy:

- The following table shows the correlation between energy content of PDHM and the growth outcomes.
-

Table 24: Correlation between energy and growth parameters

Pairs	Pearson Correlation (r)	P-value
Energy (kcal/100gm) vs average birth weight gain (grams)	0.273	0.367
Energy (kcal/100gm) vs average length gain (cm)	0.064	0.836
Energy (kcal/100gm) vs average head circumference gain (cm)	0.253	0.405
Energy (kcal/100gm) vs average mid arm circumference gain (cm)	0.110	0.720

2. Carbohydrates:

- The following table shows the correlation between carbohydrate content of PDHM and the growth outcomes-

Table 25: Correlation between carbohydrates and growth parameters

Pairs	Pearson Correlation (r)	P-value
Carbohydrates (g/dl) vs Average Birth Weight Gain (grams)	-0.417	0.156
Carbohydrates (g/dl) vs Average Length Gain (cm)	-0.408	0.167
Carbohydrates (g/dl) vs Average Head Circumference Gain (cm)	-0.490	0.089
Carbohydrates (g/dl) vs Average Mid Arm Circumference Gain (cm)	-0.289	0.338

3. Protein:

- The following table shows the correlation between protein content of PDHM and the growth outcomes-

Table 26: Correlation between proteins and growth parameters

Pairs	Pearson Correlation (r)	P-value
Protein (g/dl) vs Average Birth Weight Gain (grams)	0.639	**0.019**
Protein (g/dl) vs Average Length Gain (cm)	0.554	**0.049**
Protein (g/dl) vs Average Head Circumference Gain (cm)	0.561	**0.046**
Protein (g/dl) vs Average Mid Arm Circumference Gain (cm)	0.267	0.378

There is a moderate to strong positive correlation between the protein content of PDHM and the average gain in weight, length and head circumference, with statistical significance (p=0.019, 0.049, 0.046 respectively), indicating that higher protein content is associated with greater increase in all the anthropometric parameters except mid arm circumference.

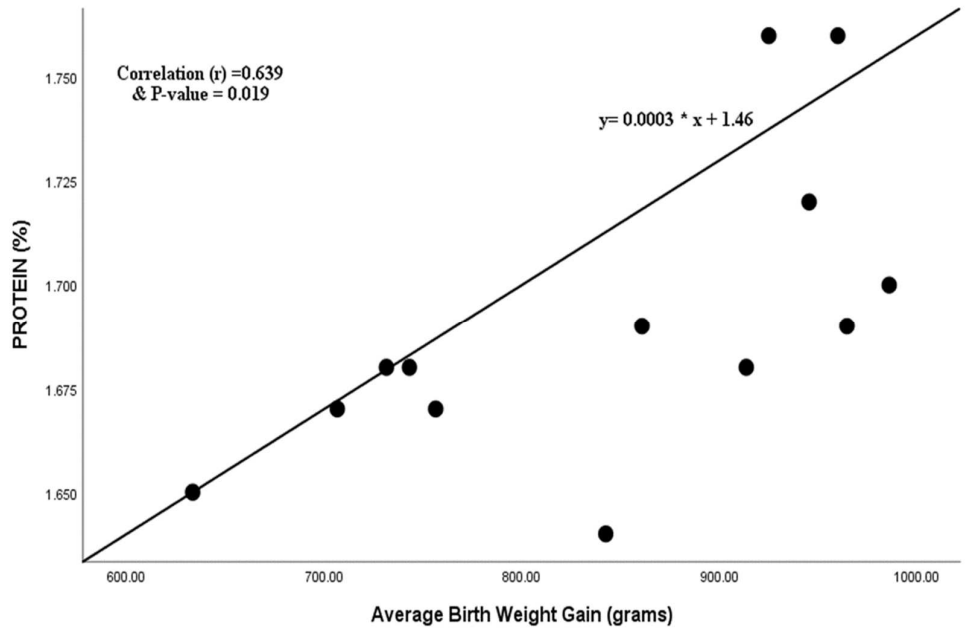


Figure 12: Scatter plot between Protein (%) and Average weight gain (grams)

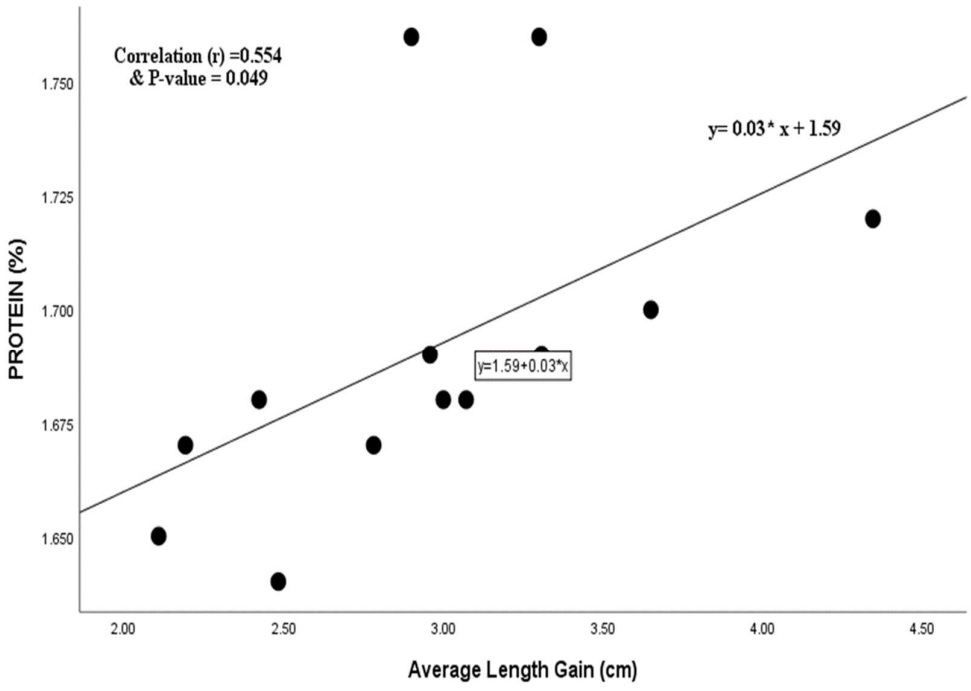


Figure 13: Scatter plot between Protein (%) and average length gain (cm)

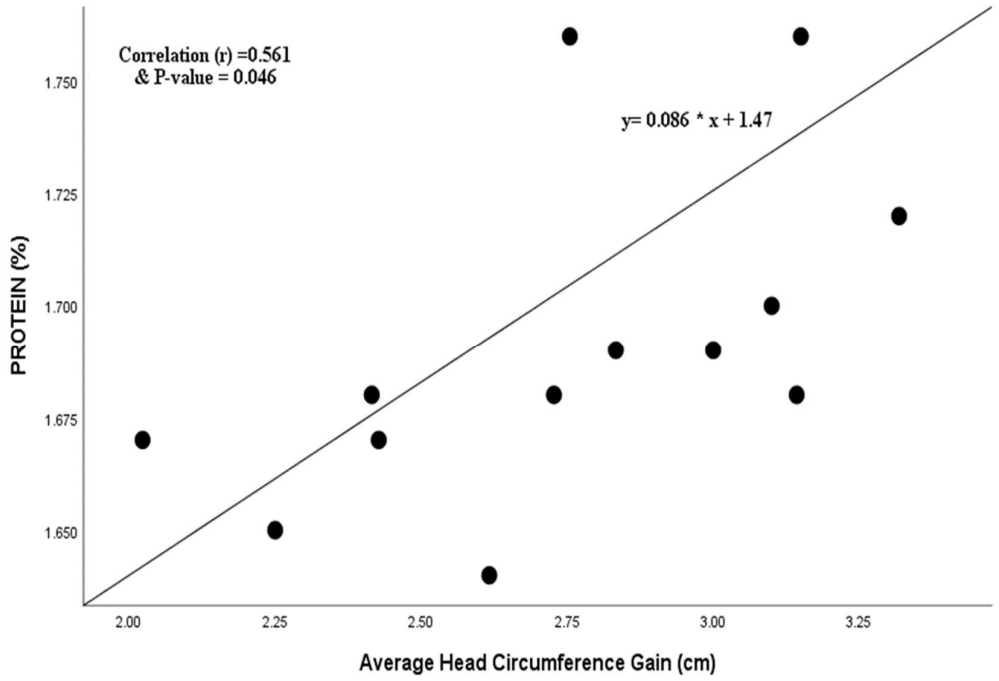


Figure 14: Scatter plot between Protein (%) and average HC Gain (cm)

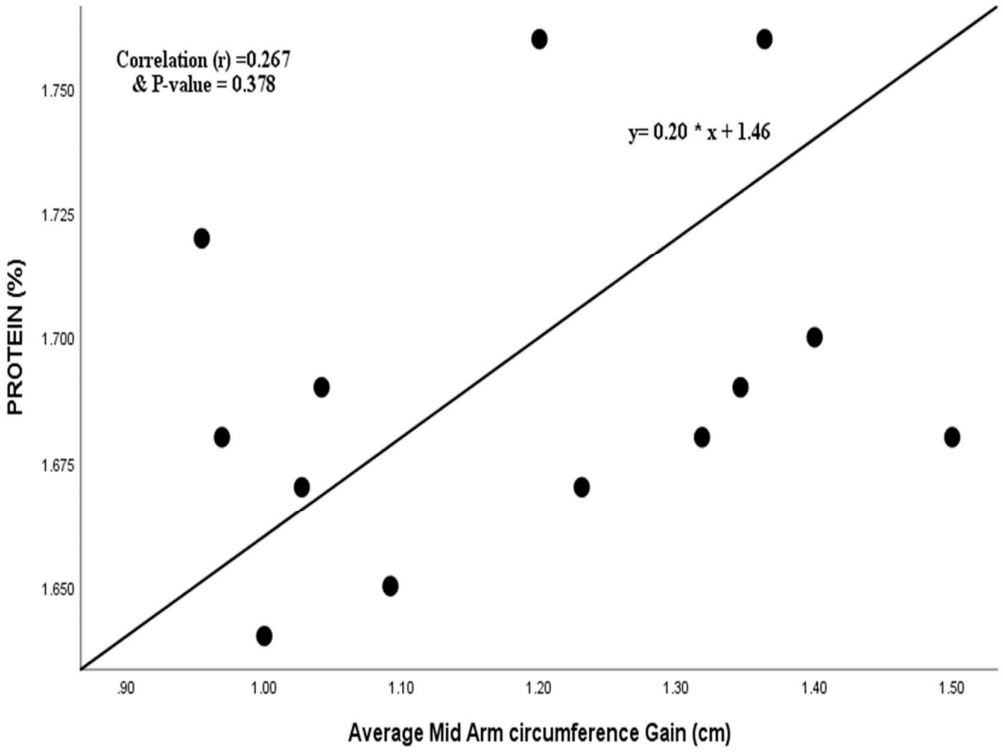


Figure 15: Scatter plot between Protein (%) and average MAC gain (cm)

4. Fats:

- The following table shows the correlation between fat content of PDHM and the growth outcomes-

Table 27: Correlation between fat and growth parameters

Pairs	Pearson Correlation (r)	P-value
Fat (g/dl) vs Average Birth Weight Gain (grams)	0.134	0.663
Fat (g/dl) vs Average Length Gain (cm)	-0.096	0.756
Fat (g/dl) vs Average Head Circumference Gain (cm)	0.093	0.762
Fat (g/dl) vs Average Mid Arm Circumference Gain (cm)	-0.084	0.785

No significant correlation was observed between the energy, carbohydrate and fat content of PDHM and the average gain in all the anthropometric parameters.

D) FACTORS AFFECTING THE GROWTH OUTCOMES

- The following tables shows the multiple linear regression analysis of the effect of various factors on the increase in anthropometric parameters over a period of 6 weeks-

1. Weight

Table 28: Factors affecting weight gain (grams)

Parameters	Beta Coefficients	95 % CI		P-value
		Lower CI	Upper CI	
(Intercept)	208.24	-689.30	1105.77	0.647
Age (Day of life)	16.42	-16.75	49.60	0.329
Maternal age (Years)	-10.45	-20.45	-0.46	**0.041**
Gravida	13.20	-23.06	49.46	0.473
Gestational age (weeks)	20.78	3.33	38.24	**0.020**
Total amount of PDHM (ml)	0.09	-0.08	0.27	0.303
Total duration of PDHM (days)	-4.38	-37.80	29.05	0.796
Gender (Baseline=Female)				
Gender male	6.51	-65.71	78.74	0.859
Mother's education (Baseline = Primary)				
Secondary	75.65	-22.31	173.61	0.129
Graduate	39.64	-89.21	168.48	0.544
Mother's Occupation (Baseline = Employed)				
Homemaker	120.71	-41.95	283.37	0.144
Socio economic status (Modified B G Prasad) [Baseline = Upper class]				
Upper middle class	-16.18	-137.53	105.16	0.792
Middle class	-117.24	-265.73	31.25	0.121
Lower middle class	206.42	16.88	395.96	**0.033**
Antenatal risk factors (Baseline = Absent)				
Present	-12.56	-113.88	88.75	0.807
Mode of delivery (Baseline = NVD)				
LSCS	15.60	-74.16	105.36	0.731
Indication for NICU admission (Baseline = Other Indications*)				
Low birth weight	25.87	-89.18	140.93	0.657

- **Maternal age** had a significant negative effect on weight gain (Beta = -10.45, 95% CI: -20.45, -0.46, P = 0.041), indicating that older maternal age is associated with reduced weight gain.
- **Gestational age** showed a significant positive effect on weight gain (Beta = 20.78, 95% CI: 3.33, 38.24, P = 0.020), suggesting that higher gestational age correlates with increased weight gain.
- **Socioeconomic status of lower middle class** showed a significant positive effect on weight gain (beta = 206.42. 95% CI:16.88,395.96, P = 0.033), indicating that infants belonging to lower middle class showed increased weight gain.

2. Length

Table 29: Factors affecting length gain (cm)

Parameters	Beta Coefficients	95 % CI		P-value
		Lower CI	Upper CI	
(Intercept)	3.955	0.256	7.655	**0.036**
Age (Day of life)	-0.021	-0.158	0.116	0.764
Maternal age (Years)	-0.031	-0.072	0.010	0.139
Gravida	0.047	-0.103	0.197	0.534
Gestational age (weeks)	-0.027	-0.113	0.059	0.532
Birth weight (gm)	0.000	-0.001	0.000	0.608
Total amount of PDHM (ml)	0.000	-0.001	0.001	0.913
Total duration of PDHM (days)	0.035	-0.106	0.175	0.627
Gender (Baseline=Female)				
Gender male	-0.247	-0.551	0.057	0.111
Mother's education (Baseline = Primary)				
Secondary	0.141	-0.263	0.545	0.490
Graduate	0.287	-0.246	0.819	0.288
Mother's Occupation (Baseline = Employed)				
Homemaker	0.723	0.048	1.398	**0.036**
Socio economic status (Modified B G Prasad) [Baseline = Upper class]				
Upper middle class	-0.129	-0.631	0.372	0.610
Middle class	-0.478	-1.090	0.134	0.125
Lower middle class	1.133	0.351	1.914	**0.005**
Antenatal risk factors (Baseline = Absent)				
Present	0.208	-0.219	0.636	0.337
Mode of delivery (Baseline = NVD)				
LSCS	-0.259	-0.630	0.112	0.170
Indication for NICU admission (Baseline = Other Indications)				
Low birth weight	-0.259	-0.839	0.321	0.378

- **Socioeconomic status of lower middle class** showed a significant positive effect on length gain (beta = 1.133.42, 95% CI:0.351,1.914, P = 0.005), indicating that infants belonging to lower middle class showed increased length gain.
- **Homemakers** as compared to other occupations of mothers showed a significant positive effect on length gain (beta = 0.723, 95% CI:0.048, 1.398, P = 0.036), indicating that infants of mothers who were homemakers showed increased length gain.

3. Head Circumference

Table 30: Factors affecting gain in HC (cm)

Parameters	Beta Coefficients	95 % CI		P-value
		Lower CI	Upper CI	
(Intercept)	2.432	-0.222	5.086	0.072
Age (Day of life)	0.016	-0.082	0.114	0.746
Maternal age (Years)	-0.027	-0.057	0.003	0.074
Gravida	0.003	-0.105	0.110	0.963
Gestational age (weeks)	0.003	-0.059	0.065	0.924
Birth weight (gm)	0.000	-0.001	0.000	0.095
Total amount of PDHM (ml)	0.000	-0.001	0.000	0.698
Total duration of PDHM (days)	0.037	-0.063	0.138	0.466
Gender (Baseline=Female)				
Gender male	0.027	-0.192	0.245	0.810
Mother's education (Baseline = Primary)				
Secondary	0.058	-0.231	0.348	0.692
Graduate	0.280	-0.102	0.662	0.149
Mother's Occupation (Baseline = Employed)				
Homemaker	0.421	-0.064	0.905	0.088
Socio economic status (Modified B G Prasad) [Baseline = Upper class]				
Upper middle class	0.035	-0.325	0.394	0.850
Middle class	0.013	-0.426	0.452	0.954
Lower middle class	0.107	-0.454	0.668	0.707
Antenatal risk factors (Baseline = Absent)				
Present	0.285	-0.022	0.591	0.069
Mode of delivery (Baseline = NVD)				
LSCS	0.166	-0.100	0.432	0.219
Indication for NICU admission (Baseline = Other Indications)				
Low birth weight	-0.230	-0.646	0.186	0.276

- None of the factors were found to have a statistically significant effect on gain in head circumference

4. Mid arm circumference

Table 31: Factors affecting gain in MAC (cm)

Parameters	Beta Coefficients	95 % CI		P-value
		Lower CI	Upper CI	
(Intercept)	0.78	-0.67	2.23	0.290
Age (Day of life)	0.01	-0.05	0.06	0.792
Maternal age (Years)	0.01	0.00	0.03	0.140
Gravida	0.00	-0.06	0.06	0.941
Gestational age (weeks)	-0.01	-0.05	0.02	0.441
Birth weight (gm)	0.00	0.00	0.00	0.314
Total amount of PDHM (ml)	0.00	0.00	0.00	0.362
Total duration of PDHM (days)	0.01	-0.05	0.06	0.776
Gender (Baseline=Female)				
Gender male	0.02	-0.10	0.14	0.749
Mother's education (Baseline = Primary)				
Secondary	0.15	0.00	0.31	0.056
Graduate	0.21	0.00	0.42	**0.047**
Mother's Occupation (Baseline = Employed)				
Homemaker	0.14	-0.13	0.40	0.306
Socio economic status (Modified B G Prasad) [Baseline = Upper class]				
Upper middle class	-0.03	-0.23	0.16	0.737
Middle class	-0.09	-0.33	0.15	0.476
Lower middle class	0.08	-0.22	0.39	0.594
Antenatal risk factors (Baseline = Absent)				
Present	-0.05	-0.22	0.12	0.544
Mode of delivery (Baseline = NVD)				
LSCS	0.10	-0.05	0.24	0.183
Indication for NICU admission (Baseline = Other Indications*)				
Low birth weight	-0.05	-0.28	0.17	0.647

- **Mother's education status of graduate** showed a significant positive effect on MAC gain (beta = 0.21, 95% CI:0.00,0.42, P = 0.047), indicating that infants of mothers who were graduates showed increased MAC gain.

E) TYPE OF METHOD OF FEEDING

- The following table gives the distribution of type of feeding of infants at different time points.

Table 32: Type of feeding

Type of feeding	At Enrolment	At Discharge	Follow-up at 2weeks	Follow-up at 6weeks
DHM	2 (1.37%)	0	0	0
DHM + MOM	144 (98.63%)	0	0	0
Lactogen	0	2 (1.37%)	2 (1.37%)	2 (1.37%)
MOM	0	144 (98.63%)	144 (98.63%)	144 (98.63%)

At enrolment, the predominant feeding method was a combination of DHM and MOM, accounting for 98.63% of infants, while a very small percentage (1.37%) received only DHM. No infants were exclusively fed with lactogen at enrolment. At discharge and during follow-up at 2 weeks and 6 weeks of life, all infants were exclusively receiving MOM (mother's own milk), constituting 98.63% of the cohort at each of these time points. A minority of infants (1.37%) were discharged on lactogen feeds and continued to use it on follow-up visits.

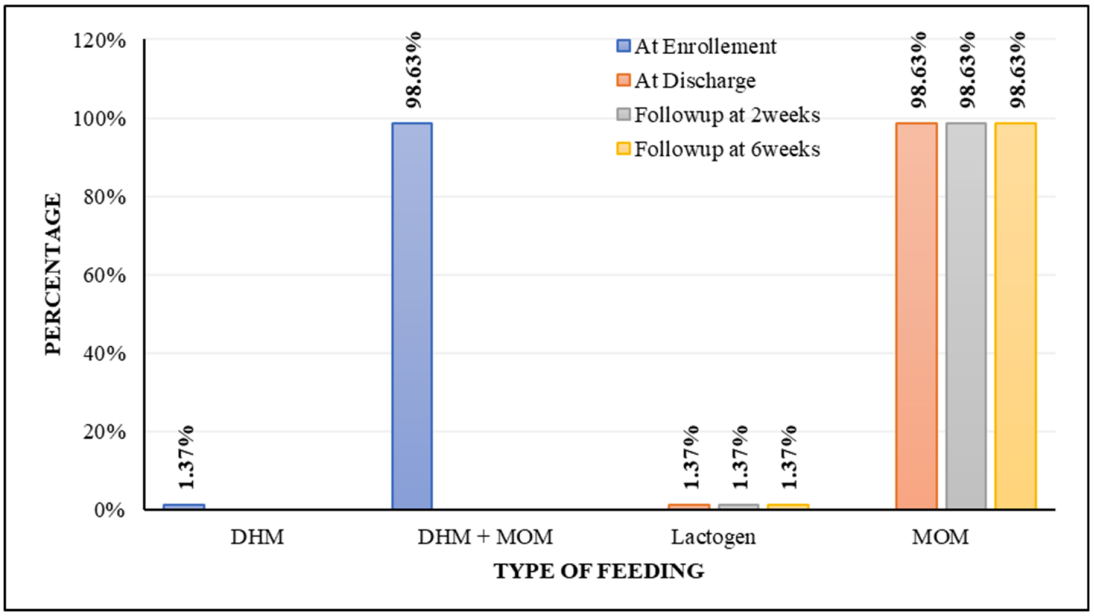


Figure 16: Distribution of type of feeding over time

- The following table gives the distribution of method of feeding of infants at different time points-

Method of Feeding	At Enrolment	At Discharge	Follow-up at 2weeks	Follow-up at 6weeks
RT FEEDS	19 (13.01%)	0	0	0
RT FEEDS + SF	1 (0.68%)	0	0	0
SF+DBF	96 (65.75%)	1 (0.68%)	0	0
SPOON FEEDS	30 (20.55%)	2 (1.37%)	2 (1.37%)	2 (1.37%)
DBF	0	143 (97.95%)	144 (98.63%)	144 (98.63%)

Table 33: Method of feeding

At enrolment, the majority of infants (65.75%) were fed using a combination of spoon feeds (SF) and direct breastfeeding (DBF), while a smaller percentage received only RT feeds (13.01%) or RT feeds combined with spoon feeds (SF) (0.68%). By the time of discharge and during follow-up at 2 weeks and 6 weeks of life, almost all infants (97.95% to 98.63%) were exclusively on direct breast feeding (DBF), with only a small proportion (1.37%) continuing to receive spoon feeds at later time points.

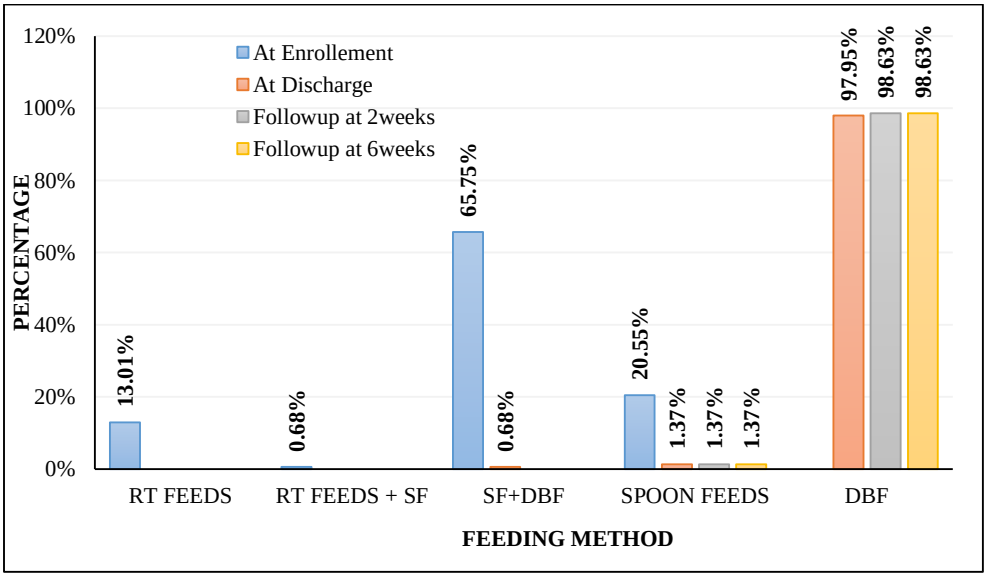


Figure 17: Distribution of method of feeding over time

F) DURATION OF HOSPITAL STAY

- The following table gives the distribution of infants according to duration of hospital stay.

Table 34: Duration of hospital stay

Variables	Sub Category	Number of subjects (%)
Total Duration of hospital stay (days)	Mean ± SD	9.97 ± 5.56
	Median (Min, Max)	8 (3, 34)

The mean duration of hospital stay for all subjects was 9.97 days (±5.56).

G) INCIDENCE OF NECROTIZING ENTEROCOLITIS AND SEPSIS

- The following table gives the distribution of infants according to complications during hospital stay.

Table 35: Complications

Variables	Sub Category	Number of infants (%)
Sepsis	No	140 (95.89%)
	Yes	6 (4.11%)
Necrotizing Enterocolitis	No	145 (99.32%)
	Yes	1 (0.68%)

The majority of infants did not experience any complication during their hospital stay. However, 4.11% of infants developed sepsis and 0.68% developed necrotizing enterocolitis.

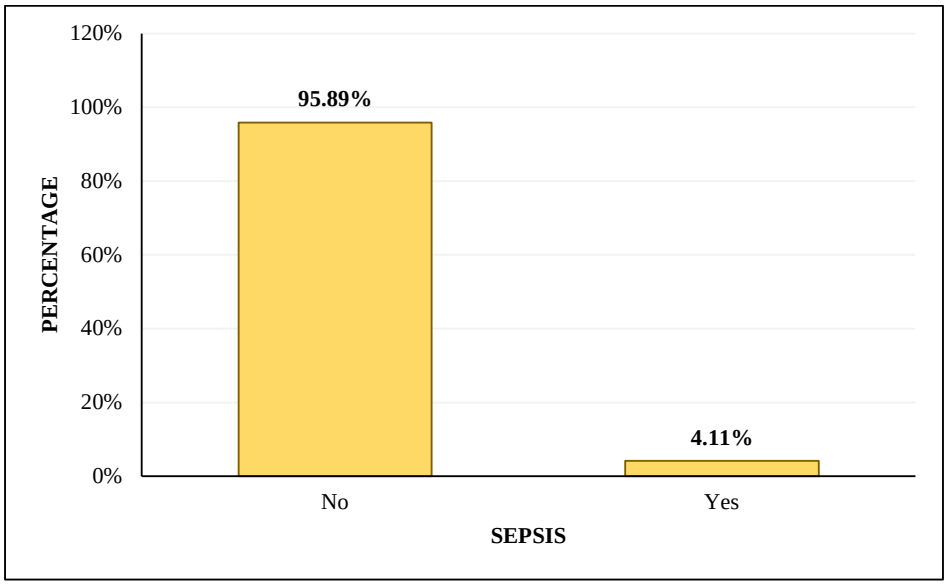


Figure 18: Incidence of sepsis

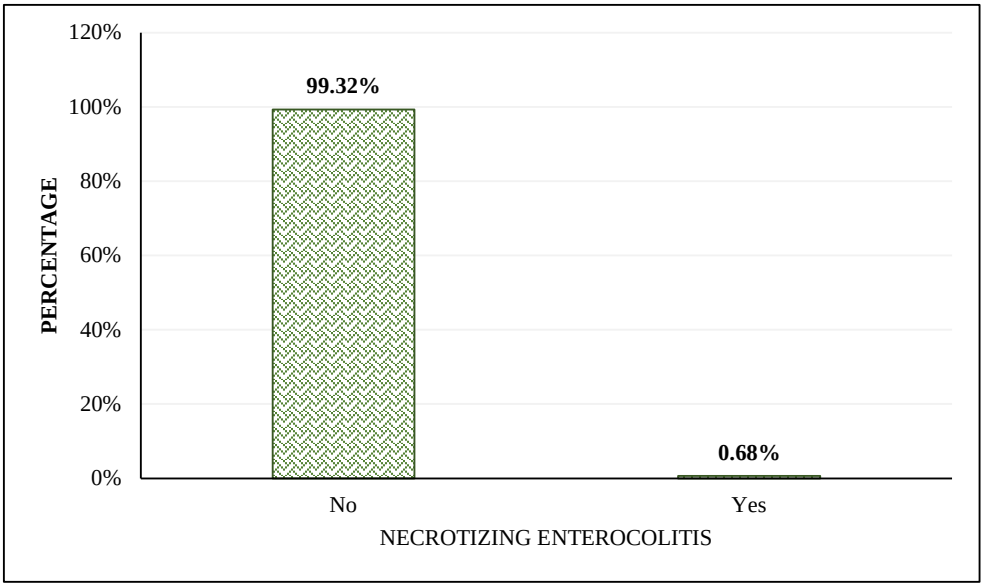


Figure19: Incidence of necrotizing enterocolitis

- The following table gives the distribution of infants with sepsis according to the blood culture-

Table 36: Blood culture reports

Organism	Number of infants
Klebsiella Pneumonia	3
Candida glabrata	1
Enterobacter sp.	1
Acinetobacter baumannii	1

DISCUSSION

Human milk is vital for the optimal growth and development of the infants because of its ideal nutrient composition. It also provides numerous advantages in terms of gastric, immune and neurodevelopmental functions. Adequate nutrition is of utmost importance and hence, WHO and UNICEF recommend early initiation of breastfeeding within 1 hour of birth, exclusive breast feeding for the first 6 months of life and continued breast feeding up to 2 years of age (40). When there is non-availability or inadequate production of mother's own milk, pasteurized donor human milk (PDHM) is recommended as the best alternative (8–11,54). PDHM confers several benefits especially in low birth weight infants. However, there is variable evidence regarding the effect of PDHM on the growth of newborn infants. The poorer growth is related to the nutrient composition of the PDHM. Therefore, analysis of the macronutrient and micronutrient composition of PDHM can be used to improve the infant feeding protocol through fortification strategies and thereby the growth of preterm infants. However, there is not much literature available on the macronutrient and micronutrient composition of PDHM especially in India and so the present study was conducted to analyse the micronutrient and macronutrient composition of PDHM and to study its effect on the growth parameters of infants.

SOCIODEMOGRAPHIC PROFILE: The sociodemographic characteristics of the infants enrolled in the study namely, age of infants at enrolment, gender, educational status and socioeconomic status of the family were similar to the earlier studies on feeding patterns of preterm infants.

The mean age of the infants at enrolment was 1.86 days (±1.1) in the study which was similar to the observations in several other studies conducted to know the

effect of PDHM on growth. A mean age of 2 days was reported by Colaizy et al. in a retrospective cohort study to determine the effect of human milk (MOM and DHM) on in-hospital growth of VLBW infants (65). Similar observations of the mean age of 1-2 days were reported by earlier in-house studies on impact of PDHM on growth of infants (162,164). On the contrary, an infant growth study by Bramer et al. on the use of PDHM in non-hospitalized newborns reported that the age of infants at 1st PDHM feed at enrolment ranged from 2 – 119 days (80).

The gender distribution of the infants observed in the study with a male preponderance (52.05%) was similar to the observations reported by several studies conducted on PDHM both in developed and developing countries(65,98). A male preponderance (57%) in the study participants was reported by Belfort et al. in a study to determine the association between macronutrient intake and growth outcomes (98). A cohort study on the impact of donor milk on short and long-term growth of VLBW infants by Hoban R et al. reported a similar observation with 54% males (20). Several Indian studies on the feeding patterns of preterm infants admitted in NICU have also reported a male preponderance (99).

Majority of the mothers of the enrolled infants (85.62%) belonged to the Hindu religion which was similar to the sociodemographic profile of Karnataka according to the NFHS-5 which stated that 88% were Hindus (42). Other studies conducted in similar settings have also observed similar findings. A study on the incidence of NEC in preterm infants receiving PDHM by Saurabh et al. also showed that 83.26% of the mothers of infants enrolled in the study belonged to Hindu religion (163). The majority of the families (94.29%) of infants who received PDHM in the study by Rohan et al. belonged to Hindu religion (162).

In the present study, 58.22% of the mothers had secondary education and 95.21% were homemakers. A study from Karnataka to assess the understanding and attitude of postnatal lactating mothers regarding human milk banking reported a 52% of the mothers having secondary education (100). Several studies on human milk banking have reported a similar observation regarding the occupation of the mothers as homemakers. A study on the risk factors associated with preterm delivery by Dahman et al. also reported 95.8 % of the mothers to be homemakers which was similar to our study (101). A questionnaire survey from south India on the perceptions of lactating mothers on human milk banking reported that 52% were homemakers (102). A study on the knowledge and attitude of Indian parous women towards human milk banking by Kaur et al. showed that 60% of the mothers were homemakers (103).

In our study, most of the mothers (58.22%) belonged to upper middle class as per the modified BG Prasad scale of socioeconomic status which is similar to many other Indian studies (100,103). The history of consanguineous marriage was seen in only 12.33% cases which was lesser than the prevalence of consanguineous marriage in Karnataka (26.4%) as per NFHS-5 (42) and study by Bellad et al. where 24.1% prevalence of consanguineous marriage in 4 PHCs of Belagavi district in Karnataka was reported (104).

MATERNAL PREGNANCY PROFILE:

Maternal age – The mean maternal age in our study was observed to be 25.7 ±3.73 years. This observation is similar to another study conducted by Ziem et al. in Ghana to determine antenatal risk factors that predispose to NICU admissions which noted that 57.2 % of the mothers enrolled in the study belonged to the age group of 20-30 years (105) Similar mean maternal age of 26 years was reported by

Velmurugan et al. in his study of perceptions of lactating mothers on human milk banking (102). The mean maternal age was found to be 23 years in a prospective observational study conducted by Yadav et al. in Mandya, Karnataka on the maternal risk factors associated with preterm birth (106). A community based longitudinal study on the risk factors associated with preterm birth in rural Mysore, Karnataka observed that women between the age of 25-29 years had a higher odd's ratio (OR=0.74) for preterm delivery (107).

On the contrary, studies on human milk (PDHM and MOM) showed higher mean maternal age. In a retrospective community observational study conducted by Bramer et al. which analysed the growth parameters and feeding in term infants who received PDHM, it was observed that the mean age of mothers of the infants was 38.9 years which is higher than observed in our study (80). In another study conducted by Belfort et al. on the macronutrient intake from human milk and growth in preterm infants, the mean maternal age was noted to be 34 years (98).

The difference in the mean maternal age in these studies from developed countries when compared to Indian studies may be related to the different social factors namely age at marriage and conception.

Gravida - In our study, there were equal number of primigravida and multigravida mothers which was similar to the observation of 46 % of the mothers being primigravida by Belfort et al. and Bramer et al. (80,98). Majority (62.24%) of the mothers delivering preterm were reported to be primigravida in a Karnataka study on infant feeding practices (108).

On the contrary, the retrospective study by Koullali et al. reported that the risk of preterm delivery increased with an increase in parity (109).

Antenatal risk factors – In our study, several antenatal risk factors were identified - twin/triplet pregnancy being the highest (32.88%) followed by intrauterine growth restriction (IUGR) (28.77%) and preeclampsia and eclampsia (23.97 %). Several studies and meta-analysis determined this association of twin/triplet pregnancy with increased incidence of stillbirths, preterm deliveries and increased admission to NICUs (110–112). Some of the studies on the etiological factors of preterm deliveries reported anemia to be the most common risk factor (111–113). On the contrary, PROM causing oligohydramnios was reported to be the most common cause of spontaneous preterm delivery in several other studies (106,114–116). Some of the South Indian studies have reported PIH as the most common risk factor causing preterm delivery (117–119).

BIRTH PROFILE:

Mode of delivery - The most common mode of delivery in the study was caesarian section, accounting for 78.77% cases. Similar observations of higher rate of caesarian sections as a risk factor associated with preterm delivery have been reported by several studies from both developed and developing countries. (99,120,121). The higher rate of LSCS in the present study can be attributed to the high risk pregnancies with complications being referred to our hospital as it is a tertiary care centre.

Gestational age – The mean gestational age of the infants was 35.91± 2.34 weeks in our study. Few other studies on PDHM also showed similar observations (80). A study conducted by Bramer at al. on the use of PDHM in non-hospitalized newborns showed that the mean gestational age at birth was 37.5 weeks (80). The studies on PDHM conducted earlier in the same hospital reported similar observations with the mean gestational age of 34-36 weeks (162,164).

Contrary to these observations, several other studies on PDHM showed lower mean gestational ages (19,25,65,78). In a study by Costa et al. on the tolerance of preterm formula versus PDHM, the mean gestational age reported was 30.2 weeks (19). Corpeleijn et al. reported a mean gestational age of 28.4 weeks in his study on the effect of PDHM on severe infections and mortality in VLBW infants (29). Colaizy et al. reported a mean gestational age of 27 weeks in his cohort study on human milk and growth outcomes (65). Majority of the infants enrolled in our study were stable and low birth weight which may be the reason for higher gestational age noted in our study compared to other studies.

Birth weight – The mean birth weight of the infants enrolled in the present study was 2008.15 grams. In a study by Quigley at al. to determine the effect of feeding with formula compared with PDHM on growth and development of infants, it was reported that all the infants enrolled had a birth weight of <2500 grams which was similar to our study (18). The study on the use of PDHM in non-hospitalized infants by Bramer et al. showed that the mean birth weight of infants enrolled in the study was 3300 grams (80). Contrary to our observations, most of the studies analysing the effect of feeding patterns on the growth outcomes reported gestational age of <32 weeks and birth weight < 1500 grams (17,19,25,65,98) .In a study to determine the associations of macronutrient intake from human milk with growth outcomes by Belfort et al. showed that the mean birth weight of the infants was 1104 grams (98). Jeong et al. reported <1500 grams of mean birth weight of the infants enrolled in a retrospective study in Korea to study the effects of exclusive PDHM feeding on morbidity and growth of preterm infants (17). The retrospective cohort study analysing the growth in VLBW infants fed PDHM by Colaizy et al. showed that the mean birth weight was 889 grams (65). A randomized trial on the tolerance of

preterm formula versus PDHM by Costa et al. reported that the mean birth weight of the very preterm infants enrolled in the study was 1342 grams and 1365 grams in the groups receiving preterm formula and PDHM respectively (19). In an audit on the growth of preterm newborns fed PDHM predominantly versus those fed MOM in the NICU by Lloyd et al., it was noted that the mean birth weight of the infants was 980 grams in the PDHM group and 930 grams in the MOM group (25).

Indication for NICU admission – The most common indication for NICU admission in our study was low birth weight (85.62%) followed by neonatal hyperbilirubinemia (19.86%) and respiratory distress syndrome (12.33%). A study on the indications and clinical profile of neonatal admissions by Khasawneh et al. from Jordan with a larger sample size reported similar observations where the most common indication for NICU admission was low birth weight / preterm (33.4%) followed by respiratory distress syndrome (28.9%) and hyperbilirubinemia (10.7%) (121). Katke et al. from Mumbai reported similar observations of LBW (30.6%) being the most common indication for NICU admission followed by RDS (23.7%), in a study on indications and risk factors in LSCS and it's effect on early peri-natal morbidity and mortality rate (122). In contrast, a study conducted to identify and quantify risk factors and causes of NICU admission of term neonates by Talisman et al. reported that the most common indication for NICU admission in term neonates was meconium aspiration syndrome followed by hypoxic ischemic encephalopathy (123). In our study, all the infants admitted in NICU who had received PDHM were enrolled irrespective of the birth weight and gestational age. This difference in the inclusion criteria resulted in different observations as compared to majority of studies reporting, with enrolment of infants with gestational age <32 weeks and birth weight < 1500 grams.

DONOR MOTHER PROFILE:

The mean gestational age of the donor mothers in our study was 38.47 (± 1.34) weeks and the mean maternal age was 23.69 (± 2.9) years. The post-natal day at which human milk was donated was a mean of 4.61 (±1.6) days in our study.

The study on human milk banking by Xiaoshan et al. from China reported 66.7% of term births in the donor mothers (124). Similarly, the study by Kumar et al. in India noted that 75.51% of the donor mothers had given birth to term infants (125). These observations were similar to the findings in our study.

A south Indian study by Kumar et al. to share the experience of human milk banking in a tertiary care centre, reported the mean age of the donor mothers to be 23.53 (± 3.27) years, similar to our study (125). Meghwal et al. in his study on human milk banking from Rajasthan, also reported the age group of donor mothers between 20–25 years (167).

Contrary to our observations, a study on 8 year experience of human milk banking from China reported the age of donor mothers between 25-30 years (124). A similar study on human milk banking from Korea also reported the age distribution of donors ranging from 20 to 45 years, with 69.8% belonging to 30-39 years of age (126).

This difference in the donor maternal age in other studies compared to Indian studies may be due to the practice of early marriage and early pregnancy in India (125).

Jang et al. from Korea also reported that 60.7% of the donors typically started to donate breast milk 1-3 months postpartum which was different from our study (126).

FEEDING PROFILE:

Volume of feeding – In the present study, the mean volume of PDHM consumed by the infants enrolled in the study was 382.26 ml ± 302.91.

Similarly, in a study on the effect of pooled PDHM v/s unpooled PDHM on the short-term growth parameters of preterm infants conducted by Aabha et al., the total amount of PDHM consumed in both group was comparable (421.45 +/- 360.79 ml in unpooled group v/s 373.59 +/- 292.25 ml in pooled group) (p=0.47) (164). In the study by Rohan et al. on impact of PDHM on feeding patterns and growth parameters following discharge from hospital up to 6 months of age, the total volume of PDHM given was 354.72 ± 114.49 ml which was similar to our study (162).

However, many other studies showed that the volume of PDHM consumption was lesser by the infants. The clinical audit in Australia in 2012-2013 conducted by Lloyd et al. to study the growth of preterm infants fed predominantly PDHM versus those fed MOM in the NICU reported that the mean quantity of milk consumed in the PDHM group was 196.4 ml +/- 9.95 ml and the MOM group was 183.1ml +/- 12.01ml (p = 0.391) (25). Costa et al., in his randomized controlled trial conducted in 2015 to assess the tolerance of preterm formula versus PDHM in very preterm infants reported that the mean volume of PDHM consumption was 52.8 +/- 37.5 ml/kg/day (19). Since most of the infants enrolled in our study were stable with a mean gestational age of 35.91 weeks and mean birth weight of 2008.15 grams, the volume of PDHM consumption was more compared to other studies where preterm infants with gestational age < 32 weeks and birth weight < 1500 grams were enrolled.

Duration of feeding **–** The mean duration of PDHM consumption in our study was 4.03 ± 1.75 days. In the study by Saurabh et al. on the incidence of NEC in preterm infants consuming PDHM, the mean duration to PDHM feeding was

5.19±3.93 days which was similar to our study (163). Another study by Aabha et. al on the effect of pooled vs unpooled PDHM on preterm growth outcomes reported that the mean duration of exclusive PDHM feeding (2.73 +/- 2.61 days v/s 2.48 +/- 2.97 days, p=0.65), mixed feeding (MOM+PDHM) (3.78 +/-2.80 days v/s 3.19 +/- 2.00 days, p=0.23) ,duration of exclusive MOM feeding (2.31 +/-3.10 days v/s 2.44 +/- 3.84 days,p=0.85) and days taken to switch to MOM (3.67 +/-2.81 days v/s 3.25 +/- 2.31 days, p=0.42) between both the groups was comparable (164). As per Rohan et al. in his study on the impact of PDHM on growth, the mean duration of PDHM feeding was 4.05 ±2.61 days, similar to our study (162).

Several other studies have reported a mean duration of PDHM feeding of > 5 days. A retrospective study conducted by Alyahya et al. on donated human milk use reported that the mean duration of PDHM feeds was 14 days in the very preterm, 10 days in the late preterm and 9 days in the term infant group (127). Another study on the use of PDHM across neonatal networks in England by Battersby et al. reported that the mean duration of PDHM feeds was 8.5-10.5 days (128). A RCT conducted by Adhisivam et al. in South India to study the effect of fortified versus unfortified PDHM on necrotizing enterocolitis reported the mean duration of PDHM feeding to be 8.5 +/- 5.1 days (78).

The infants included in our study were stable with mean gestation age of >34 weeks and mean birth weight of >2000 grams and hence, the duration of PDHM feeds were shorter. Since, the suck and swallow reflex is well established by 34 weeks, there was an early switching over to MOM and shorter duration of PDHM feeds.

PRIMARY OUTCOME:

MICRONUTRIENT ANALYSIS OF PDHM:

There are limited number of studies on the micronutrient composition of pasteurized donor human milk, especially in India. There is also a lack of literature on the accepted reference values for all the micronutrients present in PDHM.

Micronutrients in PDHM can be analysed using gas and liquid chromatography, atomic absorption spectroscopy (AAS) or inductively coupled plasma spectroscopy–atomic emission spectroscopy (ICP-AES). In our study, the method of micronutrient analysis used was atomic absorption spectroscopy (AAS) whereas in the study by Leyvraz et al. to determine the calcium, zinc and vitamin D content in breast milk, the analytical method used was ICP-MS (31).

A. **Zinc –** In our study, the mean zinc content of PDHM was 0.42 +/- 0.15 mg/dl with the values varying from a range of 0.15 - 0.67 mg/dl.

   Another study on micronutrient composition of PDHM reported similar zinc levels as mentioned in table (37). In the study by Amy Gates et al. to analyse the nutrient composition of pooled DHM and compare it with that of preterm human milk, the mean zinc levels were determined to be 0.23 ± 0.07 mg/dl which is similar to the results of our study (129). A study conducted by Goes et al. in Brazil to determine the nutrient composition of banked human milk and the influence of processing on zinc distribution in milk, reported that processing of the milk samples did not affect most of the nutrients but caused a significant fall ($p=0.04$) in the distribution of zinc (130)**.**

Table 37: Micronutrient content of PDHM

Micronutrient	Present study	Study by Gates et al.
Zinc (mg/dl)	0.42 +/- 0.15	0.23 ± 0.07
Calcium (mg/dl)	213.68 ± 57.7	25.8 ± 2.7
Iron (mcg/dl)	0.1 ± 0.03	NA

Leyvraz et al., in a systematic review for determining the calcium, zinc and vitamin D content in human milk reported that the mean zinc levels of breast milk was 0.26 mg/dL (31). There was a significant difference in the levels of zinc in different stages of lactation. It was high at the start and then decreased rapidly until it reached a plateau. Some of the factors affecting the zinc levels were age of mother, feeding practices and zinc supplementation in mother (31). A Brazilian study by Trinta et al. reported a median zinc level of 2.9mg/L after 2 weeks postpartum in mothers who delivered between 28 and 36 weeks gestation compared to 1.2 mg/L in mothers who delivered at term. Hence, zinc levels were reported to be higher in preterm milk but limited data is available (131).

Table 38: Micronutrient content of human milk

Micronutrient	Perrin et al.	Leyvraz et al.	Trinta et al.	Friel et al.
Zinc (mg/dl)	0.2 - 0.4	0.26	0.29 (preterm) 0.12 (term)	NA
Calcium (mg/dl)	NA	261	NA	NA
Iron (mcg/dl)	NA	NA	NA	0.2 - 0.4

Normally, the average zinc levels in colostrum ranges from 0.4 to 0.9 mg/dL and falls to 0.2 – 0.4 mg/dL by one to two weeks after delivery (132,133).

The PDHM analysed in the present study was donated at a mean of 4.61 (±1.6) days postpartum and hence, the zinc concentration in the present study was similar to the normal reference ranges of colostrum.

B. **Calcium –** In our study, the mean calcium content of pasteurized donor human milk was 213.68 ± 57.7 mg/dL with the values ranging from 107.5 – 320 mg/dL.

   Amy Gates et al. observed a mean calcium levels of 250.8 ± 20.7 mg/L in the analysis of pooled DHM which is similar to the present study (129). The systematic review on the micronutrient content of human milk by Leyvraz et al. reported a mean calcium concentration of 261mg/dl (31) (table 38). The level of calcium in human milk can vary from a range of 84 to 462 mg/L with a median concentration of 252 mg/L (134). It was noted that the concentration of calcium was almost constant with a very slow decrease over time. There were no significant differences in calcium concentration between adolescent and adult mothers, lactation stages, preterm and term infants, exclusive and mixed breastfeeding, with or without calcium supplementation (31).

   The mean calcium levels observed in the present study is similar to the references ranges of human milk mentioned by previous studies (table 38).

C. **Iron** – In the present study, the mean iron content in PDHM was 0.1 ± 0.03 mcg/dl with the values ranging from 0.01 mcg/ml to 0.14 mcg/ml. In a study by Friel et al., the concentration of iron in human colostrum was reported to be approximately 0.8 μg/dL and in mature breast milk was 0.2–0.4 μg/dL which was higher than the mean iron levels reported in the present study. Although the concentration of iron in human milk is low, it is found to be independent of the

mother's iron status and cannot be increased through maternal diet or iron supplementation. The small quantity of iron present in human milk is sufficient for the neonate due to its high bioavailability (135).

SECONDARY OUTCOMES:

1) MACRONUTRIENT ANALYSIS OF PDHM:

The macronutrients in human milk can be analysed using mid infrared human milk analyser (MIR-HMA) or the conventional chemical methods. In our study, method of macronutrient analysis was mid infrared spectroscopy (MIR).

Perrin et al. in his study to investigate donor human milk composition globally to develop effective strategies for the nutritional care of preterm infants, used the conventional Mojonnier ether extraction method for fat analysis, Kjeldahl method for protein analysis, Megazyme enzymatic method for lactose analysis, high pressure liquid chromatography (HPLC) for HMO analysis and ICP-MS for micronutrient analysis (136). In the study by Piemontese et al., protein, lactose, lipids and energy levels in the DHM pools were analysed before and after pasteurization using mid-infrared spectroscopy (Miris human milk analyzer) (57). The study on the comparison of macronutrient contents in human milk measured using mid-infrared human milk analyser vs. chemical reference methods by Zhu et al. concluded that human milk analyser (HMA) might be used to analyse macronutrients in human milk with acceptable accuracy and precision after recalibrating fat and protein levels of field samples (137).

A. Energy – The mean energy content of PDHM obtained in the present study was 61.09 ± 3.05 kcal/dL with the values ranging from 56.97 - 70.04 kcal/dL. Similar observation of energy content of PDHM (60.37 ± 8.41 kcal/dL) was reported by Mills et al (138). A systematic review on the nutritional composition by Perrin et al. reported an energy composition of PDHM ranging from 49.3kcal/dL to 69.3 kcal/dL which showed an almost 2-fold difference in the calorie levels of PDHM and is similar to the present study (36). Amy Gates et al. in his study to analyse the nutrient composition of pooled DHM, reported the mean energy levels to be 69 ± 5 kcal/dl (129).

The mature human milk normally contains 65–70 kcal/100 mL of energy of which about 50% is supplied by fat and 40% by carbohydrates (32). A study on the effect of holder pasteurization and frozen storage on macronutrients and energy content of human milk by García-Lara et al, observed a significant fall in the fat (3.5%) and energy content (2.8%) after pasteurization (58). In the present study, the mean energy level in PDHM was observed to be lower than the normal reference values of breast milk which could be due to the effect of pasteurisation.

Table 39: Macronutrient content of PDHM

Macronutrient	Present study	Mills et al.	Perrin et al.	Walter et al.	Gates et al.
Energy (kcal/dl)	61.09 ± 3.05	60.37±8.41	49.3 - 69.3	NA	69 ± 5 kcal/dl
Carbohydrates (g/dl)	7.47 ± 0.21	7.09 ± 0.44	NA	NA	7.6 ± 0.3
Protein (g/dl)	1.69 ± 0.03	0.89 ± 0.24	0.8 - 3.2	0.7-1.96	1.01± 0.12
Fat (g/dl)	1.94 ± 0.13	2.99 ± 0.96	1.8 - 4.1	1.46 – 9.39	3.8 ± 0.6

B. Carbohydrates – In the present study, the mean carbohydrate content of PDHM was 7.47 ± 0.21 g/dl with the values ranging from 6.84 - 7.68 g/dl. Several studies reporting the macronutrient composition have noted similar values in the PDHM (Table 39). Gates et al. in the analysis of nutrient composition of PDHM reported a mean carbohydrate concentration of 7.6 ± 0.3 g/dl (129). Similar study by Mills et al. reported the mean carbohydrate content of PDHM to be 7.09 ± 0.44 g/dL (138).

Carbohydrate normally comprises about 7% (60–70 g/L) of breast milk. It is a prominent macronutrient in human milk. Among carbohydrates, lactose is the major constituent followed by human milk oligosaccharides (HMOs) (32,33). In a systematic review by Perrin et al., the lactose content in PDHM was between 5.7 - 8.6 g/dl showing a 1.5-fold difference and the total human milk oligosaccharides (HMOs) was between 6.6 and 12.6 g/L (36). A recent review on lactose content of human milk showed that carbohydrates were not significantly affected by holder's method of pasteurization, even by using different analytical techniques (57). The concentration of lactose is the least variable of all the macronutrients in human milk (3). This explains the mean carbohydrate content of 7.47g/dl in the present study, which lies in the normal reference range of human milk.

C. Protein – The mean protein content of PDHM in our study was 1.69 ± 0.03 g/dl with the values ranging from 1.64 – 1.76 g/dl. Another study by Perrin et al. reporting the macronutrient composition of PDHM showed that the mean values of the protein content were between 0.8 g/dL and 3.2 g/dL (36), which is similar to the present study. Similar study by Mills et al. reported similar observations of mean protein content of 0.89 ± 0.24 g/dL (138). The mean protein content in PDHM was reported to be 1.01± 0.12 g/dl by Gates et al (129). Gestational age,

stage of lactation of the donor mothers and the milk processing methods including pasteurisation influence the protein content of the PDHM (36). Walter et al. studied the variability of PDHM composition between different batches and reported the mean crude protein content to be 1.16 g/dL, ranging from 0.7 to 1.96 g/dL (139).

Table 40: Macronutrient content of human milk

Macronutrient	American academy of paediatrics (AAP) reference values
Energy (kcal/dl)	65–70 kcal/dl
Carbohydrates (%) / (g/dl)	7 %
Protein (%) / (g/dl)	1 %
Fat (%) / (g/dl)	3.8 %

D. Fat - The mean fat content of PDHM in our study was 1.94 ± 0.13 g/dl with the values ranging from 1.77 – 2.26 g/dl.

As mentioned in table (39), in a systematic review by Perrin et al. the mean values of fat in PDHM ranged between 1.8 and 4.1 g/dL (36). In a study by Walter et al., the mean fat content was reported to be 3.85 g/dl, ranging from 1.46 to 9.39 g/dl. Gestational age at birth was identified as a predictor for fat content (139). In the study by Gates et al. on the PDHM composition, the mean fat content was reported to be 3.8 ± 0.6 g/dl (129). In the study by Mills et al., a mean fat content of 2.99 ± 0.96 g/dL was reported in PDHM (138).

Colostrum contains 15–20 g/L of fat, but this amount gradually increases, and mature milk contains almost 35-40 g/L (3.8%) fat. Its levels are 2–3 times higher in hindmilk than in foremilk. Fat content in human milk is closely related to maternal diet and weight gained during pregnancy (32).

This observation is consistent with the current literature on human milk composition which suggests that fat composition is highly variable between and within women and is influenced by a variety of factors including maternal diet and how the sample was collected (complete versus partial breast expression) (36).

2) GROWTH

In the present study, we observed that PDHM had a positive impact on the growth outcomes. The mean weight significantly increased at 2 weeks [2038.58 ± (509.26) grams] & 6 weeks [2850.51 (±501.48) grams] follow up compared to at enrolment [1981.37 (±425.21) grams]. The mean length significantly increased at 2 weeks [46.87 (± 2.86) cm] & 6 weeks [49.13 (± 2.88) cm] follow up compared to at enrolment [46.2 (± 2.89) cm]. The mean head circumference significantly increased at 2 weeks [32.22 (± 1.86) cm] & 6 weeks [34.33 (± 1.76) cm] follow up compared to at enrolment [31.61(±1.89) cm]. The mean mid arm circumference significantly increased at 2 weeks [8.12 (± 0.85) cm] & 6 weeks [9.04 (± 0.85) cm] follow up compared to at enrolment [7.86 (±0.86) cm].

Very few studies have reported a similar observation of positive effect of PDHM on the growth outcomes of neonates. The studies which have reported a positive effects on growth are conducted on PDHM, in a same setting as ours. A significant increase in all growth parameters including weight, length, head circumference and mid upper

arm circumference in neonates receiving PDHM was observed in these studies (162,163,164).

Many of the studies noted no difference in improvement of growth outcomes either short term or long term with the use of PDHM (20,28,80). Sisk et al. in a study to assess growth in preterm infants who were predominantly fed MOM, PDHM, or preterm formula, reported that in PDHM fed VLBW newborns the growth metrics from birth to discharge remained unaffected (27). In Fang L et al.'s study no difference in daily weight gain and head circumference was noted in both the preterm formula (PF) and PDHM groups (28). Bramer et al. in his study, examined the growth of non-hospitalized infants who were exclusively fed DHM and demonstrated that the growth velocity of infants was unaffected by DHM (80).

Kim et al. in his Korean study on the effects of exclusive DHM feeding on morbidity and growth outcomes reported that the DHM group showed a lower rate of gain in all the anthropometric parameters (17). In a retrospective analysis by R. Chowning et al., the impact DHM on the growth outcomes was investigated and revealed slower growth rates in weight and HC at discharge (14). In a retrospective clinical audit by Lloyd M et al. on the growth of preterm infants fed predominantly PDHM versus those fed MOM, transient slow growth but evident catch-up growth by discharge and no difference by 3 months of age was seen in the PDHM group (25).

Some studies comparing pasteurized donor human milk (PDHM) with preterm formula (PF) have reported better growth parameters with PF in preterm babies than PDHM (15,18,21,69).

In a RCT by Adhisivam et al. in south India on the effect of fortified versus unfortified PDHM on incidence of NEC and growth parameters, no significant difference in growth parameters was noted in the 2 groups (78).

ESTABLISHMENT OF MILK FLOW:

A milk flow process was established to trace the pooled sample of PDHM collected from a set of donor mothers to the infants who received the respective sample of PDHM. Analysis of the macronutrient and micronutrient content of this pooled PDHM sample was used to assess the growth outcomes of the infants fed with the respective sample of PDHM (36,37). Since donor mother characteristics are the strong predictors of nutrient composition which in turn can define the growth outcomes, information regarding the donor mother characteristics was compared with respective recipient infants and correlated with their growth outcomes. This process of tracing the milk flow from the donor mothers to the recipient infants and to their growth outcomes by analysis of the nutrient content of PDHM is first of its kind.

Factors affecting the nutrient composition of PDHM:

1. **Donor mother characteristics:** In the present study, Pearson correlation analysis was done between the donor mother characteristics namely, mean gestational age, mean maternal age and mean postnatal day of breast milk donation with each of the micronutrient and macronutrient content in PDHM.

   Gestational age of donor mother: A negative correlation was observed between protein content in PDHM and the mean gestational age of the donor mothers in the study (p value = 0.023), suggesting that with an increase in the gestational age, the protein content of PDHM decreased significantly. Similar protein variability with the gestational age of the donor mothers was reported by Perrin et al. in his systematic review of macronutrient composition of PDHM (36). The protein content of milk obtained from mothers who deliver preterm is significantly higher

than that of mothers who deliver at term (3). In the present study the mean gestational age was 38.47 (± 1.34) weeks and hence the micronutrient and macronutrient concentrations were in the reference ranges of term milk.

Age of donor mother: The present study reported a mean maternal age of 23.69 (± 2.9) years. The Pearson correlation analysis did not show significant variations in the nutrient composition of PDHM with change in maternal age in this study (p value > 0.05)

Stage of lactation: It is identified as a predictor for the protein content of human milk (139). Our study reported that an increase in the postnatal day of breastmilk donation was association with a fall in the levels of zinc, energy, protein and fat in PDHM (p value = 0.028, 0.032, 0.021, 0.049 respectively). In a systematic review by Leyvraz et al. to determine the calcium, zinc and vitamin D content of human milk, a significant difference in the levels of zinc in different stages of lactation was noted. It was high at the start and then rapidly fell until a plateau was reached. Some of the factors affecting the zinc levels were maternal age, breastfeeding practices and maternal zinc supplementation (31). Colostrum contains the highest amount of fat and this amount gradually decreases. The postnatal day at which human milk was donated in this study was a mean of 4.61 (±1.6) days and thereby, it was noted that the nutrient levels were similar to the normal reference ranges of colostrum. Its levels are 2–3 times higher in hindmilk than in foremilk. Fat content in human milk is closely related to maternal diet and weight gained during pregnancy (32). The normal values of macronutrients in different stages of lactation is mentioned in table 41 (140).

Table 41: Macronutrient content in different stages of lactation

Macronutrient	Transition milk	Mature milk	Extended milk
Energy (kcal/oz)	19.2 (± 1.9)	18.7 (± 2.4)	20.0 (± 2.7)
Lactose (g/dl)	7.2 (± 0.2)	7.2 (± 0.2)	7.2 (± 0.3)
Protein (g/dl)	1.3 (± 0.2)	1.0 (± 0.2)	1.2 (± 0.2)
Fat (g/dl)	3.5 (± 0.7)	3.5 (± 0.9)	4.0 (± 1.0)

2. **Method of nutrient analysis** – The standard methods of high performance liquid chromatography could not be used for the analysis of micronutrients. In the present study, atomic absorption spectroscopy (AAS) was the method of analysis. However, for macronutrient analysis the mid infrared based human milk analyser was used. There was also a lack of Indian reference values to compare the micronutrients of PDHM
3. **Effect of milk banking processes** – Although proper precautions are taken in order to not affect the composition of DHM, the various processes in human milk banking result in some alteration in the nutrient composition. Inconsistency in milk collection methods including incomplete breast expression in milk bank leads to loss of fat. The impact of milk banking processes like pooling, mixing and multiple container transfers may influence the distribution of nutrients in PDHM. There is also documented loss of some bioactive factors and nutrients during pasteurization and storage (36). In the present study, the energy content was lower than the reference range may be due to the effect of pasteurisation but other micronutrients and macronutrients were in the normal reference ranges, indicating that pasteurisation and other milk banking processes did not significantly affect

their levels in PDHM. Vieira et al.'s study on the effect of holder pasteurization, freezing and thawing on the macronutrient concentration of human milk reported that the mean protein concentration following pasteurization dropped by 3.9% (35).

Factors affecting the growth outcomes:

1. Nutrient composition of PDHM

In the present study, Pearson correlation analysis was done between the micronutrient and macronutrient composition of PDHM and each of the anthropometric parameters of the infants. A moderate to strong positive correlation between the protein content of PDHM and the average gain in all the anthropometric parameters, with statistical significance ($p<0.05$) was reported, indicating that higher protein content is associated with greater increase in all the anthropometric parameters mid arm circumference (MAC). However, the other nutrients present in PDHM did not significantly affect the growth outcomes.

Protein and energy in the diet are major determinants of physical growth for preterm infants (98). A previous longitudinal study with repeated measures of milk macronutrient content in Taiwan found that the protein content (g/dL) of human milk was positively associated with growth velocity to hospital discharge, a finding that is consistent with our results (24).

2. Sociodemographic and maternal factors

A multivariate analysis was performed to study all the covariables - sociodemographic and maternal factors affecting the growth outcomes. It was reported in the present study that the gestational age at birth showed a significant positive effect on weight gain, suggesting that higher gestational age at birth

correlated with increased weight gain. On the contrary, maternal age had a significant negative effect on weight gain, indicating that older maternal age is associated with reduced weight gain in our study. The socioeconomic status of lower middle class showed a significant positive effect on weight and length gain, indicating that infants belonging to lower middle class showed increased weight and length gain. Length gain of infants was also found to be more if mothers were homemakers. A significant impact on the MAC gain of infants was reported when the mothers were graduates.

The improved growth outcomes observed in our study among infants who received PDHM feeding are likely attributed to the fact that the average gestational age (GA) of the infants was 35.91 weeks, classifying them as late preterm, in contrast to the majority of studies which focused on infants with GA ≤ 30 weeks. GA appears to play an independent role in influencing various growth outcomes. Infants with a higher GA tend to exhibit more normal short-term growth patterns, possibly due to facing less severe clinical challenges and having lower nutritional demands compared to infants born with lower GA and birth weights. Lower gestational age results in poor weight gain and head growth in infancy (165).

Many studies show that adverse obstetrical and perinatal outcomes are associated with women of advanced maternal age (141). Majority of these outcomes can be explained through the physio-pathological changes secondary to aging in the female reproductive system and aging-associated comorbidities. However, advanced maternal age is identified as an independent risk factor according to current evidence (166). A constant rise in obstetric and neonatal morbidity was observed with maternal age after 30 years (141) Advanced maternal age is also known to be associated with increased incidence of still births (142).

On the other hand, single young mothers were known to have unsatisfactory antenatal care and small for gestational age infants. Very young maternal age was also associated with higher risk of prematurity, major congenital malformations and perinatal mortality. More studies are needed to ascertain the cause of these adverse outcomes (143–145).

3) TYPE AND METHOD OF FEEDING

Type of feeding:

In the present study, the predominant feeding method at enrolment was a combination of pasteurized donor human milk (PDHM) and mother's own milk (MOM), accounting for 98.63% of infants, while a very small percentage (1.37%) received only PDHM. At discharge and during follow-up at 2 weeks and 6 weeks of life, all infants were exclusively receiving MOM (mother's own milk), constituting 98.63% of the cohort at each of these time points. Only a minority of infants (1.37%) were discharged on lactogen feeds and continued to use it on follow-up visits. A population-based cohort study examining the influence of donor human milk availability on breast milk utilization by Agata et al. reported similar observations showing a 10% rise in breast milk feeding at discharge from NICU (89).

Williams et al. evaluated maternal breastfeeding rates before and after the introduction of donor human milk (DHM) in a systematic review. They reported a notable decrease in the percentage of feeds consisting of mother's own milk (MOM) following the introduction of DHM which was in contrast to the findings of our study (146).

The majority of mothers of preterm infants face challenges in providing sufficient milk to meet their infants' nutritional needs in the early days (147–151)

Recent research indicates that delayed lactogenesis II, the onset of mature milk production, is common among mothers of preterm babies (152–154). This delay often results in reduced milk expression volume by preterm mothers, increasing the likelihood of PDHM feeding during the initial days (150,152–155). However, this delay typically corrects itself within 1-2 weeks, leading to higher rates of direct breastfeeding (DBF) by the time of discharge and follow-up.

Another contributing factor to this trend is the association between lower segment cesarean section (LCSC) and decreased let-down reflex, which can further delay breast milk expression. The initial separation of preterm infants from their mothers during NICU stays can also hinder the proper activation of the let-down reflex, thereby increasing the reliance on PDHM feeding upon admission. However, as these factors are gradually mitigated, rates of maternal milk provision tend to increase by the time of discharge and follow-up.

Method of feeding:

In our study, the majority of infants (65.75%) were fed using a combination of spoon feeds (SF) and direct breastfeeding (DBF) at enrolment while majority (98.63%) were on direct breast feeds at discharge as well as on follow up at 2 and 6 weeks.

Studies examining breastfeeding rates before and after the establishment of a human milk bank concluded that the opening of such a facility did not decrease exclusive breastfeeding (EBF) rates at discharge (146,156). Similarly, a study by Adhisivam et al. from India noted an enhancement in breastfeeding rates following the establishment of a milk bank, with rates rising from 34% to 74% after six months (157).

The predominant method of feeding for most infants in our study at the time of enrolment was spoon feeding (SF), which subsequently transitioned to direct breastfeeding (DBF). This shift can be attributed to the inclusion of stable infants with an average gestational age of 35.91 weeks in our study. Hence, the establishment of milk banks were associated with higher breastfeeding rates at discharge and follow up.

4) DURATION OF HOSPITAL STAY

In our study, the mean duration of hospital stay for all the enrolled infants was 9.97 ± 5.56 days. Similar observations were obtained from a systematic review and meta-analysis conducted by Rui Yang et al. in 2020, which studied the impact of DHM on the duration of hospitalization in VLBW newborns and reported a significant reduction in length of hospital stay to a mean value of 11.72 days for infants receiving DHM (158).

This was in contrast to the results of a single-centre randomized non-inferiority controlled trial by Costa et al., which investigated the tolerance of preterm formula versus pasteurized donor human milk (PDHM) in very preterm infants with gestational age (GA) of ≤32 weeks. Their study reported an average hospital stay length of 37.5+/-17.5 days for infants (19).

One possible explanation for the differences observed in our study is the enrolment of stable infants with a mean gestational age of 35.91 weeks, who required a shorter duration of PDHM feeding. Hence, the early transition to maternal milk may have contributed to early discharge from the neonatal intensive care unit (NICU). Furthermore, PDHM is known to offer several advantages in preterm infants, including a reduced risk of comorbidities such as late-onset sepsis (LOS), necrotizing

enterocolitis (NEC), bronchopulmonary dysplasia (BPD), and intraventricular haemorrhage (IVH), consequently leading to decreased rates of hospital-acquired infections and shorter hospital stay.

5) INCIDENCE OF NECROTIZING ENTEROCOLITIS AND SEPSIS

In our study, majority of infants did not experience any complication during their hospital stay. However, 4.11% of infants developed sepsis and 0.68% developed necrotizing enterocolitis.

Several other studies have reported the lower incidence of NEC in preterm infants receiving human milk (MOM/PDHM) compared to formula feeds (14,16,18,87,89). A study conducted by Sullivan et al. in 2010 reported a 77% reduction in NEC in preterm infants fed a human milk diet compared to those who were fed human milk supplemented with cow-milk-based infant formula products (159).

Shoji et al. supported the theory that breast milk possesses antioxidant properties, thereby exerting a protective effect against NEC (88). In a study by Lapidaire et al., it was observed that for every 10% increase in MOM and DHM intake, there was an approximately 8% and 12% lower likelihood of NEC/sepsis, respectively. Conversely, a 10% increase in term formula (TF) intake was associated with a 12% increase in the likelihood of NEC/sepsis (90).

In contrast, Schanler et al. found no significant effect of donor milk on the incidence of late-onset sepsis or NEC. Their study, however, only considered cases of sepsis or NEC that occurred after a significant amount of enteral nutrition (50 mL/kg daily) had been tolerated, which typically occurs after 16 to 18 days (160).

A south Indian study by Adhisivam et al. from a tertiary care hospital demonstrated no increase in incidence of NEC in the fortified PDHM group compared to unfortified PDHM group and concluded that standard fortification of PDHM does not increase the incidence of NEC among preterm neonates (78).

The frequency of NEC in the present study was significantly low among the neonates who received PDHM demonstrating the positive role of human milk in the prevention of NEC compared to formula feeding. DHM contains human milk oligosaccharides which withstands the low pH of infants stomach and reaches distal small intestine and colon where it acts as a probiotic and helps beneficial bacteria to thrive along with suppressing harmful bacteria, it also has anti-adhesives that blocks the attachment of viral and bacterial pathogens thus counteracting dysbiosis at the early stage of NEC (161).

STRENGTHS OF THE STUDY

This is the first Indian study to analyse the micronutrient and macronutrient composition of pasteurized donor human milk, using standard methods. It is the also the first study to corelate the micronutrient and macronutrient content of PDHM with donor mother characteristics and growth outcomes of infants by establishing a milk flow process. Charting the milk flow helped to trace the milk from the donor mothers to the recipient infants and to their growth outcomes by analysis the nutrient content of PDHM.

LIMITATIONS OF THE STUDY

The limitation of this study is that it is a single centric study with a small sample size. There is also a lack of literature on standard reference values to compare the micronutrient and macronutrient levels. Long term follow up of the infants was not done and was beyond the scope of the study.

RECOMMENDATIONS

Multicentric studies with larger sample size and long term follow up to study the effect of micronutrient and macronutrient composition of PDHM on growth and developmental outcomes of infants is recommended.

CONCLUSION

This observational longitudinal study conducted in a NICU of a tertiary care teaching hospital, to analyse the micronutrient and macronutrient composition of pasteurized donor human milk observed the nutrient composition of PDHM in the normal expected range of human milk reference values. The pasteurized donor human milk (PDHM) had a positive impact on short term growth in infants at 6 weeks. The higher protein concentration in PDHM resulted in greater increase in all the anthropometric parameters when nutrient content was traced to the growth outcomes with the help of a milk flow process. The correlation between donor mother characteristics namely donor gestational age, donor maternal age and postnatal day of milk donation and the nutrient composition of PDHM showed that the increased levels of protein in PDHM was associated with lower gestational age and early postnatal day. Multivariate analysis of other covariables affecting the growth outcomes in infants showed that higher gestational age at birth correlated with increased weight gain and older maternal age was associated with reduced weight gain. The incidence of necrotizing enterocolitis and sepsis was significantly low with the use of PDHM, thereby resulting in shorter duration of hospital stay in the study. However, a multicentric RCT with a large sample size for a longer duration is recommended to confirm the findings of our study.

SUMMARY

The observational longitudinal study was conducted from January 2023 to December 2023 in the Neonatal Intensive Care Unit of Department of Pediatrics, KLES Dr. Prabhakar Kore hospital and Medical Research Centre, attached to Jawaharlal Nehru Medical College, Belagavi. A total of 13 batches of PDHM obtained from the human milk bank - AMRUTHA were analysed for micronutrients and macronutrients. The PDHM from each batch was distributed among the neonates eligible and enrolled in the study. Hence, a milk flow was established by tracing the milk from the donor mothers to the recipient infants and to their growth outcomes. Of 688 neonates admitted during the study period, 402 were given PDHM. Out of the 402 neonates who received PDHM, the first 200 neonates were screened. 20 neonates were excluded from the study in view of congenital malformations, birth asphyxia and early onset sepsis. A total of 180 neonates were considered eligible and were enrolled and analysed in the study. Of the 180 neonates enrolled, 8 deaths were noted during hospital stay. Of the 172 survivors, 26 neonates were lost to follow up after discharge. Hence, 146 neonates were enrolled and followed up completely till 6 weeks of life. The data was analysed and the important findings of the study are summarized as below.

1. Sociodemographic profile:

- The mean age of the infants at enrolment was 1.86 days (±1.1).
- Majority of the infants were males (52.05%) and belonged to the Hindu religion (85.62%).
- Majority of the mothers were homemakers (95.21%) with secondary education (58.22%).

- A vast majority of the families belonged to upper middle class of the socioeconomic scale (58.22%).
- Only a few families reported consanguineous marriages among parents (12.33%), while the majority did not (87.67%).

2. Maternal pregnancy profile:

- The mean age of the mothers was 25.7 years and the study included an equal number of multigravida (50%) and primigravida (50%) mothers.
- All mothers had antenatal visits and scans during their pregnancies.
- Various antenatal risk factors were identified: twin/triplet pregnancy being the highest (32.88%) followed by intrauterine growth restriction (IUGR) (28.77%) and preeclampsia and eclampsia (23.97%).

3. Birth profile:

- Majority of deliveries were conducted via LSCS, accounting for 78.77% of cases, while vaginal deliveries constituted 21.23%.
- The mean gestational age at birth was 35.91 weeks (± 2.34) with a mean birth weight of 2008.15 grams (±436.15).
- The most common indication for NICU admission was low birth weight (85.62%) followed by neonatal hyperbilirubinemia (19.86%) and respiratory distress syndrome (12.33%).

4. Feeding profile:

- Majority of infants (91.1%) were given PDHM because the mother had insufficient milk secretion while only 5.48% received it due to the mother not being healthy to breastfeed.
- Most infants (78.77%) received < 500 ml of PDHM, with only 17.81% receiving between 500-1000 ml, and 3.42% receiving over 1000 ml.
- The mean total amount of PDHM consumed by the subjects was 382.26 ml (±302.9) with a mean total duration of 4.03 days (±1.75).

5. Donor mother profile:

- The mean gestational age of the donor mothers was 38.47 (± 1.34) weeks and the mean maternal age was 23.69 (± 2.9) years.
- The post-natal day at which human milk was donated was a mean of 3.89 days.

6. Micronutrient composition of PDHM:

- Mean calcium content was 213.68 ±57.7mg
- Mean zinc content was 0.42 mg ± 0.15 mg
- Mean iron content was 0.1 mg ± 0.03 mg

7. Macronutrient composition of PDHM:

- Mean energy content was 61.09 ±3.05 kcal
- Mean carbohydrate content was 7.47 ± 0.21 %
- Mean protein content was 1.69 ± 0.03 %
- Mean fat content was 1.94 % ± 0.13 %

8. Growth outcomes:

- Weight - At enrolment, the mean weight was 1981.37 (±425.21) grams. At discharge it decreased to 1974.11 grams (±432.36) and on follow up at 2 weeks and 6 weeks, it significantly increased to 2038.58 ± (509.26) grams and 2850.51 (±501.48) grams, respectively.

- Length - At enrolment, the mean length was 46.2 (± 2.89) cm. There was a marginal increase in the mean length to 46.62 (± 2.76) cm at discharge. On follow-up at 2 weeks and 6 weeks, there was a significant increase in mean length to 46.87 (± 2.86) cm and 49.13 (± 2.88) cm respectively.

- Head circumference - At enrolment, the mean HC of the infants was 31.61(±1.89) cm. There was a marginal increase in mean HC to 31.99 (± 1.73) cm at discharge. The mean HC then increased significantly to 32.22 (± 1.86) cm and 34.33 (± 1.76) cm on follow up at 2 weeks and 6 weeks respectively.

- Mid arm circumference - At enrolment, the mean MAC of the infants was 7.86 (±0.86) cm. There was a marginal increase in mean MAC to 8.04 (± 0.77) cm at discharge. The mean MAC then increased significantly to 8.12 (± 0.85) cm and 9.04 (± 0.85) cm on follow up at 2 weeks and 6 weeks respectively.

9. A milk flow process was established to trace the pooled sample of PDHM collected from a set of donor mothers to the recipient infants and to their growth outcomes by analysis of the nutrient content of PDHM. Analysis of the macronutrient and micronutrient content of this pooled PDHM sample was used to assess the growth outcomes of the infants and was correlated with the donor mother characteristics.

10. Lower gestational ages were associated with higher protein content and early postnatal days of milk donation were associated with increased levels of zinc, energy, protein and fats according to the correlation analysis done in the study.

11. The study reported that micronutrients like calcium, zinc and iron composition of the PDHM did not significantly affect the growth outcomes in infants enrolled in the study.

12. In case of macronutrients, a moderate to strong positive correlation between the protein content of PDHM and the average gain in all the anthropometric parameters indicated that higher protein content was associated with greater increase in all the anthropometric parameters. Energy, carbohydrates and fats did not significantly affect the growth outcomes.

13. Other factors affecting the growth outcomes:

- Maternal age had a significant negative effect on weight gain, indicating that older maternal age was associated with reduced weight gain.
- Gestational age showed a significant positive effect on weight gain, suggesting that higher gestational age correlated with increased weight gain.
- Socioeconomic status being lower middle class was associated with increased weight and length gain in infants.
- Mother's occupation being homemaker was associated with increased length gain in infants.
- Mother's education being graduate was associated with increased MAC gain in infants.

14. Type of feeding:

- At enrolment, the predominant feeding method was a combination of pasteurized donor human milk (DHM) and mother's own milk (MOM), accounting for 98.63% of infants, while a very small percentage (1.37%) received only PDHM.

- At discharge and during follow-up at 2 weeks and 6 weeks of life, all infants were exclusively receiving MOM (mother's own milk), constituting 98.63%.

- A minority of infants (1.37%) were discharged on lactogen feeds and continued to use it on follow-up visits.

15. Method of feeding:

- At enrolment, the majority of infants (65.75%) were fed using a combination of spoon feeds (SF) and direct breastfeeding (DBF), while a smaller percentage received only RT feeds (13.01%) or RT feeds combined with spoon feeds (SF) (0.68%).

- By the time of discharge and during follow-up at 2 weeks and 6 weeks of life, almost all infants (97.95% to 98.63%) were exclusively on direct breast feeding (DBF), with only a small proportion (1.37%) continuing to receive spoon feeds.

16. The mean duration of hospital stay for all the infants enrolled in the study was 9.97 days (±5.56).

17. The majority of the infants did not experience any complication during their hospital stay.

- However, 4.11% of infants developed sepsis and 0.68% developed necrotizing enterocolitis.

- The most common organism isolated in blood culture was Klebsiella pneumonia.

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ANNEXURES – I

INFORMED CONSENT FORM

"TO ANALYSE THE MICRONUTRIENT COMPOSITION OF PASTEURIZED POOLED DONOR HUMAN MILK (DHM) - A ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY."

Principle Investigator:

REG. NO. BM0121016

Post Graduate Student

Department of Paediatrics

J. N. Medical College, Belagavi-10.

Co-Investigator:

DR. _____

Professor,

Department of Paediatrics,

J.N. Medical College, Belagavi-10.

Objective: TO ANALYSE THE MICRONUTRIENT COMPOSITION OF PASTEURIZED POOLED DONOR HUMAN MILK (DHM) - A ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY.

Introduction: When their own mothers' milk is unavailable or not sufficient to satisfy the requirements of babies, donor human milk (DHM) is the best alternative. DHM can reduce the incidence of late onset sepsis, Bronchopulmonary dysplasia, Retinopathy of Prematurity (ROP) and Necrotizing Enterocolitis (NEC) as well as improve feeding tolerance, shorten the length of hospital stay and reduce the medical costs. Hence, we are studying its effect on growth of infants.

Explanation of procedure: First, milk is expressed from donor mothers using a hospital grade breast pump. The breast milk collected from 5 mothers is collected and pooled in 1 bottle. The pooled donor human milk is then pasteurized using Sterifeed Pasteurizer . The Pasteurized pooled donor human milk will be sent for analysis of micronutrients and macronutrients. The micronutrients which will be analysed are iron, calcium, zinc,

vitamin A and vitamin D using an Atomic Absorption Spectrometer (AAS) in the Micronutrient Analysis lab in Jawaharlal Nehru Medical college building. The same milk sample is dispensed to recipients and their preliminary details are noted. Later, babies who have consumed this pasteurized donor human milk are followed up in NICU/KMC at discharge and 6 weeks of life.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups.

However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact: REG. NO. BM0121016, Department of Paediatrics, KAHER University's J.N Medical College, If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

STATEMENT OF CONSENT

I am making a voluntary decision to participate in the study ***"TO ANALYSE THE MICRONUTRIENT COMPOSITION OF PASTEURIZED POOLED DONOR HUMAN MILK (DHM) - A ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY."***. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE – II – PROFORMA

PROFORMA FOR DATA COLLECTION

SCREENING PROFORMA

HOSPITAL PATIENT NUMBER

SCREENING NUMBER

DATE

NAME OF THE PARTICIPANT :

B/O FIRST NAME

MIDDLE NAME

LAST NAME

AGE-

GENDER-

ADDRESS-H.NO.

STREET

TALUKA

DISTRICT

CONTACT NUMBER OF PARENTS

GENERAL QUESTIONNAIRE

- AGE OF THE MOTHER AT DELIVERY -
- DATE OF DELIVERY-

- GESTATIONAL AGE AT DELIVERY - WKS DAY

- MODE OF DELIVERY – | | | | |

- BIRTH WEIGHT AT DELIVERY- KG

- WEIGHT AT TIME OF SCREENING- KG

GENERAL QUESTIONNAIRE

INCLUSION CRITERIA

1. REQUIRING NICU OR KMC ADMISSION ☐Yes ☐No
   If yes indication

I.	LOW BIRTH WEIGHT	YES	NO
II.	RESPIRATORY DISTRESS SYNDROME	YES	NO
III.	MAS	YES	NO
IV.	HYPERBILIRUBINEMIA	YES	NO
V.	FEEDING DIFFICULTIES	YES	NO
VI.	OBSERVATION	YES	NO
VII.	HYPOGLYCEMIA	YES	NO
VIII.	ANY OTHER	YES	NO

2. STABLE Yes No
   - on ventilator support, Yes No
   - on parentral nutrition Yes No

3.Requiring DONOR MILK- Yes No

If yes , indication

I. Mother not alive	YES	NO
II. Mother is not well/ not physically healthy for breastfeeding	YES	NO
III. . Mother not having enough secretions	YES	NO
IV. Baby not maintaining RBS despite mother's feed	YES	NO
V. Baby is has not developed suck reflex	YES	NO
VI. Others	YES	NO

EXCLUSION CRITERIA

1. Congenital malformation especially cardiac anomalies	Yes		No	
2. Birth asphyxia	Yes		No	
3. Anomalies of GI tract.	Yes		No	
4. Any surgical intervention in last 1 month	Yes		No	
5. Necrotising enterocolitis	Yes		No	
6. Pateint not giving consent	Yes		No	

Eligible as per above mentioned criteria - ☐ Yes ☐ No

If eligible, CONSENT

- Does the mother/father assent to participate? ☐ Yes ☐ No
- Has the consent form been signed? ☐ Yes ☐ No
- If Consent given , Enrollment

Yes ☐

No ☐

Subject number- ☐☐☐

NAME OF THE INVESTIGATOR-

SIGNATURE-

PROFORMA

"TO ANALYSE THE MICRONUTRIENT COMPOSITION OF PASTEURIZED POOLED DONOR HUMAN MILK (DHM) - A ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY"

Subject No- IP No-

Date-

I. SOCIO-DEMOGRAPHIC DATA

1. Identification Number :
2. In patient Number :
3. Date of admission :
4. Name :
5. Religion: Hindu Muslim Sikh Others
6. Address:
7. Age:
8. Gender: Male Female Ambiguous
9. Mother`s Name:
10. Mother`s education: Illiterate Primary Secondary Graduate

Post Graduate

11. Mother`s Occupation: Home maker Employed Self employed
12. Father`s Name:
13. Father`s education: Illiterate Primary Secondary Graduate

Post Graduate

14. Number of member`s at home:
15. Income:
16. Per capita:

17. Socio economic status according to the Modified B.G. Prasad`s Classification
    a. Upper class (> Rs. 5775)
    b. Upper middle class (Rs 2887 to 5774)
    c. Middle class (Rs. 1773 to 2886)

d. Lower middle class (Rs 866 to 1732)

e. Lower class (< Rs 866)

18. Marriage: Consanguinous Non- Consanguinous

19. General information given by: Mother Father

20. Written informed consent Yes No

II. MATERNAL HISTORY :

a. Gravida:

III. Birth history

1. Mode of Delivery: FTNVD LSCS Instrumental delivery

2. Gestational age in weeks:

IV. Indication of NICU Admission

1. Indication

a. Low birth weight Yes/No

b. Respiratory distress syndrome Yes/ No

c. MAS Yes/No

d. Hyperbilirubinemia Yes/No

e. Feeding difficulties Yes/No

f. Observation Yes/No

g. Hypoglycemia Yes/No

h. Any other Yes/No

2. Examination

a. General physical examination

i. Heart rate:

ii. Respiratory rate:

iii. CFT:

iv. Temperature:

3. Anthropometry

	Measured	Expected
Weight (Kg)		
Length (cm)		
Head Circumference (cm)		
Mid Upper Arm Circumference (cm)		

4. Head to toe examination:

a. Face

b. Eyes

c. Ears

d. Oral cavity

e. Neck

f. Chest

g. Abdomen

h. Extremities

j. Congenital markers

k. Skin

5. Other systems

a. Cardiovascular system

b. Respiratory system

c. Per Abdomen

d. Central nervous system

6. Feeding Pattern

a. Type of Feeding

b. Method of feeding

c. Frequency of feeding

7. Date of starting PDHM:

Total duration of PDHM given:

8. Hospital stay

a. NICU: days

b. NICU +KMC: days

c. KMC: days

d. Post natal: days

9. Anthropometry at Discharge/ 2 weeks :

	Measured	Expected
Weight (kg)		
Length (cm)		
Head Circumference (cm)		
Mid upper arm circumference (cm)		

10. Types of feeds at discharge

a. Breast feed Yes/ No

b. If yes,

i. Direct breast feed Yes/ No

ii. Expressed breast milk Yes/No

iii. Both Yes/No

11. PDHM Yes/No

a. If yes, amount: ml/day

12. Formula Yes/No

a. If yes, amount: ml/day

13. Presence of complications at discharge

a. Necrotizing enterocolitis Yes/No

b. Sepsis Yes/No

Baby`s follow up at 6 weeks :

FEEDING PATTERNS -

a. Type of feeding

b. Method of feeding

c. Frequency of feeding

ANTHROPOMETRY-

	Measured	Expected
Weight (kg)		
Length (cm)		
Head Circumference (cm)		
Mid Upper Arm Circumference(cm)		

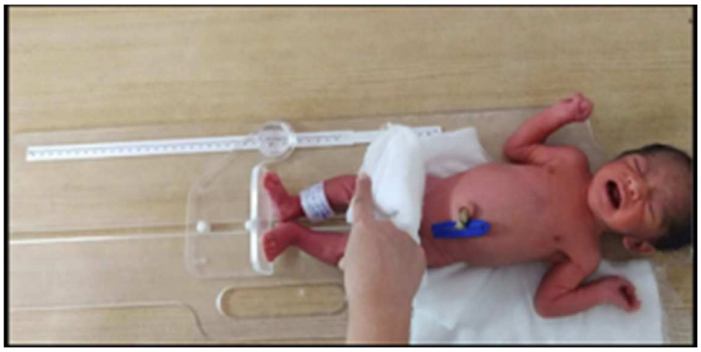
Presence of complications -

Necrotising Enterocolitis Yes/No

Sepsis Yes/No

ANNEXURE 3 – PHOTOGRAPHS FOR THESIS

Photograph 1: Essae-BS-250 electronic weighing scale used for measuring weight



Photograph 2: Infantometer was used to measure the length

Photograph 3: Head circumference was measured using Schorr's tape

Photograph 4: Hospital grade electric breast pump

Photograph 5: Milk containers

= Photograph 6: Sterifeed pasteurizer

Photograph 7: Refrigerator

Photograph 8: Deep freezer

ANNEXURE 4 - KEY TO MASTERCHART

Sex

F- Females

M- Males

Religion

1 – Hindu

2 – Muslim

3 – Sikh

4 – Others

Mother's and Father's education

1 – Illiterate

2 – Primary

3 – Secondary

4 – Graduate

5 – Post graduate

Mother's occupation

1- Homemaker

2- Employed

3- Self employed

Socio economic status

1 – Upper class

2 – Upper middle class

3 – Middle class

4 – Lower middle class

5 – Lower class

Antenatal risk factors

1 - Elderly primi > 35 years

2 - Short statured < 145 cm

3 - Preeclampsia and eclampsia

4 - Anemia

5 - Gestational diabetes mellitus

6 - Previous still birth, intra uterine death

7 - Previous caesarean section

8 - Grand multipara

9 - Preterm premature rupture of membranes

10 - IUGR

11 - Twins/ triplets

12 - Placenta previa/placenta abruption

13 - Rh isoimmunization

14 - Congenital malformations

Mode of delivery

LSCS – Lower segment caesarean section

VD – Vaginal Delivery

Indication of NICU admission

1 - Low birth weight

2 - Respiratory Distress syndrome

3 - Meconium Aspiration Syndrome

4 - NNH

5 - Feeding difficulty

6 - Observation

7 - Hypoglycemia

8 - Any other / Kangaroo mother care

Indication of starting PDHM

1 – Mother not alive

2 – Mother not physically healthy

3 – Mother not having enough secretions

4 – Baby not maintaining RBS despite mother's feed

5 – Baby has not developed suck reflex

6 - Others

Feeding history

MOM – Mother's own milk

DHM – Donor human milk

SF – Spoon feeds

RTF – Ryle's tube feeding

DBF – Direct breast feed

Serial Number	Identification Number	In patient Number	Date of admision	Date of discharge	Demographic data: Age (Day of life)	Gender	Religion	Mother's education	Mother's occupation	Father's education	Socio economic status (Modified B G Prasad)	Consanguineous marriage in parents	Maternal history: Maternal age (Years)	Gravida	Antenatal visits and scans	Antenatal risk factors	Birth history: Mode of delivery	Gestational age (weeks)	Birth weight(gm)	Indication for NICU admission	Feeding history: Type of feeding At enrollment	Type of feeding At discharge	Type of feeding F/U at 2 weeks	Type of feeding F/U at 6 weeks	Indication for starting PDHM	Batch number of DHM	Date of starting PDHM	Total amount of PDHM (ml)	Total duration of PDHM (days)
1	100	1167311	28-01-2023	03-02-2023	1	M	1	4	1	5	2	N	24	1	Y	NONE	VD	39+6	2900	6	DHM+MOM	MOM	MOM	MOM	3	83	28-01-2023	300ML	3
2	101	1167676	30-01-2023	10-02-2023	1	F	1	3	1	3	3	N	23	2	Y	9, 11	VD	33+4	1540	1	DHM+MOM	MOM	MOM	MOM	3	83	30-01-2023	175ML	3
3	102	1167677	30-01-2023	10-02-2023	1	F	1	3	1	3	3	N	23	2	Y	9, 11	VD	33+4	1550	1	DHM+MOM	MOM	MOM	MOM	3	83	30-01-2023	165ML	3
4	105	1167991	31-01-2023	06-02-2023	1	M	1	2	1	3	4	N	26	2	Y	7	LSCS	40+1	2100	1	DHM+MOM	MOM	MOM	MOM	3	83	02-01-2023	220ML	2
5	106	1168257	02-02-2023	15-02-2023	1	F	1	4	1	3	4	N	28	1	Y	10	LSCS	37+4	2170	1,8	DHM+MOM	MOM	MOM	MOM	2,3	83	02-01-2023	330ML	4
6	107	1168449	02-02-2023	09-02-2023	1	M	1	3	1	2	2	N	23	2	Y	10	VD	39+3	1700	1	DHM+MOM	MOM	MOM	MOM	3	83	02-02-2023	230ML	4
7	108	1168496	03-02-2023	12-02-2023	1	F	2	2	1	3	3	N	22	1	Y	3,5,9	LSCS	32+1	1540	1	DHM+MOM	MOM	MOM	MOM	2	83	03-02-2023	380ML	3
8	110	1169292	07-02-2023	15-02-2023	1	M	1	2	1	3	3	Y	20	1	Y	9	VD	33+6	2000	1	DHM+MOM	MOM	MOM	MOM	3	83	07-02-2023	120ML	2
9	111	1170254	10-02-2023	18-02-2023	2	F	2	4	1	4	1	N	22	2	Y	10,11	LSCS	36+4	1800	1	DHM+MOM	MOM	MOM	MOM	3	83	11-02-2023	530ML	6
10	112	1170255	10-02-2023	18-02-2023	2	F	2	4	1	4	1	N	22	2	Y	10,11	LSCS	36+4	1630	1	DHM+MOM	MOM	MOM	MOM	3	83	11-02-2023	570ML	6
11	113	1172462	21-02-2023	16-03-2023	1	M	1	4	1	4	2	N	31	1	Y	11,12	LSCS	31+3	1280	1,2	DHM+MOM	MOM	MOM	MOM	2,3	83	22-02-2023	330ML	4
12	114	1172021	20-02-2023	28-02-2023	3	M	1	3	1	3	1	N	18	1	Y	11	LSCS	36+2	2120	1	DHM+MOM	MOM	MOM	MOM	3	84	23-02-2023	190ML	2
13	115	1172024	20-02-2023	28-02-2023	3	F	1	3	1	3	1	N	18	1	Y	11	LSCS	36+2	2000	1	DHM+MOM	MOM	MOM	MOM	3	84	23-02-2023	190ML	2
14	116	1173165	25-02-2023	04-03-2023	1	M	1	3	1	2	4	N	21	2	Y	11	LSCS	36+5	1690	1	DHM+MOM	MOM	MOM	MOM	3	84	25-02-2023	240ML	2
15	117	1173167	25-02-2023	04-03-2023	1	M	1	3	1	2	4	N	21	2	Y	11	LSCS	36+5	1750	1	DHM+MOM	MOM	MOM	MOM	3	84	25-02-2023	210ML	2
16	118	1179279	28-03-2023	08-04-2023	1	M	1	4	1	4	1	N	28	1	Y	11	LSCS	33+6	1590	1,2	DHM+MOM	MOM	MOM	MOM	3	84	28-03-2023	270ML	4
17	119	1179282	28-03-2023	08-04-2023	1	M	1	4	1	4	1	N	28	1	Y	11	LSCS	33+6	1780	1,2	DHM+MOM	MOM	MOM	MOM	3	84	28-03-2023	270ML	4
18	120	1179287	28-03-2023	08-04-2023	1	F	1	4	1	4	1	N	28	1	Y	11	LSCS	33+6	1500	1,2	DHM+MOM	MOM	MOM	MOM	3	84	28-03-2023	250ML	4
19	121	1179498	29-03-2023	06-04-2023	1	M	1	4	1	4	1	N	23	2	Y	10	LSCS	38+6	2650	6	DHM+MOM	MOM	MOM	MOM	3	84	29-03-2023	160ML	3
20	122	1179084	27-03-2023	10-04-2023	2	F	1	2	1	2	3	N	23	1	Y	10	LSCS	32	1300	1,2	DHM+MOM	MOM	MOM	MOM	3	84	28-03-2023	130ML	3
21	123	1181828	10-04-2023	19-04-2023	1	F	1	2	1	4	2	N	20	1	Y	11	LSCS	32+1	1500	1	DHM+MOM	MOM	MOM	MOM	3	84	10-04-2023	860ML	8
22	124	1181829	10-04-2023	19-04-2023	1	M	1	2	1	4	2	N	20	1	Y	11	LSCS	32+1	1500	1	DHM+MOM	MOM	MOM	MOM	3	85	10-04-2023	600ML	7
23	125	1182127	11-04-2023	19-04-2023	1	M	1	3	1	4	2	N	26	4	Y	7,12	LSCS	32+4	1850	1,2	DHM+MOM	MOM	MOM	MOM	2,3	85	11-04-2023	180ML	3
24	126	1182189	11-04-2023	26-04-2023	1	M	1	3	1	4	2	N	30	1	Y	11	LSCS	31+5	1200	1,2	DHM+MOM	MOM	MOM	MOM	3	85	11-04-2023	210ML	3
25	128	1183124	15-04-2023	23-04-2023	1	F	1	4	1	4	2	N	34	2	Y	5,11,13	LSCS	35+1	1930	1,4	DHM+MOM	MOM	MOM	MOM	3	85	15-04-2023	745ML	8
26	129	1183127	15-04-2023	23-04-2023	1	F	1	4	1	4	2	N	34	2	Y	5,11,13	LSCS	35+1	1670	1,4	DHM+MOM	MOM	MOM	MOM	3	85	15-04-2023	465ML	5
27	131	1183173	15-04-2023	22-04-2023	3	F	1	3	1	4	2	N	27	2	Y	3,4,12,13	LSCS	34	1500	1	DHM+MOM	MOM	MOM	MOM	2	85	17-04-2023	180ML	4
28	132	1183358	17-04-2023	22-04-2023	1	M	1	3	1	4	2	N	30	3	Y	7,12	LSCS	33+3	2000	1	DHM+MOM	MOM	MOM	MOM	2,3	85	17-04-2023	180ML	3
29	134	1184768	24-04-2023	06-05-2023	1	M	1	4	1	4	2	N	28	1	Y	NONE	VD	32+1	1600	1,2	DHM+MOM	MOM	MOM	MOM	3	85	24-04-2023	75ML	3
30	135	1187060	04-05-2023	09-05-2023	1	M	1	3	1	4	2	N	23	1	Y	9,13	LSCS	37	2400	1	DHM+MOM	MOM	MOM	MOM	3	85	04-05-2023	90ML	2
31	136	1185798	28-04-2023	06-05-2023	6	M	1	3	1	4	1	N	26	2	Y	5,7	LSCS	37+3	2500	4	DHM+MOM	MOM	MOM	MOM	3	85	04-05-2023	200ML	3
32	137	1188507	12-05-2023	19-05-2023	2	F	1	3	1	4	2	N	26	1	Y	10	LSCS	40+3	2020	1,4	DHM+MOM	MOM	MOM	MOM	3	22	13-05-2023	210ML	2
33	140	1189432	17-05-2023	01-06-2023	4	M	1	3	1	4	4	N	26	1	Y	5	LSCS	39+4	3030	8	DHM+MOM	MOM	MOM	MOM	3	22	21-05-2023	120ML	2
34	141	1190275	21-05-2023	01-06-2023	1	F	2	4	1	4	2	N	28	7	Y	3,6,8	LSCS	33+2	1350	1	DHM+MOM	MOM	MOM	MOM	3	22	21-05-2023	680ML	9
35	142	1190208	20-05-2023	25-05-2023	2	F	1	4	1	4	2	N	26	1	Y	10	VD	37+4	1840	1	DHM+MOM	MOM	MOM	MOM	3	22	21-05-2023	100ML	2
36	143	1189923	19-05-2023	25-05-2023	3	M	1	3	1	4	2	N	26	1	Y	NONE	VD	36+3	2300	1,4,7	DHM+MOM	MOM	MOM	MOM	3	22	22-05-2023	300ML	3
37	144	1190615	22-05-2023	02-06-2023	2	F	1	3	1	4	2	N	26	1	Y	3,4,11	LSCS	35+3	1900	1,4	DHM+MOM	MOM	MOM	MOM	3	22	24-05-2023	840ML	7
38	145	1190616	22-05-2023	02-06-2023	2	F	1	3	1	4	2	N	26	1	Y	3,4,11	LSCS	35+3	1400	1,4	DHM+MOM	MOM	MOM	MOM	3	22	23-05-2023	1080ML	9
39	146	1191703	27-05-2023	06-06-2023	1	F	1	3	1	4	2	N	24	2	Y	10	LSCS	39+4	2000	1,4	DHM+MOM	MOM	MOM	MOM	2	22	27-05-2023	520ML	5
40	147	1191691	27-05-2023	02-06-2023	2	M	1	3	1	4	3	N	26	1	Y	10	VD	36+5	1860	1	DHM+MOM	MOM	MOM	MOM	3	22	28-05-2023	300ML	3
41	148	1191837	28-05-2023	04-06-2023	1	F	1	4	1	4	1	N	27	1	Y	3,5,11	LSCS	36	2000	1	DHM+MOM	MOM	MOM	MOM	3	22	28-05-2023	330ML	3
42	149	1191838	28-05-2023	04-06-2023	2	F	1	4	1	4	1	N	27	1	Y	3,5,11	LSCS	36	2040	1	DHM+MOM	MOM	MOM	MOM	3	22	29-05-2023	280ML	3
43	151	1192045	29-05-2023	08-06-2023	2	F	1	3	1	3	2	N	32	3	Y	7,10,11	LSCS	35+5	1900	1,4	DHM+MOM	MOM	MOM	MOM	3	22	30-05-2023	330ML	3
44	152	1192043	29-05-2023	08-06-2023	4	M	1	3	1	3	2	N	32	3	Y	7,10,11	LSCS	35+5	1970	1,4	DHM+MOM	MOM	MOM	MOM	3	22	02-06-2023	420ML	5
45	155	1192944	01-06-2023	12-06-2023	3	M	1	4	1	4	2	Y	28	1	Y	NONE	LSCS	39	2460	8	DHM+MOM	MOM	MOM	MOM	3	27	03-06-2023	200ML	3
46	156	1193433	04-06-2023	10-06-2023	1	M	1	3	1	4	2	N	29	3	Y	3,5,11	LSCS	34+3	2400	1,4	DHM+MOM	MOM	MOM	MOM	3	27	04-06-2023	320ML	4
47	157	1193434	04-06-2023	10-06-2023	1	M	1	3	1	4	2	N	29	3	Y	3,5,11	LSCS	34+3	2200	1,4	DHM+MOM	MOM	MOM	MOM	3	27	04-06-2023	330ML	3
48	158	1190275	05-06-2023	10-06-2023	1	M	1	2	1	2	4	N	32	2	Y	9	NVD	33+3	1970	1,4	DHM+MOM	MOM	MOM	MOM	3	27	05-06-2023	520ML	5
49	159	1193765	05-06-2023	10-06-2023	2	M	1	4	1	4	2	N	29	1	Y	2,10	LSCS	38+3	2600	6	DHM+MOM	MOM	MOM	MOM	3	27	06-06-2023	190ML	3
50	160	1193796	06-06-2023	10-06-2023	1	F	1	3	1	4	2	N	20	1	Y	10	VD	38+6	1900	1	DHM+MOM	MOM	MOM	MOM	3	27	06-06-2023	290ML	3
51	161	1193774	05-06-2023	12-06-2023	1	M	1	4	1	4	2	N	22	2	Y	NONE	LSCS	38+2	2260	1	DHM+MOM	MOM	MOM	MOM	3	27	06-06-2023	210ML	3
52	162	1193791	06-06-2023	11-06-2023	2	F	1	3	1	3	1	N	21	2	Y	NONE	VD	40+3	3030	3	DHM+MOM	MOM	MOM	MOM	3	27	07-06-2023	240ML	3
53	163	1194045	07-06-2023	13-06-2023	1	F	2	4	1	4	2	Y	21	1	Y	NONE	LSCS	39+3	2600	6	DHM+MOM	MOM	MOM	MOM	3	27	07-06-2023	270ML	3
54	164	1193918	06-06-2023	19-06-2023	2	F	1	3	1	4	2	N	22	2	Y	5,11	LSCS	34+2	1700	1	DHM	LACTOGEN	LACTOGEN	LACTOGEN	2	27	07-06-2023	1470ML	9
55	165	1193428	07-06-2023	14-06-2023	1	M	2	3	1	4	3	Y	29	3	Y	3,4	LSCS	35+6	2100	1	DHM+MOM	MOM	MOM	MOM	3	27	07-06-2023	380ML	4
56	166	1193336	03-06-2023	10-06-2023	5	M	1	3	1	4	2	N	23	1	Y	3,10	LSCS	39+5	2800	8	DHM+MOM	MOM	MOM	MOM	3	38	08-06-2023	300ML	3
57	167	1193916	06-06-2023	19-06-2023	3	F	1	3	1	4	2	N	22	2	Y	5,11	LSCS	34+2	1600	1	DHM	LACTOGEN	LACTOGEN	LACTOGEN	2	38	08-06-2023	1470ML	8
58	168	1194728	09-06-2023	15-06-2023	1	F	4	4	1	5	1	N	37	1	Y	1,3	LSCS	34+1	1600	1	DHM+MOM	MOM	MOM	MOM	3	38	09-06-2023	300ML	4
59	169	1194824	10-06-2023	18-06-2023	1	F	1	3	1	4	2	N	22	2	Y	7,11	LSCS	36	2300	1	DHM+MOM	MOM	MOM	MOM	3	38	10-06-2023	240ML	4
60	170	1194827	10-06-2023	18-06-2023	1	M	1	3	1	4	2	N	22	2	Y	7,11	LSCS	36	2520	6	DHM+MOM	MOM	MOM	MOM	3	38	10-06-2023	150ML	3
61	171	1194762	09-06-2023	17-06-2023	2	F	1	3	1	3	2	N	20	1	Y	NONE	LSCS	39+2	2200	1	DHM+MOM	MOM	MOM	MOM	3	38	10-06-2023	300ML	3
62	172	1195051	12-06-2023	17-06-2023	1	F	1	3	1	4	3	N	24	1	Y	2	VD	37+1	2600	8	DHM+MOM	MOM	MOM	MOM	3	38	12-06-2023	300ML	3
63	176	1195790	14-06-2023	23-06-2023	2	M	1	4	1	4	2	N	19	1	Y	3,10	LSCS	34+5	1600	1	DHM+MOM	MOM	MOM	MOM	3	38	15-06-2023	620ML	5
64	178	1196081	15-06-2023	22-06-2023	3	M	2	2	1	3	2	Y	27	3	Y	7	LSCS	37+2	1820	1	DHM+MOM	MOM	MOM	MOM	3	38	17-06-2023	270ML	3
65	179	1196562	18-06-2023	23-06-2023	1	M	1	3	1	4	2	N	30	1	Y	3	LSCS	38	2100	1	DHM+MOM	MOM	MOM	MOM	3	38	18-06-2023	310ML	3
66	180	1195468	13-06-2023	28-06-2023	7	F	1	3	1	3	2	N	28	2	Y	7,9,13	LSCS	30	1360	1	DHM+MOM	MOM	MOM	MOM	3	38	20-06-2023	200ML	5
67	185	1198959	27-06-2023	07-07-2023	1	F	1	2	1	2	2	Y	31	1	Y	10,13	LSCS	36+5	1980	1	DHM+MOM	MOM	MOM	MOM	3	38	27-06-2023	180ML	4
68	186	1198041	23-06-2023	06-07-2023	4	M	1	3	1	4	2	N	39	1	Y	3,5,11	LSCS	33+6	2000	1,4	DHM+MOM	MOM	MOM	MOM	3	41	27-06-2023	240ML	5
69	187	1198046	23-06-2023	06-07-2023	4	F	1	3	1	4	2	N	39	1	Y	3,5,11	LSCS	33+6	1800	1,4	DHM+MOM	MOM	MOM	MOM	3	41	27-06-2023	210ML	3
70	191	1198702	26-06-2023	07-07-2023	3	F	1	3	1	4	2	N	27	1	Y	10	LSCS	38+6	1920	1	DHM+MOM	MOM	MOM	MOM	3	41	29-06-2023	180ML	3
71	192	1199305	29-06-2023	24-07-2023	1	M	1	3	1	3	2	N	27	1	Y	10,11,13	LSCS	33+1	1600	1,2	DHM+MOM	MOM	MOM	MOM	3	41	30-06-2023	330ML	7
72	193	1199306	29-06-2023	24-07-2023	1	M	1	3	1	3	2	N	27	1	Y	10,11,13	LSCS	33+1	1600	1,2	DHM+MOM	MOM	MOM	MOM	3	41	30-06-2023	180ML	6
73	194	1199308	29-06-2023	24-07-2023	1	F	1	3	1	3	2	N	27	1	Y	10,11,13	LSCS	33+1	1400	1,2	DHM+MOM	MOM	MOM	MOM	3	41	30-06-2023	70ML	4
74	201	1201402	09-07-2023	19-07-2023	2	F	1	4	1	4	2	N	24	2	Y	7,9	LSCS	35+2	1800	1	DHM+MOM	MOM	MOM	MOM	3	41	10-07-2023	440ML	5
75	202	1201790	10-07-2023	16-07-2023	2	M	1	4	1	4	1	N	25	1	Y	10	LSCS	37+4	1800	1	DHM+MOM	MOM	MOM	MOM	3	14	11-07-2023	300ML	3

Serial Number	Method of feeding: At enrollment	At discharge	F/U at 2 weeks	F/U at 6 weeks	Anthropometric parameters: Weight (grams) At enrollment	At discharge	F/U at 2 weeks	F/U at 6 weeks	Length (cm) At enrollment	At discharge	F/U at 2 weeks	F/U at 6 weeks	Head circumference (cm) At enrollment	At discharge	F/U at 2 weeks	F/U at 6 weeks	Mid arm circumference (cm) At enrollment	At discharge	F/U at 2 weeks	F/U at 6 weeks	Complications: Sepsis	Necrotizing Enterocolitis	Total duration of hospital stay (Days)
1	SF+DBF	DBF	DBF	DBF	2890	2860	3130	4700	50	50	51	56.5	35	35	36	39	10	10	10	11	NONE	NONE	5
2	SPOON FEEDS	DBF	DBF	DBF	1540	1440	1560	2020	42	43	43	46	30	30.5	30.5	33	7.5	7.5	7.5	8	NONE	NONE	11
3	SPOON FEEDS	DBF	DBF	DBF	1550	1480	1580	2100	44	45	45	48.8	30	30.5	30.5	33.5	8	8	8	9	NONE	NONE	10
4	SF+DBF	DBF	DBF	DBF	2100	2090	2400	3100	46	46	47	50.5	34	34	35	37.5	9	9	9.5	10	NONE	NONE	6
5	SF+DBF	DBF	DBF	DBF	2170	2260	2260	3200	50	52	52	56	32	32	32	35	7.5	8	8	8.5	NONE	NONE	13
6	SF+DBF	DBF	DBF	DBF	1700	1780	1780	2860	45	46	46	49	29	30	30	33	8	8.5	8.5	9	NONE	NONE	12
7	RT FEEDS + SF	DBF	DBF	DBF	1540	1560	1560	2300	40	41	41	44	28	29	29	32	7	7	7	7.5	NONE	NONE	18
8	SPOON FEEDS	DBF	DBF	DBF	2000	1940	2150	2890	49	49	50	53	33	33	34	36	8	8	8	9	NONE	NONE	8
9	SF+DBF	DBF	DBF	DBF	1760	1700	1910	2820	41	41.5	42	44	30	30	30.5	33	8	8	8.5	9	NONE	NONE	8
10	SF+DBF	DBF	DBF	DBF	1610	1680	1890	3200	45	45.5	46	50	30	30	30.5	33	7.5	7.5	8	9	NONE	NONE	8
11	RT FEEDS	SF + DBF	DBF	DBF	1280	1190	1060	1420	38	38	38	40	28	29	28	30.5	5.5	6	5.5	6.5	CANDIDA	NONE	23
12	SF+DBF	DBF	DBF	DBF	2050	2250	2250	3200	51	52	52	55	33	34	34	36	9	9	9	10	NONE	NONE	13
13	SF+DBF	DBF	DBF	DBF	1960	2200	2200	3000	46	47	47	50	31	32	32	34	8.5	9	9	9.5	NONE	NONE	13
14	SPOON FEEDS	DBF	DBF	DBF	1690	2040	2040	3200	41	42	42	45	32	32.5	32.5	34.5	7.5	8	8	9	NONE	NONE	17
15	SPOON FEEDS	DBF	DBF	DBF	1750	2020	2020	2900	43	44	44	47	31	32	32	34.5	8	8.5	8.5	9	NONE	NONE	17
16	RT FEEDS	DBF	DBF	DBF	1590	1620	1620	2100	45	45.5	45.5	47	31	32	32	34.5	6.5	7	7	7.5	NONE	NONE	11
17	RT FEEDS	DBF	DBF	DBF	1780	1800	1800	2380	45	46	46	47.5	31	32	32	34	6.5	7	7	8	NONE	NONE	11
18	RT FEEDS	DBF	DBF	DBF	1500	1480	1480	2100	45	45	45	48	30	31	31	33.5	6.5	7	7	8	NONE	NONE	11
19	SF+DBF	DBF	DBF	DBF	2650	2640	2640	3800	52	53	53	55	33	34	34	36	10	10.5	10.5	11.5	NONE	NONE	8
20	RT FEEDS	DBF	DBF	DBF	1260	1290	1290	2160	42	43	43	45	28	28.5	28.5	31	7	7	7	8	NONE	NONE	14
21	SPOON FEEDS	DBF	DBF	DBF	1500	1380	1380	2290	45	45	45	48.5	29	29	29	32.5	8	8	8	9	NONE	NONE	9
22	SPOON FEEDS	DBF	DBF	DBF	1500	1350	1350	2210	44	44.5	44.5	46.5	29	30	30	33	7.5	8	8	8.5	NONE	NONE	9
23	RT FEEDS	DBF	DBF	DBF	1850	1810	1990	3010	48	48	48.5	51.5	30	30	31	33	8	8	8.5	9.5	NONE	NONE	8
24	RT FEEDS	DBF	DBF	DBF	1200	1300	1300	2230	42.5	43.5	43.5	46	28	28	28	30	7	7.5	7.5	8.5	NONE	NONE	15
25	SPOON FEEDS	DBF	DBF	DBF	1930	1960	1840	2840	43	44	44	47	30	31	31	33	7.5	8	8	9	NONE	NONE	20
26	SPOON FEEDS	DBF	DBF	DBF	1670	1840	1680	2680	45.5	46.5	46.5	48.5	30	31	31	33.5	7.5	8	8	9	NONE	NONE	20
27	SPOON FEEDS	DBF	DBF	DBF	1500	1580	1580	2620	44	45	45	48	29	30	30	32.5	8	8	8	9	KLEBSIELLA. P	NONE	18
28	SPOON FEEDS	DBF	DBF	DBF	2000	1920	2160	3200	44	44	45	48	30	30	31	34	8.5	8.5	8.5	9.5	NONE	NONE	5
29	RT FEEDS	DBF	DBF	DBF	1600	1550	1550	2220	41	42	42	45	28.5	29	29	31	7	7.5	7.5	8.5	NONE	NONE	12
30	SF+DBF	DBF	DBF	DBF	2400	2350	2620	3520	44	44	45	47.5	33	33	34	36	8.5	8.5	9	10.5	NONE	NONE	5
31	SF+DBF	DBF	DBF	DBF	2380	2440	2680	3480	51	51	52	55.5	33	33	33.5	35.5	9	9	9.5	10.5	NONE	NONE	8
32	SF+DBF	DBF	DBF	DBF	2000	2030	2200	3200	43	43.5	43.5	46	30	30	31	33	8	8	8.5	9	NONE	NONE	7
33	SF+DBF	DBF	DBF	DBF	2900	3240	3240	4100	54	55	55	58	35	36	36	38	9	9	9	10.5	NONE	NONE	15
34	SPOON FEEDS	DBF	DBF	DBF	1350	1220	1310	2200	44.5	45.5	45.5	48	31	32	32	34	7	7	7	8.5	NONE	NONE	11
35	SF+DBF	DBF	DBF	DBF	1840	1770	1820	2620	40	40	41	43.5	30	30	31	33	8	8	8	9	NONE	NONE	5
36	SF+DBF	DBF	DBF	DBF	2300	2190	2420	3400	46	46	47	50	30	30	30.5	33	8.5	8.5	8.5	9.5	NONE	NONE	6
37	SF+DBF	DBF	DBF	DBF	1890	1870	1960	3020	46	46.5	46.5	50	31	32	32	34	9	9.5	9.5	10.5	NONE	NONE	11
38	SF+DBF	DBF	DBF	DBF	1380	1350	1410	2260	43	44	44	46.5	28	29	29	31.5	7.5	8	8	9.5	NONE	NONE	11
39	SF+DBF	DBF	DBF	DBF	2000	1870	1990	3020	46	46	46	48.5	31.5	32	32	34.5	8	8	8	9.5	NONE	NONE	10
40	SF+DBF	DBF	DBF	DBF	1860	1900	2100	2940	46	46	46	48.5	33	33.5	34	36	8	8	8.5	9.5	NONE	NONE	6
41	SF+DBF	DBF	DBF	DBF	2000	1920	2100	2960	46	46	46	49	31.5	32	32.5	34.5	8.5	8.5	9	10	NONE	NONE	7
42	SF+DBF	DBF	DBF	DBF	2010	1860	2020	2830	46	46.5	46.5	49	31	31	31	34	8	8	8	9	NONE	NONE	7
43	SF+DBF	DBF	DBF	DBF	1860	1860	1930	2700	45	45.5	45.5	48	31	32	32	33.5	7.5	8	8	8.5	NONE	NONE	10
44	SF+DBF	DBF	DBF	DBF	1890	1880	2000	2900	45	46	46	48.5	31	32	32	34	8	8.5	8.5	9.5	NONE	NONE	10
45	SF+DBF	DBF	DBF	DBF	2420	2340	2380	3200	45	46	46	48	34	35	35	37.5	9.5	10	10	11	NONE	NONE	12
46	SF+DBF	DBF	DBF	DBF	2400	2280	2440	3280	49	49	49.5	52	33	33	34	36	9	9	9.5	10.5	NONE	NONE	6
47	SF+DBF	DBF	DBF	DBF	2200	2090	2250	3090	49	49	49	51.5	32	32	32	35	8	8	8	9	NONE	NONE	6
48	SPOON FEEDS	DBF	DBF	DBF	1970	2000	2180	3150	46	46	47	50	31	31	31.5	33	8	8	8.5	9.5	NONE	NONE	5
49	SF+DBF	DBF	DBF	DBF	2570	2460	2640	3240	49	49	49.5	51.5	33	33	33.5	35.5	9	9	9.5	10.5	NONE	NONE	5
50	SF+DBF	DBF	DBF	DBF	1900	1890	2090	2860	44	44	45	48	33	33	33	35.5	8	8	8.5	9.5	NONE	NONE	5
51	SF+DBF	DBF	DBF	DBF	2260	2260	2400	3200	48	48	48	50.5	34	34	34.5	36.5	8.5	8.5	8.5	9.5	NONE	NONE	7
52	SF+DBF	DBF	DBF	DBF	3000	2890	3040	3830	50	50	50.5	53	33	33	33.5	35.5	9.5	9.5	10	11	NONE	NONE	5
53	SF+DBF	DBF	DBF	DBF	2600	2650	2810	3600	48	48	48	50	34.5	34.5	35	37	10	10	10.5	11.5	NONE	NONE	6
54	SPOON FEEDS	SPOON FEEDS	SPOON FEEDS	SPOON FEEDS	1680	1580	1580	2570	44	45	45	47.5	28	29	29	31	7.5	7.5	7.5	8.5	NONE	NONE	13
55	SF+DBF	DBF	DBF	DBF	2100	2100	2240	3250	52	52	53	55	32	32.5	32.5	35	8	8	8	9	NONE	NONE	7
56	SF+DBF	DBF	DBF	DBF	2700	2790	2930	3820	52	52	52.5	55	35	35	35.5	38	10	10	10	10.5	NONE	NONE	7
57	SPOON FEEDS	SPOON FEEDS	SPOON FEEDS	SPOON FEEDS	1520	1470	1470	2380	45	46	46	48	27	28	28	31	7	7.5	7.5	8.5	NONE	NONE	13
58	SPOON FEEDS	DBF	DBF	DBF	1600	1380	1560	2360	45	45.5	45.5	47.5	32	32	32	34	8	8	8	9	NONE	NONE	6
59	SF+DBF	DBF	DBF	DBF	2300	2240	2360	3180	49	49	49.5	52	32	32	32.5	34.5	9	9	9.5	10	NONE	NONE	8
60	SF+DBF	DBF	DBF	DBF	2520	2580	2680	3420	48	48	48	50	33.5	33.5	34	36.5	9	9	9	10	NONE	NONE	8
61	SF+DBF	DBF	DBF	DBF	2200	2120	2240	3050	48	48	48.5	50	33	33	33.5	35.5	8	8	8.5	9	NONE	NONE	8
62	SF+DBF	DBF	DBF	DBF	2600	2500	2680	3490	49	49	49.5	52	35	35	35.5	38	8.5	8.5	8.5	9.5	NONE	NONE	5
63	SPOON FEEDS	DBF	DBF	DBF	1600	1430	1580	2560	44	45	45	48	30	30	30	34	7	7.5	7.5	8.5	NONE	NONE	9
64	SF+DBF	DBF	DBF	DBF	1730	1680	1820	2700	46	46	46	48.5	32	32	32.5	35	7.5	7.5	7.5	8	NONE	NONE	7
65	SF+DBF	DBF	DBF	DBF	2000	1890	2130	3000	48	48	48.5	51.5	33	33	33.5	36	8	8	8	9	NONE	NONE	5
66	RT FEEDS	DBF	DBF	DBF	1300	1420	1420	2200	43	44	44	46	28	28	28	30	6	6.5	6.5	7.5	NONE	NONE	15
67	SF+DBF	DBF	DBF	DBF	1980	1740	1850	2650	44	44.5	44.5	48	31	31.5	31.5	33	7.5	7.5	7.5	8.5	NONE	NONE	10
68	SPOON FEEDS	DBF	DBF	DBF	1880	1810	1840	2680	50	51	51	52	30	31	31	33	6.5	7	7	8	NONE	NONE	13
69	SPOON FEEDS	DBF	DBF	DBF	1680	1680	1700	2500	50	50.5	50.5	52	28	29	29	31.5	7	7.5	7.5	8.5	NONE	NONE	13
70	SF+DBF	DBF	DBF	DBF	1850	1790	1880	2630	48	49	49	51	32	32.5	32.5	34	7.5	8	8	9	NONE	NONE	11
71	RT FEEDS	DBF	DBF	DBF	1600	1700	1480	2350	46	48	47	50	33	34	33.5	36.5	7	8	7.5	9	NONE	NONE	25
72	RT FEEDS	DBF	DBF	DBF	1600	1650	1400	2280	46	47	47	49	33	34	34	36	7.5	8	8	9	NONE	NONE	25
73	RT FEEDS	DBF	DBF	DBF	1400	1380	1280	1980	43	44	44	46.5	32	33	33	35	7	7.5	7.5	8.5	NONE	NONE	25
74	SF+DBF	DBF	DBF	DBF	1760	1750	1840	2820	44	44.5	44.5	48	30	31	31	34	7.5	8	8	8.5	NONE	NONE	10
75	SF+DBF	DBF	DBF	DBF	1750	1680	1900	2720	48	48	48	51	32	32	32	35	7	7	7	8	NONE	NONE	6

76	203	1202103	11-07-2023	26-07-2023	2	M	1	2	1	4	2	N	32	4	Y	7,8	LSCS	34+6	1500	1,2	DHM+MOM	MOM	MOM	MOM	MOM	2	14	12-07-2023	330ML	3	SPOON FEEDS	DBF	DBF	DBF	1460	1490	1490	2390	42	43	43	45	29	29.5	29.5	32	6	6	6	7.5	ENTEROBACTER	NONE	15
77	204	1202432	13-07-2023	26-07-2023	1	F	2	3	1	4	2	Y	23	3	Y	7,10	LSCS	37+5	1400	1	DHM+MOM	MOM	MOM	MOM	MOM	3	14	13-07-2023	470ML	5	SF+DBF	DBF	DBF	DBF	1400	1480	1480	2460	45	46	46	48	31	32	32	34	6	6.5	6.5	7.5	NONE	NONE	13
78	205	1201482	10-07-2023	15-07-2023	3	M	2	3	1	4	2	N	32	7	Y	3,8	LSCS	34+3	2050	1	DHM+MOM	MOM	MOM	MOM	MOM	2,3	14	13-07-2023	240ML	2	SPOON FEEDS	DBF	DBF	DBF	1980	1890	2070	2890	48	48	49	51.5	32	32	33	35	8	8	8.5	9.5	NONE	NONE	5
79	206	10006732	11-09-2023	05-10-2023	2	F	1	3	1	4	3	N	26	3	Y	3	LSCS	31	1220	1,2	DHM+MOM	MOM	MOM	MOM	MOM	2	14	12-09-2023	485ML	6	RT FEEDS	DBF	DBF	DBF	1220	1240	1050	2150	43	44	44	46	29	30	30	32	6.5	7	7	8	NONE	NONE	24
80	207	10006940	12-09-2023	23-09-2023	1	F	1	3	1	4	2	N	20	1	Y	3,11	LSCS	33+2	1800	1	DHM+MOM	MOM	MOM	MOM	MOM	3	14	12-09-2023	930ML	6	SPOON FEEDS	DBF	DBF	DBF	1800	1880	1950	2960	44	44.5	44.5	48	32	32.7	32.7	34	7	7.5	7.5	8.5	NONE	NONE	11
81	208	10006941	12-09-2023	23-09-2023	1	F	1	3	1	4	2	N	20	1	Y	3,11	LSCS	33+2	1900	1	DHM+MOM	MOM	MOM	MOM	MOM	3	14	12-09-2023	670ML	5	SPOON FEEDS	DBF	DBF	DBF	1900	1840	1915	2850	46	46.7	46.7	48.5	30	30.5	30.5	33	7	7.5	7.5	8	NONE	NONE	11
82	209	10007095	13-09-2023	21-09-2023	1	F	1	4	1	4	2	N	23	1	Y	NONE	LSCS	36+3	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	2	14	13-09-2023	1000ML	5	SF+DBF	DBF	DBF	DBF	2300	2280	2280	3450	44	44.5	45	47	31	30	31.3	33.4	8	8	8.5	9.5	NONE	NONE	8
83	210	10007214	13-09-2023	24-09-2023	2	M	1	3	1	4	2	N	26	1	Y	9	VD	36+6	2250	1	DHM+MOM	MOM	MOM	MOM	MOM	3	14	14-09-2023	180ML	3	SF+DBF	DBF	DBF	DBF	2250	2320	2380	3170	46	46.8	46.8	47.9	32	32.5	32.5	34.5	8	8.5	8.5	9.5	NONE	NONE	11
84	211	10007713	16-09-2023	06-10-2023	1	M	1	3	1	4	2	Y	26	1	Y	9	LSCS	31+1	1920	1,2	DHM+MOM	MOM	MOM	MOM	MOM	3	14	16-09-2023	180ML	3	RT FEEDS	DBF	DBF	DBF	1920	2000	1900	2760	44	45	44.7	46,2	30	31	31	32.4	7.5	8	8	9	KLEBSIELLA P	NONE	20
85	213	10007898	16-09-2023	30-09-2023	2	F	1	3	1	3	3	N	28	1	Y	3,10	LSCS	36+2	1700	1	DHM+MOM	MOM	MOM	MOM	MOM	3	14	17-09-2023	870ML	10	SF+DBF	DBF	DBF	DBF	1700	1680	1680	2600	44	44.5	44.5	46.8	31	32	32	34	7	7	7	8	NONE	NONE	14
86	214	10007901	17-09-2023	05-10-2023	1	M	1	3	1	4	2	N	26	3	Y	3,10	LSCS	32+1	1300	1,2	DHM+MOM	MOM	MOM	MOM	MOM	3	64	17-09-2023	520ML	6	RT FEEDS	DBF	DBF	DBF	1300	1380	1280	2160	43	44	44	45.5	28	29	29	31.8	6.5	7	7	7.5	KLEBSIELLA P	NONE	18
87	215	10007953	17-09-2023	26-09-2023	1	M	1	4	1	4	2	N	25	1	Y	NONE	VD	33+6	1680	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	17-09-2023	130ML	4	SPOON FEEDS	DBF	DBF	DBF	1680	1590	1640	2340	44	44.5	44.5	45.7	29	29.5	29.5	31.2	7	7	7	8	NONE	NONE	9
88	216	10007967	18-09-2023	28-09-2023	1	M	1	3	1	3	3	N	23	2	Y	NONE	VD	36+3	2100	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	18-09-2023	380ML	4	SF+DBF	DBF	DBF	DBF	2100	2120	2180	2980	48	48.5	48.5	50	33	33.5	33.5	35.5	8	8	8	9	NONE	NONE	10
89	217	10007928	15-09-2023	22-09-2023	3	M	1	3	1	4	2	N	28	3	Y	7	LSCS	39+3	3200	4	DHM+MOM	MOM	MOM	MOM	MOM	3	64	18-09-2023	280ML	3	SF+DBF	DBF	DBF	DBF	3120	3190	3310	4100	51	51.5	52	54.4	34	34.5	35	37	9	9	9	10	NONE	NONE	7
90	218	10007832	16-09-2023	22-09-2023	3	F	2	3	1	4	3	Y	23	3	Y	10	LSCS	35+5	2000	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	19-09-2023	150ML	3	SF+DBF	DBF	DBF	DBF	1950	1960	2070	2830	47	47	47.5	49	32	32	32.3	34.6	8	8	8	9	NONE	NONE	6
91	219	10007946	17-09-2023	06-10-2023	3	F	1	3	1	4	2	Y	27	1	Y	3,4,10	LSCS	32+5	1300	1,2	DHM+MOM	MOM	MOM	MOM	MOM	3	64	20-09-2023	210ML	4	RT FEEDS	DBF	DBF	DBF	1220	1270	1250	2000	43	43.7	43.7	45	29	30	30	31.7	7	7.5	7.5	8.5	NONE	NONE	19
92	220	10008246	20-09-2023	23-09-2023	1	M	2	3	1	3	2	N	22	1	Y	3,10	VD	36+4	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	20-09-2023	150ML	3	SF+DBF	DBF	DBF	DBF	2300	2220	2400	3180	48	48	48.5	50.5	34	34	34	36.3	8.5	8.5	8.5	9.5	NONE	NONE	3
93	221	10008335	20-09-2023	27-09-2023	2	M	1	3	1	4	2	N	24	1	Y	NONE	LSCS	39+3	2520	3,4	DHM+MOM	MOM	MOM	MOM	MOM	3	64	21-09-2023	180ML	5	SF+DBF	DBF	DBF	DBF	2500	2480	2600	3380	50	50	50.5	52.8	34	34	34	36.5	8.5	8.5	8.5	9.5	NONE	NONE	7
94	222	10008511	21-09-2023	26-09-2023	1	F	1	3	1	4	3	N	20	1	Y	NONE	VD	36+6	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	21-09-2023	180ML	4	SF+DBF	DBF	DBF	DBF	2300	2220	2400	3190	49	49	49.8	51.5	33	33	33.5	35.5	8.5	8.5	8.5	9	NONE	NONE	5
95	223	10008512	21-09-2023	28-09-2023	1	M	1	3	1	3	2	N	25	1	Y	10	LSCS	36+6	2100	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	21-09-2023	180ML	4	SF+DBF	DBF	DBF	DBF	2100	2040	2190	2970	48	48	48.5	50.2	32	32	32.5	34	8.5	8.5	8.5	9	NONE	NONE	7
96	224	10008851	22-09-2023	06-10-2023	1	M	1	4	1	4	2	N	28	4	Y	3,5,7,8	LSCS	32	1500	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	22-09-2023	510ML	7	RT FEEDS	DBF	DBF	DBF	1500	1580	1580	2440	45	46	46	48.5	30	31	31	33	7.5	8	8	9	NONE	NONE	14
97	225	10008985	22-09-2023	26-09-2023	2	F	1	3	1	4	3	N	28	1	Y	NONE	VD	37+5	1900	1	DHM+MOM	MOM	MOM	MOM	MOM	3	64	23-09-2023	210ML	3	SF+DBF	DBF	DBF	DBF	1880	1820	1940	2740	46	46	46.5	48.7	31.5	31.5	31.5	33.8	7.5	7.5	7.5	8.5	NONE	NONE	4
98	227	10009162	23-09-2023	27-09-2023	2	M	1	2	1	3	3	N	24	2	Y	10	VD	37	2100	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	24-09-2023	240ML	3	SF+DBF	DBF	DBF	DBF	2100	2010	2120	2920	47	47	47.5	49.7	32	32	32.3	34.8	8	8	8	8.5	NONE	NONE	4
99	228	10009308	24-09-2023	29-09-2023	2	F	1	3	1	4	3	N	28	1	Y	NONE	LSCS	36+4	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	25-09-2023	390ML	5	SF+DBF	DBF	DBF	DBF	2260	2190	2370	3190	48	48	48.8	51	33	33	33.7	35.2	8.5	8.5	9	9.8	NONE	NONE	5
100	229	10009196	23-09-2023	29-09-2023	2	F	1	3	1	4	2	N	28	1	Y	NONE	LSCS	37	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	25-09-2023	210ML	4	SF+DBF	DBF	DBF	DBF	2260	2250	2380	3140	47	47	47.5	49.5	34	34	34.5	36	8.5	8.5	8.5	9.5	NONE	NONE	6
101	230	10009757	26-09-2023	02-10-2023	2	F	1	4	2	4	2	N	27	2	Y	5,7	LSCS	36+6	1980	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	27-09-2023	230ML	4	SF+DBF	DBF	DBF	DBF	1950	1900	1970	2760	46	46	46.5	48.8	33.5	33.5	34	35.2	8	8	8	9	NONE	NONE	6
102	231	10010093	27-09-2023	31-10-2023	1	F	1	3	1	4	2	N	28	3	Y	11	LSCS	32+5	1520	1,2	DHM+MOM	MOM	MOM	MOM	MOM	3	67	27-09-2023	600ML	7	RT FEEDS	DBF	DBF	DBF	1520	1820	1500	2080	43.5	44.7	44	47	29	30.5	29.4	31.5	7	8	7.5	8	NONE	NONE	34
103	232	10010094	27-09-2023	31-10-2023	2	M	1	3	1	4	2	N	28	3	Y	11	LSCS	32+5	1820	1,2	DHM+MOM	MOM	MOM	MOM	MOM	3	67	28-09-2023	690ML	8	RT FEEDS	DBF	DBF	DBF	1800	2060	1760	2360	44.5	45.5	45	47.8	30	31	30.4	33	7.5	8	8	8.5	ACINETOBACTER B	NONE	34
104	233	10010141	27-09-2023	02-10-2023	3	M	2	3	1	4	3	Y	23	2	Y	NONE	VD	37+3	3000	4	DHM+MOM	MOM	MOM	MOM	MOM	3	67	30-09-2023	210ML	3	SF+DBF	DBF	DBF	DBF	2870	2850	3010	3590	51	51	51.5	53	34	34	34	35.5	9	9	9.3	10	NONE	NONE	5
105	237	10009492	25-09-2023	03-10-2023	4	F	2	3	1	4	2	Y	26	1	Y	3,10	LSCS	37+3	2000	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	01-10-2023	360ML	3	SF+DBF	DBF	DBF	DBF	1920	1940	2020	2710	46.5	46.5	46.8	48.8	32	32	32.3	34.5	8	8	8	9	NONE	NONE	8
106	238	10010637	30-09-2023	05-10-2023	2	M	1	4	2	4	1	N	27	1	Y	NONE	LSCS	37+3	2420	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	01-10-2023	290ML	3	SF+DBF	DBF	DBF	DBF	2400	2340	2480	3220	48	48	48.5	50.5	33	33	33.5	35.5	8	8	8	9	NONE	NONE	5
107	239	10010636	30-09-2023	06-10-2023	2	F	1	3	1	4	2	N	29	1	Y	3,10	LSCS	39+2	1800	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	02-10-2023	570ML	4	SF+DBF	DBF	DBF	DBF	1760	1720	1840	2670	43	43	43.5	46	31	31	31.7	34	7.5	7.5	7.5	8.5	NONE	NONE	6
108	240	10010607	25-09-2023	10-10-2023	5	F	1	3	1	4	3	N	28	2	Y	3,10	VD	37+4	2200	1	DHM+MOM	MOM	MOM	MOM	MOM	3	67	30-09-2023	1710ML	8	SF+DBF	DBF	DBF	DBF	2100	2280	2280	3120	47	48	48	50	32	32.8	32.8	35	7.5	8	8	9	NONE	NONE	15
109	241	10010766	30-09-2023	13-10-2023	2	M	1	3	1	4	2	N	26	3	Y	7	LSCS	37+5	2620	8	DHM+MOM	MOM	MOM	MOM	MOM	3	68	01-10-2023	240ML	3	SF+DBF	DBF	DBF	DBF	2600	2740	2740	3390	50	51.5	51.5	52.3	33	33.7	33.7	34.5	8	8	8	8.5	NONE	NONE	13
110	242	10010154	01-10-2023	04-10-2023	4	F	1	4	2	4	2	N	24	1	Y	NONE	LSCS	37+3	2700	4	DHM+MOM	MOM	MOM	MOM	MOM	3	68	01-10-2023	270ML	3	SF+DBF	DBF	DBF	DBF	2580	2640	2750	3330	50	50	50.5	51.5	33	33	33	35.5	8	8	8	9	NONE	NONE	7
111	243	10010967	02-10-2023	15-10-2023	1	M	1	3	1	3	2	N	28	1	Y	3	LSCS	34+2	1720	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	02-10-2023	2320ML	10	SPOON FEEDS	DBF	DBF	DBF	1720	1790	1790	2590	44	45	45	47.3	31	31.5	31.5	33	7.5	8	8	8.8	NONE	NONE	13
112	244	10011666	05-10-2023	10-10-2023	2	M	1	3	1	4	2	N	27	2	Y	10	VD	38+6	2200	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	06-10-2023	210ML	4	SF+DBF	DBF	DBF	DBF	2160	2120	2290	2950	46	46	46.7	47.8	32	32	32.5	33.5	8.5	8.5	9	9	NONE	NONE	5
113	245	10011728	05-10-2023	11-10-2023	2	F	1	2	1	4	3	N	26	2	Y	11	LSCS	37	2120	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	06-10-2023	610ML	5	SF+DBF	DBF	DBF	DBF	2100	2020	2260	3040	47	47	47.5	48.8	33	33	34	35.5	8.5	8.5	9	9.8	NONE	NONE	6
114	246	10011960	06-10-2023	11-10-2023	1	M	1	3	1	4	2	N	29	3	Y	7,10	LSCS	38+4	2200	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	06-10-2023	450ML	4	SF+DBF	DBF	DBF	DBF	2200	2110	2.32	2960	47	47	47.4	48.8	33	33	33.5	35	8	8	8	9	NONE	NONE	5
115	247	10012410	08-10-2023	15-10-2023	1	M	1	3	1	3	3	N	22	3	Y	7	LSCS	35+4	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	08-10-2023	420ML	3	SF+DBF	DBF	DBF	DBF	2300	2270	2410	3030	47	47	47.7	50	33	33	33	36.5	8.5	8.5	8.5	9	NONE	NONE	7
116	248	10012121	06-10-2023	13-10-2023	2	F	1	2	1	4	3	N	24	2	Y	11	VD	37+6	2200	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	07-10-2023	390ML	3	SF+DBF	DBF	DBF	DBF	2160	2180	2300	2930	47.5	47.5	48	50	32	32	32.3	34.8	8	8	8	9	NONE	NONE	7
117	249	10012120	06-10-2023	13-10-2023	2	F	1	2	1	4	3	N	24	2	Y	11	VD	37+6	2210	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	07-10-2023	370ML	3	SF+DBF	DBF	DBF	DBF	2180	2170	2280	2880	46.5	46.5	47	49.5	33	33	33.5	35.2	8	8	8	8.5	NONE	NONE	7
118	250	10012340	08-10-2023	15-10-2023	1	F	1	3	1	3	3	N	26	1	Y	3	LSCS	32+3	1560	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	08-10-2023	360ML	3	SPOON FEEDS	DBF	DBF	DBF	1560	1580	1580	2210	44	44.5	44.5	46.5	29	30	30	31.7	6.5	7	7	8	NONE	YES	14
119	251	10012211	07-10-2023	13-10-2023	2	M	1	2	1	3	4	N	28	2	Y	3,10	LSCS	36+3	2200	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	08-10-2023	240ML	4	SF+DBF	DBF	DBF	DBF	2170	2150	2260	2860	47	47	47.3	49	32.5	32.5	33	34.7	8	8	8	9	NONE	NONE	6
120	252	10012452	08-10-2023	21-10-2023	2	F	1	4	2	4	2	N	22	2	Y	11	LSCS	33+3	1820	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	09-10-2023	620ML	6	SPOON FEEDS	DBF	DBF	DBF	1800	1840	1840	2630	45	45.7	45.7	48	31	31.5	31.5	34	7	7	7	8.5	NONE	NONE	13
121	253	10012453	08-10-2023	21-10-2023	2	F	1	4	2	4	2	N	22	2	Y	11	LSCS	33+3	1980	1	DHM+MOM	MOM	MOM	MOM	MOM	3	68	09-10-2023	710ML	6	SPOON FEEDS	DBF	DBF	DBF	1950	1960	1960	2690	45.5	46.3	46.3	48.5	32	33	33	35	7	7	7	8	NONE	NONE	13
122	254	10011561	04-10-2023	10-10-2023	4	M	1	3	1	3	3	N	27	1	Y	NONE	LSCS	38+2	3010	4	DHM+MOM	MOM	MOM	MOM	MOM	3	71	09-10-2023	460ML	3	SF+DBF	DBF	DBF	DBF	2960	3000	3110	3800	52	52	52.5	54.5	35	35.5	36	37.7	8.5	8.5	8.5	9.5	NONE	NONE	8
123	255	10011724	05-10-2023	11-10-2023	4	M	1	2	1	4	3	N	26	2	Y	11	LSCS	37	2400	1,4	DHM+MOM	MOM	MOM	MOM	MOM	3	71	10-10-2023	250ML	2	SF+DBF	DBF	DBF	DBF	2320	2350	2500	3110	48.5	48.5	48.5	50	33.5	33.5	34	35.5	8	8	8	9	NONE	NONE	6
124	257	10012743	09-10-2023	15-10-2023	1	F	2	3	1	3	3	Y	27	3	Y	7,11	LSCS	35+1	1940	1,4	DHM+MOM	MOM	MOM	MOM	MOM	3	71	09-10-2023	340ML	3	SF+DBF	DBF	DBF	DBF	1940	1840	1920	2520	44	44	44.5	46	33	33	33.5	35.5	8	8	8	9	NONE	NONE	6
125	258	10012745	09-10-2023	15-10-2023	1	F	2	3	1	3	3	Y	27	3	Y	7,11	LSCS	35+1	1560	1,4	DHM+MOM	MOM	MOM	MOM	MOM	3	71	09-10-2023	330ML	3	SF+DBF	DBF	DBF	DBF	1560	1470	1520	2140	43	43	43.5	44.8	30	30	30	32	7	7	7	8	NONE	NONE	6
126	259	10012464	09-10-2023	15-10-2023	2	M	1	2	1	3	3	N	22	1	Y	NONE	VD	35+2	1980	1	DHM+MOM	MOM	MOM	MOM	MOM	3	71	10-10-2023	290ML	4	SF+DBF	DBF	DBF	DBF	1960	1870	2020	2610	44	44	44.5	45.8	31.5	31.5	32	33.3	8	8	8	9	NONE	NONE	6
127	262	10013113	11-10-2023	19-10-2023	2	M	1	4	1	4	2	N	25	2	Y	7,11	LSCS	35+5	1820	1	DHM+MOM	MOM	MOM	MOM	MOM	3	71	12-10-2023	360ML	3	SF+DBF	DBF	DBF	DBF	1800	1710	1840	2520	44	44.5	44.8	46.5	30.5	31	31	33	7.5	7.5	7.8	9	NONE	NONE	8
128	263	10013114	11-10-2023	19-10-2023	2	M	1	4	1	4	2	N	25	2	Y	7,11	LSCS	35+5	2140	1	DHM+MOM	MOM	MOM	MOM	MOM	3	71	12-10-2023	400ML	3	SF+DBF	DBF	DBF	DBF	2100	2030	2150	2830	46.5	46.5	47	49.5	32	32	32.3	35	8	8	8.5	9.5	NONE	NONE	8
129	264	10013247	11-10-2023	15-10-2023	2	F	1	4	1	4	2	Y	27	2	Y	7	LSCS	37+3	2030	1	DHM+MOM	MOM	MOM	MOM	MOM	3	71	12-10-2023	300ML	3	SF+DBF	DBF	DBF	DBF	2000	1950	2100	2750	47	47	47.5	49.3	33	33	33.8	35.7	8	8	8.5	9.3	NONE	NONE	4
130	265	10013110	11-10-2023	16-10-2023	3	F	1	3	1	4	2	N	24	1	Y	3	VD	37+6	2200	1,4	DHM+MOM	MOM	MOM	MOM	MOM	3	71	13-10-2023	240ML	4	SF+DBF	DBF	DBF	DBF	2140	2110	2200	2840	47.5	47.5	48	49.3	32	32	32.3	34	8.5	8.5	8.5	9.3	NONE	NONE	5
131	266	10013370	11-10-2023	17-10-2023	3	F	2	2	1	3	3	Y	23	2	Y	7	LSCS	39+1	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	3	71	13-10-2023	300ML	4	SF+DBF	DBF	DBF	DBF	2260	2210	2370	3010	48	48	48.3	50.3	33	33	33.5	35.3	8	8	8	9	NONE	NONE	6
132	267	10013846	13-10-2023	27-10-2023	2	M	1	3	1	4	2	N	25	4	Y	3,6,10	LSCS	36	1900	1	DHM+MOM	MOM	MOM	MOM	MOM	3	71	14-10-2023	340ML	3	SF+DBF	DBF	DBF	DBF	1870	1990	1990	2540	44.5	45	45	46.5	31	31.8	31.8	33	8	8.5	8.5	9.5	NONE	NONE	14
133	268	10013962	15-10-2023	21-10-2023	3	M	2	3	1	3	3	Y	22	2	Y	NONE	VD	38+6	2500	4	DHM+MOM	MOM	MOM	MOM	MOM	3	71	17-10-2023	240ML	2	SF+DBF	DBF	DBF	DBF	2000	2060	2200	2710	50	50	50.5	51.8	35	35	35.3	36.5	9.5	9.5	9,5	10	NONE	NONE	6
134	269	10014700	17-10-2023	23-10-2023	2	M	1	2	1	3	3	N	24	1	Y	NONE	VD	37	2500	4	DHM+MOM	MOM	MOM	MOM	MOM	3	73	15-10-2023	360ML	3	SF+DBF	DBF	DBF	DBF	2480	2460	2590	3150	49.5	49.5	50	51.3	34	34	34.3	35.8	9	9	9	10	NONE	NONE	6
135	270	10014954	18-10-2023	30-10-2023	1	F	1	4	1	4	2	N	26	1	Y	3,4	LSCS	35+6	1560	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	18-10-2023	520ML	5	SF+DBF	DBF	DBF	DBF	1560	1460	1500	2220	43.5	44	44	46	30.5	31	31	32.5	6.5	7	7	8	NONE	NONE	8
136	271	10014551	17-10-2023	24-10-2023	1	M	1	2	1	3	3	N	24	2	Y	NONE	LSCS	36+2	2360	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	19-10-2023	400ML	4	SF+DBF	DBF	DBF	DBF	2360	2300	2440	3140	49	49	49.3	51.3	34	34	34.5	36	8.5	8.8	9	10	NONE	NONE	7
137	272	10015219	19-10-2023	26-10-2023	2	F	1	3	1	3	2	N	25	2	Y	7,10	LSCS	36	2050	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	19-10-2023	380ML	3	SF+DBF	DBF	DBF	DBF	2020	1990	2100	2690	48	48.3	48.5	50	33	33	33.3	34.8	8	8	8	9	NONE	NONE	7
138	273	10015269	19-10-2023	25-10-2023	1	M	2	2	1	3	3	Y	22	1	Y	NONE	LSCS	36+2	2300	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	20-10-2023	300ML	3	SF+DBF	DBF	DBF	DBF	2300	2240	2360	2940	48.5	48.5	48.8	50.5	33.5	33.5	34	35.3	8	8	8	9	NONE	NONE	6
139	274	10015290	20-10-2023	26-10-2023	3	M	1	2	1	2	3	N	23	1	Y	NONE	LSCS	40+2	3300	4	DHM+MOM	MOM	MOM	MOM	MOM	3	73	22-10-2023	240ML	2	SF+DBF	DBF	DBF	DBF	3260	3230	3365	3985	52.5	52.5	53	54.3	35	35	35.5	37.3	9	9	9.3	10	NONE	NONE	6
140	275	10015426	20-10-2023	25-10-2023	1	M	1	3	1	3	3	N	24	1	Y	10	VD	37+5	2000	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	22-10-2023	300ML	3	SF+DBF	DBF	DBF	DBF	2000	1930	2100	2640	47.5	47.5	48	49.3	33	33	33.3	34.8	7.5	7.5	8	9	NONE	NONE	5
141	276	10015872	23-10-2023	28-10-2023	2	M	2	4	2	4	1	N	27	2	Y	10	VD	37+2	2000	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	22-10-2023	280ML	2	SF+DBF	DBF	DBF	DBF	1980	1920	2100	2700	47.5	47.5	48	49	32	32	32.5	33.5	8	8	8	8.8	NONE	NONE	5
142	277	10015526	21-10-2023	26-10-2023	2	M	1	4	1	4	1	N	29	3	Y	4	LSCS	36+3	3300	4	DHM+MOM	MOM	MOM	MOM	MOM	3	73	22-10-2023	270ML	3	SF+DBF	DBF	DBF	DBF	3280	3250	3420	3940	52	52	52.5	54.5	35.5	35.5	36	37.5	9	9	9	10	NONE	NONE	5
143	278	10015538	21-10-2023	03-11-2023	1	M	2	3	1	3	3	N	28	4	Y	6,9	LSCS	33+4	1900	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	22-10-2023	520ML	4	SPOON FEEDS	DBF	DBF	DBF	1900	1980	1980	2800	46.5	47	47	49	32.5	33	33	34.5	7.5	8	8	9	NONE	NONE	13
144	279	10015985	25-10-2023	08-11-2023	3	F	1	3	2	4	2	N	25	3	Y	3,4,7	LSCS	34	1650	1,4	DHM+MOM	MOM	MOM	MOM	MOM	3	73	25-10-2023	600ML	5	SF+DBF	DBF	DBF	DBF	1570	1600	1600	2440	43	44	44	46	30	31	31	33	6	6.5	6.5	7.5	NONE	NONE	14
145	280	10016217	25-10-2023	03-11-2023	1	M	1	3	1	3	3	N	23	1	Y	10	LSCS	36+1	1960	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	26-10-2023	480ML	4	SF+DBF	DBF	DBF	DBF	1960	1890	2000	2680	47	47.5	47.8	49.3	32	32.5	32.5	34.3	8	8	8.3	9.5	NONE	NONE	9
146	281	10016619	27-10-2023	05-11-2023	1	F	1	2	1	3	2	N	29	4	Y	7,10	LSCS	33+5	1550	1	DHM+MOM	MOM	MOM	MOM	MOM	3	73	27-10-2023	600ML	5	SPOON FEEDS	DBF	DBF	DBF	1550	1500	1590	2290	43	43.5	43.5	45.5	31	31.5	31.5	33	6.8	7	7	8	NONE	NONE	9

Energy