

**“TO STUDY THE EFFECT OF POOLED PASTEURIZED
DONOR HUMAN MILK VERSUS UNPOOLED
PASTEURIZED PRETERM DONOR HUMAN MILK ON THE
SHORT TERM GROWTH PARAMETERS OF PRETERM
INFANTS - A ONE YEAR RANDOMIZED CONTROLLED
TRIAL AT KLE, DR.PRABHAKAR KORE HOSPITAL AND
MRC, BELAGAVI.”**

BY

REG NO. – BM0120001

Dissertation

Submitted to

KAFER, Belagavi, Karnataka,

In partial fulfilment of the requirements for the degree of

M. D. (Doctor of Medicine)

IN

PAEDIATRICS


**DEPARTMENT OF PAEDIATRICS,
JAWAHARLAL NEHRU MEDICAL COLLEGE
BELAGAVI, KARNATAKA**

JUNE/JULY – 2023

KLE Academy of Higher Education and Research, Belagavi,
Karnataka


Endorsement by the HOD, Principal/Head
of the Institution

This is to certify that the dissertation entitled “To study the effect of Pooled Pasteurized Donor Human Milk versus Unpooled Pasteurized Preterm Donor Human Milk on the short term growth parameters of preterm infants - A ONE YEAR RANDOMIZED CONTROLLED TRIAL AT KLE, DR.PRABHAKAR KORE HOSPITAL AND MRC, BELAGAVI” is a bonafide and genuine research work carried out by REG NO. BM0120001


Dr. TANMAYA METGUD M.D.
Professor & Head,
Department of Paediatrics,
J. N. Medical College,
Nehru Nagar,
Belagavi-590010

Date: 2/1/2023
Place: Belagavi.




Dr.N.S.MAHANTASHETTI M.D.
Principal,
J.N.Medical College,
Nehru Nagar,
Belagavi-590010.

PRINCIPAL
J.N. Medical College,
BELAGAVI- 590 010
Date: 2/1/2023
Place: Belagavi.

UNDERTAKING


I REG NO. – BM0120001 here by declare that the information and the data mentioned in my dissertation entitled “TO STUDY THE EFFECT OF POOLED PASTEURIZED DONOR HUMAN MILK VERSUS UNPOOLED PASTEURIZED PRETERM DONOR HUMAN MILK ON THE SHORT TERM GROWTH PARAMETERS OF PRETERM INFANTS - A ONE YEAR RANDOMIZED CONTROLLED TRIAL AT KLE, DR.PRABHAKAR KORE HOSPITAL AND MRC, BELAGAVI.” belongs to me and is original. I am aware of the definition of plagiarism as detailed below.

- An act or instance of using are closely imitating the language and thoughts of another author without authorization and the representation of that authors work as one’s own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another’s words, thoughts or ideas as one’s own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the dissertation prepared by me is original one and does not involve plagiarism anywhere. In case at a later stage, it is found that I have indulged in plagiarism, then, I am solely responsible for the same and the institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the university.

Date: 2/1/2023

Place: Belagavi.



ANTI- PLAGIARISM CHECK – ACCEPTANCE LETTER



JAWAHARLAL NEHRU MEDICAL COLLEGE

(Recognized by Medical Council of India, New Delhi)

Accredited 'A+' Grade by NAAC (3rd Cycle)

Placed in Category 'A' by MHRD (GoI)



Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 - 2471350



0831 - 2470759



www.jnmc.edu


principal@jnmc.edu

Ref No: MDC/PG/


Date: 31-12-2022.

ACCEPTANCE LETTER

The softcopy of thesis entitled: "TO STUDY THE EFFECT OF POOLED PASTEURIZED DONOR HUMAN MILK VERSUS UNPOOLED PASTEURIZED PRETERM DONOR HUMAN MILK ON SHORT TERM GROWTH PARAMETERS OF PRETERM INFANTS FOLLOWING DISCHARGE FROM HOSPITAL UPTO FOUR WEEKS OF AGE" has been submitted for Anti-Plagiarism check through Turnitin software. The scan has been carried out and the scanned output reveals a match percentage of 10% which is within the acceptable limits of 10% as per the guidelines given by UGC.


Guide.




Dr. (Mrs.) N.S. Mahantashetti.
Chairperson-Anti-plagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BM0120001
Postgraduate Student,
2020-21 Batch,
Department of Paediatrics,
J. N. Medical College, Belagavi.

PRINCIPAL
J.N. Medical College,
BELAGAVI- 590 010

ETHICAL CLEARANCE



K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed-to-be-University)

Accredited 'A' Grade by NAAC (2nd Cycle)

Placed in Category 'A' by MHRD (Govt)

**JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)**

Website: <http://www.jnmc.edu>
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office 2472550

Principal: 2471701

Fax No +91 (0)831 - 2470759

Ref: MDC/DOME/148

Date: 25/01/2021

To,

Dr. Aabha Dhand
PG student in Paediatrics,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"TO STUDY THE EFFECT OF POOLED HUMAN DONOR MILK V/S UNPOOLED PRETERM HUMAN DONOR MILK ON THE SHORT TERM GROWTH OF PRETERM BABIES AT KLE HOSPITAL, BELAGAVI - A RANDOMIZED CONTROL TRIAL "**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Smita Sonoli)
Member Secretary

JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Harsha Hegde)
Chairman,

JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

LIST OF ABBREVIATIONS

PDHM	-	Pasteurized donor human milk
MOM	-	Mothers own milk
EBF	-	Exclusive breast feeding
HMB	-	Human milk bank
HM	-	Human milk
EBM	-	Expressed breast milk
KMC	-	Kangaroo mother care
LBW	-	Low birth weight
VLBW	-	Very low birth weight
ELBW	-	Extremely low birth weight
LSCS	-	Lower segment caesarean
LOS	-	Late onset sepsis
NEC	-	Necrotizing enterocolitis
ROP	-	Retinopathy of prematurity
BPD	-	Bronchopulmonary dysplasia
NMR	-	Neonatal Mortality Rate
IMR	-	Infant Mortality Rate
NG	-	Nasogastric tube
RT	-	Ryle's tube
NICU	-	Neonatal intensive care unit
NVD	-	Normal vaginal delivery
PTVD	-	Preterm vaginal delivery
OG	-	Orogastric
PIH	-	Pregnancy induced hypertension

PPROM	-	Preterm premature rupture of membranes
RCTs	-	Randomized controlled trial
WHO	-	World Health Organization
NFHS	-	National Family Health Survey
UNICEF	-	United Nations International Children's Education Fund
UN	-	United Nations
NICHHD	-	National Institute of Child Health and Human Development
CLMC	-	Comprehensive Lactational Management Centre
LCPUFA	-	Long Chain Polyunsaturated Fatty Acids
EUGR	-	Extra Uterine Growth Restriction
SGA	-	Small for gestational age
CNS	-	Central nervous system
DNA	-	Deoxyribonucleic Acid
LDL	-	Low density lipoprotein
CGA	-	Corrected gestational age
PUFA	-	Polyunsaturated Fatty Acids
DHA	-	Docosahexaenoic Acid
EPA	-	Eicosapentaenoic Acid
TGF	-	Transforming growth factor
EGF	-	Epidermal growth factor
IGF	-	Insulin like growth factor
GCSF	-	Granulocyte- colony stimulating factor
VEGF	-	Vascular endothelial growth factor
MFGE8	-	Milk fat globulin EGF and Factor 8
HB-EGF	-	Heparin-Binding EGF

BDNF	-	Brain Derived Neutrophic Factor
GDNF	-	Glial cell line derived neutrophic factor
IQ	-	Intelligent Quotient
PIQ	-	Performance Intelligent Quotient
HMBANA	-	Human Milk Bank Association of North America
HoP	-	Holder's Pasteurization
HTST	-	High temperature short time
SD	-	Standard deviation
TNF	-	Tumor Necrosis Factor
p	-	Probability
vs	-	Versus
wk	-	Week
d	-	Days
cm	-	Centimetre
cumm	-	Cubic millimetre
Kg	-	Kilograms
gm	-	Grams
GA	-	Gestational age
IgA	-	Immunoglobulin A
CFU	-	Colony forming unit
HC	-	Circumference
PF	-	Preterm formula
BW	-	Birth weight
MM	-	Mother's milk
°C	-	Degree Celsius

IL	-	Interleukin
IQR	-	Interquartile range
min	-	Minutes
mL	-	Milliliter
L	-	Liter
n	-	Total number
i.e	-	That is
%	-	Percentage
etc	-	et cetera
Ga	-	gestational age

ABSTRACT

Background and objectives

Adequate nutrition is of outmost importance for ensuring the optimal growth and development especially in preterm infants. Breast milk with its ideal nutrient composition is known to achieve the optimal growth and development. Feeding difficulties and inadequate milk production are the major challenges in providing optimal nutrition for the preterm infants since most of the mothers of preterm infants experience delayed lactogenesis II and are unable to provide adequate milk to their infants during the initial days. Under these circumstances when mothers milk is not available, WHO and UNICEF recommends using Pasteurized Donor Human Milk (PDHM) as the next best infant feeding option. Pasteurized donor human milk (PDHM) is the pooled donor mothers milk which is distributed through human milk bank. Human milk bank systematically collects, pasteurizes, stores and distribute the donor human milk. PDHM is known to confer several advantages in preterm infants including feeding tolerance, reducing risk of Late Onset Sepsis (LOS), Necrotizing Enterocolitis (NEC), Bronchopulmonary Dysplasia (BPD) and Retinopathy of Prematurity (ROP). However, effect of PDHM in improving the growth parameters in preterm infants is controversial. Several studies have reported that babies fed with PDHM have no significant difference in gain in weight, length and head circumference at discharge when compared to preterm formula fed infants. The human milk composition of mothers who have given birth to preterm infants is different from that of mothers delivering term infants with protein content as high as 4.1 ± 2.1 g/100 ml in early preterm milk. Also the processes like freezing, thawing and pasteurization are known to modify the macronutrient content of donor human milk. PDHM

dispensed in human milk banks is a combination of pooled milk from both preterm and term donor mothers of different gestational age postpartum, which may affect the donor human milk composition and in turn may affect the growth outcomes of infants. This variability in human milk composition along with effect of pasteurization and routine practice of pooling both term and preterm milk together may be attributed to the effects on growth of preterm infants fed with PDHM .There is a lack of evidence to show the effect of pooled pasteurized preterm milk on the growth outcomes of infants. Therefore we conducted a RCT to study the effect of pooled pasteurized donor human milk versus unpooled pasteurized (pooling of only preterm donor milk) preterm donor human milk on the short term growth parameters (weight, length , head-circumference and mis- upper arm circumference) of preterm infants.

Materials and methods

This randomized controlled trial was conducted from December 2021 to August 2022 in the Neonatal Intensive Care Unit of Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. A total of 98 stable preterm infants (49 in each group) requiring PDHM anytime during their hospital stay were enrolled and analyzed during the study period. Baseline demographics, along with anthropometric parameters and feeding details were collected at the start of the study in a pre-designed and pre-tested proforma. Participants were then randomized into two groups, **Group A (intervention group)** - Preterm infants receiving unpooled preterm pasteurized donor human milk, and **Group B** – Preterm infants receiving pooled pasteurized donor human milk using computer generated random numbers. The pasteurized donor milk was provided from the human milk bank attached to the level III NICU of KLES Dr. Prabhakar Kore

Hospital and Medical Research Centre, Belagavi. The neonates were then followed up in the hospital for growth parameters namely weight, length, head circumference and mid upper arm circumference along with type of feeding, method of feeding, amount of feeding , duration of feeding and frequency of feeding during NICU stay ,at discharge, and during follow-up visits at 2 weeks and 4 weeks of life. The results were analyzed using statistical software R and Microsoft Excel and based on graphical representation and p values. A probability value (p-value) of less than or equal to 0.05 at 95 % confidence interval was considered statistically significant.

Results

During the study period a total of 98 preterm infants (49 in each group) were enrolled and analysed in the study. Of the 49 analysed in the pooled PDHM group , 46 preterm infants survived , and 3 deaths were noted. Of the 46 survivors, 2 preterm infants were lost to follow up at 2 weeks and 7 were lost to follow up at 4 weeks. Of the 49 analyzed in the unpooled preterm PDHM group , 48 preterm infants survived , and 1 death was noted. Of the 48 survivors, 1 preterm infant was lost to follow up at 2weeks and 1 was lost to follow up at 4 weeks. In the present study , the maternal characteristics namely maternal age (25.10 years v/s 23.84 years, p=0.09), maternal education (73.47% v/s 55.10%,p=0.15) maternal occupation (97.96% v/s 97.96%,p=0.00), maternal pregnancy profile (59.18% v/s 65.31 % ,p=0.24) , antenatal risk factors associated with preterm delivery , birth history namely , mean gestational age (34.42 weeks v/s 34.51weeks, p=0.80),mean weight at birth (1824.90 gm v/s 1769.59 gm, p=0.46), mode of delivery (63.27 % v/s 61.22 %,p=0.83) ,APGAR Score(7.96 v/s 7.88 ,p=0.43), day of enrollment (1.714days v/s 1.531)(p=0.71) except gender (**p=0.04**) , indications for NICU

admission (100% v/s 97%), and indication for starting PDHM (93.88% v/s 87.76%) , all were comparable between both the groups. The mean maternal age observed in both the groups during the study was 24.47 years. Most of the mothers (64.29%) were educated upto secondary level and were homemakers (97.96%).Most of the mothers in the study (62.24%) were primigravida and the most common antenatal risk factor associated with preterm delivery was PPROM (40.5%) followed by severe intra-uterine growth restriction (IUGR) (32.65 %).The mean gestational age of the preterm infants in the study was observed to be 34.46 weeks with the mean weight at birth of 1797.24 gm. The most common mode of delivery observed was C-section (62.24%) with mean APGAR score at birth of 7.92 and mean day of enrollment of 1.622 days. Majority (51.02%) of preterm infants in the study were males. However a significant difference was observed in gender in between the two groups (**p=0.04**). Most of preterm infants in unpooled preterm PDHM group were females (59.18 %) and in pooled PDHM group were males (61.22%). The most common indication for admission to NICU was Low Birth Weight (98.98%) followed by Kangaroo Mother care (81.63%) and the most common indication for starting PDHM in preterm infants was decreased breast milk secretions in the mother's delivering preterm infants (90.82 %) followed by Poor Maternal health status (19.39 %). When analyzing and comparing primary outcomes namely weight, length , head circumference , and mid upper arm circumference at different time points and across the total length of hospital stay and follow ups , no much significant difference was observed in growth parameters between both the groups i.e from enrollment till the end of 4 weeks follow up ($p>0.05$). Although significant difference for specific growth parameters was noticed within the same group.The total % increase in mean weight (30.47% v/s 26.14%) (**p= 0.0001 v/s p=0.0001**) and mean mid-upper arm

circumference(4.11 % vs 3.42 %) (**p=0.0001 v/s p=0.0001**) as observed from the beginning i.e baseline value at enrollment till end of 4 weeks within the same group i.e preterm unpooled PDHM group in this study was found to be significant. Similarly significant difference in total % increase in mean length (4.36 % v/s 3.45%)(**p=0.001 v/s p=0.001**)and mean head circumference (5.91 % v/s 5.76 %) (**p=0.0001 v/s p=0.0001**) was observed from the beginning i.e baseline value at enrollment till end of 4 weeks within the same group i.e pooled PDHM group in this study. Secondary outcomes namely ,feeding patterns, length of hospital stay and rate of blood infections were comparable between both the groups at all time points during the study period. The mean amount of PDHM across different time points i.e at enrollment (55.96 +/- 37.79 litres v/s 47.94 +/- 31.75 litres p=0.25), at discharge (61.43 +/- 48.09 litres v/s 60.00 +/- 61.29 litres p=0.93), at 2 weeks follow up (106.00 +/- 63.54 litres v/s 138.33 +/- 122.71 p=0.64), at 4weeks follow up (300 litre v/s 360 litres) and total amount of PDHM (421.45 +/- 360.79 litre v/s 373.59 +/- 292.25 litres) (p=0.47) consumed by preterm infants during total length of hospital stay between both groups were comparable. Other secondary outcomes like the mean duration of feedings namely 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), days taken to switch to MOM (3.67 +/-2.81 days v/s 3.25 +/- 2.31 days, p=0.42), mean frequency of feeding across different time points i.e at enrollment (9.98 ±2.46 feeds/day in vs 9.84±2.65 feeds/day, p= 0.84), at discharge (10.69 ± 1.37 feeds/day to 10.67 ±1.42 feeds/ day, p=0.96), at 2 weeks follow up (12.26 ± 1.51 feeds/day to 12.14 ±1.56 feeds/ day, p=0.90), at 4 weeks follow up (13.45 ± 1.79 feeds/day to 13.67 ±1.56 feeds/ day, p=0.69) and method of

feeding i.e at enrollment , majority(41.86%) of the preterm infants in the study were on SF (42.55% v/s 41.08%p=0.39), at discharge shift was noted to SF + DBF (79.79%) as method of feeding in both groups(81.25% v/s 78.26% p= 0.98), at 2 weeks follow up increasing trend was observed for DBF(29.67%) as method of feeding in both group (25.53 % v/s 34.09% p=0.29) and by 4 weeks significant increase in DBF rates was observed (43.02%) (40.43 % v/s 46.15% p=0.80) though overall rate of SF + DBF remained high at 55.81 % (59.57% v/s 51.28% p=0.80) all were comparable between both the groups. In the present study the mean duration of hospital stay in both groups (9.65+/-6.64 days and 9.10 +/- 6.49 days p=0.68) and % of blood infections at enrollment between both the groups at enrollment (10.42% v/s 6.25% p=1.00) were also found to be comparable.

Conclusion .

Although exclusive unpooled pasteurized preterm milk (pooled milk of 5 donor mothers delivering preterm) showed better overall outcomes within the same group for total % increase in weight and mid upper arm circumference of preterm infants by the end of 4 weeks, and pooled pasteurized donor human milk(pooled milk of 5 donor mothers including both preterm and term mothers) showed better overall outcome for total % increase in length and head circumference of preterm infants by the end of 4 weeks, however, when comparing growth outcomes of preterm infants between both the groups no much significant difference was noted for weight, length , head circumference and mid upper arm circumference. Therefore segregating donor human milk based on gestational age postpartum to provide exclusive unpooled pasteurized preterm milk especially for preterm infants admitted in NICU to meet their milk based needs and achieve better short term growth outcomes considering

compositional variation of human milk , and preterm milk to be naturally enriched with higher protein content as compared to term milk is not useful . Therefore wherever possible, pooled pasteurized donor human milk i.e pooled milk of both preterm and term delivering mothers should be given to preterm infants.

Keywords: Preterm, Pasteurized Donor Human Milk, Human Milk Bank, Banked Pooled Pasteurized Donor Human Milk.

CONTENTS

Sr. No.	Topic	Page No.
1.	INTRODUCTION	1-4
2.	OBJECTIVES	5
3.	REVIEW OF LITERATURE	6-45
4.	MATERIAL AND METHODS	46-52
5.	RESULTS	53-79
6.	DISCUSSION	80-103
7.	CONCLUSION	104
8.	SUMMARY	105-110
9.	BIBLIOGRAPHY	111-137
10.	ANNEXURES	138-155
	ANNEXURES I – CONSENT FORM	138-140
	ANNEXURE II – PROFORMA	141-151
	ANNEXURE III – PHOTOGRAPHS	152
	ANNEXURE IV – KEY TO MASTER CHART	153-155
	ANNEXURE V – MASTERCHART	

LIST OF TABLES

TABLE. NO.	DESCRIPTION	PAGE NO.
1	Comparison Of Maternal Parameters Between Unpooled And Pooled Group	55
2	Comparison Of Maternal Pregnancy Profile Between Unpooled And Pooled Group	56-57
3	Comparison Of Baseline Birth History Between Unpooled And Pooled Group	59
4	Comparison Of Mean Weight At Different Time Points Between Unpooled And Pooled Group.	62
5	Comparison Of % Gain In Mean Weight At Different Time Points Between Unpooled And Pooled Group	63
6	Comparison Of Mean Length (In Cm) At Different Time Points Between Unpooled And Pooled Group	64
7	Comparison Of % Gain in Mean Length (In Cm) At Different Time Points Between Unpooled And Pooled Group	65
8	Comparison Of Mean Head Circumference (In Cm) At Different Time Between Unpooled And Pooled Groups.	66
9	Comparison Of % Gain In Mean Head Circumference (In Cm) At Different Time Between Unpooled And Pooled Groups	67
10	Comparison Of Mean Upper Arm Circumference (In Cm) At Different Time Points Between Unpooled And Pooled Group.	68

11	Comparison Of % Gain In Mean Upper Arm Circumference (In Cm) At Different Time Points Between Unpooled And Pooled Group	69
12	Comparison Of Total Amount Of PDHM Between Unpooled And Pooled Group	71
13	Comparison of Mean Amount of PDHM between Unpooled and Pooled group At Different Time Points	72
14	Comparison Of Change of Mean Frequency Of Feeding Between Unpooled And Pooled Group	75
15	Comparison Of Length Of Hospital Stay Between Unpooled And Pooled Group	79

LIST OF FIGURES

FIGURE NO.	DESCRIPTION	PAGE NO.
1	Classification Of Neonates (Based On Gestational Age)	7
2	Classification Of Neonates (Based On Birth Weight)	7
3	Macronutrient Composition Of Human Milk	16
4	Human milk composition of preterm and term milk	41
5	CONSORT diagram for screening and enrolment of preterm newborn	54

LIST OF GRAPHS

GRAPH NO.	DESCRIPTION	PAGE NO.
1	Comparison Of Baseline Birth History Between Unpooled And Pooled Group	60
2	Comparison Of Indication For NICU Admission Between Unpooled And Pooled Group	61
3	Comparison Of Indication For Starting PDHM Between Unpooled And Pooled	70
4	Comparison of Duration of PDHM between Unpooled and Pooled group	73
5	Comparison Of Mean Frequency Of Feeding Between Unpooled And Pooled Group	74
6	Comparison Of Type Of Feeding Between Unpooled And Pooled Group	77
7	Comparison Of Method Of Feeding Between Unpooled And Pooled Group	78

LIST OF PHOTOGRAPHS

PHOTO NO.	DESCRIPTION	PAGE NO.
1	Essae-BS-250 electronic weighing scale used for measuring weight.	152
2	Infantometer was used to measure the length	152
3	Head circumference was measured using Schorrs tape	152

INTRODUCTION

Annually, approximately 15 million babies are born preterm, indicating a global preterm birth rate of about 10.6%^{1,2,3,6,7,9}. Across regions, Sub-Saharan Africa and South Asia report overwhelming majority accounting to about 60%-80% of preterm births reported worldwide^{1,2,3,6,7,9}. India is a major contributor to the global preterm birth statistics and contributes 23 to 24% to the global average^{4,5,7,8,10,11}. The prevalence of preterm birth in India is between 13-17% and 28.25% in Karnataka and 8.6% in Belagavi^{4,5,7,8,10,11}.

Every year almost 1 million children die due to preterm birth and related complications, making it the leading cause of childhood mortality^{2, 11}. Hypoglycemia, infection, respiratory distress syndrome, bronchopulmonary dysplasia, apnea of prematurity, jaundice, necrotizing enterocolitis, gastro-esophageal reflux, patent ductus arteriosus, retinopathy of prematurity, intra-ventricular hemorrhage, anemia of prematurity and feeding difficulties are the complications related to prematurity. Of all the complications, feeding difficulties account for a major proportion and have been estimated to range between 25% and 45%^{12, 13, 14, 15, 25,26,27,28}. Shortened period of intra-uterine development, diminished suck and swallow co-ordination, immunocompromised state, with immature physiological organ system functions, along with decreased breast milk production by mother's delivering preterm have been attributed to cause the feeding difficulties observed in preterm infants^{14,15,16,17,19,22,26,27}.

Adequate nutrition is of utmost importance for ensuring the optimal growth and development especially in preterm infants^{20,21,25,26,28}. Human milk with its ideal nutrient composition is known to achieve the optimal growth and development with improvement in both short term and long term outcomes in preterm infants^{12,13,15,20,21,25,26,27}. Lactose present in human milk serves as the principle source of energy ensuring adequate weight gain in preterm infants. Oligosaccharides like Fucose along with immunoglobulin's IgA, IgM, IgG and growth factors like EGF,IGF help in development of defense mechanism and thereby decrease incidence of sepsis and NEC in preterm infants^{12,13,15,20,21,22}. Whey proteins present in the human milk is easily digestible and is known to put less solute load on the developing immature gut of a preterm infant and hence leads to decreased feed intolerance and earlier achievement of full enteral feeding^{12,13,14,15,17}. Polyunsaturated Fatty Acids (PUFA) like Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) found abundantly in human milk also helps in achieving adequate neuroplasticity of brain of preterm infants in early years of life and play a significant role in strengthening neuro-cognitive outcomes in long term, along with retinal maturity^{12,13,15,20,21,22}. World health organization (WHO), UNICEF and other related organisations emphasise on the importance of optimal nutrition for adequate growth and development in preterm infants and recommends exclusive breastfeeding for the first 6 months of life followed by continued breastfeeding for up to 2 years of age^{23,24}.

Feeding difficulties and inadequate milk production are the major challenges in providing optimal nutrition to the preterm infants. Most of the mothers of preterm infants are unable to provide adequate milk to meet the nutritional needs of their infants in the initial days^{14,15,16,17,19}. Recent studies have shown that, delayed lactogenesis II is common in mothers of preterm babies^{30,32,33}. Delay in the onset of

lactogenesis II will lead to decreased volume of milk expression and also babies are less likely to exclusively breast feed at 4-6 weeks postpartum^{17,29, 30,31,32,33}. Under these circumstances when mothers milk is not available, WHO and UNICEF recommends using Pasteurized Donor Human Milk (PDHM) as the next best infant feeding option^{23,34,35}.

Pasteurized donor human milk (PDHM) is the pooled donor mother's milk which is distributed through human milk bank^{40, 85}. Human milk bank systematically collects, pasteurizes, stores and distribute the donor human milk. PDHM is known to confer several advantages in preterm infants^{36,37,38,39,40,46,85}. Several meta-analysis and systematic reviews have demonstrated advantages of PDHM when compared to formula feeding^{41,42,43,44,53,54,55,56,58}. PDHM is known to increase feeding tolerance, reduce the risk of developing Late onset sepsis (LOS), Necrotizing enterocolitis (NEC), Bronchopulmonary dysplasia (BPD) and Retinopathy of prematurity (ROP) in preterm infants^{41,42,43,44,45,46,47,50,55,56,58}. However, the effect of PDHM in improving the growth parameters in preterm infants is controversial^{45,49,51,52,55,56,58,62,68,69,70,71,72,73}

Several studies have reported that babies fed with PDHM have no significant difference in gain in weight, length and head circumference at discharge when compared to preterm formula fed infants.^{41,50,55,56,57,58,60,74,76,80}

The human milk composition of mothers who give birth to preterm infants is different from that of mothers delivering term infants⁸¹. Studies have shown that early preterm human milk contains more protein and lower carbohydrate levels. Early pre-term milk is known to be naturally enriched with proteins⁸¹. Several studies have reported protein content as high as 4.1 ± 2.1 g/100 ml in early preterm milk^{81, 82}. A study in Turkey comparing changes in breast milk macronutrient content during the

first month in preterm and term infants reported , higher fat, energy and carbohydrate content in term milk when compared to preterm milk by day 14 and day 28 postpartum ,concluding that human milk composition is not only variable with gestation but also over time in the same mother ⁸³ . Similar variations were also observed for lactose content and long chain polyunsaturated fatty acids (LCPUFA) which were found to be higher in term milk ⁸⁴.The donor milk processing like freezing, thawing and pasteurization also is known to modify the macronutrient content of donor human milk, particularly reducing the content of lipids and proteins ^{86,87,88,89,90,91,92,93,94,95,96} . This variability in human milk composition along with effect of pasteurization on the donor milk may be attributed to the effects on growth of preterm infants fed with PDHM .

PDHM dispensed in human milk banks is a combination of pooled milk from both preterm and term donor mothers of different gestational age postpartum, which may affect the donor human milk composition and in turn may affect the growth outcomes of infants ⁹⁷. There is a lack of evidence to show the effect of pooled pasteurized preterm milk on the growth outcomes of infants. A recent Cochrane review of all the available RCTs related to growth effects in VLBW babies receiving banked donor preterm milk v/s banked donor term milk found no studies to demonstrate the effect of pooled preterm PDHM on growth outcomes in preterm infants⁹⁷. Therefore we conducted a RCT to study the effect of pooled human donor milk v/s unpooled (pooling of only preterm donor milk) preterm human donor milk on the short term growth parameters of preterm infants.

OBJECTIVES

PRIMARY

To study the effect of Pooled Pasteurized Donor Human Milk v/s Unpooled Pasteurized Preterm Donor Human Milk on the short term growth parameters (weight, length, head circumference and mid upper arm circumference) of preterm infants following discharge from hospital up to 4 weeks of age.

SECONDARY

To study the

1. Duration of hospital stay.
2. Amount and duration of receiving PDHM, MOM and time taken to transit.
3. Method, type and frequency of feeding patterns.

REVIEW OF LITERATURE

HISTORICAL IMPORTANCE

Nurturing babies is an issue which is inextricably bound to all species in nature. From ancient times until today, the importance of breastfeeding has been well appreciated and understood and even reflected in history, art and religion all around the world so much so that, in prehistoric times, the goddesses of motherhood were presented with naked breasts¹⁰⁴. The earliest Indian scripture, the Vedas, which represent the primary sacred texts of ancient times (1800 BC), mention milk and breast to be symbolic of longevity and nectarine sweetness. While the breasts have been conceived as a pitcher full of nectar (milk) in Atharva Veda, the other ancient Ayurveda texts like Charka Samhita (400-200 BC), the Sushruta Samhita (400BC), and Kashyap Samhita, illustrate the importance of breastfeeding, and consider it to be having vivifying power and property^{104,105,106, 107}.

INTRODUCTION

Preterm birth is defined by WHO as a live birth that occurs before 37 completed weeks of gestation, or fewer than 259 days from the first date of a woman's last menstrual period and low birth weight (LBW) babies as babies who weigh less than 2500gm at the time of birth¹.

• **CLASSIFICATION OF NEONATES (BASED ON GESTATIONAL AGE)**²²

Sub – Groups	Weeks	Days
Extremely preterm	< 28 weeks	195
Early preterm	< 34weeks	237
Late preterm	Between 34 0/7 and 36 6/7	238 to 258
Term	Between 37 0/7 and 42 6/7	259 to 293

Figure 1. Classification of Neonates (Based On Gestational Age) ²²

• **CLASSIFICATION OF NEONATES (BASED ON BIRTH WEIGHT)** ²²⁻

Sub- Groups	Weight
Low birth weight (LBW)	< 2,500 grams
Very low birth weight (VLBW)	<1500g
Extremely low birth weight (ELBW)	<1000g

Figure 2 . Classification of Neonates (Based On Birth Weight) ²²

EPIDEMIOLOGY

According to global statistics reported by WHO including , “BORN TOO SOON” report, annually, approximately 15 million babies are born preterm which account for more than 1 in 10 of all the babies born globally, indicating a global preterm birth rate of about 10.6% ^{1,2,3,6,7,9}. The burden of prematurity is particularly high in low and middle income countries namely sub Saharan Africa and South-east Asia ^{1,2,3,4,7,10}. The Indian Foundation for Premature Babies together with WHO reported, overwhelming contribution of India (23-24%) in premature birth statistics across globe, with every 1 in 6th live born baby in India being born preterm accounting to an estimated prevalence rate of 13-17% in India ^{1,2,,3,4,5,6,7,8,9,10,11} .The prevalence of premature birth across India also shows varied trends with rates of 28.25% in Karnataka and 8.6% in Belagavi ^{5,8,10,11,108}. A recent review article in Indian context, studying prevalence and associated risk factors associated with preterm births in India, reported highest frequency of preterm birth from Tamil Nadu (28.25%) and Maharashtra (6.1%) ^{5,8,9,108,109,111}.

As per the current statistics , prematurity has now become the leading cause of under 5 mortality worldwide surpassing pneumonia both in number and magnitude ^{1,2,4,6,7, 9,10} . Every year almost 1 million children die due to preterm birth and related complications and India accounts to 20% of neonatal and 17% to the infant mortality rates globally ^{2, 4,10,11}. As per the NFHS -5 report Infant Mortality Rate (IMR) is observed to be 21% per 1000 live births in Karnataka and it is this overwhelming data and number that the issue of prematurity has been recognized as global health burden so much so that United Nation in its last meeting held on adoption of Sustainable Developmental Goals 3 addressed it as a major agenda and formulated

target #3.2 aiming to end all preventable causes of deaths of newborn and childhood mortality under 5 by 2030 ^{2,112}.

SHORT TERM COMPLICATIONS AND RISK FACTORS ASSOCIATED WITH FEEDING DIFFICULTIES IN PRETERM INFANTS

Prematurity is known to cause both short term and long term complications in preterm infants. The third trimester of pregnancy is known to be the period of rapid organ growth and maturation in a developing fetus. High energy and adequate nutritional supplementation is required during this period to keep the development process working harmoniously and is therefore also called period of high nutritional accretion by the developing fetus. It is also the period of development of oromotor skill co-ordination and adequate suck reflex to prepare the developing fetus for survival in the extra-uterine environment ^{14, 28}.

Since preterm infants are born before this period of adequate maturation and development of their organ systems, along with less established feeding mechanisms, they are more prone for complications, like hypoglycemia, infection, respiratory distress syndrome, bronchopulmonary dysplasia, apnea of prematurity, jaundice, necrotizing enterocolitis, gastroesophageal reflux, patent ductus arteriosus, retinopathy of prematurity, intraventricular hemorrhage, and anemia of prematurity, all related to specific organ system development. In addition to the specific organ system vulnerability the early extra-uterine exposure and oxidative stress of the altered environment puts extra stress on preterm infants leading to extra uterine growth restriction (EUGR) and thereby sequelae to the associated long term complications like lung disorders, hypertension, metabolic disorders etc. later in adult life ^{12,15,16,17,28}. Of all the complication related to prematurity, feeding difficulties

leading to sub-optimal nutrition account for a major proportion and have been estimated to range anywhere between 25% and 45% ¹⁴.

As per a review article analyzing and understanding the dietary transitional patterns in preterm infants published by Carla Lucchi Pagliaro et al in 2015, preterm infants were found to be more prone for feeding difficulties¹⁴. Immature oral reflexes, reduced sucking ability, early and easy fatigability, along with incoordination while sucking, swallowing and breathing were all attributed to be the cause of feeding difficulties. Shorter duration of intra-uterine development, immuno-compromised state, immature gastrointestinal and other organ system functioning, and associated co-morbidities like small for gestational age (SGA) were also reported ¹⁴.

Preterm infants usually have long hospital stays owing to their inadequate intra-uterine growth and therefore are more prone to frequent exposure to harmful sensory stimuli during hospital stay like prolonged intubation time, upper airway aspiration and repeated feeding tube insertions. All these stimuli make them more vulnerable and prone to feeding issues and hence decreased acceptability to full volume feed by oral route exclusively ^{14, 17, 18, 113}.

Mothers delivering preterm infants have decreased milk production during early post natal period. The average breastfeeding rate of preterm infants during hospitalization in developed countries is estimated between 13–49% ¹⁸. In Indian scenario, an observational study conducted in a tertiary care NICU reported, only 44 (45.4%) of the 97 enrolled preterm, VLBW babies, were exclusively breastfed at discharge ¹¹⁴. A recent study published by Xiurong Yu in 2019 reported that only 50% of mothers who delivered prematurely were able to express sufficient milk to meet their infant's needs at 6 weeks postpartum and were 3 times more at risk of not

producing adequate milk in comparison to mothers delivering at term gestation^{29,32}. A study conducted by Dingding Dong et al in 2022, studying the lactational status and breastfeeding challenges in mother's delivering preterm, reported 51.4% of the mothers experienced delayed lactogenesis II (i.e. failure to initiate milk production within 72 hour postpartum) and hence significantly lower daily expressed milk volume until Day 7 postpartum and consequently lower exclusive breastfeeding rates by their preterm infants at 3 months of corrected age³³. Early post natal separation due to NICU admission, lack of maternal and neonatal physical interaction, interrupted feeding sessions, type of delivery, family support along with maternal health factors were also reported to be the additional factors leading to decreased milk production in mothers delivering preterm³³. Similar findings were also observed in a recent study by Xin Jiang and Hui Jiang et al, which reported significant decline in exclusive breastfeeding rates at 6 months post discharge in mothers delivering preterm¹⁸.

LONG TERM COMPLICATIONS ASSOCIATED WITH PREMATURETY AND EFFECT OF SUB-OPTIMAL NUTRITION

As per a recent article aimed at understanding the relationship between preterm nutrition and neurodevelopment outcome by Alyson Margaret Skinner et al, cerebellar development, synaptic connectivity, myelination, and neuroplasticity of premature infant's brain is very sensitive to nutrition in the first few weeks of life and the reason stated is that, the preterm neonate's brain is the most metabolically demanding organ and consumes the largest amount of energy and nutrients for its function and programmed growth and maturation. Starting in the prenatal period and continuing until the second year of life, the development of the cerebral white and

gray matter structures of the central nervous system (CNS) involves many processes as stated above and sub optimal nutrition is known to hamper these processes¹⁵.

Sub optimal nutrition causes decrease in cell replication cycles , total brain DNA, myelin sheath formation, restricted dendritic arborization and eventually reduced connectivity between neurons thereby impairing motor and cognitive development and social abilities in preterm infants¹⁵. Similar findings were reported by Katherine E Chetta et al, in her study on outcomes with human milk intake in preterm infants¹⁰¹. A follow-up study to determine the beneficial effects of breast milk ingestion by infants with extremely low birth weight in the NICU on developmental outcomes at 30 months of corrected age conducted by National Institute of Child Health and Human Development (NICHD) also reported that, the use of mother's milk is associated with clear gains in Bayley scores- an approximate increase of 0.59 Bayley Mental Developmental Index points, 0.56 Psychomotor Developmental Index points, and 0.99 Behavioral Index Points at 30 months of age for every +10 mL/kg/day average increase in mother's milk given in the NICU ¹¹⁵.

Doctrine of Child Development also states that infants with preterm, LBW are at an increased risk of coronary heart disease, hypertension, and type 2 diabetes in adulthood ¹¹⁶. Several studies have shown an association between preterm birth and risks of developing metabolic syndromes in early to mid-adulthood all related to suboptimal neonatal nutrition ^{15,16,25,26,27,28}. A large cohort follow up study from Sweden which included 4 million subjects conducted between 1973 and 2015 and followed up for mortality rates till 2017, reported a strong inverse association between gestational age at birth and mortality. Those born preterm or early term had 5-fold and 1.3-fold increased risk of mortality, respectively, relative to those born at

full-term, all related to mid adulthood endocrine and metabolic disorders ^{26,118}. Epidemiologic studies and meta-analysis review of previous cohorts have consistently linked preterm birth with higher blood pressure in adulthood, increased risk of type 1 and type 2 diabetes, almost 2 fold increased risk of lipid disorders and 3 fold increased risk of chronic kidney disease ,and chronic lung disorders owing to reduced pulmonary function capacity and immune system immaturity^{15,16,25,26,27,28,117}. Attributable risks estimated using the data from this large cohort reported that the preterm and extremely preterm birth, accounted for an estimated 20.8 and 52.8 additional deaths per 100,000 person-years at ages18–45 years respectively , compared with full-term birth ^{26,118}. Indicating that preterm birth is independently associated with modestly increased all-cause mortality in early to mid-adulthood.

Adequate feeding and optimal nutrition is of outmost importance for ensuring better short term and long term outcomes in preterm infants. WHO committee for recommendations on optimal feeding options for low birth weight and preterm infants along with National Neonatology Forum of India recommends breastfeeding or mother's own expressed milk (MOM) as the first best and preferred infant feeding option for all newborns including those born small for gestational age (SGA), preterm, or VLBW (very low birth weight) admitted in NICU and emphasise exclusive breastfeeding for the first 6 months of life followed by continued breastfeeding for up to 2 years of age ^{23,24}.

BENEFITS ASSOCIATED WITH HUMAN MILK CONSUMPTION IN PRETERM INFANTS

Human milk with its specific macro and micronutrient content along with innumerable bio-active factors, long chain polyunsaturated fatty acids (LCPUFA) ,

oligosaccharides, growth factors, chemokines and cytokines is known to provide several advantages in neonates especially preterm neonates and help in overcoming preterm birth related complication like ^{19,20,21,63,64}.

1. Decreased incidence of late onset sepsis (LOS)
2. Reduced incidence of Necrotizing enterocolitis (NEC), secretory immunoglobulin's, lipases, lysozymes, growth factors, and oligosaccharides all play a role in protective mechanism.
3. Reduced incidence of retinopathy of prematurity (ROP).
4. Reduced incidence of hospital re-admission.
5. Decreased long-term risk of metabolic syndrome and lower low density lipoprotein (LDL) levels compared to those receiving formula feeding.
6. Better neurodevelopmental outcomes.
7. Decreased feed intolerance.
8. Earlier achievement to full enteral feeds.

Studies comparing growth outcomes in preterm infants supplemented with unpasteurized breast milk v/s pasteurized breast milk have shown better growth outcomes, including better weight gain velocities with unpasteurized breast milk. An observational study analyzing longitudinal growth outcomes and morbidities in 90 extremely preterm infants born between 2013 to 2015 in Gothenburg, Sweden comparing mother's milk v/s pasteurized donor human milk consumption reported that unpasteurized mother's milk correlated positively with almost all z-scores for

weight, length, head circumference at postnatal age of 28days¹¹⁹. Another retrospective study conducted by Lloyd M et al in 2019 also reported similar observation when comparing mother's own milk with pasteurized donor human milk, and reported better weight growth velocity at 34+1weeks post menstrual age in group supplemented with mother's own milk as compared to group supplemented with pasteurized donor human milk⁸⁰. As per a recent review by Jacopa Cerasani et al in 2020, studying human milk feeding and preterm infant's growth and body composition, analyzing 91 articles over a period of 11 years that is 2009 to 2020 reported a broader reduction in the weight z scores from birth to discharge in group receiving >75% human milk as compared to those receiving < than 75%¹²⁰.

When analyzing reasons for the above observation, a positive correlation was observed between consumption of human milk and adipose mass deposition in late preterm infants fed sole human milk diet, which strengthened on reaching term corrected gestational age (CGA)¹²¹. Study by Piemontese et al reported higher percentage of lean mass deposition at term corrected gestational age (CGA) in very low birth weight (VLBW) infants fed with human milk at more than equal to 50% of total milk volume as compared to VLBW infants fed with human milk at less than 50% of total milk volume¹²¹. Mol et al studied and compared preterm infants with term infants for body mass composition, for mother's own milk or preterm formula and showed at term CGA, the group with VLBW infants who were fed with mother's own milk had analogous body composition to full term infants as compared to group with VLBW infants fed with preterm formula(PF)¹²³. Therefore human milk feeding with its positive correlation with fat free mass deposition in preterm infants, contributes positively to recovery of body composition and hence better growth outcomes in preterm infants in the long run¹²⁰.

THE COMPOSITION OF HUMAN BREAST MILK ^{19,20,21,22}

Breast milk with its ideal nutrient composition is known to support the development and growth of preterm infant in the most precise and natural way.

Human milk is known to contain the following:

- **MACRONUTRIENT COMPOSITION OF HUMAN MILK** ^{19,20,21,22}

Component	Contribution (in %)
Water	87
Protein	1
Lipids	4
Carbohydrates	7

Figure 3. Macronutrient Composition of Human Milk

- **CARBOHYDRATES** - Lactose, being the most common carbohydrate and the principal source of energy found in colostrum. Apart from lactose, human milk also contains oligosaccharides which are abundant and help in defense mechanism (Fucose), facilitating brain development (sialic acid) and acting as a probiotic agent (facilitating commensal organism growth) ²².
- **PROTEINS** - The protein component of human milk is in the form of WHEY and CASEIN. Breast milk is whey protein predominant which makes it easily digestible and puts less solute load on the gut of the neonate. The average whey: casein ratio in breast milk is 60:40 and major whey proteins are Lactalbumin (40%), Lactoferrin (30%) and IgA (15-20 %) ²².

- **LIPIDS** - Human milk contains lipids in the form of unsaturated fat, essential fatty acids, phospholipids. Human milk differs from milk of other mammals in that it has a higher proportion of PUFA (Poly Unsaturated Fatty Acids) such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), which play a key role in brain and retinal development ²².

Human milk also confers impeccable immunity by providing for anti-inflammatory, anti-infective and anti- allergen properties via presence of following :

- **BIOACTIVE MOLECULES** - Bioactive dietary components are substances that "change biological processes or substrates, hence influencing body function or condition, and thereby health"⁵⁵. The mammary epithelium synthesizes and secretes some bioactive components in human milk, while other bioactive components are collected from maternal serum and transferred via the mammary epithelium via receptor-mediated transport⁵⁶. Important bioactive factors present in human milk are ²¹ :
 - a. Immunoglobulins including, IgG, IgM, sIgA
 - b. Cytokines such as TGF- β , IL-6, IL-7, IL-8, IL-10, IFN- γ , TNF α .
 - c. Chemokines (e.g., G-CSF, Macrophage Migratory Inhibitory Factors)
 - d. Growth factors (e.g., EGF, VEGF, IGF, Erythropoietin).
 - e. Hormones (e.g., Somatostatin, Calcitonin)
 - f. Anti-microbial (e.g., Lactoferrin, Lactadherin /MFG E8)
 - g. Metabolic hormones (e.g., Adiponectin, leptin, Ghrelin)
 - h. Cells such as macrophages, stem cells.

• **GROWTH FACTORS**

- (i) **EGF** - The maturation and repair of the intestinal mucosa are both dependent on EGF. EGF is resistant to digestive enzymes and low pH, allowing it to cross through the stomach and into the intestines, where it promotes DNA synthesis, cell division, water and glucose absorption, and protein synthesis in enterocytes. In the newborn gut, EGF has a number of protective mechanisms in place, including:
- a. Reversing TNF-induced changes in intestinal and hepatic tight junction proteins.
 - b. Suppression of programmed cell death²¹.
- (ii) **Heparin-binding growth factor (HB-EGF)**- is the primary growth factor responsible for the resolution of damage caused by hypoxia, reperfusion injury, hemorrhagic shock / injury during resuscitation, and necrotizing enterocolitis²¹.
- (iii) The enteric nervous system, which depends on **brain-derived neurotrophic factor (BDNF)** and **glial cell-line derived neurotrophic factor (GDNF)** for development, is also immature in the neonatal gut. BDNF can enhance peristalsis, a function which is frequently impaired in the pre-term gut. GDNF from breast milk improves neuron survival and growth²¹.
- (iv) Human milk contains **IGF-I and IGF-II**, as well as IGF binding proteins and IGF-specific proteases. Colostrum has the highest levels, which gradually decrease throughout lactation. IGF-1 may also have a function in enterocyte survival after oxidative stress induces intestinal damage²¹.

- **LEPTINS** - play an important role in the postnatal growth of infants by regulating energy conversion and infant BMI, as well as hunger regulation^{21,124}.

PASTEURIZED DONOR HUMAN MILK

When mothers own milk (MOM) is not available, WHO recommends using pasteurized donor human (PDHM) as the next best alternative for infant feeding especially for low birth weight infants^{23,34,35}. Pasteurized Donor Human Milk (PDHM) is defined as a pooled donor milk which is distributed through human milk banks to neonates who may or may not be the biological child of the donor mother⁷⁹. The concept of donor human milk dates back to ancient times, popularly known as “wet nursing” and described in ayurveda as the concept of “Dhaatri” in which lactating mothers act as donors for babies whose mothers are unable to produce enough milk due to variety of reasons ^{104,105,106, 107}. With advancement the concept of “Dhaatri” declined and was replaced by modern day HUMAN MILK BANKING. Pasteurized human milk is distributed by human milk banks which systematically collects, pasteurizes, stores and distribute the donor human milk ^{36,37,38,39,40,46,79, 85}.

The first human milk bank in the world was started in Vienna , Austria in 1909, under the noble guidance of Theodar Escherich , followed by Boston and many more continued to open throughout US and European regions. The first human milk bank was started by Armida Fernandez , in the year 1989 at Sion Hospital , Mumbai , India .In the last decade there is increase in the number of HMBs throughout India ^{34,35,36,40}.

Government of India in 2017 released national guidelines on lactational management centers in public health facility and addressed human milk banks in India

under the umbrella heading of Comprehensive Lactation Management Centers (CLMC) depending upon the level of health facilities where these units were established⁹⁸. As of 2022 statistics, India reports nearly 90 milk banks across various states and regions both in private and public sector^{34,35,36,40}.

PDHM is known to offer several advantages to preterm infants. Several meta-analysis and systematic reviews have demonstrated advantages of PDHM when compared to formula feeding^{41,42,43,44,53,54,55,56,58}. PDHM is known to increase feeding tolerance, reduce the risk of developing Late onset sepsis (LOS), Necrotizing enterocolitis (NEC), Bronchopulmonary dysplasia (BPD) and Retinopathy of prematurity (ROP) in preterm infants^{41-50,55,56,58}. Cristefalo et al in his randomized controlled trial comparing the effect of formula feed v/s PDHM, reported shorter duration of parenteral nutrition in infants fed PDHM as compared to formula feeds⁴³. The study also reported that the incidence of surgical evidence of NEC was as high as 21% in formula fed group as against PDHM group which showed only 3% incidence⁴³. Another study by Yang et al reported, shorter duration of hospital stay in preterm infants fed PDHM v/s formula feed⁴⁷. Lapidaire and Lucas et al conducted a randomized controlled trial to study cognitive outcomes and NEC in preterm infants with human milk and reported that increase in mother's breast milk or PDHM intake was associated with lower chances of NEC and thereby better neurological outcomes in terms of better full scale IQ and PIQ at age 7 and 30 years¹²⁵.

THE HUMAN MILK BANK

Human milk banks are repositories of donated human milk focused to provide donor milk to high risk newborn babies admitted in the NICU when MOM is not available. Milk banks receive milk from donors, process it, and store it, up until use¹²⁶. Most commonly milk from multiple donors is pooled, although some banks pool milk only of individual donors (single-donor banks)¹²⁶. Usually, milk provided by milk banks undergoes pasteurization. Once pasteurized, milk is placed in small (100-150 mL) containers and is stored frozen and then later distributed as per requirement and demand¹²⁶. The national CLMC guidelines for the systematic functioning and management of human milk banks are as follows:

REQUIREMENTS TO SET UP HUMAN MILK BANK¹²⁷

The certain set of requirements for establishment and operation of human milk banks includes-

1) **INFRASTRUCTURE**¹²⁷-

- i) **LOCATION** - Human milk banks are primarily focused to provide donor milk to high risk newborns admitted in neonatal unit. Also presence of human milk banks in the NICUs is associated with elevated rates of exclusive breast feeding rates in VLBW and preterm babies. Therefore a location in close proximity or even inside the boundaries of neonatal unit is preferred.
- ii) **SPACE** - The minimum requirement as per guidelines is a partitioned room of 250 square feet, big enough to be able to comfortably accommodate at least the equipment required for milk banking, along with a work area for the technician to carry out all administrative formalities

such as enrollment, screening of donors and counseling etc. and some storage space for records. Ideally there should be

- **Administrative Area**- to carry out all administrative formalities such as enrollment and screening of donors. It would also serve as dispensing center for DHM from the milk banks.
- **Counseling Area**- to facilitate registration and counseling sessions with lactation management counsellors in milk banks, most milk banks have counseling area and reception area combined.



RECEPTION AND COUNSELLING AREA



MILK EXPRESSION AREA

- **Milk Expression Area**- This area should include milk expression stations for mothers with privacy for milk expression and should be equipped with electric breast pumps. Provision of music/television and a crèche is helpful in reducing stress of mothers. Teaching videos of KMC, expression of breast milk and advantages of breast milk feeding should be shown under supervision of milk

bank staff. The other equipment in this area should include a deep-freezer (400L) and a refrigerator (190L) for storage of raw breast milk. The milk expression area should also include a nursing station with provision of wall cabinets for storage of autoclaved milk collection bottles and sterile tubing etc.

- **Cleaning/Autoclave Room**- It should be located adjacent to milk expression area. Cleaning and autoclaving arrangements should be available here to clean the used milk bottles and tubing and seal them for reuse.
- **Milk Processing/Storage Area**- Entry and exit to this area should have glass/swing door to reduce risk of infection. This area should accommodate equipment namely refrigerator(190L), laminar air flow, deep freezer(400L), water bath, electronic sealing machine etc. for carrying out key processes namely pooling of donor human milk, preparing aliquots, cooling of milk for pre pasteurized storage, thawing and pasteurization. This area should be under strict temperature control with negative airflow and should have access for CLMC technician to conduct milk processing activities.



MILK STORAGE AREA

- **Microbiological Laboratory**-The microbiological laboratory should be present to carry out the cultures and other procedures to test the milk for ensuring safety of processed milk. The central lab of the health facility where CLMC is established should carry out the laboratory tests to screen the donor mothers.
- **Milk Storage Area** - This area should be equipped with two deep freezers (a vertical of 400L and a Horizontal of 300L) for storage of post-pasteurized milk. Preterm and term milk should be stored in separate compartments. This area should also be under strict temperature control with negative airflow.

2) **EQUIPPMENTS REQUIRED** ¹²⁷

- a. **Pasteurizer/Shaker Water Bath** - It is a device that carries out heat treatment of donor milk. The donor human milk which is collected is pasteurized using the Holder Pasteurization Method, which involves heating the milk in a sealed container to 62.5°C and holding it there for 30 minutes, then rapidly chilling it in a specified device before dispensing it for use by the receiver. This process helps get rid of all the infective agents and making sure milk is free from any bacterial endotoxin and safe for consumption. Alternate to this is the use of a shaker water bath with a micro-processor controlled temperature regulator, an electronic time device, and a shaker speed controller for pasteurization of donor milk. The breast milk in the container is heated through the steam and hot water in water shaker bath. To avoid coagulation of the milk and to ensure even distribution of heat, the tray on which breast milk containers are placed is shaken. The size of the shaker water bath varies as per the need of the milk bank, with tray capacity varying between 9-24 flasks/stainless steel containers of 200-400ml capacity.



PASTEURIZATION MACHINE

- b. **Deep Freezer**- A deep freezer to store the milk at -20C is essential in the milk bank. It is desirable to have deep freezer with digital display of the temperature inside the deep freezer with an alarm setting if the temperature rises above the set temperature. A separate freezer for preprocessed milk is needed to keep the donated raw milk which awaits pasteurization. It is desirable to have two deep freezers for processed milk. First for storage of the milk till the post-pasteurization milk culture reports are available. This freezer should be locked at all times with access only to the technician, so that no milk is accidentally used till the culture reports are available. The second deep freezer is used for storage of the pasteurized milk once the culture reports are negative and the milk is considered safe for distribution.



DEEP FREEZER

- c) **Refrigerator**-Separate refrigerators are required to store the milk till the whole day's collection is over and the milk is ready to be mixed and pooled for further processing and also for thawing the milk before being dispatched.



REFRIGERATOR

- d) **Hot Oven /Autoclave**- A hot air oven / autoclave in the milk bank or centralized sterile service department is essential for sterilizing the containers used for collection from donors, containers for pasteurization, storage and the test tubes needed for sending milk culture samples to the microbiology laboratory.
- e) **Breast Milk Pump** -For milk banking, hospital grade electric pumps are preferred as they result in better volumes of expressed milks and are relatively painless and comfortable to use. Breast pumps can be a source of infection and hence they should be cleaned properly.



BREAST MILK PUMP

- f) **Containers**- For collection and storing the milk, single use hard plastic containers of polycarbonates, pyrex or propylene are used across the world. However, in Indian experience, cylindrical, wide-mouthed stainless steel containers of about 200 ml capacity with tight fitting/ screwed caps are equally effective. They are easily available, and are durable, easy to clean and autoclave.



CONTAINER/STORAGE BOTTLES

- 3) **GENERATOR/UN-INTERUPPTED POWER SUPPLY**¹²⁷ – Every milk bank should have a dedicated source of uninterrupted power supply in the form of generator or inverter.
- 4) **OTHER DESIRABLE EQUIPMENTS**¹²⁷- It includes infra-red spectroscopy based milk analyzer.
- 5) **STAFF AND ADMINISTRATION**¹²⁷- Human milk banks should have a panel of consultants to guide overall development and functioning.
- a) **Director**- Person who is overall in charge of milk bank activities and administration. Usually the head of neonatal services whose role is planning, developing, implementing and evaluating milk bank services.

- b) **Milk Bank Officer/Chief Operating Officer**- Usually a doctor from public health and responsible for overall day to day functioning , administration, training and promotion and updating milk bank , taking consents from donors, and recipients and taking decisions on medical and technical aspects of the milk bank unit. He is supposed to report to the director.

- c) **Lactation Management Nurses**- There should be at least one dedicated lactation nurse whose primary job is to help mothers with lactation problems, to motivate mothers to donate milk ,to organize the milk collection, to dispatch the donated milk to the milk bank and to ensure cleaning and disinfection and sterilization of pumps and other equipment's required.

- d) **Milk Bank Technician**- He looks after all day to day activities in the milk bank, is responsible for pasteurization of milk , microbiological surveillance, collection of culture reports, maintenance of records and disbursement of milk.

- e) **Milk Bank Attendant**- His job is to clean and sterilize the milk containers and breast milk pumps, to transport the milk to the milk bank from the collection sites, to take samples for culture to the microbiology department and to collect the reports, to maintain hygiene level in office and other rooms.

- f) **Microbiologist**- His job is to carry out the cultures and send reports to the milk bank.

- g) **Receptionist/Record Keeper**- There job is to counsel women who come to the milk bank and maintain counseling register containing data updated about women counseled, who agreed to donate, who will have to come back to milk bank. There work is to fill the Donor Record Files and Recipients Record Files. Also to maintain inventory of stationary items and maintain comfort and decorum of the milk bank.

As per CLMC guidelines, if it is not feasible to have the above mentioned staff, the basic minimum staff requirement includes- a Lactation Management Nurse, Milk Bank Technician (who can maintain records of collection and distribution), Sister in charge of NICU (who can take up the job of coordinating collection and transport of milk to milk bank), Regular Hospital Attendant (who can take up the job of cleaning), Unit Head (for overall functioning of the milk bank). Microbiology work can be shared by the regular hospital microbiology staff.

6. **FUNDS**¹²⁷- 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.
7. **HEALTHY DONORS** ¹²⁷- Healthy lactating mothers who would be willing to donate.

PROCESSING OF DONOR HUMAN MILK ¹²⁷- The process of processing of donor human milk involves, collection, pooling, pre-pasteurization testing, pasteurization, post-pasteurization testing and finally storage before dispensing. The fresh raw breast milk collected over the day is kept in pre-processed milk freezer/refrigerator. Caution should be taken not mix the newly collected fresh raw milk with the frozen milk already collected since it can result in defreezing with hydrolysis

of triglycerides . If mixing fresh raw breast milk to frozen raw breast milk previously collected from same donor, care should be taken to chill the fresh raw milk before adding to frozen milk. For sick or preterm babies it is advisable to use a new container for each session of pumping. Whole day's collection is then processed by mid-day so that pre-processing cultures can be sent in time for microbiological testing.

Mixing & Pooling: Before the microbiological testing of milk collection is done, donated breast milk from multiple donors is pooled. Milk is transferred from donations collected in small containers to larger glass flasks/pasteurizer containers. Each pool (which usually includes milk from 3 to 5 donors) is thoroughly mixed to ensure an even distribution of milk components. Names & ID of donors in each pool is also recorded for future needs.

Microbiological Screening Of Donated Milk: Microbiological screening of donated and pooled milk is done as soon as possible both after and before pasteurization according to the protocol of the bank. The aim is to maintain contamination level at zero at all possible areas.

Pre-Pasteurization Culture: Wherever possible pre-pasteurization culture of the collected raw breast milk should be sent in order to know the extent of contamination and the efficacy of pasteurization process. Before treatment, there are no set levels for colony count levels, but a rough guide is as follows:

- <103CFU/mL: milk is used
- >105CFU/mL: milk is not used
- 103to 105CFU/mL: milk is only used if organisms are skin commensals.

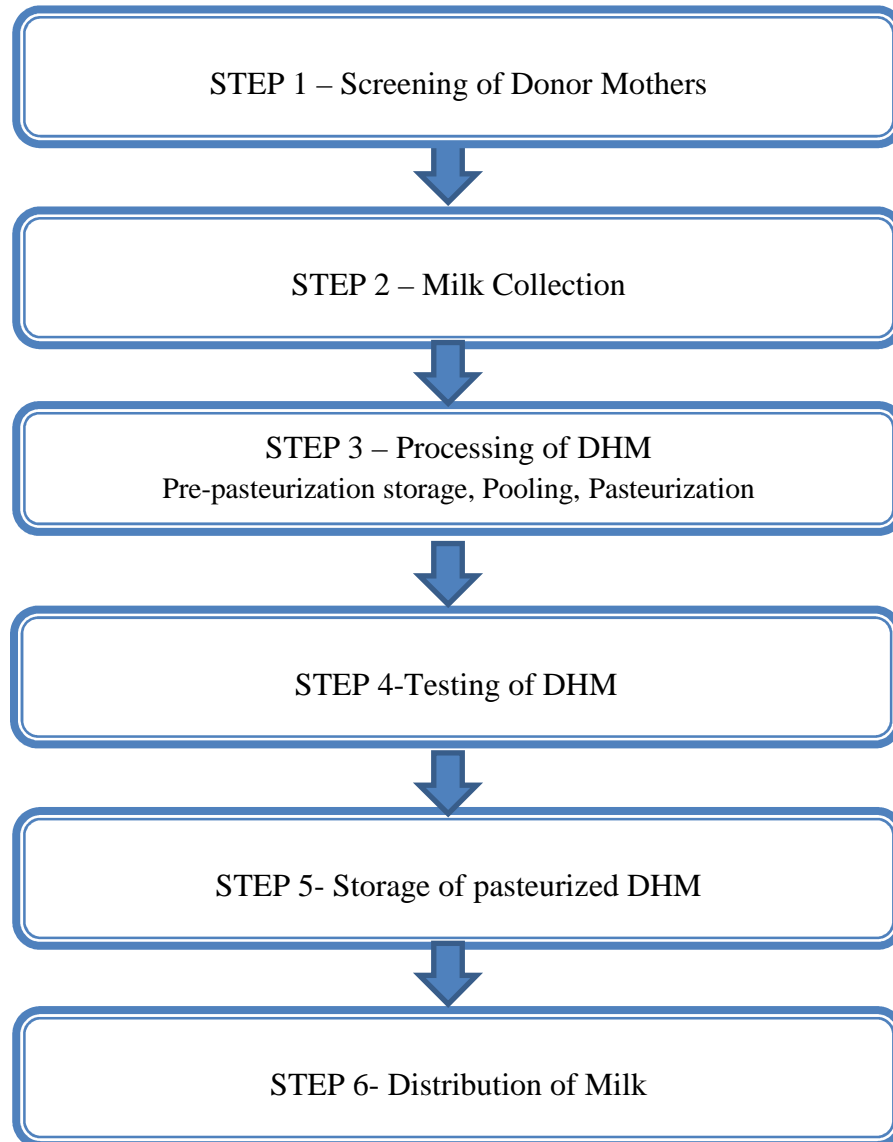
Heavily contaminated milk is discarded because pasteurization process is not effective on heavily contaminated sample. Heavily contaminated milk with specific bacteria (e.g. *S. aureus*, *E.coli*) may contain enterotoxins and thermostable enzymes even after pasteurization, expert panel has selected 105 CFU/mL for total bacterial count, 104 CFU/mL for Enterobacteriaceae and *S. aureus* as threshold values which are in accordance with milk banks operating worldwide.

Pasteurization of Donated Breastmilk¹²⁷: The fresh raw donated milk is immediately pasteurized after collection. Holder method of pasteurization using 62.5°C for 30 minutes is widely used. Properties are better preserved at this temperature without compromising bacteriological safety. Recently, other safer methods of pasteurization with better preservation of nutrients and other properties, like flash heat treatment, HTST (High Temperature Short Time: 72°C for 16 seconds) and ultra violet irradiation are also coming up for use in human milk banks.

The stainless steel containers for the pasteurizer/shaker-water bath is cleaned & sterilized before use. The fresh raw donated breast milk of individual donor or multiple donors collected in small sterilized containers is poured in the larger sterilized stainless steel containers suitable to size of the pasteurizer. Containers should not be filled more than four-fifths the volume to allow for expansion of milk when heated. The containers are then placed in their specific slots inside the machine tray and clamped to avoid spillage. The bath canner is filled with water enough to submerge ¼ to ½ portions of the steel containers. The temperature is set at 62.5°C; time is set to 30 minutes and shaking speed set to Level 1. After 30 minutes, containers are taken out, sealed tight and allowed to cool rapidly. Samples for post pasteurization culture are then drawn from each container and containers are kept in

the deep freezer at -20°C. Each containers of same batch of pasteurization are placed in one labeled batch tray and it is stored in the freezer designated for post-pasteurization milk awaiting cultures report which is not distributed till culture reports come negative. After each batch of pasteurization the containers are sent for autoclaving after cleaning.

Post Pasteurization Culture- It is done in every set-up before the milk is dispensed. No amount of growth is acceptable. 1 to 2 mL of milk from each batch should be sent to microbiology lab for culture from each container. If the test report is positive then the entire pasteurized batch/container's pool is discarded depending on culture reports of other containers of the same batch of pasteurization. The containers with negative culture reports are transferred to deep freezer designated for culture negative pasteurized milk ready for disposal. The six basic steps involved in HUMAN MILK BANKING are summarized in the algorithm given below ^{35,38,85,99-} .



POOLING OF DONOR HUMAN MILK^{127,93:}

Human milk composition is dynamic, and changes over the course of a feed, over the course of a day, and over the course of lactation. For these reasons, DHM banks routinely pool milk from multiple donors in order to limit extreme variation. Criteria used to select individual donations for pooling are not standardized and vary among milk banks. Currently, milk postpartum age (age of the infant at the time milk was expressed) is not typically taken into account when pooling individual donations.

Normally before the microbiological testing of milk collection is done, donated breast milk from multiple donors is pooled. Milk is transferred from donations collected in small containers to larger glass flasks/pasteurizer containers. Each pool (which usually includes milk from 3 to 5 donors) is thoroughly mixed. Names & ID of donors in each pool is recorded for future use. This pooled milk is then subjected to pasteurization and further processing. Studies have shown pooling of milk from multiple donors help in evenly distributing milk components throughout the aliquot. A study by Young et al analyzing the impact of milk bank pooling practices on concentrations and variability of bioactive components of donor human milk reported less variability in IgA and insulin concentration in milk with more than 2 pooled donors. Pools with only one donor had lower total IgA and lower insulin concentrations than pools with at least 2 donors ($p < 0.05$) thereby optimizing bioactive components in donated milk and decreased compositional variability in produced DHM pools^{93,128}. Another study by Young et al studying effect of pooling practices and time postpartum of milk donations on the energy, macronutrient, and zinc concentrations of resultant Donor Human Milk Pools concluded that Donor human milk pools were lower in calories than is normally assumed and these

relatively low caloric density and protein concentrations need to be considered when providing DHM to low birth weight and premature infants ¹²⁹.

PASTEURIZED DONOR HUMAN MILK AND GROWTH IN PRETERM INFANTS

Reports of the effect of PDHM on the growth parameters in preterm babies vary in different settings and have been controversial. Studies conducted have shown better growth in infants fed predominantly MOM as compared to PDHM.

A retrospective analysis study conducted by R Chowning et al in 2016 analyzing 550 VLBW infants for the effect of human donor milk on prevention of necrotizing enterocolitis and postnatal growth reported slower growth rates in weight and head-circumference at discharge following introduction of DHM when MOM was not available⁴⁹. Another retrospective case control study conducted in 2012 by Giulliani F et al, to study and evaluate short term advantages of MOM as a sole diet compared to donor milk as a sole diet, in terms of growth, anti-infectious properties, feeding tolerance, NEC, and ROP prevention in a population of VLBW infants born in a tertiary care center reported, no significant difference in clinical outcomes for infants belonging to MOM group compared with PDHM group. Only a slight but not statistical significant difference in growth was observed in favor of maternal milk ¹³⁰. Lloyd M et al in 2019 also reported similar observation when comparing mother's own milk with pasteurized donor human milk, and reported better weight growth velocity at 34+1 weeks post menstrual age in group supplemented with mother's own milk as compared to group supplemented with pasteurized donor human milk but showed evident catch-up growth by discharge. By 12 months of age no difference was observed in between the two groups. ⁸⁰. An observational study analyzing

longitudinal growth outcomes and morbidities in 90 extremely preterm infants born between 2013-2015 in Gothenburg , Sweden comparing mother's milk v/s pasteurized donor human milk consumption reported that unpasteurized mother's milk correlated positively with almost all z-scores for weight, length, head circumference at postnatal age of 28days¹¹⁹. As per a recent review by Jacopa Cerasani et al in 2020, studying human milk feeding and preterm infant's growth and body composition, analyzing 91 articles over a period of 11 years that is 2009 to 2020 reported a broader reduction in the weight z scores from birth to discharge in group receiving >75% human milk as compared to those receiving < than 75% ¹²⁰. A positive correlation was observed between consumption of human milk and adipose mass deposition in late preterm infants fed sole human milk diet, which strengthened on reaching term corrected gestational age (CGA) ¹²⁰. Study by Piemontese et al reported higher percentage of lean mass deposition at term corrected gestational age (CGA) in very low birth weight (VLBW) infants fed with human milk at more than equal to 50% of total milk volume as compared to VLBW infants fed with human milk at less than 50% of total milk volume ¹²². Mol et al studied and compared preterm infants with term infants for body mass composition, for mother's own milk or preterm formula and showed at term CGA, the group with VLBW infants who were fed with mother's own milk had analogous body composition as compared to group with full term infants ¹²³.

When comparing PDHM feeding v/s formula feeding in preterm infants for growth outcomes, studies report mixed results. Schanler and Richard et al conducted a randomized control trial on 243 extremely premature infants (<30weeks), comparing donor milk v/s preterm formula as substitute for MOM and found the rates of weight gain differed between DHM and PF group and was higher in PF group ,

though there was no difference in head circumference gains between the two groups⁵¹. Similar findings were reported by Lucas A et al in his study on early feeding of low birth weight infant and its effect on growth and Tyson et al in his study on growth, metabolic response, and development in VLBW infants fed banked human milk v/s enriched formula^{55,59,132}. Brownell et al, also reported a rate of decline in weight gain by 0.17g/kg/day for every 10% increase in DHM intake⁷³. Weight gain increased significantly with increasing formula milk intake. On the other hand head circumference growth decreased as compared to infants fed MOM.

Another retrospective study, conducted by Kim L Chung in 2017 reviewing 132 infants of birthweights (BWs) < 1500g and gestational ages (GAs) < than 32 weeks, studying the effects of exclusive donor human milk feeding in a short period after birth on morbidity and growth of preterm infants during hospitalization reported, lower values of weight, head circumference and height in PDHM group as compared to PF group on achieving full enteral feeding of 130ml/kg, but showed similar growth results at 36 weeks PMA⁵². Fang L et al conducted a prospective cohort, analyzing the effects of preterm donor milk (DM) on growth, feeding tolerance, and severe morbidity in very-low-birth-weight infants (VLBW) infants whose mothers could not produce enough milk, and reported no difference in daily weight gain and weekly head growth of VLBW in both PF and PDHM group concluding preterm DHM does not affect the growth of VLBW infants. Though the length of hospital stay and time to achieve full parenteral nutrition was lower in PDHM group⁴¹. Similar findings were reported in studies by Costa et al, Canizo Vazquez D et al^{58,131}. Sisk et al also conducted similar study to evaluate necrotizing enterocolitis and growth in preterm infants fed predominantly maternal milk, pasteurized donor milk, or preterm formula and reported MM and PDHM feedings, given until 34 weeks postmenstrual

age, were associated with lower rates of NEC in very low birth weight infants without interfering with growth. There were no differences in growth metrics from birth to hospital discharge⁵⁰. Hoban et al in his retrospective cohort also reported slower rates of decline in short-term growth trajectories for weight and length in VLBW infants fed higher formula proportions⁷². A randomized controlled trial (RCT) conducted in a tertiary care teaching hospital, in south India in 2018 by Adhisivam et al to study the effect of pasteurized donor human milk on 80 healthy preterm neonates randomized into groups with fortified PDHM and unfortified PDHM respectively and followed up for 28 days or discharge whichever was earlier for growth outcomes, reported no significant difference in growth parameters like weight gain and increase in head circumference among neonates in the fortified versus the unfortified group⁷⁷.

Quigley et al in 2019 conducted a meta-analysis review comparing formula v/s PDHM for feeding of preterm and LBW infants, showed formula fed infants were found to regain birth weight earlier and have higher in-hospital rates of weight gain, linear growth, and head growth than infants who received donor breast milk however at the cost of increased risk and incidence of NEC subsequently⁷¹. Meta-analysis by Fe Yu MD et al also reported similar results¹³³. Several studies conducted by Connor et al and Morley et al have demonstrated better short-term weight gain and head circumference (HC) growth with MOM supplemented with fortified DM rather than formula^{40,89}.

Dempsey et al in 2019 conducted a systemic review of randomized and quasi-randomized trials comparing banked donor preterm milk v/s banked donor term milk to study growth and development outcomes in VLBW infants and reported no literature evidence to demonstrate the effect of pooled preterm PDHM on growth

outcomes in preterm infants⁹⁷. 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 in conducting such.

DIFFERENCE IN COMPOSITION OF PRETERM AND TERM MILK

Human milk composition is known to differ with gestation, with days, hours, and also overtime in the same mother. The specific nutritional requirements of the preterm infants are met through special composition of preterm milk. Preterm infants because of their additional nutritional needs have higher in built nutritional accretion rates to accommodate the extra calories, protein, fats etc. for better growth and development.

One of the earliest studies conducted by Atkinson et al, in 1978 and 1981 at the Departments of Nutrition and Food Science and of Pediatrics, University of Toronto, Canada analyzed serial 24 hour milk collections from a group of preterm and term mothers, and found a significantly higher total nitrogen and protein nitrogen content in preterm milk which decreased during the first month of lactation. It also reported a 20-30% higher energy content in preterm milk during early lactation compared to term milk, due to a 20-30% higher concentration of fat and lipid in preterm milk¹³⁴. The findings of Gross et al who conducted a similar study in Durham in 1980 were also similar and in addition concluded, the milk produced during the first month following parturition by mothers delivering between 28 and 36 weeks of gestation contained significantly higher concentrations of nitrogen, sodium, and chloride, and lower concentrations of lactose than milk produced by mothers delivering at term.¹³⁵ Many other studies came up over the years with similar

findings .In 2014 , Dominica A Gidrewicz and Tanis R Fenton , conducted a systematic review and meta-analysis of the 41 observational studies comparing preterm and term milk for breast milk nutrient content (energy, macronutrient (protein, lactose, fat) and mineral content) and highlighted higher preterm milk protein content of 2.2 g/dL and a fat content of 2.6 g/dL compared to term milk protein content of 1.8 g/dL and a fat content of 2.2 g/dL in the first week of life ¹³⁶. Similar findings were observed in a systemic review published in American Society for Parenteral and Enteral Nutrition by Gates et al in 2020 called “Review of preterm human milk nutrient composition”, where 27 articles published between January 1950 and December 2019 studying macronutrients and micronutrient content of preterm human milk were analyzed. The article stated, the energy density increased sharply over the first 7–14 days in preterm milk, after which energy density was largely consistent. The preterm milk was found to be more energy dense or that energy density was equivalent with the differences likely due to the influence of timing and technique of sample collection ⁸².Based on the aggregate data from 19 available reports of this review, fat content in preterm human milk appeared to increase nearly 2-fold over the first 2 weeks after birth and was relatively flat in mature preterm human milk. According to a latest review by Zhu Jing et al in May 2022,early pre-term milk is naturally enriched with bile salt-stimulated lipase, lipoprotein lipase and protein, with protein content as high as 4.1 ± 2.1 g/100 ml¹¹⁰ owing to the high protein accretion rates by preterm , LBW babies which reflected the nutrition needs of the preterm infant. ⁸¹ Another study in Turkey comparing changes in breast milk macronutrient content during the first month in preterm and term infants reported , higher fat, energy and carbohydrate content in term milk when compared to preterm

milk by day 14 and day 28 postpartum ,concluding that human milk composition is not only variable with gestation but also over time in the same mother⁸³.

The transition of milk composition over weeks between term and preterm milk is shown in Figure 4.

Nutrient	TERM	PTM 1 st week	PTM 2 nd week	PTM 3 rd week	PTM 4 TH week	PTM 5 th week	PTM 6 th week
Protein (g)	1.1	2.3	1.9	1.6	1.5	1.4	1.3
Sodium (mmol)	0.6	1.7	1.3	1.2	0.9	0.8	0.8
Potassium(mmol)	1.5	1.7	1.5	1.3	1.3	1.2	1.2
Calcium(mmol)	0.8	0.7	0.7	0.7	0.7	0.7	0.7
Phosphorous(mmol)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Energy(kcal)	67	64	67	67	67	67	67

Figure 4. Human Milk Composition of Preterm and Term Milk

PASTEURIZATION AND HUMAN MILK COMPOSITION

Human milk processing involves processes like pasteurization, freezing, thawing, distributing milk into different bottles, all of which are known to modify the composition of donor human milk. These procedures reduce the macronutrient and biological composition of the donor milk, resulting in slow growth of infants fed with

PDHM. Inadequacy of standard PDHM fortification (particularly with regard to protein), loss of lipase activity during pasteurization and loss of fat during handling are the main reasons for this slower growth noted in preterm infants receiving PDHM^{88,89,90}. A study conducted in “Hospital 12 de Octubre” (Madrid, Spain) by Gracia Lara et al in 2013 to study the effect of HoP and frozen storage on macronutrients and energy content of breast milk analysed 59 raw milk samples donated by 28 fresh donor mothers and stored for less than 24 hrs at 5°C in refrigerator. Milk was analysed prior to freezing, and after freezing for 90 days at -20°C, mimicking NICU standard handling of maternal milk and reported time-dependent decreases for fat and energy content after freezing, which was clinically significant when considering the nutritional needs of preterm infants⁸⁷. Secondary analysis of milk through the donor milk cycle: raw milk(thawed after storage at -20°C), post-pasteurized milk prior to freezing, and post-pasteurized milk frozen at -20°C for 180 days reported significant decreases in fat and total energy content after pasteurization and before freezing but stable lactose and nitrogen content. Frozen storage resulted in additional significant decreases over the 180-day period for fat, energy and lactose content, but nitrogen remained stable. Total fat content was therefore decreased by 6.2% by the combination of pasteurization and frozen storage for 90 days. As fat is far more calorically dense at 9 kcal/g than carbohydrate or protein (4 kcal/g), the impact of fat loss on energy content was found to be significant^{86,87}.

Another study conducted by Ahrabi et al assessing lactoferrin and secretory IgA levels by enzyme-linked immunosorbent assay (ELISA) before and after freezing at -20°C in raw milk samples that were never pasteurized, reported that “freezer storage up to nine months does not affect lactoferrin or secretory IgA”¹³⁷.

Viera et al performed an in vitro study to demonstrate the effects of donor milk processing and NICU feeding practices on nutrient content in a step-wise fashion. Milk samples from 57 donors were analysed at each step: raw (frozen), post-HoP prior to freezing, post-HoP after thawing. It also included analysis of milk after mock tube-feeding, subjecting post HoP thawed milk to two conditions: gravity bolus and one-hour infusion and demonstrated fat and protein decreased at each step in the process, with the largest losses between raw and HoP (-6% fat and -4% protein). Further losses were seen with bolus feeding (-5% additional fat loss and -3% additional protein loss), compared to one-hour infusion (-40% additional fat loss and -10% additional protein loss)⁹¹. Castro et al performed a similar study of the effect of the infusion time of pasteurized DM feedings through 5-french feeding tubes, demonstrating that longer infusions led to greater nutrient loss. Milk fed by gravity bolus lost no nutrients, but milk fed over 30 minutes, 60 minutes, and 4 hours lost significant fat and thus energy. Energy decreased 8% at 30 minutes, 11% at 60 minutes, and a startling 29% after four hours⁶⁶.

Pasteurization also affects bioactive compounds in human milk, oligosaccharides and glycosaminoglycans are not depleted by HoP. Human milk immunoglobulin's are likely degraded by HoP, bile salt stimulated lipase, lipoprotein lipase, and amylase, and all are negatively affected by HoP to some degree^{86,92,128}. Czank et al., investigated different pasteurisation temperatures with long exposures and found that temperature rather than holding time had a greater influence in retaining the bioactivity of the human milk proteins. For example, a slight increase in temperature from 57 °C to 58 °C decreased the retention of activity of the proteins more than when a similar proportional change in holding time was employed. While thermal inactivation of bacteria occurs during pasteurisation, the broad action

of heat also disrupts proteins through thermally-induced denaturation. Also freezing itself can affect raw human milk due to rupture of the fat globule membrane, causing cream formation, as well as protein changes including alterations in casein micelle stability and altered quaternary structure of whey proteins.⁹²

POOLING AND DONOR HUMAN MILK COMPOSITION

Human milk composition is dynamic, and changes over the course of a feed, over the course of a day, and over the course of lactation. For these reasons, DHM banks routinely pool milk from multiple donors in order to limit extreme variation. Studies have shown pooling of milk from multiple donors help in evenly distributing milk components throughout the aliquot. A study by Young et al analyzing the impact of milk bank pooling practices on concentrations and variability of bioactive components of donor human milk reported less variability in IgA and insulin concentration in milk with more than 2 pooled donors. Pools with only one donor had lower total IgA and lower insulin concentrations than pools with at least 2 donors ($p < 0.05$) thereby optimizing bioactive components in donated milk and decreased compositional variability in produced DHM pools^{93,128}. Another study by Young et al studying effect of pooling practices and time postpartum of milk donations on the energy, macronutrient, and zinc concentrations of resultant donor human milk pools concluded that donor human milk pools were lower in calories than is normally assumed and these relatively low caloric density and protein concentrations need to be considered when providing DHM to low birth weight and premature infants¹²⁹.

NEED FOR STUDY

The variability in human milk composition along with effect of pasteurization and routine practice of pooling both term and preterm milk together may be attributed to the controversial effects on growth of preterm infants fed with PDHM. There is a lack of evidence to show the effect of pooled pasteurized preterm milk on the growth outcomes of infants. Therefore we conducted a RCT to study the effect of pooled pasteurized donor human milk versus unpooled pasteurized (pooling of only preterm donor milk) preterm donor human milk on the short term growth parameters (weight, length, head-circumference and mid- upper arm circumference) of preterm infants.

METHODOLOGY

The study was conducted from December 2021 to August 2022 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.

Study Design:

Randomized controlled trial.

Study Duration:

December 2021 to August 2022

Study Place:

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

Source Of Data :

Preterm infants admitted in the Neonatal Intensive Care Unit of Department of Pediatrics, KLES Dr. Prabhakar Kore hospital and Medical Research Centre, Belagavi.

Sample Size:

The sample size was calculated from the formula as mentioned below by taking power as 80% and an effect size as 0.6

$$x = \frac{2(Z_{\alpha/2} + Z_{\beta})^2}{\left(\frac{|\mu_1 - \mu_2|}{\sigma}\right)^2}$$

$$d = \frac{|\mu_1 - \mu_2|}{\sigma}$$

Where

μ_1 = mean of the first group,

μ_2 = mean of the second group

σ^2 = common error variance,

$Z_{\alpha/2}$ values = 1.96 and

Z_{β} values = 0.84.

Confidence interval level= 95%

Power =80%

Calculated sample size was 45 in each group assuming 10% lost to follow-up.

The actual sample size per group was 49 in each group.

Selection Criteria: -

Inclusion Criteria-

1. Stable preterm infants admitted to NICU, receiving PDHM, any time during their stay .(Preterm birth as defined by WHO - a live birth that occurs before 37 completed weeks of gestation, or fewer than 259 days from the first date of a woman's last menstrual period)¹

Exclusion Criteria-

Preterm neonates with

1. Major congenital anomalies.
2. Birth asphyxia
3. Surgical anomalies of gastrointestinal
4. Necrotizing enterocolitis
5. Patient not giving consent

Method Of Data Collection-

The study was conducted after obtaining institutional ethical clearance. Informed consent was obtained from the parents /caregivers of the eligible preterm infants. The preterm infants fulfilling the selection criteria were enrolled for the study. At enrollment, 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 of the infant like birth weight, age at enrollment, weight, mode of delivery, gestational age, APGAR score, and indication for NICU admission and indication for starting PDHM were recorded on a pre-designed and pre-tested proforma.

Randomization: After enrollment, the participants were randomized to group A & group B using computer generated random numbers

Group A (intervention) - Preterm infants receiving unpooled preterm pasteurized donor human milk,

Group B – Preterm infants receiving pooled pasteurized donor human milk.

Intervention : Preterm infants in the interventional group (Group A) received unpooled PDHM that is pooled preterm pasteurized donor milk from five mothers who had delivered preterm infants, while preterm infants in (Group B) received pooled that is pooled mixed pasteurized donor milk from five mothers who had delivered predominantly term & preterm infants. The pasteurized donor milk was provided from the human milk bank “AMRUTHA” attached to the Level III NICU of KAHER Dr.Prabhakar Kore charitable hospital, Belagavi which was established in 2018. Breast milk was collected under sterile precautions using hospital grade breast pumps from healthy mothers who have delivered preterm and term infants, after screening and obtaining informed written consent. Processing ,storage and dispensing of the pooled sample of DHM was done as per the National CLMC guidelines⁹⁸.The infants received mothers own milk once it was available.

The neonates were followed up in the hospital for growth parameters namely weight, length, head circumference and mid upper arm circumference along with type of feeding, method of feeding, amount of feeding, duration of feeding and frequency of feeding during NICU stay, at discharge, and during follow-up visits at 2weeks and 4 weeks .

OUTCOMES ASSESSED:

PRIMARY OUTCOMES¹⁰³:

Included the growth parameters namely, weight, length, head circumference and mid upper arm circumference at admission, at discharge and at follow up visits at 2 weeks and 4 weeks of life.

1. **Weight:** The neonate`s weight was measured on Essae-BS-250 electronic weighing scale with precision of 0.001 kg. Neonate 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.
2. **Length:** The neonate`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.
3. **Head Circumference:** Neonate`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.
4. **Mid Upper Arm Circumference:** Neonate`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

SECONDARY OUTCOMES:

1. Feeding Details :

a) **Type Of Feeding** – Exclusive Breast feeding/mothers own milk (MOM)/Pasteurized donor human milk (PDHM), Formula Milk Feeding and Mixed 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”⁹⁹.

Formula Feeds: “An artificial substitute for breast milk intended for feeding infants, using cow’s milk as a base, supplemented with vitamins and minerals”¹⁰⁰.

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”¹⁰¹.

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”.¹⁰²

- c) **Frequency of Feeding:** Number of feeds per day.
- d) **Duration of Feeding:** Total number of days receiving MOM, PDHM or Mixed feeding.
- e) **Amount of Feeding:** Amount in (ml) Of PDHM, MOM or Mixed feeding.

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

Statistical Analysis:

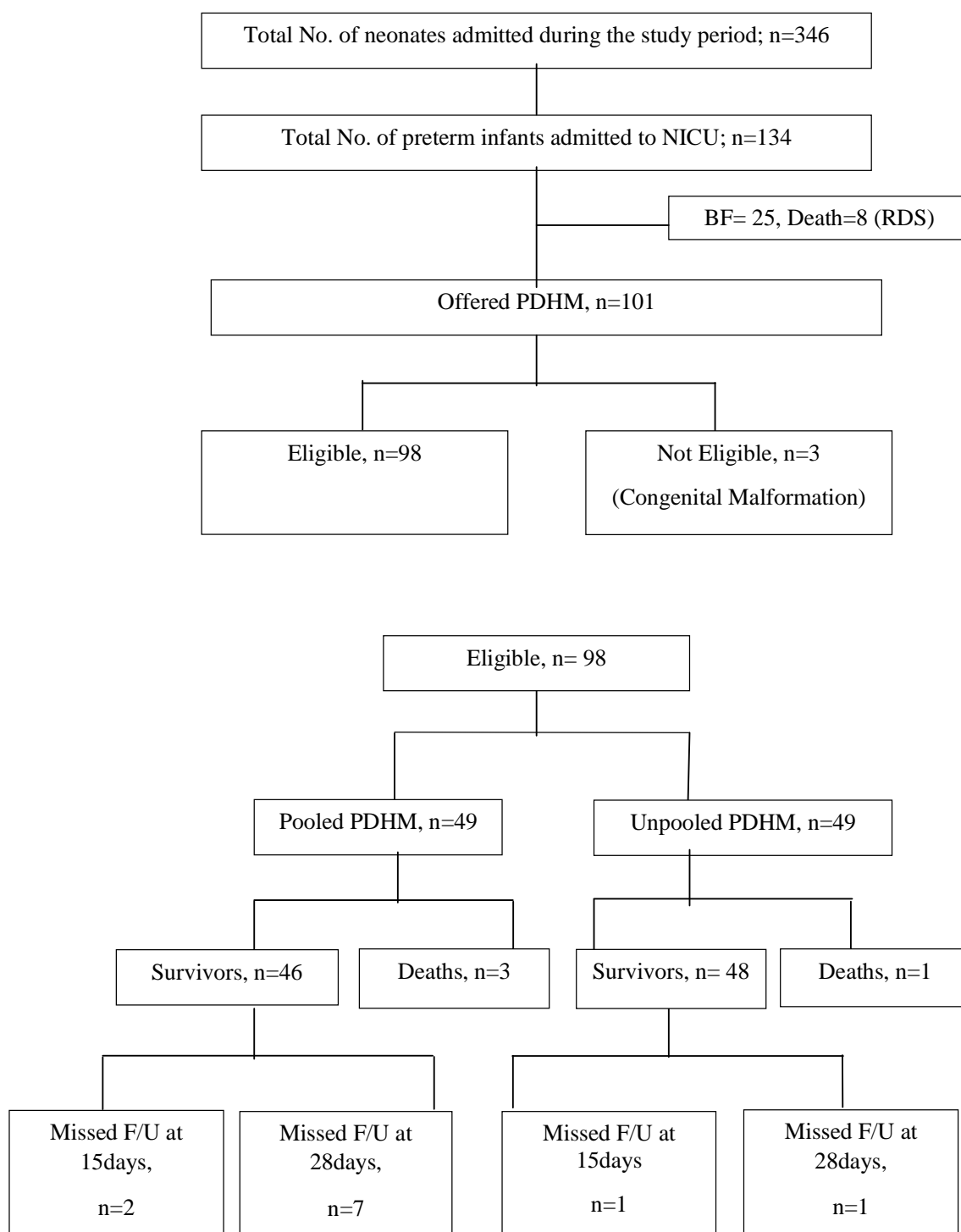
Data obtained was coded and collected and stored in Microsoft Excel spreadsheet. Data was analyzed using statistical software R and Microsoft Excel. The continuous variables were given in mean \pm SD/median (range). Categorical variables were represented by frequency. To check the dependency between attributes Chi-square test was used. To compare mean/distribution over groups t-test/ANOVA/Mann-Whitney test/Kruskal-Wallis test was used. To compare the mean/distribution within the group at 2 or more time points paired t-test/Repeated measures of ANOVA/Wilcoxon’s test/Friedman’s test was used. To compare the paired nominal data at two time points within the group, McNemar’s test was used. To check the normality of variables Quantile-Quantile (QQ) plot/Shapiro-Wilk’s test was used. A probability value (P-value) of less than or equal to 0.05 at 95 % confidence interval was considered statistically significant.

RESULTS

The study was conducted from December 2021 to August 2022 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 346 neonates were admitted in NICU during the study period of which 134 were preterm infants. Of the 101 preterm infants who received PDHM, 3 preterm infants were excluded from the study in view of congenital malformations (CHD+ GIT). A total of 98 preterm infants were enrolled (49 in each group) and were analyzed in the study. Of the 49 analyzed in the pooled PDHM group, 46 preterm infants survived, and 3 deaths were noted. Of the 46 survivors, 2 preterm infants were lost to follow up at 2 weeks and 7 were lost to follow up at 4 weeks. Of the 49 analyzed in the unpooled PDHM group, 48 preterm infants survived, and 1 death was noted. Of the 48 survivors, 1 preterm infant was lost to follow up at 2 weeks and 1 was lost to follow up at 4 weeks (Fig 5)

Figure 5. CONSORT diagram for screening and enrolment of preterm newborn



I. MATERNAL PARAMETERS

In the present study, maternal parameters were comparable between unpooled and pooled groups (Table 1) .The mean age of the mother was 24.47 years (25.10 years v/s 23.84 years=0.09). Majority of the mothers (64.29%) had received education up to secondary educational level (73.47% v/s 55.10%,p=0.15).Majority of the mothers (97.96%) were observed to be homemakers (97.96% v/s 97.96%,p=0.00).

Table 1: Comparison of Maternal Parameters Between Unpooled and Pooled Group

Profile	Unpooled	%	Pooled	%	Total	%	χ^2	p-value
Maternal age								
Mean+/-SD	25.10	4.46	23.84	2.77	24.47	3.75	1.6870	0.0949
Maternal education								
Illiterate	1	2.04	2	4.08	3	3.06	5.2520	0.1540
Primary	5	10.20	4	8.16	9	9.18		
Secondary	36	73.47	27	55.10	63	64.29		
Graduate & above	7	14.29	16	32.65	23	23.47		
Mother occupations								
Homemaker	48	97.96	48	97.96	96	97.96	0.0000	1.0000
Employed	1	2.04	1	2.04	2	2.04		

II. MATERNAL PREGNANCY PROFILE

In the present study, maternal history and antenatal risk factors associated with preterm delivery were comparable between unpooled and pooled groups (Table 2). Majority of the mothers (62.24%) were primigravida (59.18% v/s 65.31% ,p=0.24). The most common antenatal risk factor associated with preterm delivery was PPRM 40.5 % (45.5% v/s 36.20%) followed by severe intra-uterine growth restriction (IUGR), (32.65 % v/s 32.65 % ,p=0.38).

Table 2: Comparison of Maternal Pregnancy Profile Between Unpooled And Pooled Group

Maternal History	Unpooled	%	Pooled	%	Total	%	Statistic	p-value
Gravida								
Primi	29	59.18	32	65.31	61	62.24	$\chi^2=4.1480$	0.2460
Multi-G	15	30.61	10	20.41	25	25.51		
Multi-P	2	4.08	6	12.24	8	8.16		
Multi-L	3	6.12	1	2.04	4	4.08		
Total	49	100.00	49	100.00	98	100.00		

Antenatal History	Unpooled	%	Pooled	%	Total	%	Statistics	p-value
Risk Factors								
Elderly primigravida	3	6.12	1	2.04	4	4.08	Z score =0.28	0.3868
Short Stature	0	0.00	1	2.04	1	1.02		
Pre-eclampsia and eclampsia	10	20.41	10	20.41	20	20.41		
Anaemia	2	4.08	10	20.41	12	12.24		
GDM	2	4.08	5	10.20	7	7.14		
Previous still birth or IUD	0	0.00	0	0.00	0	0.00		
Previous LSCS	2	4.08	2	4.08	4	4.08		
Grand multipara	0	0.00	0	0.00	0	0.00		
PPROM	36	45.5	33	36.2	69	40.5		
IUGR	16	32.65	16	32.65	32	32.65		
Twins	6	12.24	10	20.41	16	16.33		
Rh Isoimmunization	0	0.00	0	0.00	0	0.00		
Placenta previa /abruptio	2	4.08	1	2.04	3	3.06		
Congenital Malformations	0	0.00	2	4.08	2	2.04		

III. BASELINE BIRTH HISTORY

In the study, mean gestational age, mean weight at birth, mode of delivery, APGAR score and day of enrollment were comparable except gender, between unpooled and pooled groups.

Majority (51.02%) of preterm infants in the study were males. However a significant difference was observed in gender in between the two groups ($p=0.04$). Majority of preterm infants in unpooled group were females (59.18 %) and in pooled group were males (61.22%).

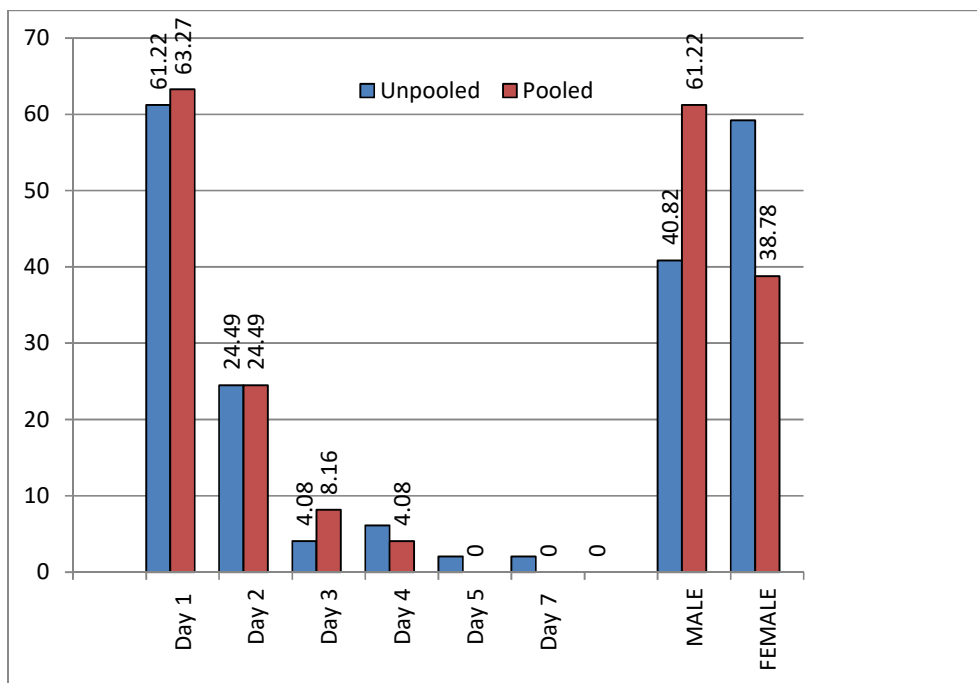
The mean gestational age of the preterm infants in the study was 34.46 weeks (34.42 weeks in unpooled group v/s 34.51weeks in pooled group, $p=0.80$).The mean weight at birth was 1797.24 gm (1824.90 gm in unpooled group v/s 1769.59 gm in pooled group, $p=0.46$).The most common mode of delivery observed was C-section (62.24%) and was similar between unpooled and pooled groups (63.27 % v/s 61.22 %, $p=0.83$). The mean APGAR score at birth of the preterm infants was 7.92 and was similar between both the groups (7.96 v/s 7.88, $p=0.43$) (Table 3)

Table 3: Comparison of Baseline Birth History Between Unpooled And Pooled Group

Profile	Unpooled	%	Pooled	%	Total	%	χ^2	p-value
Gender								
Male	20	40.82	30	61.22	50	51.02	4.0830	0.0430*
Female	29	59.18	19	38.78	48	48.98		
Gestational age								
Mean+/-SD	34.42	2.04	34.51	1.66	34.46	1.85	T=-0.2447	0.8072
Mode of delivery								
LSCS	31	63.27	30	61.22	61	62.24	$\chi^2 = 0.0430$	0.8350
NVD	18	36.73	19	38.78	37	37.76		
Birth wt (in grams)								
Mean+/-SD	1824.90	397.31	1769.59	352.33	1797.24	374.59	T=-0.7290	0.4678
5 min APGAR								
Mean+/-SD	7.96	0.54	7.88	0.48	7.92	0.51	T=0.7889	0.4321
Total	49	100.00	49	100	98	100		

Majority (62.24%) of the preterm infants were enrolled at day one of life (61.22% in unpooled group v/s 63.27% in pooled group, p=0.71). The mean day of enrollment was 1.622 (1.714days in unpooled group v/s 1.531 in pooled group) (p=0.71), (Graph 1).

Graph 1: Comparison of Baseline Birth History Between Unpooled And Pooled Group

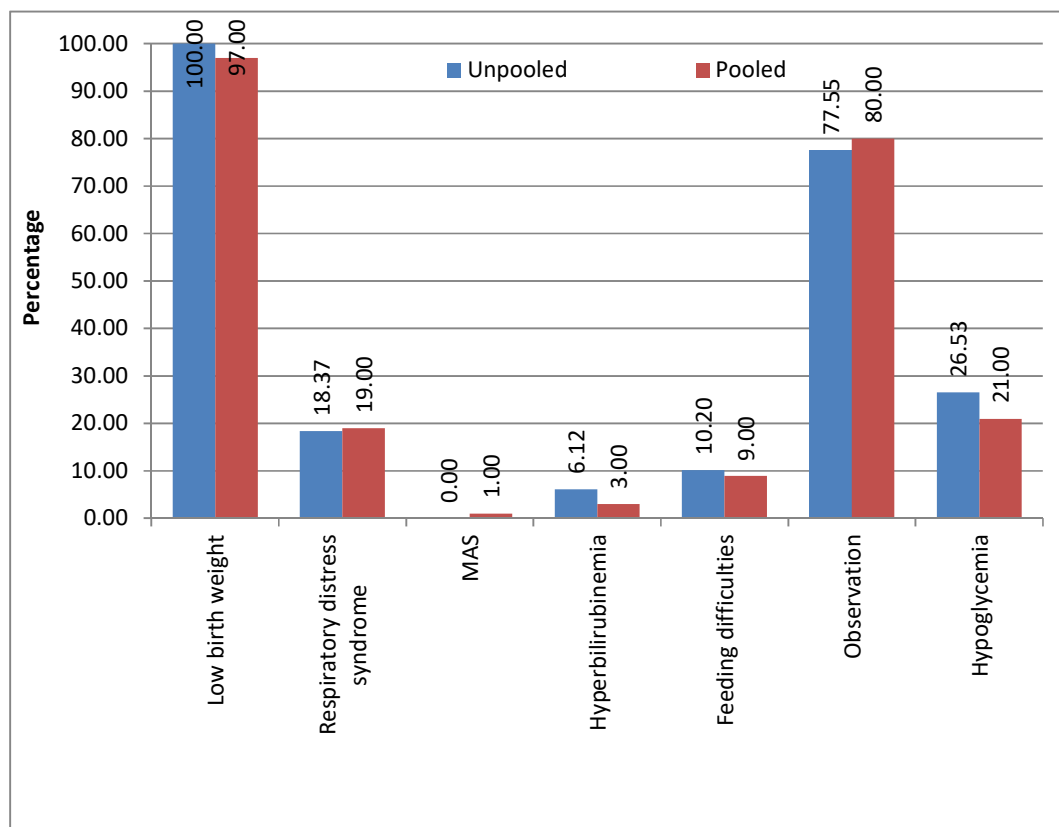


IV. INDICATION FOR NICU ADMISSION

In the present study, indications for NICU admission were comparable between both the groups.

The most common indication for admission of preterm infants to Neonatal Intensive Care Unit was Low Birth Weight (98.98%) (100% in unpooled group v/s 97% in pooled group) followed by Kangaroo Mother care (81.63%) and were similar in unpooled and pooled groups (77.55% in unpooled group v/s 80 % in pooled group).(Graph 2)

Graph 2: Comparison of Indication For NICU Admission Between Unpooled And Pooled Group



V. PRIMARY OUTCOMES

A. GROWTH PARAMETER- WEIGHT

At enrolment, baseline mean weight (1793 ± 344 gm in Unpooled group v/s 1738 ± 315 gm in Pooled group, $p=0.41$) was similar between the two groups. At Discharge, transient decline in the mean weight was observed in Unpooled group (1773 ± 302 gm v/s $2106 \text{ gm } \pm 619$ gm, $p=0.38$) compared to weight gain in infants of the Pooled group .Increase in the mean weight of preterm infants from the baseline value was observed within both the groups at 2 weeks (1958 ± 410.5 gm v/s 1898 ± 389 gm, $p=0.47$) and at 4 weeks follow up (2357 ± 433 gm v/s 2212 ± 556 gm , $p=0.17$), but no significant difference in weight gain was observed between the two groups (Table 4),

Table 4: Comparison of Mean Weight At Different Time Points Between Unpooled And Pooled Group.

Time points	Unpooled		Pooled		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
At enrolment	1793.67	344.04	1738.57	315.83	0.8259	0.4109
At discharge	1773.54	302.17	2106.74	2619.09	-0.8755	0.3836
F/U at 15 days	1958.72	410.56	1898.41	389.60	0.7178	0.4748
F/U at 4 weeks	2357.87	433.11	2212.69	556.36	1.3604	0.1774
At enrolment -At discharge	-30.83	101.47	333.04	2554.92	-0.9862	0.3266
At enrolment -15days	147.45	125.79	135.00	148.50	0.4324	0.6665
At enrolment -4 weeks	546.60	144.09	454.49	399.80	1.4700	0.1453

The total percentage increase in mean weight from the weight at enrolment value to 4 weeks observed was significant within the groups. The difference noted was higher in Unpooled group (30.47% v/s 26.14%) (**p= 0.0001 vs p=0.0001**) (Table 5)

**Table 5: Comparison of % Gain in Mean Weight at Different Time Points
Between Unpooled And Pooled Group**

Groups	Changes from At enrolment to	% of change	t-value	P-value	F-value	P-value	Effect size
Unpooled	At discharge	-1.72	2.1051	0.0407*	287.574	0.0001*	0.8620
	F/U at 15 days	8.22	-8.0358	0.0001*			
	F/U at 4 weeks	30.47	-26.0064	0.0001*			
Pooled	At discharge	19.16	-0.8841	0.3813	45.1120	0.0001*	0.5430
	F/U at 15 days	7.76	-6.0301	0.0001*			
	F/U at 4 weeks	26.14	-7.0992	0.0001*			

B. GROWTH PARAMETER - LENGTH

At enrolment, baseline mean length (44.59 ± 2.5 cm in Unpooled group v/s 44.78 ± 2.40 cm in Pooled group $p=0.70$) was similar between the two groups. Increase in mean length of preterm infants, from the baseline value was observed in both groups, at discharge (45.07 ± 2.15 cm v/s 45.27 ± 1.98 cm $p=0.65$), at 2 weeks (45.65 ± 2.48 cm v/s $45.81.78 \pm 2.30$ cm $p=0.75$) and by 4 weeks follow up (46.23 ± 3.07 cm v/s 46.76 ± 2.35 $p=0.377$) respectively but no significant difference in increase in mean length was observed between the two groups. (Table 6)

**Table 6: Comparison of Mean Length (In Cm) at Different Time Points
Between Unpooled And Pooled Group**

Time points	Unpooled		Pooled		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
At enrolment	44.59	2.52	44.78	2.40	-0.3776	0.7066
At discharge	45.07	2.15	45.27	1.98	-0.4506	0.6534
F/U at 15 days	45.65	2.48	45.81	2.30	-0.3189	0.7506
F/U at 4 weeks	46.23	3.07	46.76	2.35	-0.8868	0.3777
At enrolment -At discharge	0.37	0.56	0.33	0.53	0.3771	0.7070
At enrolment -15days	0.95	0.31	0.97	0.32	-0.1918	0.8484
At enrolment -4 weeks	1.54	2.13	1.95	0.44	-1.2035	0.2321

The total percentage increase in mean length from enrollment value to 4 weeks observed was significant within the groups. The difference noted was higher in Pooled group (4.36 % v/s 3.45%) ($p=0.001$ v/s $p=0.001$) (Table 7)

Table 7: Comparison Of % Gain in Mean Length (In Cm) at Different Time Points Between Unpooled And Pooled Group

Groups	Changes from At enrolment to	% of change	t-value	P-value	F-value	P-value	Effect size
Unpooled	At discharge	0.84	-4.5833	0.0001*	16.8980	0.0001*	0.2690
	F/U at 15 days	2.14	-20.9466	0.0001*			
	F/U at 4 weeks	3.45	-4.9513	0.0001*			
Pooled	At discharge	0.74	-4.2527	0.0001*	217.852	0.0001*	0.8510
	F/U at 15 days	2.16	-19.9867	0.0001*			
	F/U at 4 weeks	4.36	-27.4343	0.0001*			

C. GROWTH PARAMETER – HEAD-CIRCUMFERENCE

At enrolment, baseline mean head circumference (31.10 ± 1.80 cm in Unpooled v/s 31.26 ± 1.58 cm $p=0.64$) was similar between the two groups. Increase in mean head circumference of preterm infants from the baseline value was observed in both groups at discharge (31.45 ± 1.57 cm v/s 31.71 ± 1.21 cm, $p=0.36$), at 2 weeks (32.04 ± 1.88 v/s 32.30 ± 1.45 cm, $p=0.47$) and by the end of 4 weeks (32.95 ± 1.85 cm v/s 33.17 ± 1.35 cm $p=0.52$) respectively, but no significant difference in increase in head circumference was observed between the two groups. (Table 8)

Table 8: Comparison of Mean Head Circumference (In Cm) at Different Time Between Unpooled And Pooled Groups.

Time points	Unpooled		Pooled		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
At enrolment	31.10	1.80	31.26	1.58	-0.4657	0.6425
At discharge	31.45	1.57	31.71	1.21	-0.9158	0.3622
F/U at 15 days	32.04	1.88	32.30	1.45	-0.7201	0.4733
F/U at 4 weeks	32.95	1.85	33.17	1.35	-0.6335	0.5281
At enrolment -At discharge	0.28	0.43	0.35	0.57	-0.6468	0.5193
At enrolment -15days	0.88	0.36	0.99	0.38	-1.3609	0.1770
At enrolment -4 weeks	1.79	0.44	1.85	0.42	-0.5869	0.5589

The total percentage increase in mean head circumference from mean head circumference at enrolment value to 4 weeks observed was significant within the group .The difference noted was higher in Pooled group (5.91 % v/s 5.76 %)(**p=0.0001 v/s p=0.0001**) (Table 9)

Table 9 : Comparison of % Gain In Mean Head Circumference (In Cm) at Different Time Between Unpooled And Pooled Groups

Groups	Changes from At enrolment to	% of change	t-value	P-value	F-value	P-value	Effect size
Unpooled	At discharge	0.90	-4.5413	0.0001*	255.732	0.0001*	0.8480
	F/U at 15 days	2.84	-16.8083	0.0001*			
	F/U at 4 weeks	5.76	-27.8337	0.0001*			
Pooled	At discharge	1.11	-4.1744	0.0001*	216.814	0.0001*	0.8510
	F/U at 15 days	3.16	-17.2366	0.0001*			
	F/U at 4 weeks	5.91	-27.7128	0.0001*			

D. GROWTH PARAMETER - MID UPPER ARM -CIRCUMFERENCE

At enrollment, baseline mean mid upper arm circumference (7.50 \pm 0.44 cm in Unpooled group and 7.50 \pm 0.46 cm in Pooled group p=1.00) was similar between the two groups. Increase in mean mid upper arm circumference from the baseline value of preterm infants was observed in both groups, at discharge (7.54 \pm 0.44 cm v/s 7.54 \pm 0.45 cm p=0.99), at 2 weeks (7.66 \pm 0.42 cm v/s 7.64 \pm 0.42 cm p=0.86) and by the end of 4 weeks (7.82 \pm 0.38cm v/s 7.74 \pm 0.42 cm p=0.34) follow up respectively, but no significant difference in increase in mid upper-arm circumference was observed between the two groups.(Table 10)

Table 10 : Comparison Of Mean Upper Arm Circumference (In Cm) at Different Time Points Between Unpooled And Pooled Group.

Time points	Unpooled		Pooled		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
At enrolment	7.50	0.44	7.50	0.46	0.0000	1.0000
At discharge	7.54	0.44	7.54	0.45	-0.0097	0.9923
F/U at 15 days	7.66	0.42	7.64	0.42	0.1728	0.8632
F/U at 4 weeks	7.82	0.38	7.74	0.42	0.9452	0.3472
At enrolment -At discharge	0.03	0.12	0.02	0.10	0.4068	0.6851
At enrolment -15days	0.15	0.23	0.15	0.23	0.0250	0.9801
At enrolment -4 weeks	0.31	0.27	0.26	0.25	0.9224	0.3590

The total percentage increase in mean mid upper arm circumference from mean mid upper arm circumference at enrolment value to 4 weeks observed was significant within the group. The difference noted was higher in Unpooled group (4.11 % vs 3.42 %) ($p=0.0001$ v/s $p=0.0001$) (Table 11)

Table 11 : Comparison of % Gain In Mean Upper Arm Circumference (In Cm) At Different Time Points Between Unpooled And Pooled Group

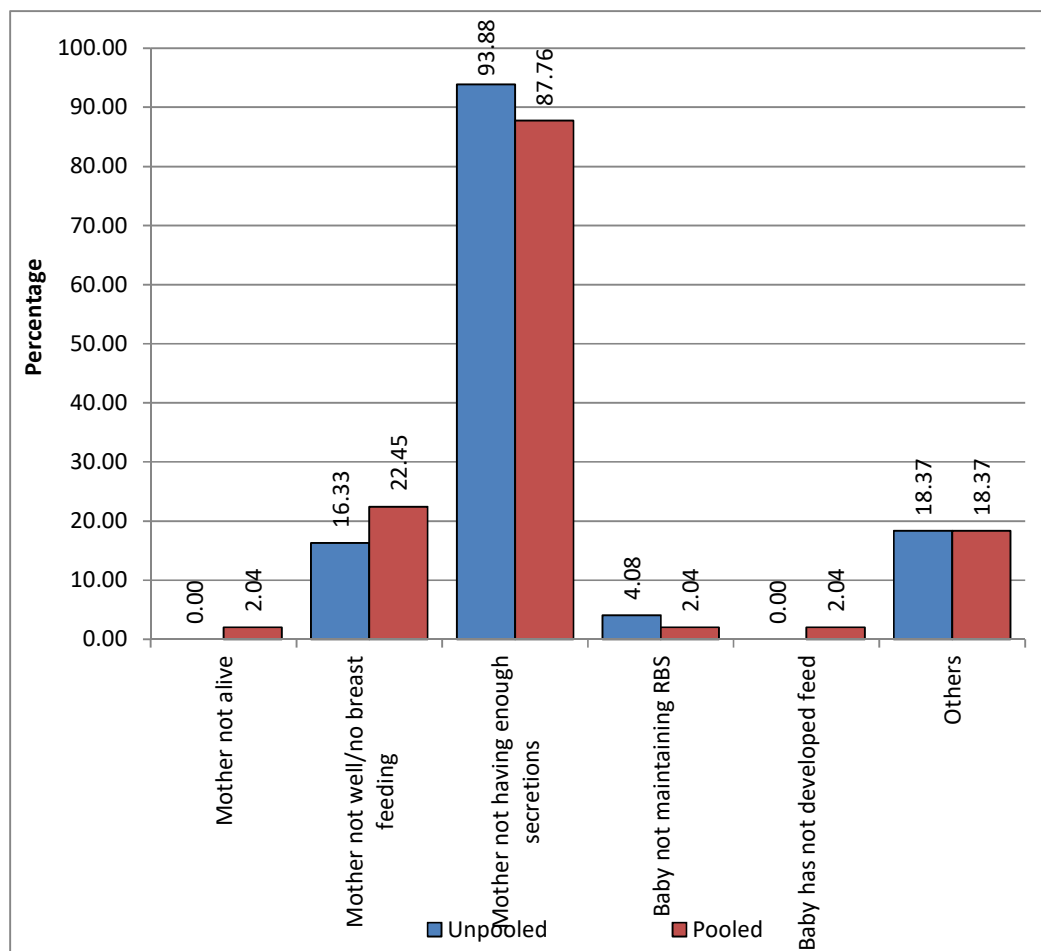
Groups	Changes from At enrolment to	% of change	t-value	P-value	F-value	P-value	Effect size
Unpooled	At discharge	0.42	-1.7701	0.0832	34.897	0.0001*	0.4310
	F/U at 15 days	1.99	-4.4176	0.0001*			
	F/U at 4 weeks	4.11	-7.9248	0.0001*			
Pooled	At discharge	0.29	-1.4298	0.1595	21.911	0.0001*	0.3600
	F/U at 15 days	1.97	-4.2279	0.0001*			
	F/U at 4 weeks	3.42	-6.2450	0.0001*			

VI. FEEDING DETAILS

A. INDICATION FOR STARTING PDHM

The major indication for starting PDHM in preterm infants was decreased breast milk secretions in the mother’s delivering preterm infants 90.82 % (93.88% in unpooled group v/s 87.76% in pooled group), followed by poor maternal health status 19.39 % (16.33 % unpooled group v/s 22.45% pooled group). In the present study indication for starting PDHM were comparable between both the groups (Graph 3)

Graph 3 : Comparison Of Indication For Starting PDHM Between Unpooled And Pooled



B. TOTAL AMOUNT OF PDHM

The total amount of PDHM consumed in both group was comparable (421.45 +/- 360.79 litre in unpooled group v/s 373.59 +/- 292.25 litres in pooled group) (p=0.47) (Table 12)

Table 12: Comparison of Total Amount Of PDHM Between Unpooled And Pooled Group

Parameters	Unpooled		Pooled		t-value	p-value
	Mean	SD	Mean	SD		
Total Amount of PDHM (ml)	421.45	360.79	373.59	292.25	0.7215	0.4724

C .MEAN AMOUNT OF PDHM

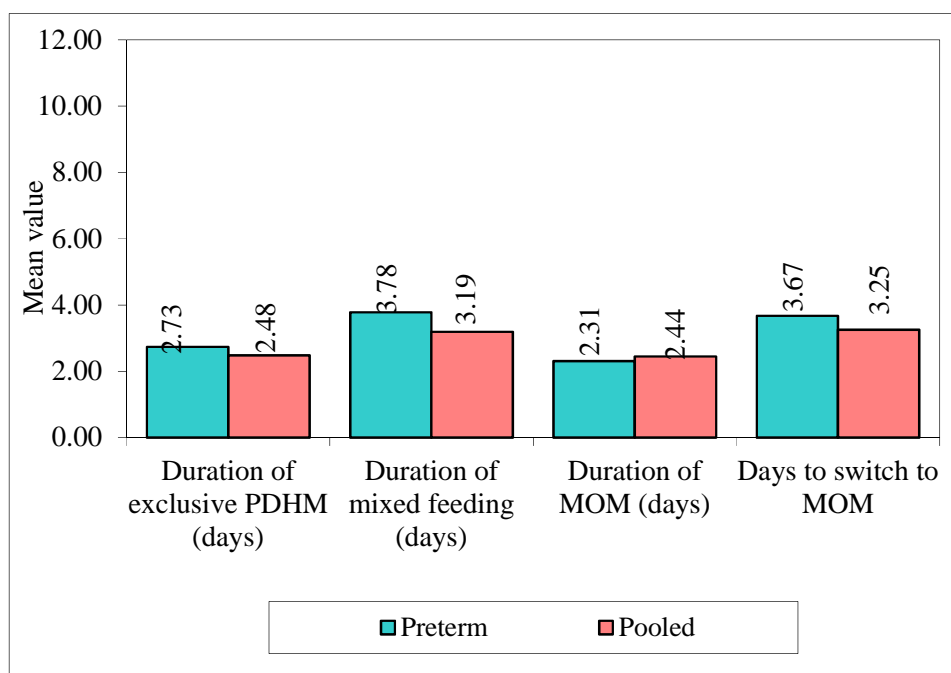
The mean amount of PDHM consumed by preterm infants across different time points between unpooled and pooled group was comparable (Table 5). At enrollment (55.96 +/- 37.79 litre v/s 47.94 +/- 31.75 litres p=0.25), at discharge (61.43 +/- 48.09 litre v/s 60.00 +/- 61.29 litres p=0.93), at 2 weeks (106.00 +/- 63.54 litre v/s 138.33 +/- 122.71 p=0.64) and at 4weeks (300 litre v/s 360 litres).The total amount of PDHM consumed from enrolment till end of 4 weeks was higher in pooled group (360 litres v/s 300 litres) (Table 13)

Table 13: Comparison of Mean Amount of PDHM between Unpooled and Pooled group At Different Time Points

Time points	Unpooled		Pooled		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
At enrolment	55.96	37.79	47.94	31.75	1.1375	0.2581
At discharge	61.43	48.09	60.00	61.29	0.0861	0.9318
F/U at 15 days	106.00	63.54	138.33	122.71	-0.4792	0.6446
F/U at 4 weeks	300.00	-	360.00	-	-	-
At enrolment -At discharge	13.24	59.69	17.17	61.32	-0.2171	0.8292
At enrolment -15days	92.50	56.91	101.00	112.98	-0.1373	0.8942
At enrolment -4 weeks	288.00	-	300.00	-	-	-

D .DURATION OF PDHM

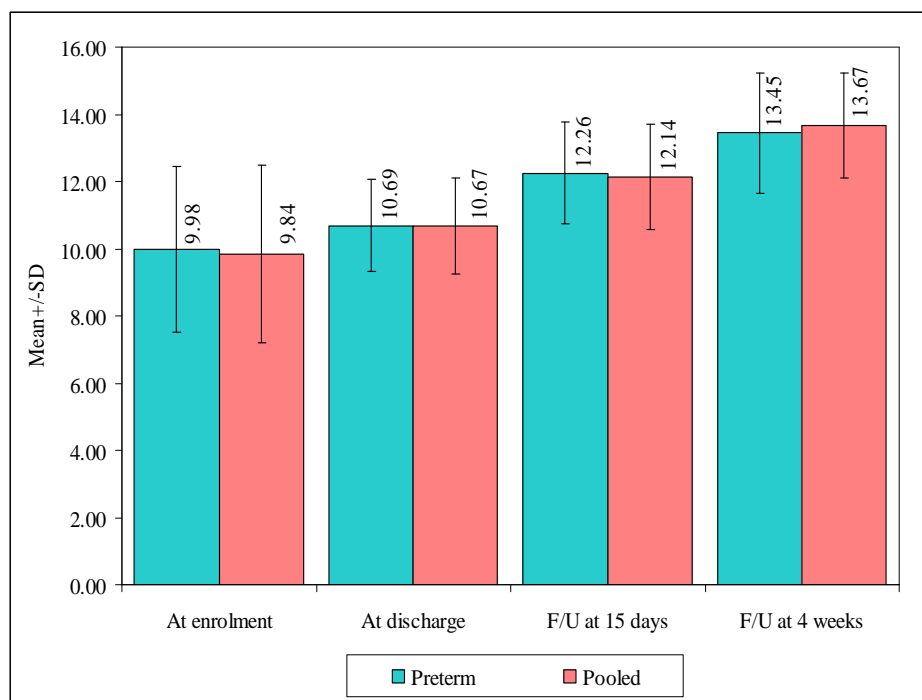
The mean duration of exclusive PDHM feeding (2.73 +/- 2.61 days v/s 2.48 +/- 2.97 days, p=0.65), mixed feeding (MOM+PDHM) (3.78 +/-2.80 days v/s 3.19 +/- 2.00 days, p=0.23) ,duration of exclusive MOM feeding (2.31 +/-3.10 days v/s 2.44 +/- 3.84 days,p=0.85) and days taken to switch to MOM (3.67 +/-2.81 days v/s 3.25 +/- 2.31 days, p=0.42) between both the groups was comparable (Graph 4).

Graph 4: Comparison of Duration of PDHM between Unpooled and Pooled**group**

E .FREQUENCY OF FEEDING

The mean frequency of feeding in preterm infants in unpooled and pooled group across different time points was comparable. The mean frequency at enrolment was (9.98 ± 2.46 feeds/day v/s 9.84 ± 2.65 feeds/day, $p= 0.84$), at discharge was (10.69 ± 1.37 feeds/day to 10.67 ± 1.42 feeds/ day, $p=0.96$), at 2 weeks was (12.26 ± 1.51 feeds/day to 12.14 ± 1.56 feeds/ day, $p=0.90$) and at 4 weeks was (13.45 ± 1.79 feeds/day to 13.67 ± 1.56 feeds/ day, $p=0.69$) respectively. (Graph 5).

Graph 5 : Comparison of Mean Frequency of Feeding Between Unpooled And Pooled Group



The total percentage increase in frequency of feeding from enrolment value to 4 weeks observed was significant within the groups .The difference noted was higher in pooled group (33.47% v/s 40.14%) (p= **0.0001** vs p=**0.0001**). (Table 14)

Table 14 : Comparison Of Change of Mean Frequency Of Feeding Between Unpooled And Pooled Group

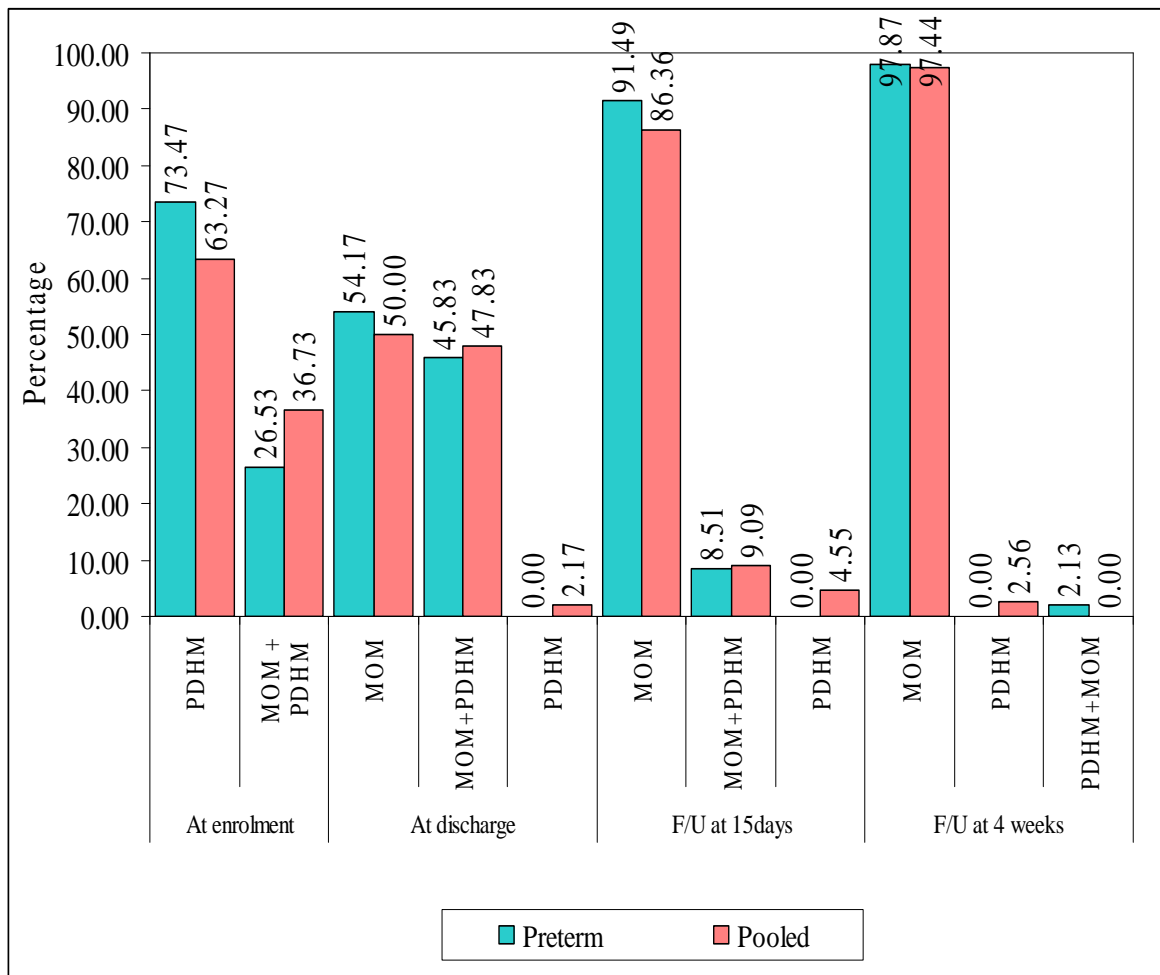
Groups	Changes from At enrolment to	% of change	T-value	Z-value	P-value	Friedma n's ANOVA	P-value
Preterm	At discharge	6.26	294.00	1.1094	0.2672	56.5747	0.0001*
	F/U at 15 days	21.53	31.00	4.4580	0.0001*		
	F/U at 4 weeks	33.47	24.00	5.0250	0.0001*		
Pooled	At discharge	7.07	206.00	1.0845	0.2781	56.9814	0.0001*
	F/U at 15 days	22.64	46.00	4.4060	0.0001*		
	F/U at 4 weeks	40.14	0.00	4.8599	0.0001*		

F .TYPE OF FEEDING

- 1) At enrolment, majority (68.37 %) of the preterm infants in the unpooled and pooled group were exclusively on PDHM (73.47 % v/s 63.27% p=0.27).
- 2) At discharge, majority (52.13%) were on MOM (54.17% v/s 50.00% p=0.95). The rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 2.17% p= 0.95).
- 3) At 2 weeks period , similar trends of increased MOM rates and decreasing PDHM rates were observed in both group with majority (89.01%)of preterm infants on MOM in both the groups (91.49% v/s 86.36% p=0.98). The rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 2% p= 0.98).
- 4) At 4 weeks almost all (97.67%) of preterm infants were on MOM in both the groups (97.87% v/s 97.44 % p=0.89). The rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 1% p= 0.89). (Graph 6). Both the groups were comparable for type of feeding at different time points.

Graph 6 : Comparison of Type Of Feeding Between Unpooled And Pooled

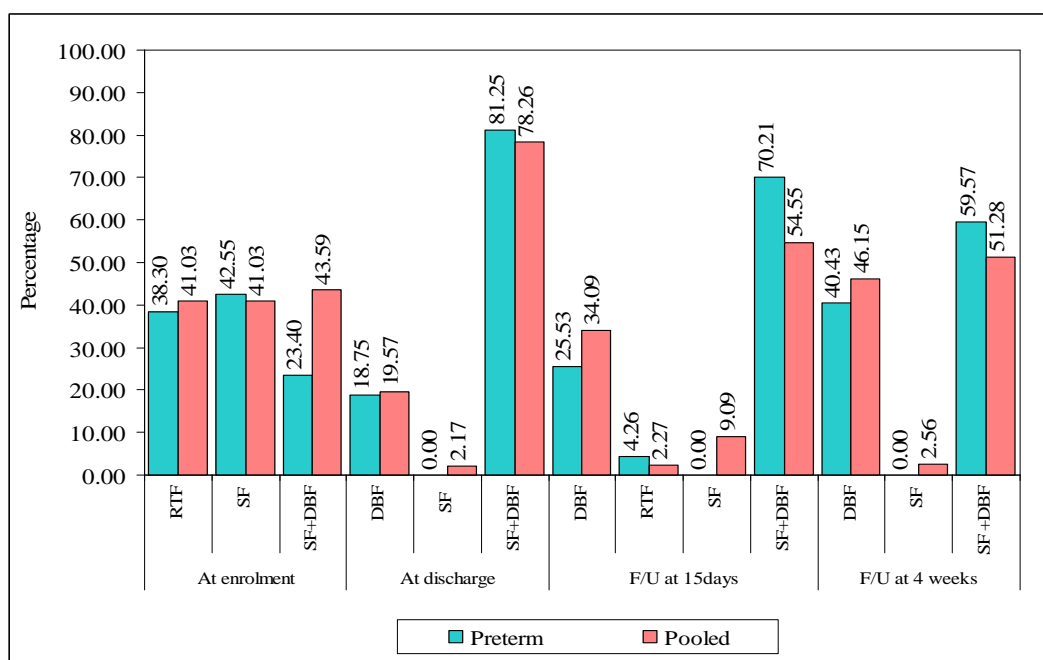
Group



G. METHOD OF FEEDING

1. At enrolment, majority (41.86%) of the preterm infants in the study were spoon feeding (42.55% v/s 41.08% $p=0.39$).
2. At discharge shift was noted to SF + DBF (79.79%) as method of feeding in both groups (81.25% v/s 78.26% $p= 0.98$). Increase in rate of exclusive DBF (19.15%) as method of feeding was noted in both groups (18.75% v/s 19.57% $p=0.98$)
3. Increasing trend was observed for DBF(29.67%) as method of feeding at 2 weeks follow up in both group (25.53 % v/s 34.09% $p=0.29$)
4. And by 4 weeks significant increase in DBF rates were observed (43.02%) (40.43 % v/s 46.15% $p=0.80$) overall rate of SF + DBF remained high at 55.81 % (59.57% v/s 51.28% $p=0.80$)(Graph 7)

Graph 7 : Comparison Of Method Of Feeding Between Unpooled And Pooled Group



VII SECONDARY OUTCOMES**A . LENGTH OF HOSPITAL STAY**

In the present study the mean duration of hospital stay in both unpooled and pooled group was comparable (9.65+/-6.64 days and 9.10 +/- 6.49 days p=0.68) (Table 15)

Table 15 :Comparison of Length Of Hospital Stay Between Unpooled And Pooled Group

Parameters	Preterm		Pooled		t-value	p-value
	Mean	SD	Mean	SD		
Duration of hospital stay	9.65	6.84	9.10	6.49	0.4092	0.6833

DISCUSSION

Prematurity has been recognized as global health burden with the leading cause of under 5 mortality worldwide^{1,2,4,6,7, 9,10}. Adequate nutrition is of outmost importance for ensuring the optimal growth and development especially in preterm infants^{20,21,25,26,28}. Human milk with its ideal nutrient composition is known to achieve the optimal growth and development with improvement in both short term and long term outcomes in preterm infants^{12,13,15,20,21,25,26,27}. Feeding difficulties and inadequate milk production by mothers delivering preterm are the two main reasons stated for sub-optimal nutrition in preterm infants and have long term growth, neurodevelopment and metabolic implications. Under these circumstances of inadequate milk production and non-availability of mothers own milk ,Pasteurized Donor Human Milk (PDHM) is recommended as the second best infant feeding option ^{23,34,35}. PDHM is known to confer several advantages in preterm infants^{41,42,43,44,53,54,55,56,58} . However , the effect of PDHM in improving the growth outcomes in preterm infants is controversial^{45,49,51,52,55,56,58,62,68,69,70,71,72,73} . Most milk banks provide pooled donor human milk (term plus preterm milk) and human milk composition is different for different gestation, which may affect the donor human milk composition and in turn may affect the growth outcomes of infants⁹⁷. Processes like pasteurization , freezing and thawing are also known to alter the composition of human milk by acting on the lipases and other bio-active components along with macronutrients^{86,87,88,89,90,91,92,93,94,95,96} . There is a lack of clear evidence with no studies to demonstrate the effect of pooled pasteurized preterm milk on the growth outcomes of infants⁹⁷. Therefore present study was conducted to study the effect of pooled

donor human milk v/s unpooled (pooling of only preterm donor milk) preterm donor human milk on the short term growth parameters of preterm infants.

General Maternal Characteristics- In the present study, maternal characteristics namely, maternal age, occupation and maternal educational status were comparable between unpooled and pooled groups.

A. **MATERNAL AGE** -The mean maternal age of the preterm infants enrolled in the our study was observed to be 24.47 years (25.10 years v/s 23.84 years, $p=0.09$). Several studies exploring the risk factors associated with preterm delivery from low and middle income countries have reported similar observations . In a retrospective case-control study from Yemen ,by Dahman et al, analyzing records of 591 live-born deliveries to explore risk factors associated with preterm delivery reported that the preterm delivery was common in the maternal age group of 14-25 years ¹³⁸ .In an observational study from Nepal ,to evaluate the incidence, risk factors and consequences of preterm birth in Nepal, reported, 83.5% of the mother in the study were between age group of 20-35 yrs¹³⁹. A community-based prospective cohort study of 1977 antenatal mothers from India conducted to explore different risk factors for PTB reported mean maternal age of 24.6 ± 2.5 years (48.2%), similar to our observations ¹⁴⁰. In a community based longitudinal study carried out in rural Mysore, Karnataka, India conducted for a period of one year March 2015- February 2016 by Rashmi et al on 257 antenatal mothers, studying risk factors associated with preterm birth reported women aged 25-29 years to have higher OR (Odd's Ratio=0.74) for preterm delivery¹⁴¹. Similar observation was reported by a single-center, observational

cohort study conducted by Madore L.S et al comparing in-hospital weight gain, and neurodevelopment outcomes in preterm infants supplemented with predominantly (>50%) DBM (donor breast milk) to those fed only MOM (mother's own milk) or supplemented with predominantly (>50%) preterm formula (PF) reported the mean maternal age in the study to be around 29 years ⁷⁸. On the contrary, a recent Indian review by Devi T et al published in 2021 analyzing 9 Indian studies, studying prevalence and associated risk factors of preterm birth in India reported maternal age of <20 years and > 40years with increased risk for preterm delivery ^{5,11, 111,142,143}.

B. MATERNAL EDUCATION STATUS: In our study, most of the mothers (64.29%) were educated up to secondary educational level (73.47% v/s 55.10%,p=0.15). Similar observations were reported by other Indian studies conducted to evaluate the risk factors for preterm delivery. Abhishek Gurung et al in his study of preterm birth in Nepal reported 68.1% mothers to have received secondary education¹³⁹. A hospital based cross sectional questionnaire study assessing the knowledge and attitude of post natal lactating mothers regarding human milk banking at Yenepoya Medical College and Hospital, Karnataka, reported 52% of the mothers had received high school education¹⁴⁴. However, a recent review of 9 Indian studies evaluating the prevalence and associated risk factors of preterm birth reported, mothers with low educational status mostly illiterate and primary educated to have increased risk of preterm deliveries than mothers with higher educational status^{145,149,150}.

- C. **MATERNAL OCCUPATION** :In our study most of the mothers (97.96%) were observed to be homemakers (97.96% v/s 97.96%,p=0.00). Similar observations were reported by Dahman et al from Yemen, analyzing records of 591 live-born deliveries and studying the risk factors associated with preterm delivery, reported 95.8 % of the mothers to be homemakers ¹³⁸.

Maternal Pregnancy Profile - In the present study, maternal pregnancy profile namely, gravida status and antenatal risk factor associated with preterm delivery were comparable between unpooled and pooled groups.

- A. **GRAVIDA STATUS** - Most of the mothers delivering preterm infants in our study (62.24%) were primigravida (59.18% v/s 65.31 %, p=0.24). Similar observations were reported by several Indian studies. A descriptive longitudinal follow-up study of infant feeding practices in a tertiary care hospital in Karnataka, reported that most of the mothers delivering preterm infants in the study (62.24%) were primigravida¹⁵¹. A retrospective hospital based study by Ahankari et al observed that the odds of preterm delivery and LBW(low birth weight) were increased in primigravida compared to multigravida women¹¹¹. Contrary to our observations several studies have reported increased prevalence of preterm delivery in multigravida pregnant women. Bouchra Koullali et al in his retrospective study to investigate associations between parity and the risk of spontaneous preterm birth of large number of pregnant women, noted increased risk for spontaneous preterm birth in women with increase in parity ¹⁵². Similar observation of increased risk of preterm delivery with increase in parity was reported by S J Etuk et al

from Nigeria¹⁵³. Several Indian studies have also reported similar observations^{109,141}.

B. ANTENATAL RISK FACTORS - The most common antenatal risk factor associated with preterm delivery in our study was PPRM (preterm premature rupture of membrane) with oligohydramnios (45.5% v/s 36.2% ,p=0.38) followed by severe intra-uterine growth restriction (IUGR), (32.65 % v/s 32.65 % ,p=0.38). Similar observations of PPRM causing oligohydramnios has been reported to be significantly associated with preterm delivery by several Indian studies^{108,146,147,154,155}. It is known that patients with PPRM and oligohydramnios are at increased risk of microbial invasion of the amniotic cavity causing intra-amniotic inflammation. Also rupture of membranes causes release of local inflammatory mediators and imbalance between matrix metalloproteins and chemical messengers with, increased collagenase and protease activity, ultimately causing increased intrauterine pressure and therefore preterm delivery^{157,158}. Contrary to these observations few of the south Indian studies have reported PIH (pregnancy induced hypertension) as the most common antenatal risk factor causing preterm birth in India. Devi et al in a recent review of 9 Indian studies has reported PIH (21.45%) as the most common risk factor for preterm birth^{5,11}. A similar observation has been reported by CR Rao et al from Karnataka in his case control study¹⁴⁸. A systemic review and meta-analysis of 9 studies and total 27,119 participants, by Getaneh Muluaem et al, concluded that among the risk factors associated with preterm birth, PIH was in increasing trend and mothers with PIH were nearly 4.7 times more likely to give preterm birth than those with no PIH¹⁵⁶.

Baseline Birth History -In the present study, baseline birth history namely, mean gestational age, mean weight at birth, mode of delivery, Apgar score and day of enrollment were comparable, between unpooled and pooled groups except for gender.

A. **MEAN GESTATIONAL AGE**- The mean gestational age of the preterm infants in our study was 34.46 weeks (34.42 weeks in unpooled group v/s 34.51 weeks in pooled group, $p=0.80$) and similar in both the groups. This is in contrast to majority of the studies from both Indian and International literature which have reported mean gestational age of \leq to 32 weeks. A non inferiority randomized controlled trial conducted by Costa et al analyzing 70 preterm infants for tolerance of preterm formula versus pasteurized donor human milk in very preterm infants reported majority of infants with gestational age (GA) of ≤ 32 weeks⁵⁸. A retrospective clinical audit conducted by Lloyd M et al to study growth of preterm infants fed predominantly pasteurised donor human milk v/s those fed mother's own milk also collected data from the medical records of a cohort of preterm infants with gestational age \leq to 30 weeks⁸⁰. Debora et al in her study, analyzing availability of donor milk for very preterm infants and risk of necrotizing enterocolitis along with growth outcomes and rates of breastfeeding also reported mean gestational age of 29.5 ± 2.3 weeks⁷⁶. Similar observation is reported by several Indian studies. Adhisivam et al in his randomized controlled trial (RCT) study in a tertiary care teaching hospital, in south India studying the effect of fortified v/s unfortified pasteurized donor human milk on 80 healthy preterm neonates reported a mean gestational age (GA) of 31.82 weeks⁷⁷. Rohan Sapra et al in his longitudinal observational study conducted in level III NICU (neonatal intensive care unit), of a tertiary care hospital from North Karnataka, studying feeding patterns and growth parameters

in newborns receiving PDHM following discharge from hospital up to 6 months of age also reported mean GA of 35.72weeks¹⁵⁹. Saurabh et al in his hospital based cross sectional study in a similar setting, studying the incidence of NEC among preterm infants receiving PDHM, reported mean gestational age between 28 week to 34 week by Modified Ballard score¹⁶⁰.

- B. **BIRTH WEIGHT** - The mean weight at birth of the preterm infants in our study was 1797.24 gm (1824.90 gm in unpooled group v/s 1769.59 gm in pooled group, p=0.46). This is in contrast to majority of the studies as most studies analyzing the effect of feeding patterns on growth parameters, have preterm infants with mean GA < 32 weeks, and therefore mean weight of < 1500gm. A hospital based retrospective study, conducted by Kim , Eun Jeong MD et al in in Korea, between January 2011 and December 2016 studying the effects of exclusive donor human milk feeding on morbidity and growth of preterm infants during hospitalization reviewed 132 infants with birth weights (BW) <1500 g and gestational ages (GAs) of less than 32 weeks ⁵². Another retrospective cohort study conducted by Ahreen Allana et al in Kings County Hospital Center (KCHC) in Brooklyn, New York, over four years, two years before and two years after the introduction of donor human milk analyzing use of donor human milk in preterm, very-low-birth-weight (VLBW) infants for the rates of necrotizing enterocolitis, duration of parenteral nutrition (PN), growth, culture-positive sepsis, length of hospital stay, and mortality, analyzed 190 infants with a birth weight of less than 1500 g ¹⁶¹. Similar observations were also reported by several Indian studies^{77,159,160} .

C. **MODE OF DELIVERY**- In the study the most common mode of delivery observed was LSCS (lower segment caesarian section) (62.24%) (63.27 % v/s 61.22 %,p=0.83) which is similar to observations noted by others. A retrospective analysis from a large tertiary referral center from Australia, to evaluate the effect of mode of delivery on neonatal outcome among preterm infants in different birth weight categories reported higher incidence of LSCS and better outcomes with LSCS in AGA (appropriate for gestational age) preterm infants¹⁶². A case control study from Iran reported the odds ratio of preterm delivery in women with a history previous C-section delivery increased by 2.09 folds¹⁶³. Similar observations of majority of preterm deliveries by LSCS (67%) is also reported by an Indian study¹⁶⁴.

It is known that the rate of cesarean section is significantly higher in the preterm period, as several criteria must be ensured to enable vaginal delivery for preterm infants namely regular labor contractions, intrapartum fetal cardiotocography without pathological features, absence of congenital fetal anomalies , appropriate neonatal birth weight, etc. to name a few along with nil maternal comorbidities¹⁶². The higher rate of LSCS in our study can be attributed to the referral of high-risk antenatal cases with complications to our hospital since it is a tertiary care facility and increased occurrence of complications in preterm delivery in our study.

D. **GENDER OF THE PRETERM INFANT**- Majority (51.02%) of preterm infants in the study were males. However a significant difference was observed in gender in between the two groups (p=0.04). Majority of preterm infants in unpooled group were females (59.18 %) and in pooled group were males (61.22%). This is similar to the study by Hassan N. et al in 2019, conducted to

outline the morbidity and mortality pattern of preterm infants admitted to NICU of a tertiary care center in Western Uttar Pradesh (UP) and reported that, 74.5% of neonates were boys¹⁶⁴. Similar observation was reported by Saurabh et al in his study in the Indian setting (56.11%) analyzing effect of PDHM on incidence of NEC¹⁶⁰. Costa et al, Sisk et al, also reported similar observation of male gender preponderance in their respective studies^{50,58}.

E. **APGAR SCORE** - In our study the mean APGAR score at birth of the preterm infants was 7.92 and was similar between both the groups (7.96 v/s 7.88, p=0.43) This was in accordance with, a population based cohort study from Norway by D Mosteret al which aimed at analyzing the combination of a low five minute Apgar score and symptoms of neonatal encephalopathy with minor impairments at school age reported 75% of the neonates with Apgar scores of 7-10¹⁶⁵. Similar observation was reported by a hospital based retrospective study, conducted by Kim, Eun Jeong MD et al in Korea, studying the effects of exclusive donor human milk feeding on morbidity and growth of preterm infants during hospitalization and reported mean Apgar score of 7.1 +/- 1.4⁵². The higher Apgar scoring in our study could be because, majority of the preterm infants in our study were stable preterm infants belonging to mean gestational age of 34.4 weeks and mean birth weight of 1797.24gm with no associated co-morbidities.

F. **DAY AT ENROLLMENT**

In the present study, we observed that majority (62.24%) of the preterm infants were enrolled at day one of life (61.22% in unpooled group v/s 63.27% in pooled group, p=0.71). This observation is similar to another Indian study conducted by Rohan Sapra et al in North Karnataka.

G. INDICATION FOR NICU ADMISSION

In the study, the most common indication for admission to NICU was low birth weight (98.98%) (100% v/s 97%) followed by kangaroo mother care (81.63%) (77.55% v/s 80 %). This is similar to observation reported by a hospital based, cross- sectional descriptive study by Khasawneh et al conducted in Jordan between September 2016 and September 2018 analyzing a total of 1444 infants which reported 39 % of the preterm infants admitted to NICU was because of LBW ¹⁶⁶. Similar observation was reported in an Indian study by Dayanithi et al determining prevalence of LBW and premature births and their associated maternal factors ¹⁶⁷. Other south Indian studies by Rohan et al and Saurabh et al also reported similar observation.

Feeding Profile

A. **AMOUNT OF FEEDING** – In the study the total amount of PDHM consumed in both groups was high. The total amount of PDHM (421.45 +/- 360.79 litre v/s 373.59 +/- 292.25 litres) (p=0.47) and the mean amount of PDHM consumed by preterm infants across different time points between both groups were comparable. At enrollment (55.96 +/- 37.79 litre v/s 47.94 +/- 31.75 litres p=0.25), at discharge (61.43 +/- 48.09 litre v/s 60.00 +/- 61.29 litres p=0.93), at 2 weeks (106.00 +/- 63.54 litre v/s 138.33 +/- 122.71 p=0.64) and at 4weeks (300 litre v/s 360 litres). However majority of the studies report lesser volumes of PDHM utilization by preterm infants. A mono-centric randomized non-inferiorty controlled trial conducted by Costa et al between January 2015 to August 2015 assessing tolerance of preterm formula versus PDHM in very preterm infants reported the amount of PDHM consumed to be (52.8+/- 37.5

ml/kg /day) in PDHM group⁵⁸.Lloyd M et al , also conducted a retrospective clinical audit in , Australia between 1 January 2012 and13 April 2013, to study growth of preterm infants fed predominantly pasteurised donor human milk v/s. those fed mother's own milk in the neonatal intensive care unit, and reported total milk intake for the PDHM group to be (196.4 ml +/- 9.95 ml) compared with that of the MOM group (183.1ml +/- 12.01ml, $P=0.391$) which is in contrast to our study⁸⁰.

Majority of our babies enrolled in our study were stable preterm infants with mean gestational age of 34.4 weeks and mean weight of 1797.24gm and therefore the consumption of PDHM is more compared to other studies which have preterm infants belonging to $GA \leq$ to 32 weeks and birth weight of less than 1500gm.

B. DURATION OF FEEDING- In our study the mean duration of feedings namely 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, $p=0.42$) were comparable between both the groups. This is in contrast to several studies reporting mean duration of PDHM feeding \geq to 5 days. A hospital based retrospective cohort study conducted by Melesa et al in in Euthopia from August 2018 to 2019, to determine time taken to reach full enteral feeding among low-birth-weight neonates reported the median time to achieve full enteral feeding as 8 days ¹⁷⁰. Similar observation has been reported in many European studies with mean duration of PDHM feeding ranging between 8.5 -10.5 days ^{168,169}. A randomized controlled trial (RCT) conducted

in a tertiary care teaching hospital, in south India in 2018 by Adhisivam et al to study the effect of fortified v/s unfortified pasteurized donor human milk on 80 healthy preterm neonates for incidence of NEC, sepsis, mortality, duration of hospital stay, and time taken to reach full enteral feeds and growth outcomes, reported mean duration of PDHM feeding of 8.5 ± 5.1 days⁷⁷. Several other Indian studies have also reported similar observation^{159,160}.

The shorter duration of PDHM feeding of preterm infants in our study is due to inclusion of stable, preterm, SGA babies with adequate gestation and baseline weight measurements. Suck and swallow reflexes are well established by 34 weeks therefore leading to early switching over to mother's milk (3.67 ± 2.81 days in unpooled group and 3.25 ± 2.31 days in pooled group respectively) which could also explain the reason for shorter duration of PDHM feeding.

C. **TYPE OF FEEDING** - In our study the type of feeding in preterm infants in both the group across different time points was comparable. At enrollment, majority (68.37 %) of the preterm infants in both the groups were exclusively on PDHM (73.47 % v/s 63.27% $p=0.27$). At discharge, the rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 2.17% $p= 0.95$) and majority (52.13%) were on MOM (mother's own milk) (54.17% v/s 50.00% $p=0.95$). And by the end of 4 weeks almost all (97.67%) of preterm infants were on MOM in both the groups (97.87% v/s 97.44 % $p=0.89$). The rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 1% $p= 0.89$). Similar observation has been reported by a population based cohort by Agata Kantorowska et al studying the impact of donor human milk availability on breast milk use and NEC (necrotising enterocolitis) rates from 2007 to 2013 in California and has reported 10% increase in breast milk feeding at NICU

discharge¹⁷¹. This is in contrast to Williams et al who conducted a systematic literature review of studies in October 2014 that assessed maternal breastfeeding rates before and after the introduction of DHM and found that presence of a human milk bank (HMB) and the usage of PDHM in NICU and reported a significant decrease in the percentage of feeds that were MOM after the introduction of DHM.

Most of the mothers of preterm infants are unable to provide adequate milk to meet the nutritional needs of their infants in the initial days^{14,15,16,17,19}. Recent studies have shown that, delayed lactogenesis II is common in mothers of preterm babies^{30,32,33}. Delay in the onset of lactogenesis II will lead to decreased volume of milk expression by preterm mothers and therefore more chances of PDHM feeding during the initial days.^{17,29, 30,31,32,33} This delay in lactogenesis gets corrected by 1-2 weeks and therefore increased rates of DBF by discharge and follow up. Another reason for the above observation is LCSC(Lower segment cesarian section) is associated with decrease in let down reflex and therefore delay in breast milk expression. The initial separation of preterm infants from their mothers during NICU stay causes no proper activation of let down reflex and is also one reason, for PDHM feeding rates high on admission which is eventually taken over by MOM by discharge and follow up as these factors are mitigated.

- D. **METHOD OF FEEDING** - In our study the method of feeding in preterm infants in both the group across different time points was comparable. At enrollment , majority (41.86%) of the preterm infants in the study were on SF (spoon feeds) (42.55% v/s 41.08% $p=0.39%$) .At discharge shift was noted to SF + DBF(direct breast feeding) (79.79%) as method of feeding in both

groups(81.25% v/s 78.26% p= 0.98). Increase in rate of exclusive DBF (19.15%) as method of feeding was noted in both groups (18.75% v/s 19.57% p=0.98).At 2 weeks follow up increasing trend was observed for DBF(29.67%) as method of feeding in both group (25.53 % v/s 34.09% p=0.29).And by 4 weeks significant increase in DBF rates was observed (43.02%) (40.43 % v/s 46.15% p=0.80) though overall rate of SF + DBF remained high at 55.81 % (59.57% v/s 51.28% p=0.80). Similar trend of increase in BF rates by discharge was observed in studies by Bramer et al and Underwood et al ^{20,172}. Torres et al in his study on preterm infants with weight of < or = 1500 g or GA< 32 weeks gestation, analyzing BF rates before and after the opening of the human milk bank, reported ,that opening a milk bank did not reduce EBF rates at discharge¹⁷⁵.An Indian study by Adhisivam et al also reported an improvement in breastfeeding rates after opening of milk bank at 6months from 34% to 74% ⁷⁷.

Most of the infants in our study were on SF at enrolment, which later changed to DBF. This is due to inclusion of stable, preterm infants in our study with mean gestational age of 34.4 weeks. Although sucking and swallowing activities are present by 28 weeks intra-partum , the coordination does not develop fully until about 32 to 34 weeks and therefore preterm infants born at less than 32 weeks gestation, cannot sustain full oral feeds. Since majority of the preterm infants in our study belonged to gestational age of 34 weeks, SF was started. WHO also recommends starting SF as it helps in reaching full enteral feeds faster and early hospital discharge when compared to RT feeding¹.

It was also observed that type of feeding at following visits had significant correlation with method of feeding at each visit as, DBF rates increased as MOM was available.

Also PDHM availability promotes a breastfeeding - friendly environment which boost breastfeeding rates and therefore higher rates of DBF at discharge and follow up visits.

FREQUENCY OF FEEDING- In our study the mean frequency of feeding in preterm infants in both the group across different time points were comparable. At enrolment (9.98 ± 2.46 feeds/day in v/s 9.84 ± 2.65 feeds/day , $p= 0.84$).At discharge (10.69 ± 1.37 feeds/day to 10.67 ± 1.42 feeds/ day, $p=0.96$). At 2 weeks (12.26 ± 1.51 feeds/day to 12.14 ± 1.56 feeds/ day, $p=0.90$) .At 4 weeks (13.45 ± 1.79 feeds/day to 13.67 ± 1.56 feeds/ day, $p=0.69$). Increase in frequency of feeding was observed during follow up visits.This is in contrast to a randomized clinical trial conducted by Hussain et al in VLBW (very low birth weight) neonates in evaluating and comparing volume advancement v/s frequency advancement as feeding methods in VLBW infants and reported decrease in frequency of feeds after discharge from hospital ¹⁷⁴. Kumar et al in a recent systemic review and meta-analysis of 6 RCTs in PGI,Chandigarh studying effect of three-hourly versus two-hourly feeding interval in stable preterm infants also reported three hourly interval to be a safer option ¹⁷³. The possible explanation of increased feeding frequency at discharge and subsequent follow ups in our study is because, our NICU is associated with Human Milk Bank with proper lactation management counsellors which has affected the feeding frequency in our study both at discharge and at follow up. Also majority of the infants in our study at discharge and follow up visits

at 2 weeks and 4 weeks were on DBF +SF causing combined frequency rates to be high in our study. Also since this study is largely based on preterm infants, and a short interval (1–2 hour) feeding schedule delivers smaller volume per feed and is therefore easily tolerated by preterm infants.

LENGTH OF HOSPITAL STAY - In our study the mean duration of hospital stay in both groups was comparable (9.65+/-6.64 days and 9.10 +/- 6.49 days $p=0.68$). The average length of hospital stay ranged between 3 days to 15 days. This is similar to observation seen in a systematic review and meta-analysis conducted by Rui Yang et al studying the effect of donor human milk on the length of hospital stay in very low birth weight infants in 2020 analyzing 136 articles which reported, that meta-analysis of eight observational studies showed significant reduction in length of hospital stay for infants receiving DHM (donor human milk) (- 11.72 days; 95% CI - 22.07,-1.37 days; $p = 0.03$). This is in contrast to mono-centric randomized non-inferiority controlled trial conducted by Costa et al assessing tolerance of preterm formula versus PDHM in very preterm infants with gestational age (GA) of ≤ 32 weeks which reported an average length of hospital stay for infants to be 37.5+/- 17.5 days⁵⁸. The possible explanation for this observation in our study is because of enrollment of stable, preterm babies with mean gestational age of 34 weeks requiring shorter duration of PDHM feeding. Also early switching over to mother's milk (3.67 +/-2.81 days in unpooled group and 3.25 +/- 2.31 days in pooled group respectively) and increased rate of EBF at discharge is also one reason for early discharge from NICU. Also PDHM is known to confer several advantages in preterm infants with decreased risk of comorbidities like LOS, NEC, BPD, IVH etc. leading to decreased rate of hospital acquired infections and therefore shorter in-hospital stay^{41- 47,50,55,56,58}.

Primary Outcomes

The growth parameters of preterm infants in our study , namely mean weight, length , head-circumference, mid upper arm circumference did not show significant difference between the unpooled (exclusive pooled preterm milk) and pooled group (pooled milk of both preterm and term) following discharge from hospital up to 4 weeks of age. However the total percentage increase in growth parameters namely mean weight, length, head circumference and mid upper arm circumference from the baseline value at enrolment to 4 weeks observed was significant within the groups. Our observations infer that PDHM had a positive effect on the growth parameters irrespective whether exclusive preterm pooled milk was given or pooled milk(both term and preterm milk combined) was given .

A recent Cochrane review by Dempsey et al in 2019 analyzing all the available RCTs related to growth effects in VLBW babies receiving banked donor preterm milk v/s banked donor term milk found no studies to demonstrate the effect of exclusive pooled preterm PDHM on growth outcomes in preterm infants⁹⁷. The limitation stated in the Cochrane review for conduct of such studies was decreased breast milk production in mothers delivering preterm. We did not encounter this limitation in our study, and therefore this study is first and one of its kind and therefore we found no studies to compare with our results.

However majority of the studies reported in the literature studying the effect of PDHM on the growth outcomes of preterm infants have shown controversial results.

Very few studies have reported similar observation of improved growth outcomes in neonates receiving PDHM when compared to either MOM or PF

(preterm formula) at discharge and at follow up visit . A recent longitudinal observational study conducted by Rohan Sapra et al in level III NICU of a tertiary care hospital situated in North Karnataka, between January 2020 to December 2021 studying feeding patterns and growth parameters in 70 newborns receiving PDHM feeding following discharge from hospital up to 6 months of age, reported improvement in all growth parameters including weight, length , head circumference and mid upper arm circumference in neonates receiving PDHM by the time of discharge up to 6 months of age, implying PDHM has a positive impact on early and late postnatal growth¹⁵⁹.

Another Indian cross sectional study by Saurabh et al based in similar setting , studying the incidence of NEC along with secondary outcomes as growth parameters among 221 preterm infants receiving PDHM , reported significant increase in weight(4.61%), height(3.15%), and head circumference (4.07%) at discharge ($p<0.001$)¹⁶⁰.

However majority of the studies conducted to show the effect of PDHM on growth outcomes in preterm infants when compared to MOM or PF have noted no difference in improvement of growth outcomes either short term or long term.

Several studies have compared PDHM with MOM to analyze the advantages of PDHM on various parameters in preterm infants. A retrospective analysis of 550 VLBW infants by R Chowning et al for the effect of human donor milk on prevention of necrotizing enterocolitis and postnatal growth outcomes reported slower growth rates in weight and head-circumference at discharge following introduction of DHM ⁴⁹.A retrospective study, conducted by Kim, Eun Jeong MD et al in neonatal intensive care unit (NICU) of a hospital in Korea, studying the effects of

exclusive donor human milk feeding on morbidity and growth outcomes of preterm infants during hospitalization reviewed 132 VLBW infants and reported ,that DHM group demonstrated a comparatively lower rate of weight gain, head circumference increment, and height increment from birth to the age at which an enteral feeding volume of 130 mL/kg/d was achieved. However there were no significant difference noted in these values at 36 weeks postmenstrual age between both groups¹⁷⁷. A retrospective clinical audit conducted by Lloyd M et al, to study growth of preterm infants fed predominantly pasteurised donor human milk v/s. those fed mother's own milk in preterm infants (≤ 30 weeks gestational age) receiving either ≥ 28 d of PDHM (n 53) or ≥ 28 d of their mother's own milk (MOM, n 43) with standard fortification observed transient slow growth in PDHM group but evident catch-up growth by discharge. By 3 months of age no difference was observed in between the two groups⁸⁰.

A retrospective case control study conducted in 2012 by Giulliani F et al, to study and evaluate short term advantages of MOM as a sole diet compared to donor milk as a sole diet , in terms of growth, anti-infectious properties, feeding tolerance, NEC, and ROP prevention in a population of VLBW infants born in a tertiary care center reported , no significant difference in clinical outcomes for infants belonging to MOM group compared with PDHM group. Only a slight but not statistical significant difference in growth was observed in favor of maternal milk¹³⁰.

An observational study analyzing longitudinal growth outcomes and morbidities in 90 extremely preterm infants born between 2013-2015 in , Sweden by Lund et al comparing mother's milk v/s pasteurized donor human milk consumption reported that unpasteurized mother's milk correlated positively with almost all z-

scores for weight, length, head circumference at postnatal age of 28 days and at PMA at 32 and 36 weeks. Infants consuming > 80% of MOM had more favorable z scores at PMA at 32 and 36 weeks ¹¹⁹.

Studies comparing PDHM with PF (preterm formula) have reported better growth parameters with PF in preterm babies than PDHM. A randomized control trial from a NICU from Texas comparing donor milk v/s preterm formula as substitute for MOM found the rates of weight gain to be higher in PF group infants as against DHM group at discharge ⁵¹. A randomized multicenter study by Lucas et al, conducted on 502 preterm infants admitted in NICU in Cambridge, to study effect of human milk v/s formula milk for development outcomes in preterm infants reported, babies fed on preterm formula gained weight and head circumference significantly faster than those fed on banked breast milk ⁵⁹. A single-center retrospective study conducted by Brownell et al in 4 urban level NICUs, aimed at analyzing dose-response relationship between donor human milk, mother's own milk, preterm formula, and neonatal growth outcomes analyzed 314 infants records and reported that adjusted mean growth velocity for weight significantly decreased by 0.17g/ kg⁻¹ / day for every 10% increase in DHM intake, but did not vary with PF intake in neonates⁷³.

A prospective cohort conducted from August 2017 to October 2019 in a large neonatal intensive care unit in China by Fang L et al, analyzing the effects of preterm donor milk (DM) on growth, feeding tolerance, and severe morbidity in very-low-birth-weight infants, analyzed 304 preterm infants weighing <1,500 g or of gestational age <32 weeks and reported no difference in daily weight gain and weekly head growth of VLBW in both PF and PDHM group concluding preterm DHM does not affect the growth of VLBW infants⁴¹. A systemic review

conducted by Quigley M et al in 2018, analyzed 11 completed trials up to June 2017 studying the effect of formula feeding v/s donor breast milk on growth and development in preterm or low birth weight (LBW) infants and reported formula-fed infants to have higher in-hospital rates of weight gain, linear growth and head growth as compared to PDHM⁷¹.

A retrospective cohort study by Sisk et al in North Carolina also reported no differences in growth metrics from birth to hospital discharge in preterm infants fed predominantly maternal milk, pasteurized donor milk, or preterm formula⁵⁰. A monocentric randomized controlled non-inferiority trial conducted by Costa et al assessing tolerance of preterm formula versus PDHM in very preterm infants analysed 75 eligible preterm infants, and reported infants in PF group regained birth weight faster by 2 days than PDHM group⁵⁸.

A retrospective cohort conducted by Hoban et al in urban level III NICU in Chicago in two time periods: A “pre-DM” group using preterm infant formula as a supplement to MOM, and a “DM” group that supplemented MOM with pasteurized DM instead of formula between January 2011 and December 2012 analyzing 321 VLBW for anthropometric data at six time points from birth to 20–24 months corrected age in VLBW infants showed higher formula proportion was associated with slower rates of decline in short-term growth trajectories for weight and length. Though z-scores for weight and length decreased during hospitalization for DM group but it increased for all parameters including head circumference post-discharge and therefore DM can be used in early post-birth period to mitigate potential risk from formula without compromising long-term growth⁷².

A retrospective study, conducted by Kim L Chung et al in China reviewing 132 infants of birth weights (BW) <1500 g and gestational ages (GAs) of less than 32 weeks studying the effects of exclusive donor human milk feeding in a short period after birth on morbidity and growth of preterm infants during hospitalization reported lower values of weight, head circumference and height 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 ⁵².

A randomized controlled trial (RCT) conducted in a tertiary care teaching hospital, in south India in 2018 by Adhisivam et al to study the effect of fortified v/s unfortified pasteurized donor human milk on 80 healthy preterm neonates followed up for 28 days or discharge primary outcome measurement as incidence of NEC and the secondary outcomes measurements as severity of NEC, incidence of sepsis, mortality, duration of hospital stay, number of days to reach full enteral feeds and weight gain and increase in head circumference, reported no significant difference in growth parameters like weight gain and increase in head circumference among neonates in the fortified versus the unfortified group⁷⁷.

However the results in our study demonstrate better growth outcomes within the groups with PDHM feeding irrespective of exclusive preterm PDHM feeding or Pooled (term and preterm) PDHM feeding. This could be because majority of the studies showing no effect on growth outcomes with PDHM milk did not analyze growth outcomes as primary outcomes for their study. Also many factors like gestational age and amount of PDHM influence growth outcomes with PDHM feeding.

GESTATIONAL AGE - Better growth outcomes observed for preterm infants in our study with PDHM feeding is likely because the mean gestational age of the preterm infants in our study was 34.46 weeks that is late preterm as against majority of the studies with GA (gestational age) ≤ 30 weeks. GA is independently associated with some growth outcomes. Infants with higher GA experienced more normal short-term growth, likely due to a less challenging clinical course and lower nutritional requirements than infants with lower GAs and birth weights.

DOSE OF PASTEURIZED DONOR HUMAN MILK - Better growth outcomes observed for preterm infants in our study with PDHM feeding is likely because the total amount of PDHM consumed in both groups was high as against majority of the studies reported. Majority of our babies enrolled in our study were stable preterm infants with mean gestational age of 34.46 weeks and mean weight of 1797.24gm and therefore the consumption of PDHM is more compared to other studies which have preterm infants belonging to GA \leq 32 weeks and birth weight of less than 1500gm. This is because consumption of human milk is correlated positively with fat free mass deposition in late preterm infants which ultimately leads to improved metabolic and neurodevelopment outcomes¹²⁰. A recent review by Jacopa Cerasani et al in 2020, studying human milk feeding and preterm infant's growth and body composition, reported a broader reduction in the weight z scores from birth to discharge in group receiving $>75\%$ human milk as compared to those receiving $<$ than 75% ¹²⁰. Similar observation was reported by Piemontese et al in his study in Milan, who observed higher percentage of lean mass deposition at term corrected gestational age (CGA) in very low birth weight (VLBW) infants fed with human milk at more than equal to 50% of total milk volume as compared to VLBW infants fed with human milk at less than 50% of total milk volume¹²².

STRENGTHS OF THE STUDY

The strength of the study is its study design and adequate sample size .This is the first study to assess the effect of exclusive preterm PDHM v/s Pooled PDHM on short term growth parameters in preterm infants in Indian settings.

LIMITATIONS OF THE STUDY

The limitations of this study is that it is a single center study and with no long term follow up of the preterm infants, which was beyond the scope of the study.

RECOMMENDATIONS

Multicentric studies with larger sample size and long term follow up to study the effect of exclusive preterm pasteurized donor human milk on growth and developmental outcomes of preterm infants is recommended.

CONCLUSION OF THE STUDY

This randomized controlled trial (RCT) was conducted among preterm infants receiving pasteurized donor human milk (PDHM) from December 2021 to August 2022. The present study showed no difference when comparing short term growth parameters (weight, length, head-circumference, mid-upper arm circumference) of preterm infants receiving pooled pasteurized donor human milk versus unpooled pasteurized preterm donor human milk. However, significant difference was noted for various growth parameters (weight, length, head-circumference, mid-upper arm circumference) within the group suggesting that pasteurized donor human milk (PDHM) promotes short term growth parameters in preterm infants but not with exclusive preterm PDHM. Multicenteric, large sample size RCT studies for longer duration are recommended to confirm the findings of our study.

SUMMARY

The Randomized Control Trial was conducted from December 2021 to August 2022 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. During the study period a total of 346 neonates admitted in NICU were screened. Out of these 134 were preterm infants, of which 101 received PDHM. Of the 101 preterm infants who received PDHM, 3 preterm infants were excluded from the study and a total of 98 preterm infants were enrolled (49 in each group) and were analyzed in the study. Of the 49 analysed in the pooled PDHM group, 46 preterm infants survived, and 3 deaths were noted. Of the 46 survivors, 2 preterm infants were lost to follow up at 2 weeks and 7 were lost to follow up at 4 weeks. Of the 49 analyzed in the unpooled PHDM group, 48 preterm infants survived, and 1 death was noted. Of the 48 survivors, 1 preterm infant was lost to follow up at 2weeks and 1 was lost to follow up at 4 weeks. The data was analyzed and the important findings of the study are summarized as below.

1. In the present study, maternal characteristics namely maternal age, maternal education and maternal occupation were comparable between both the groups.

Mean maternal age observed was 24.47 years (25.10 years v/s 23.84 years, $p=0.09$). Most of the mothers (64.29%) were educated up to secondary level (73.47% v/s 55.10%, $p=0.15$) and were homemakers (97.96% v/s 97.96%, $p=0.00$).

2. Maternal pregnancy profile and antenatal risk factors associated with preterm delivery were comparable between both the groups.

Most of the mothers in the study (62.24%) were primigravida (59.18% v/s 65.31 %, $p=0.24$) and the most common antenatal risk factor associated with

preterm delivery was PPRM (40.50 %) (45.5% v/s 36.2%) followed by severe intra-uterine growth restriction (IUGR), (32.65 % v/s 32.65 % ,p=0.38).

3. Birth history namely, mean gestational age, mean weight at birth, mode of delivery, Apgar score and day of enrollment were comparable between both the groups except for gender which showed significant variation. Mean gestational age of the preterm infants in the study was 34.46 weeks (34.42 weeks v/s 34.51 weeks, p=0.80). The mean weight at birth was 1797.24 gm (1824.90 gm v/s 1769.59 gm, p=0.46). The most common mode of delivery observed was C-section (62.24%) (63.27 % v/s 61.22 %, p=0.83). The mean APGAR score at birth of the preterm infants was 7.92 (7.96 v/s 7.88, p=0.43). The mean day of enrollment was 1.622 (1.714 days v/s 1.531) (p=0.71).
4. Majority (51.02%) of preterm infants in the study were males. However a significant difference was observed in gender between the two groups (p=0.04). Most of preterm infants in unpooled group were females (59.18 %) and in pooled group were males (61.22%).
5. Indications for NICU admission were comparable between both the groups. The most common indication for admission of preterm infants to Neonatal Intensive Care Unit was Low Birth Weight (98.98%) (100% v/s 97%) followed by Kangaroo Mother Care (81.63%)(77.55% v/s 80 %).
6. In the study, the total percentage increase in mean weight from the weight at enrolment till end of 4 weeks was observed to be significant within the two groups. The difference noted was higher in Unpooled group (30.47% v/s 26.14%) (**p= 0.0001 v/s p=0.0001**), however increase in mean weight at different time points and across total length of hospital stay and 4 weeks follow up between the two groups was not significant.

- a) From enrolment to Discharge (-30.83gm v/s 333.04gm, $p= 0.32$)
 - b) From enrolment to 2 weeks follow up (147.45gm v/s 135.00gm , $p=0.66$)
 - c) From enrollment to 4 weeks follow up (546.60gm v/s 454.49gm , $p=0.14$)
7. In the study the total percentage increase in mean length from length at enrollment till end of 4 weeks was observed to be significant within the groups. The difference noted was higher in Pooled group (4.36 % v/s 3.45%)(**$p=0.001$** vs **$p=0.001$**), however increase in mean length at different time points and across total length of hospital stay and 4 weeks follow up between the two groups was not significant.
- a) From enrolment to Discharge (0.37 cm v/s 0.33cm , $p=0.70$)
 - b) From enrolment to 2 weeks follow up (0.95cm v/s 0.97cm , $p=0.84$)
 - c) From enrollment to 4 weeks follow up (1.54cm v/s 1.95cm , $p=0.23$)
8. In the study , the total percentage increase in mean head circumference from mean head circumference at enrolment till end of 4 weeks was observed to be significant within the group .The difference noted was higher in Pooled group (5.91 % vs 5.76 %)(**$p=0.0001$** vs **$p=0.0001$**), however increase in mean head circumference at different time points and across total length of hospital stay and 4 weeks follow up between the two groups was not significant.
- a) From enrolment to Discharge (0.28cm v/s 0.35cm , $p=0.51$)
 - b) From enrolment to 2 weeks follow up (0.88 cm v/s 0.99cm , $p=0.17$)
 - c) From enrollment to 4 weeks follow up (1.79cm v/s 1.85cm , $p=0.55$)
9. In the study, the total percentage increase in mean mid upper arm circumference from mean mid upper arm circumference at enrolment till the end of 4 weeks was observed to be significant within the groups .The difference noted was higher in Unpooled group (4.11 % vs 3.42 %) (**$p=0.0001$**

vs $p=0.0001$), however increase in mid upper arm circumference at different time points and across total length of hospital stay and 4 weeks follow up between the two groups was not significant.

- a) From enrolment to Discharge (0.03cm v/s 0.02cm , $p=0.68$)
- b) From enrolment to 2 weeks follow up (0.15cm v/s 0.15cm , $p=0.98$)
- c) From enrollment to 4 weeks follow up (0.31cm v/s 0.26cm , $p=0.35$)

10. Indication for starting PDHM was comparable between both the groups. The major indication for starting PDHM in preterm infants was decreased breast milk secretions in the mother's delivering preterm infants 90.82 % (93.88% v/s 87.76%), followed by poor maternal health status 19.39 % (16.33 % v/s 22.45%).

11. The total amount of PDHM consumed in both group was comparable (421.45 +/- 360.79 litre v/s 373.59 +/- 292.25 litres) ($p=0.47$). The mean amount of PDHM consumed by preterm infants across different time points between both groups was comparable. The total amount of PDHM consumed from enrolment till end of 4 weeks was higher in Pooled group (360 litres v/s 300 litres).

- a) At enrollment (55.96 +/- 37.79 litre v/s 47.94 +/- 31.75 litres $p=0.25$),
- b) At discharge (61.43 +/- 48.09 litre v/s 60.00 +/- 61.29 litres $p=0.93$),
- c) At 2 weeks (106.00 +/- 63.54 litre v/s 138.33 +/- 122.71 $p=0.64$) and
- d) At 4weeks (300 litre v/s 360 litres).

12. In the study the mean duration of feedings namely 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, $p=0.42$) were comparable between both the groups.

13. In the study the mean frequency of feeding in preterm infants in both the group across different time points was comparable.

- a) At enrollment (9.98 ± 2.46 feeds/day in vs 9.84 ± 2.65 feeds/day, $p=0.84$),
- b) At discharge (10.69 ± 1.37 feeds/day to 10.67 ± 1.42 feeds/day, $p=0.96$),
- c) At 2 weeks (12.26 ± 1.51 feeds/day to 12.14 ± 1.56 feeds/day, $p=0.90$)
- d) At 4 weeks (13.45 ± 1.79 feeds/day to 13.67 ± 1.56 feeds/day, $p=0.69$)

14. In the study the type of feeding in preterm infants in both the group across different time points was comparable.

- a) At enrolment, majority (68.37 %) of the preterm infants in both the groups were exclusively on PDHM (73.47 % v/s 63.27% $p=0.27$).
- b) At discharge, majority (52.13%) were on MOM (54.17% v/s 50.00% $p=0.95$).
The rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 2.17% $p=0.95$).
- c) At 2 weeks, increased MOM rates and decreasing PDHM rates were observed in both groups. Majority (89.01%) of preterm infants were on MOM in both the groups (91.49% v/s 86.36% $p=0.98$). The rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 2% $p=0.98$).
- d) At 4 weeks almost all (97.67%) of preterm infants were on MOM in both the groups (97.87% v/s 97.44 % $p=0.89$). The rate of exclusive PDHM feeding had decreased in both the groups (0% v/s and 1% $p=0.89$).

15. In the study the method of feeding in preterm infants in both the group across different time points was comparable.

- a) At enrolment, majority (41.86%) of the preterm infants in the study were on spoon feeding (SF) (42.55% v/s 41.08% $p=0.39$).

- b) At discharge shift was noted to SF + DBF (79.79%) as method of feeding in both groups (81.25% v/s 78.26% p= 0.98). Increase in rate of exclusive DBF (19.15%) as method of feeding was noted in both groups (18.75% v/s 19.57% p=0.98)
- c) At 2 weeks follow up increasing trend was observed for DBF(29.67%) as method of feeding in both group (25.53 % v/s 34.09% p=0.29)
- d) And by 4 weeks significant increase in DBF rates was observed (43.02%) (40.43 % v/s 46.15% p=0.80) though overall rate of SF + DBF remained high at 55.81 % (59.57% v/s 51.28% p=0.80).
16. In the present study the mean duration of total length of hospital stay in both groups was comparable (9.65+/-6.64 days and 9.10 +/- 6.49 days p=0.68).

BIBLIOGRAPHY

1. WHO. Preterm birth; 2018. Available from: <http://www.who.int/en/news-room/factsheets/detail/preterm-birth>.
2. Walani SR. Global burden of preterm birth. *Int J Gynaecol Obstet.* 2020;150(1):31-3. doi: 10.1002/ijgo.13195, PMID 32524596.
3. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet.* 2012;379(9832):2162-72. doi: 10.1016/S0140-6736(12)60820-4, PMID 22682464.
4. National health portal of India. Preterm Birth. URL- <http://nhp.gov.in/disease/reproductive-system/female-gynaecological-diseases/preterm-birth>.
5. Devi T, Singh H. Prevalence and risk factors associated with preterm birth in India: systematic review and meta-analysis. *Authorea Prepr.* 2020 Apr 22.
6. WHO, March of Dimes, PMNCH, Save the Children. 15 Million preterm births: priorities for action based on national, regional and global estimates. In: Howson CP, Kinney MV, Lawn J, editors. *Born too soon: the global action report on preterm birth; 2012.*
7. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: A systematic review and modelling analysis. *Lancet Glob Health.* 2019;7(1):e37-46. doi: 10.1016/S2214-109X(18)30451-0, PMID 30389451.

8. Pusdekar YV, Patel AB, Kurhe KG, Bhargav SR, Thorsten V, Garces A et al. Rates and risk factors for preterm birth and low birthweight in the global network sites in six low- and low middleincome countries. *Reprod Health*. 2020;17(Suppl 3);Suppl 3:187. doi: 10.1186/s12978-020-01029-z, PMID 33334356.
9. Cao G, Liu J, Liu M. Global, regional, and national incidence and mortality of neonatal preterm birth, 1990-2019. *JAMA Pediatr*. 2022;176(8):787-96. doi: 10.1001/jamapediatrics.2022.1622, PMID 35639401.
10. Kannaujiya AK, Kumar K, Upadhyay AK, McDougal L, Raj A, James KS et al. Effect of preterm birth on early neonatal, late neonatal, and postneonatal mortality in India. *PLOS Glob Public Health*. 2022 Jun 28;2(6):e0000205. doi: 10.1371/journal.pgph.0000205.
11. Devi T, Singh H. Prevalence and risk factors associated with preterm birth in India: systematic review and meta-analysis. *Authorea*. Apr 22, 2020.
12. Dr Garg P. Feeding of low birth weight neonates. Danone Nutricia Academy. URL- <https://danonenutriciaacademy.in/expert-article/feeding-of-low-birth-weight-neonates/>.
13. Elizabeth KE. Nutrition and child development. 5th ed [Chapter]: Infant and Young Child Feeding. P. 14-29.
14. Pagliaro CL, Bühler KE, Ibidi SM, Limongi SC. Dietary transition difficulties in preterm infants: critical literature review. *J Pediatr (Rio J)*. 2016 Jan-Feb;92(1):7-14. doi: 10.1016/j.jpeds.2015.05.004. PMID 26481169.
15. Skinner AM, Narchi H. Preterm nutrition and neurodevelopmental outcomes. *World J Methodol*. 2021 Nov 20;11(6):278-93. doi: 10.5662/wjm.v11.i6.278, PMID 34888181, PMCID PMC8613713.

16. Pravia CI, Benny M. Long-term consequences of prematurity. *Cleve Clin J Med*. 2020 Nov 23;87(12):759-67. doi: 10.3949/ccjm.87a.19108, PMID 33229393.
17. Zukova S, Krumina V, Buceniece J. Breastfeeding preterm born infant: chance and challenge. *Int J Pediatr Adolesc Med*. 2021 Jun;8(2):94-7. doi: 10.1016/j.ijpam.2020.02.003. PMID 34084879, PMCID PMC8144854.
18. Jiang X, Jiang H. Factors associated with post NICU discharge exclusive breastfeeding rate and duration amongst first time mothers of preterm infants in Shanghai: a longitudinal cohort study. *Int Breastfeed J*. 2022;17(1):34. doi: 10.1186/s13006-022-00472-x, PMID 35501877.
19. Boquien CY. Human milk: an ideal food for nutrition of preterm newborn. *Front Pediatr*. 2018 Oct 16;6:295. doi: 10.3389/fped.2018.00295, PMID 30386758, PMCID PMC6198081.
20. Underwood MA. Human milk for the premature infant. *Pediatr Clin North Am*. 2013 Feb;60(1):189-207. doi: 10.1016/j.pcl.2012.09.008. PMID 23178065, PMCID PMC3508468.
21. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. *Pediatr Clin North Am*. 2013;60(1):49-74. doi: 10.1016/j.pcl.2012.10.002, PMID 23178060.
22. Eichenwald EC, Hansen AR, Martin C, Stark AR, Jain N. Cloherty and Stark's manual of neonatal care. South Asian edition. Philadelphia: Wolters Kluwer, 2021.
23. Clinical practice guidelines: feeding of low birth weight neonates National Neonatology Forum, India; 2020. Available from. Assessed on 27 July 2021. Available from: [http://www.nnfi.org/assests/pdf/cpg-guidelines/Feeding of LBW.pdf](http://www.nnfi.org/assests/pdf/cpg-guidelines/Feeding_of_LBW.pdf).

24. Guidelines on optimal feeding of low birth-weight infants in low- and middle-income countries [internet]. Geneva, Switzerland: World Health Organization; 2011. Available from. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK298973/>.
25. Luu TM, Rehman Mian MO, Nuyt AM. Long-term impact of preterm birth: neurodevelopmental and physical health outcomes. *Clin Perinatol*. 2017 Jun;44(2):305-14. doi: 10.1016/j.clp.2017.01.003. PMID 28477662.
26. Crump C. An overview of adult health outcomes after preterm birth. *Early Hum Dev*. 2020 Nov;150:105187. doi: 10.1016/j.earlhumdev.2020.105187. PMID 32948365, PMCID PMC7480736.
27. Markopoulou P, Papanikolaou E, Analytis A, Zoumakis E, Siahianidou T. Preterm birth as a risk factor for metabolic syndrome and cardiovascular disease in adult life: A systematic review and meta-analysis. *J Pediatr*. 2019 Jul;210:69-80.e5. doi: 10.1016/j.jpeds.2019.02.041. PMID 30992219.
28. Ruys CA, van de Lagemaat M, Rotteveel J, Finken MJJ, Lafeber HN. Improving long-term health outcomes of preterm infants: how to implement the findings of nutritional intervention studies into daily clinical practice. *Eur J Pediatr*. 2021 Jun;180(6):1665-73. doi: 10.1007/s00431-021-03950-2. PMID 33517483, PMCID PMC8105221.
29. Hill PD, Aldag JC, Chatterton RT, Zinaman M. Comparison of milk output between mothers of preterm and term infants: the first 6 weeks after birth. *J Hum Lact*. 2005 Feb;21(1):22-30. doi: 10.1177/0890334404272407, PMID 15681632.

30. Henderson JJ, Hartmann PE, Newnham JP, Simmer K. Effect of preterm birth and antenatal corticosteroid treatment on lactogenesis II in women. *Pediatrics*. 2008 Jan;121(1):e92-100. doi: 10.1542/peds.2007-1107, PMID 18166549.
31. Murase M, Nommsen-Rivers L, Morrow AL, Hatsuno M, Mizuno K, Taki M et al. Predictors of low milk volume among mothers who delivered preterm. *J Hum Lact*. 2014 Nov;30(4):425-35. doi: 10.1177/0890334414543951. PMID 25063573.
32. Yu X, Li J, Lin X, Luan D. Association between Delayed Lactogenesis II and Early Milk Volume among Mothers of Preterm Infants. *Asian Nurs Res (Korean Soc Nurs Sci)*. 2019 May;13(2):93-8. doi: 10.1016/j.anr.2019.02.001, PMID 30776448.
33. Dong D, Ru X, Huang X, Sang T, Li S, Wang Y et al. A prospective cohort study on lactation status and breastfeeding challenges in mothers giving birth to preterm infants. *Int Breastfeed J*. 2022;17(1):6. doi: 10.1186/s13006-021-00447-4, PMID 35012631.
34. Tran HT, Nguyen TT, Mathisen R. The use of human donor milk. *BMJ*. 2020 Nov 17;371. doi: 10.1136/bmj.m4243.
35. WHO newsletter on WHO guidelines on human milk banking; 2022. Available from: <https://www.who.int/news-room/articles-detail/call-for-proposals-support-for-the-development-of-who-guidelines-on-donor-human-milk-banking>.
36. Infant and Young Child Feeding Chapter, Indian Academy of Pediatric, Bharadva K, Tiwari S, Mishra S, Mukhopadhyay K, Yadav B et al. Human milk banking guidelines. *Indian Pediatr*. 2014 Jun;51(6):469-74. doi: 10.1007/s13312-014-0424-x, PMID 24986283.

37. Moro GE. History of milk banking: from origin to present time. *Breastfeed Med.* 2018;13(S1):S16-7. doi: 10.1089/bfm.2018.29077.gem, PMID 29624424.
38. Nangia S, Sachdeva RC, Sabharwal V. Human Milk Banking: An Indian experience. *NeoReviews.* 2018;19(4):e201-10. doi: 10.1542/neo.19-4-e201.
39. Kumar RK, Singhal A, Vaidya U, Banerjee S, Anwar F, Rao S. Optimizing nutrition in preterm low birth weight infants-consensus summary. *Front Nutr.* 2017 May 26;4:20. doi: 10.3389/fnut.2017.00020, PMID 28603716, PMCID PMC5445116.
40. Tran HT, Nguyen TT, Mathisen R. The use of human donor milk. *BMJ.* 2020;371:m4243. doi: 10.1136/bmj.m4243.
41. Fang L, Zhang M, Wu L, Wang R, Lin B, Yao J et al. Is preterm donor milk better than preterm formula for very-low-birth-weight infants? *Food Nutr Res.* 2021 Sep 24;65. doi: 10.29219/fnr.v65.5346, PMID 34650391, PMCID PMC8494261.
42. Kantorowska A, Wei JC, Cohen RS, Lawrence RA, Gould JB, Lee HC. Impact of donor milk availability on breast milk use and necrotizing enterocolitis rates. *Pediatrics.* 2016 Mar;137(3):e20153123. doi: 10.1542/peds.2015-3123. PMID 26908696. Erratum in: *Pediatrics.* PMID: 26908696; PMC ID: PMC4771129. 2016 Jun;137(6):null:e20153123. doi: 10.1542/peds.2015-3123, PMID 26908696.
43. Cristofalo EA, Schanler RJ, Blanco CL, Sullivan S, Trawoeger R, Kiechl-Kohlendorfer U et al. Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. *J Pediatr.* 2013 Dec;163(6):1592-1595.e1. doi: 10.1016/j.jpeds.2013.07.011. PMID 23968744.

44. Kreissl A, Sauerzapf E, Repa A, Binder C, Thanhaeuser M, Jilma B et al. Starting enteral nutrition with preterm single donor milk instead of formula affects time to full enteral feeding in very low birthweight infants. *Acta Paediatr.* 2017 Sep;106(9):1460-7. doi: 10.1111/apa.13914. PMID 28498519.
45. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics.* 1999 Jun;103(6 Pt 1):1150-7. doi: 10.1542/peds.103.6.1150, PMID 10353922.
46. Colaizy TT. Donor human milk for very low birth weights: patterns of usage, outcomes, and unanswered questions. *Curr Opin Pediatr.* 2015 Apr;27(2):172-6. doi: 10.1097/MOP.000000000000201, PMID 25689453, PMCID PMC4414040.
47. Yang R, Chen D, Deng Q, Xu X. The effect of donor human milk on the length of hospital stay in very low birthweight infants: a systematic review and meta-analysis. *Int Breastfeed J.* 2020 Oct 28;15(1):89. doi: 10.1186/s13006-020-00332-6, PMID 33115488, PMCID PMC7594457.
48. Zhang J, Li MM, Yu ZB, Liu F, Liu BB, Weng L et al. [Evaluation of human milk feeding in hospitalized very low and extremely low birth weight infants]. *Zhonghua Er Ke Za Zhi.* 2020 May 2;58(5):387-91. Chinese. doi: 10.3760/cma.j.cn112140-20190828-00548, PMID 32392954.
49. Chowning R, Radmacher P, Lewis S, Serke L, Pettit N, Adamkin DH. A retrospective analysis of the effect of human milk on prevention of necrotizing enterocolitis and postnatal growth. *J Perinatol.* 2016 Mar;36(3):221-4. doi: 10.1038/jp.2015.179. PMID 26633147.

50. Sisk PM, Lambeth TM, Rojas MA, Lightbourne T, Barahona M, Anthony E et al. Necrotizing enterocolitis and growth in preterm infants fed predominantly maternal milk, pasteurized donor milk, or preterm formula: A retrospective study. *Am J Perinatol.* 2017 Jun;34(7):676-83. doi: 10.1055/s-0036-1597326. PMID 27936476.
51. Schanler RJ, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics.* 2005 Aug;116(2):400-6. doi: 10.1542/peds.2004-1974, PMID 16061595.
52. Kim EJ, Lee NM, Chung SH. A retrospective study on the effects of exclusive donor human milk feeding in a short period after birth on morbidity and growth of preterm infants during hospitalization. *Med (Baltim).* 2017 Sep;96(35):e7970. doi: 10.1097/MD.0000000000007970, PMID 28858132, PMCID PMC5585526.
53. Miller J, Tonkin E, Damarell RA, McPhee AJ, Suganuma M, Suganuma H et al. A systematic review and meta-analysis of human milk feeding and morbidity in very low birth weight infants. *Nutrients.* 2018 May 31;10(6):707. doi: 10.3390/nu10060707, PMID 29857555, PMCID PMC6024377.
54. Gross SJ. Growth and biochemical response of preterm infants fed human milk or modified infant formula. *N Engl J Med.* 1983;308(5):237-41. doi: 10.1056/NEJM198302033080501, PMID 6848932.
55. Lucas A, Morley R, Cole TJ, Gore SM. A randomised multicentre study of human milk versus formula and later development in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* 1994;70(2):F141-6. doi: 10.1136/fn.70.2.f141, PMID 8154907.

56. Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. *Lancet*. 1990;336(8730):1519-23. doi: 10.1016/0140-6736(90)93304-8, PMID 1979363.
57. Corpeleijn WE, De Waard M, Christmann V, Van Goudoever JB, Jansen-van der Weide MC, Kooi EM, et al. Effect of donor milk on severe infections and mortality in very low-birth-weight Infants: the early nutrition study randomized clinical trial. *JAMA Pediatr*. 2016;170(7):654-61. doi: 10.1001/jamapediatrics.2016.0183, PMID 27135598.
58. Costa S, Maggio L, Alighieri G, Barone G, Cota F, Vento G. Tolerance of preterm formula versus pasteurized donor human milk in very preterm infants: a randomized non-inferiority trial. *Ital J Pediatr*. 2018;44(1):96. doi: 10.1186/s13052-018-0532-7, PMID 30115086.
59. Lucas A, Morley R, Cole TJ, Gore SM, Davis JA, Bamford MF, et al. Early diet in preterm babies and developmental status in infancy. *Arch Dis Child*. 1989;64(11):1570-8. doi: 10.1136/adc.64.11.1570, PMID 2690739.
60. Morley R, Lucas A. Randomized diet in the neonatal period and growth performance until 7.5-8 y of age in preterm children. *Am J Clin Nutr*. 2000;71(3):822-8. doi: 10.1093/ajcn/71.3.822, PMID 10702179.
61. R  ih   NC, Heinonen K, Rassin DK, Gaull GE. Milk protein quantity and quality in low-birthweight infants: I. Metabolic responses and effects on growth. *Pediatrics*. 1976;57(5):659-84. PMID 7767.
62. Castellano Y  nez C, Castillo Barrio B, Mu  oz Labi  n MDC, Ortiz Movilla R, Garc  a Lara NR, Royuela Vicente A, et al. Providing very preterm infants with donor human milk led to faster breastfeeding rates but worse biometric

- gains. *Acta Paediatr.* 2019;108(4):766-7. doi: 10.1111/apa.14691, PMID 30537331.
63. American Academy of Pediatrics. Breastfeeding and the use of human milk. *Pediatrics.* 2012;129(3):e827-41. [DOI: 10.1542/peds.2011-3552; PUBMED: 22371471]. doi: 10.1542/peds.2011-3552, PMID 22371471.
64. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr.* 2010;50(1):85-91. doi: 10.1097/MPG.0b013e3181adaee0, PMID 19881390.
65. ESPGHAN Committee on Nutrition, Arslanoglu S, Corpeleijn W, Moro G, Braegger C, Campoy C et al. Donor human milk for preterm infants: current evidence and research directions. *J Pediatr Gastroenterol Nutr.* 2013;57(4):535-42. doi: 10.1097/MPG.0b013e3182a3af0a, PMID 24084373.
66. Castro M, Asbury M, Shama S, Stone D, Yoon EW, O'Connor DL, et al. Energy and fat intake for preterm infants fed donor milk Is significantly impacted by enteral feeding method. *JPEN J Parenter Enteral Nutr.* 2019;43(1):162-5. doi: 10.1002/jpen.1430, PMID 30070721.
67. Williams T, Nair H, Simpson J, Embleton N. Use of donor human milk and maternal breastfeeding rates: a systematic review. *J Hum Lact.* 2016;32(2):212-20. doi: 10.1177/0890334416632203, PMID 26887844.
68. McGuire W, Anthony MY. Formula milk versus term human milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2001;(4)(4):CD002971. doi: 10.1002/14651858.CD002971, PMID 11687169.

69. Quigley M, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2014(4). doi: 10.1002/14651858.CD002971.pub3.
70. Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2018(6). doi: 10.1002/14651858.CD002971.pub4.
71. Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2019(7):Art No.: CD002971. doi: 10.1002/14651858.CD002971.pub5.
72. Hoban R, Schoeny ME, Esquerra-Zwiers A, Kaenkumchorn TK, Casini G, Tobin G et al. Impact of donor milk on short- and long-term growth of very low birth weight infants. *Nutrients.* 2019 Jan 22;11(2):241. doi: 10.3390/nu11020241, PMID 30678256, PMCID PMC6412258.
73. Brownell EA, Matson AP, Smith KC, Moore JE, Esposito PA, Lussier MM et al. Dose-response relationship between donor human milk, mother's own milk, preterm formula, and neonatal growth outcomes. *J Pediatr Gastroenterol Nutr.* 2018 Jul;67(1):90-6. doi: 10.1097/MPG.0000000000001959, PMID 29543698.
74. Morley R, Lucas A. Randomized diet in the neonatal period and growth performance until 7.5-8 y of age in preterm children. *Am J Clin Nutr.* 2000 Mar;71(3):822-8. doi: 10.1093/ajcn/71.3.822, PMID 10702179.
75. Hård AL, Nilsson AK, Lund AM, Hansen-Pupp I, Smith LEH, Hellström A. Review shows that donor milk does not promote the growth and development of preterm infants as well as maternal milk. *Acta Paediatr.* 2019 Jun;108(6):998-1007. doi: 10.1111/apa.14702. PMID 30565323, PMCID PMC6520191.

76. Cañizo Vázquez D, Salas García S, Izquierdo Renau M, Iglesias-Platas I. Availability of donor milk for very preterm infants decreased the risk of necrotizing enterocolitis without adversely impacting growth or rates of breastfeeding. *Nutrients*. 2019 Aug 14;11(8):1895. doi: 10.3390/nu11081895, PMID 31416157, PMCID PMC6722966.
77. Adhisivam B, Kohat D, Tanigasalam V, Bhat V, Plakkal N, Palanivel C. Does fortification of pasteurized donor human milk increase the incidence of necrotizing enterocolitis among preterm neonates? A randomized controlled trial. *J Matern Fetal Neonatal Med*. 2019 Oct;32(19):3232-7. doi: 10.1080/14767058.2018.1461828. PMID 29618272.
78. Madore LS, Bora S, Erdei C, Jumani T, Dengos AR, Sen S. Effects of donor breastmilk feeding on growth and early neurodevelopmental outcomes in preterm infants: an observational study. *Clin Ther*. 2017;39(6):1210-20. doi: 10.1016/j.clinthera.2017.05.341, PMID 28576299.
79. Atio N. Child health division Ministry of Health and Family Welfare Government of India Lactation Management Centres in public health facilities; 2017.
80. Lloyd ML, Malacova E, Hartmann B, Simmer K. A clinical audit of the growth of preterm infants fed predominantly pasteurised donor human milk v. those fed mother's own milk in the neonatal intensive care unit. *Br J Nutr*. 2019 May 14;121(9):1018-25. doi: 10.1017/S0007114519000357. PMID 30947757.
81. Zhu J, Liu M, Xing Y. Preterm birth and human milk proteome: are we ready for individualized fortification? *Curr Opin Clin Nutr Metab Care*. 2022 May 1;25(3):216-22. doi: 10.1097/MCO.0000000000000824. PMID 35199659.

82. Gates A, Marin T, Leo G, Stansfield BK. Review of preterm human-milk nutrient composition. *Nutr Clin Pract*. 2021 Dec;36(6):1163-72. doi: 10.1002/ncp.10570. PMID 32862494.
83. Sahin S, Ozdemir T, Katipoglu N, Akcan AB, Kaynak Turkmen M. Comparison of changes in breast milk macronutrient content during the first month in preterm and term infants. *Breastfeed Med*. 2020 Jan;15(1):56-62. doi: 10.1089/bfm.2019.0141. PMID 31765240.
84. Narang AP, Bains HS, Kansal S, Singh D. Serial composition of human milk in preterm and term mothers. *Indian J Clin Biochem*. 2006 Mar;21(1):89-94. doi: 10.1007/BF02913072, PMID 23105575, PMCID PMC3453757.
85. Haiden N, Ziegler EE. Human milk banking. *Ann Nutr Metab*. 2016;69;Suppl 2:8-15. doi: 10.1159/000452821, PMID 28103607.
86. Piemontese P, Mallardi D, Liotto N, Tabasso C, Menis C, Perrone M et al. Macronutrient content of pooled donor human milk before and after Holder pasteurization. *BMC Pediatr*. 2019 Feb 12;19(1):58. doi: 10.1186/s12887-019-1427-5, PMID 30755170. PMCID. PMC6373.
87. García-Lara NR, Vieco DE, De la Cruz-Bértolo J, Lora-Pablos D, Velasco NU, Pallás-Alonso CR. Effect of Holder pasteurization and frozen storage on macronutrients and energy content of breast milk. *J Pediatr Gastroenterol Nutr*. 2013 Sep;57(3):377-82. doi: 10.1097/MPG.0b013e31829d4f82, PMID 23752081.
88. Adhisivam B, Vishnu Bhat B, Rao K, Kingsley SM, Plakkal N, Palanivel C. Effect of Holder pasteurization on macronutrients and immunoglobulin profile of pooled donor human milk. *J Matern Fetal Neonatal Med*. 2019 Sep;32(18):3016-9. doi: 10.1080/14767058.2018.1455089. PMID 29587541.

89. O'Connor DL, Ewaschuk JB, Unger S. Human milk pasteurization: benefits and risks. *Curr Opin Clin Nutr Metab Care*. 2015 May;18(3):269-75. doi: 10.1097/MCO.000000000000160, PMID 25769062.
90. Peila C, Coscia A, Bertino E, Cavaletto M, Spertino S, Icardi S et al. Effects of Holder pasteurization on the protein profile of human milk. *Ital J Pediatr*. 2016 Apr 7;42:36. doi: 10.1186/s13052-016-0248-5, PMID 27056486, PMCID PMC4823917.
91. Vieira AA, Soares FV, Pimenta HP, Abranches AD, Moreira ME. Analysis of the influence of pasteurization, freezing/thawing, and offer processes on human milk's macronutrient concentrations. *Early Hum Dev*. 2011 Aug;87(8):577-80. doi: 10.1016/j.earlhumdev.2011.04.016. PMID 21592688.
92. Colaizy TT. Effects of milk banking procedures on nutritional and bioactive components of donor human milk. *Semin Perinatol*. 2021 Mar;45(2):151382. doi: 10.1016/j.semperi.2020.151382. PMID 33632557.
93. Young Bridget E, Katherine M, Borman Laraine L, Rebecca H, Nancy K F. Milk Bank Pooling Practices Impact Concentrations and Variability of Bioactive Components of Donor Human Milk *Frontiers in Nutrition*, VOLUME=7 ,2020. URL=<https://www.frontiersin.org/articles/10.3389/fnut.2020.579115> DOI=10.3389/fnut.2020.579115.
94. Peila C, Moro GE, Bertino E, Cavallarin L, Giribaldi M, Giuliani F et al. The effect of holder pasteurization on nutrients and biologically active components in donor human milk: a review. *Nutrients*. 2016 Aug 2;8(8):477. doi: 10.3390/nu8080477, PMID 27490567, PMCID PMC4997390.
95. Moro GE, Billeaud C, Rachel B, Calvo J, Cavallarin L, Christen L et al. Processing of donor human milk: update and recommendations from the

- European milk bank association (EMBA). *Front Pediatr.* 2019;7:49. doi: 10.3389/fped.2019.00049, PMID 30873395.
96. Binte Abu Bakar SY, Salim M, Clulow AJ, Nicholas KR, Boyd BJ. Human milk composition and the effects of pasteurisation on the activity of its components. *Trends Food Sci Technol.* 2021;111:166-74. doi: 10.1016/j.tifs.2021.02.055.
97. Dempsey E, Miletin J. Banked preterm versus banked term human milk to promote growth and development in very low birth weight infants. *Cochrane Database Syst Rev.* 2010 Jun 16;(6);Jun 28(6):2019. doi: 10.1002/14651858.CD007644.pub2. Update in: *Cochrane Database Syst Rev.* PMID 20556782.
98. National guidelines on lactational management centres in public health facilities 2017.
99. Centre for Clinical Practice at NICE (UK). Donor breast milk banks: the operation of donor milk bank services. London: National Institute for Health and Clinical Excellence. UK; 2010 Feb (NICE Clinical Guidelines, No. 93.). Introduction Available from: Available from: <https://www.ncbi.nlm.nih.gov/books/NBK66147/> AccessDate: 15.09.2020.
100. Questions & answers for consumers concerning infant formula. Available from: p. 95 [illness/questions-answers-consumers-concerning-infant-formula#1](https://www.fda.gov/food/people-risk-foodborne-illness/questions-answers-consumers-concerning-infant-formula#1). Bibliography. Available from: [https://www.fda.gov/food/people-risk-foodborne-](https://www.fda.gov/food/people-risk-foodborne-illness/questions-answers-consumers-concerning-infant-formula#1) [cited 15.9.2020] [cited 15.9.2020]. Available from: <http://medlineplus.gov/ency/article/007235.htm>.
101. Chetta KE, Schulz EV, Wagner CL. Outcomes improved with human milk intake in preterm and full-term infants. *Semin Perinatol.* 2021 Mar;45(2):151384. doi: 10.1016/j.semperi.2020.151384. PMID 33494913.

102. Tsao YC. Paladai for feeding babies. *HK Med J.* 2018;24(1):90-1.
103. World Health Organization Training course on child growth assessment. Geneva: World Health Organization; 2008.
104. Papastavrou M, Genitsaridi SM, Komodiki E, Paliatsou S, Midw R, et al. Breastfeeding in the course of history. *J Pediatr Neonatal Care* 2(6). 2015;00096.
105. Laroia N, Sharma D. The religious and cultural bases for breastfeeding practices among the Hindus. *Breastfeed Med.* 2006 Jun 1;1(2):94-8. doi: 10.1089/bfm.2006.1.94, PMID 17661569.
106. Madhuri B, Gajbhiye PI. Breast feeding. *Int J Res Indian Med.* 2019;03.
107. Singh K, Verma B. Breast feeding- an Ayurveda perceptive. *J Homeop Ayurv. Med.* 2012;1:112. doi: 10.4172/2167-1206.1000112.
108. Kuppusamy N, Vidhyadevi A. Prevalence of preterm admissions and the risk factors of preterm labor in rural medical college hospital. *Int J Sci Stud.* 2016;4(9):125-28.
109. Fernandes SF, Chandra S. A study of risk factors for preterm labour. *Int J Reprod Contracept Obstet Gynecol.* 2015;4(5):1306-12. doi: 10.18203/2320-1770.ijrcog20150701.
110. Dayanithi M. Low birth weight and premature births and their associated maternal factors. *Int J Community Med Public Health.* 2018;5(6):2277-85. doi: 10.18203/2394-6040.ijcmph20182086.
111. Ahankari A, Bapat S, Myles P, Fogarty A, Tata L. Factors associated with preterm delivery and low birth weight: A study from rural Maharashtra, India. *F1000Research.* 2017;6:72. doi: 10.12688/f1000research.10659.1, PMID 28529697.
112. IIPS and ICF. National family health survey (NFHS-5), 2019-21. Mumbai.

113. International Institute for Population Sciences , 2019.Available from URL http://rchiips.org/nfhs/NFHS-5Reports/NFHS-5_INDIA_REPORT.pdf
114. Kamity R, Kapavarapu PK, Chandel A. Feeding problems and long-term outcomes in preterm infants-A systematic approach to evaluation and management. *Children (Basel)*. 2021 Dec 8;8(12):1158. doi: 10.3390/children8121158, PMID 34943354, PMCID PMC8700416.
115. Chauhan A, Kumar M, Tripathi S, Singh SN, Singh VK. Breastfeeding rates at discharge for very low birthweight neonates and their determinants: an observational study from a tertiary care neonatal intensive care unit in India. *J Paediatr Child Health*. 2022 Sep;58(9):1653-60. doi: 10.1111/jpc.16074. PMID 35778999.
116. Vohr BR, Poindexter BB, Dusick AM, McKinley LT, Higgins RD, Langer JC et al. Persistent beneficial effects of breast milk ingested in the neonatal intensive care unit on outcomes of extremely low birth weight infants at 30 months of age. *Pediatrics*. 2007 Oct;120(4):e953-9. doi: 10.1542/peds.2006-3227, PMID 17908750.
117. Kimm SYS. Fetal origins of adult disease: the Barker hypothesis revisited— 2004. *Curr Opin Endocrinol Diabetes*. Aug 2004;11(4):192-6. doi: 10.1097/01.med.0000140938.39925.4c.
118. Doyle LW, Anderson PJ. Adult outcome of extremely preterm infants. *Pediatrics*. Aug 2010;126(2):342-51. doi: 10.1542/peds.2010-0710, PMID 20679313.
119. Risnes K, Bilsteen JF, Brown P, Pulakka A, Andersen AN, Opdahl S, et al. Mortality among Young adults born preterm and early term in 4 Nordic nations.

- JAMA Netw Open. 2021;4(1):e2032779. doi: 10.1001/jamanetworkopen.2020.32779, PMID 33416885.
120. Lund AM, Löfqvist C, Pivodic A, Lundgren P, Hård AL, Hellström A et al. Unpasteurised maternal breast milk is positively associated with growth outcomes in extremely preterm infants. *Acta Paediatr.* 2020 Jun;109(6):1138-47. doi: 10.1111/apa.15102. PMID 31747093, PMCID PMC9541184.
121. Cerasani J, Ceroni F, De Cosmi V, Mazzocchi A, Morniroli D, Roggero P et al. Human milk feeding and preterm infants' growth and body composition: A literature review. *Nutrients.* 2020 Apr 21;12(4):1155. doi: 10.3390/nu12041155, PMID 32326178, PMCID PMC7230190.
122. Colaizy TT, Carlson S, Saftlas AF, Morriss FH, Jr. Growth in VLBW infants fed predominantly fortified maternal and donor human milk diets: A retrospective cohort study. *BMC Pediatr.* 2012;12:124. doi: 10.1186/1471-2431-12-124, PMID 22900590.
123. Piemontese P, Liotto N, Mallardi D, Roggero P, Puricelli V, Gianni ML, et al. The effect of human milk on modulating the quality of growth in preterm infants. *Front Pediatr.* 2018;6:291. doi: 10.3389/fped.2018.00291, PMID 30356677.
124. Mól N, Zasada M, Kwinta P. Does type of feeding affect body composition in very low birth weight infants? – A prospective cohort study. *Pediatr Neonatol.* 2019;60(2):135-40. doi: 10.1016/j.pedneo.2018.04.010, PMID 29784603.
125. Eilers E, Ziska T, Harder T, Plagemann A, Obladen M, Loui A. Leptin determination in colostrum and early human milk from mothers of preterm and term infants. *Early Hum Dev.* 2011;87(6):415-9. doi: 10.1016/j.earlhumdev.2011.03.004, PMID 21482454.

126. Lapidaire W, Lucas A, Clayden JD, Clark C, Fewtrell MS. Human milk feeding and cognitive outcome in preterm infants: the role of infection and NEC reduction. *Pediatr Res.* 2022 Apr;91(5):1207-14. doi: 10.1038/s41390-021-01367-z. PMID 34168271, PMCID PMC9122812.
127. Haiden N, Ziegler EE. Human milk banking. *Ann Nutr Metab.* 2016;69;Suppl 2:8-15. doi: 10.1159/000452821, PMID 28103607.
128. Young Bridget E, Katherine M, Borman Laraine L, Rebecca H, Krebs Nancy F Infant and Young Child Feeding Chapter, Indian Academy of Pediatric, Bharadva K, Tiwari S, Mishra S, Mukhopadhyay K, Yadav B, Agarwal RK, Kumar V. Human milk banking guidelines. *Indian Pediatr.* 2014 Jun;51(6):469-74. doi: 10.1007/s13312-014-0424.
129. Young BE, Murphy K, Borman LL, Heinrich R, Krebs NF. Milk bank pooling practices impact concentrations and variability of bioactive components of donor human milk. *Front Nutr.* VOLUME=7, YEAR=2020. 2020;7:579115. doi: 10.3389/fnut.2020.579115, PMID 24986283.
130. Young BE, Borman LL, Heinrich R, Long J, Pinney S, Westcott J et al. Effect of pooling practices and time postpartum of milk donations on the energy, macronutrient, and zinc concentrations of resultant donor human milk pools. *J Pediatr.* 2019 Nov;214:54-9. doi: 10.1016/j.jpeds.2019.07.042. PMID 31558278, PMCID PMC6886691.
131. Giuliani F, Prandi G, Coscia A, Cresi F, Di Nicola P, Raia M et al. Donor human milk versus mother's own milk in preterm VLBWIs: a case control study. *J Biol Regul Homeost Agents.* 2012 Jul-Sep;26(3);Suppl:19-24. PMID 23158509.

132. Cañizo Vázquez D, Salas García S, Izquierdo Renau M, Iglesias-Platas I. Availability of donor milk for very preterm infants decreased the risk of necrotizing enterocolitis without adversely impacting growth or rates of breastfeeding. *Nutrients*. 2019 Aug 14;11(8):1895. doi: 10.3390/nu11081895, PMID 31416157, PMCID PMC6722966.
133. Tyson JE, Lasky RE, Mize CE, Richards CJ, Blair-Smith N, Whyte R et al. Growth, metabolic response, and development in very-low-birth-weight infants fed banked human milk or enriched formula. I. Neonatal findings. *J Pediatr*. 1983 Jul;103(1):95-104. doi: 10.1016/s0022-3476(83)80790-2, PMID 6864403.
134. Yu F, Cao B, Wen Z, Li M, Chen H, Xie G. Is donated breast milk better than formula for feeding very low birth weight infants? A systematic review and meta-analysis. *Worldviews Evid-Based Nurs*. 2019 Dec;16(6):485-94. doi: 10.1111/wvn.12410. PMID 31743577.
135. Anderson GH, Atkinson SA, Bryan MH. Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. *Am J Clin Nutr*. 1981;34(2):258-65. doi: 10.1093/ajcn/34.2.258, PMID 7211727.
136. Gross SJ, David RJ, Bauman L, Tomarelli RM. Nutritional composition of milk produced by mothers delivering preterm. *J Pediatr*. 1980;96(4):641-4. doi: 10.1016/s0022-3476(80)80729-3, PMID 7359266.
137. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatr*. 2014 Dec 1;14(1):216. doi: 10.1186/1471-2431-14-216, PMID 25174435.
138. Ahrabi AF, Handa D, Codipilly CN, Shah S, Williams JE, McGuire MA et al. Effects of extended freezer storage on the integrity of human milk. *J Pediatr*.

- 2016;177:140-3,ISSN 0022-3476. doi: 10.1016/j.jpeds.2016.06.024, PMID 27423174.
139. Dahman HAB. Risk factors associated with preterm birth: a retrospective study in Mukalla Maternity and Childhood Hospital, Hadhramout Coast/Yemen. Sudan J Paediatr. 2020;20(2):99-110. doi:10.24911/SJP.1061575722503, PMID 32817730, PMCID PMC7423304
140. Gurung A, Wrammert J, Sunny AK, Gurung R, Rana N, Basaula YN et al. Incidence, risk factors and consequences of preterm birth – findings from a multi-centric observational study for 14 months in Nepal. Arch Public Health. 2020;78:64. doi: 10.1186/s13690-020-00446-7, PMID 32695337.
141. Trivedi P, Saxena D, Puwar T, Savaliya S, Ganguly P. A cohort study on risk factors for preterm births in rural Gujarat. Indian J Public Health. 2018 Apr-Jun;62(2):111-6. doi: 10.4103/ijph.IJPH_337_16, PMID 29923534.
142. Rashmi A, Narayanamurthy MR, Vidya GS, Vidyaxmi K, Renuka M. Risk factors for preterm birth: a community based longitudinal study in rural Mysuru, Karnataka, India. Int Journal Community Med Health . 2016-12-22 ,Vol 3. 2016;12. doi: 10.18203/2394-6040.ijcmph20164294.
143. Mahavarkar SH, Madhu CK, Mule VD. A comparative study of teenage pregnancy. J ObstetGynaecol. 2008 Aug;28(6):604-7. doi:10.1080/01443610802281831, PMID 19003655.
144. Medhi R, Das B, Das A, Ahmed M, Bawri S, Rai S. Adverse obstetrical and perinatal outcome in adolescent mothers associated with first birth: a hospital-based case-control study in a tertiary care hospital in North-East India. Adolesc

- Health Med Ther. 2016 Apr 1;7:37-doi- 10.2147/AHMT.S91853,PMID 27099539, PMCID PMC4824280.
145. Rajeesha CH, Sahana KS, Prakash RM, Saldanha. Knowledge and attitude of post natal lactating mothers regarding human milk donation,:hospital based cross sectional questionnaire study in Yenepoya Medical College and Research Center in Mangalore, Karnataka. *Interntional Journal of Applied Research*. Volume-8,Issue-3, Mar 2018. PRINT ISSN No 2249-555X.
146. Singh S, Malhotra AK. Across-sectional study on prevalence of preterm birth in medical college Jhansi(U.P). *Indian J Appl Res*. 2018;8(10):50-2.
147. Mahajan A, Magon S. Study of risk factors for preterm births in a teaching hospital: A prospectivestudy. *Int J Med Den Sc*. 2016;6:1407-12.
148. Garg S, Kaur T, Saran AS, Yadav M. A study of etiology and outcome of preterm birth at a tertiar care centre. *Int J Reprod Contracept Obstet Gynecol*. 2017;6(10):4488-91. doi: 10.18203/2320-1770.ijrcog20174429.
149. Rao CR, de Ruitter LE, Bhat P, Kamath V, Kamath A, Bhat V. A Case-Control Study on Risk Factors for preterm deliveries in a secondary care hospital, Southern India. *ISRN Obstet Gynecol*. 2014;2014:935982. doi: 10.1155/2014/935982, PMID 25006487.
150. Dayanithi M. Low birth weight and premature births and their associated maternal factors. *Int J Community Med Public Health*. 2018;5(6):2277-85. doi: 10.18203/2394-6040.ijcmph20182086.
151. Patel PK, Pitre DS, Bhooker SP. Predictive value of various risk factors for preterm labor. *Natl J Community Med*. 2015;6(1):121-5.

152. Hegde JR, Manjula L, Venkatesha KR. Infant feeding practices in an urban tertiary care hospital: A descriptive longitudinal study. *Indian J Child Health*. 2017;4:496-502.
153. 152.. Koullali B, van Zijl MD, Kazemier BM, Oudijk MA, Mol BWJ, Pajkrt E et al. The association between parity and spontaneous preterm birth: a population based study. *BMC Pregnancy Childbirth*. 2020;20(1):233. doi: 10.1186/s12884-020-02940-w, PMID 32316915.
154. Etuk SJ, Etuk JS, Oyo-Ita AE. Factors influencing the incidence of pre-term birth in Calabar, Nigeria. *Niger J Physiol Sci*. 2005 Jun-Dec;20(1-2):63-8. PMID 17220930.
155. B. Shetty M, B. M. K, Malyala M, Swarup A, Pathadan DS, Pocha S. Preterm birth: associated risk factors and outcome in tertiary care center. *Int J Reprod Contracept Obstet Gynecol*. 2017;6(8):3271-74. doi: 10.18203/2320-1770.ijrcog20173258.
156. Jamal S, Srivastava R. A retrospective analytical study of the epidemiology and causes of preterm birth. *Int J Reprod Contracept Obstet Gynecol*. 2017;6(12):5453-7. doi: 10.18203/2320-1770.ijrcog20175259.
157. Muluaem G, Wondim A, Woretaw A. The effect of pregnancy induced hypertension and multiple pregnancies on preterm birth in Ethiopia: a systematic review and meta-analysis. *BMC Res Notes*. 2019 Feb 18;12(1):91. doi: 10.1186/s13104-019-4128-0, PMID 30777132, PMCID PMC6380048
158. Vermillion ST, Kooba AM, Soper DE. Amniotic fluid index values after preterm premature rupture of the membranes and subsequent perinatal infection. *Am J Obstet Gynecol*. 2000 Aug;183(2):271-6. doi: 10.1067/mob.2000.107653, PMID 10942458.

159. Ekin A, Gezer C, Taner CE, Ozeren M. Perinatal outcomes in pregnancies with oligohydramnios after preterm premature rupture of membranes. *J Matern Fetal Neonatal Med.* 2015 Nov;28(16):1918-22. doi: 10.3109/14767058.2014.972927.PMID 25283853.
160. Sapra Rohan,(2022) .To study the feeding patterns and growth parameters of babies fed on pasteurized donor human milk(Unpublished thesis).KLE Academy of Higher Education and Research, Belagavi, Karnataka
161. Saurabh(2021).To study the incidence of NEC among preterm neonates receiving pasteurized donor human milk(Unpublished thesis). KLE Academy of Higher Education and Research, Belagavi, Karnataka
162. Allana A, Lo K, Batool M, Hand I. Impact of donor human milk in an urban NICU population. *Children (Basel).* 2022 Oct 27;9(11):1639. doi: 10.3390/children9111639, PMID 36360367, PMCID PMC9688816.
163. Holzer I, Lehner R, Ristl R, Husslein PW, Berger A, Farr A. Effect of delivery mode on neonatal outcome among preterm infants: an observational study. *Wien Klin Wochenschr.* 2017 Sep;129(17-18):612-7. doi: 10.1007/s00508-016-1150-2. PMID 28004267, PMCID PMC5599430.
164. Ghelichkhani S, Masoumi SZ, Shirzadeh AA, Khazaei S, Shahbazi F. Evaluation of maternal risk factors for preterm delivery in Fatemieh Hospital of Hamadan, Iran, 2019: A case-control study. *J Fam Med Prim Care.* 2021 Oct;10(10):3832-7.doi:10.4103/jfmpc.jfmpc_1032_21.PMID34934688, PMCID PMC8653452.
165. Hassan Najia, Mukhopadhyay S, Mohan S. Morbidity and mortality profile of preterm neonates admitted in neonatal intensive care unit of a tertiary care

- centre in Western Uttar Pradesh, India. *Int J Contemp Pediatr.* 2019-08-23;6(5). doi: 10.18203/2349-3291.ijcp20193154
166. Moster D, Lie RT, Markestad T. Joint association of Apgar scores and early neonatal symptoms with minor disabilities at school age. *Arch Dis Child Fetal NeonatalEd.* 2002 Jan;86(1):F16-21.doi: 10.1136/fn.86.1.f16, PMID 11815542, PMCID PMC1721350.
167. Khasawneh W, Sindiani A, Rawabdeh SA, Aleshawi A, Kanaan D. Indications and clinical profile of neonatal admissions: A cross-sectional descriptive analysis from a Single Academic Center in Jordan. *J Multidiscip Healthc.* 2020 Sep 23;13:997-1006. doi: 10.2147/JMDH.S275267, PMID 33061405, PMCID PMC7520145.
168. Dayanithi M. Low birth weight and premature births and their associated maternal factors. *Int J Community Med Public Health.* 2018;5(6):2277-85. doi: 10.18203/2394-6040.ijcmph20182086.
169. Alyahya W, Barnett D, Cooper A, Garcia AL, Edwards CA, Young D et al. Donated human milk use and subsequent feeding pattern in neonatal units. *Int Breastfeed J.* 2019;14:39. doi: 10.1186/s13006-019-0233-x, PMID 31507645.
170. Battersby C, Marciano Alves Mousinho R, Longford N, Modi N, UK Neonatal Collaborative Necrotising (UKNC-NEC) Study Group. Use of pasteurised human donor milk across neonatal networks in England. *Early Hum Dev.* 2018 Mar;118:32-6. doi: 10.1016/j.earlhumdev.2018.01.017. PMID 29454186.
171. Tewoldie MT, Girma M, Seid H. Determinants of time to full enteral feeding achievement among infants with birth weight 1000-2000g admitted to the

- neonatal intensive care unit of public hospitals in Hawassa city, Sidama region Ethiopian, 2019: A retrospective cohort study. PLOS ONE. 2022 Jul 28;17(7): e0271963. doi: 10.1371/journal.pone.0271963, PMID 35901065, PMCID PMC9333277.
172. Kantorowska A, Wei JC, Cohen RS, Lawrence RA, Gould JB, Lee HC. Impact of donor milk availability on breast milk use and necrotizing enterocolitis rates. Pediatrics. 2016 Mar;137(3):e20153123. doi: 10.1542/peds.2015-3123. PMID 26908696. Erratum in: Pediatrics. PMID: 26908696; PMC ID: PMC4771129. 2016 Jun;137(6):null:e20153123. doi: 10.1542/peds.2015-3123, PMID 26908696.
173. Bramer S, Boyle R, Weaver G, Shenker N. Use of donor human milk in nonhospitalized infants: an infant growth study. Matern Child Nutr. 2021 Apr;17(2):e13128. doi: 10.1111/mcn.13128. PMID 33404169, PMCID PMC7988867.
174. Kumar J, Meena J, Debata P, Sankar M, Kumar P, Shenoi A. Three-hourly versus two-hourly feeding interval in stable preterm infants: an updated systematic review and meta-analysis of randomized controlled trials. Eur J Pediatr. 2022 May;181(5):2075-86. doi: 10.1007/s00431-022-04405-y, PMID 35147747.
175. Hussain A, Rehman A, Fatima N. Comparison of volume and frequency advancement feeding protocols in very low birth weight neonates. Pak J Med Sci. 2018 Jan-Feb;34(1):78-81. doi: 10.12669/pjms.341.14092, PMID 29643883, PMCID PMC5857034.
176. Utrera Torres MI, Medina López C, Vázquez Román S, Alonso Díaz C, Cruz-Rojo J, Fernández Cooke E et al. Does opening a milk bank in a neonatal unit

- change infant feeding practices? A before and after study. *Int Breastfeed J.* 2010;5:4. doi: 10.1186/1746-4358-5-4, PMID 20211005.
177. Kim EJ, Lee NM, Chung SH. A retrospective study on the effects of exclusive donor human milk feeding in a short period after birth on morbidity and growth of preterm infants during hospitalization. *Med (Baltim).* 2017 Sep;96(35):e7970. doi: 10.1097/MD.0000000000007970, PMID 28858132, PMCID PMC5585526.

ANNEXURE I – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

To study the effect of Pooled Pasteurized Donor Human Milk v/s Unpooled Pasteurized Preterm Donor Human Milk on the short term growth of preterm infants in NICU at KLE HOSPITAL, BELAGAVI –A randomized controlled trial

Principal Investigator :Dr.

Guide- Dr

You have been asked to involve your child in the above said research to be conducted at NICU of KLE university's JN medical college hospital, Belagavi by Dr.AABHA DHAND , PG student in the Department of Pediatrics at Jawaharlal Nehru Medical College, Belagavi.

Introduction

PURPOSE OF THE STUDY:

Participation of your child will help us to evaluate growth patterns of preterm infants who have received Unpooled Pasteurised Preterm Donor Human Milk with growth patterns of preterm infants who have received Pooled Pasteurized Donor Human Milk and other observational outcome following discharge. You are free to discontinue the participation in the study at any time for any reasons and you will not be paid any reimbursement for participation in the research. Hence involving your child in the study is your voluntary decision.

Voluntary participation

Your child's participation in this study is your voluntary decision, whether or not to participate will not affect your current or future relationship with KLEs Dr. Prabhakar Kore Charitable Hospital & MRC, Belagavi.

Risk and benefits

There are no risks involved.

Reduction in morbidity and mortality.

Privacy and Confidentiality -The only people who will know that you are a research participant are member of the research team. No information about you or provided by you, during research will be disclosed to others without your written consent. When the results of the research are published or discussed in the conferences, no information will be disclosed that would reveal your identity. Any information obtained in connections with this study and that can be identified with you remain confidential and will be disclosed only with your permission.

Queries

If you have any queries you may contact Dr. _____ Post Graduate Student Department Of Pediatrics Jnmc ,Belagavi-590010 Phone No. 79829-27527

Dr. _____ MD DCH Professor Department Of Paediatrics, JNMC,Belagavi-590010

If you have any questions about your rights or research participation you may contact Chairman ethical committee -Dr. Harsha hegde, Chairperson, JNMC,IEC And Scientist D, Icmr, National Institute Of Traditional Medicine Belagavi-590010

You will be given a copy of this form for your information and to keep for your record.

TATEMENT OF CONSENT

I hereby voluntarily agree for my participation in this study. I understand that even if I have the liberty to withdraw at any time. My signature below indicates that I have read or have been told in the language I understand , about this entire consent form including the risks and benefits and have had all my questions answered. I will be given a copy of this consent form.

Signature of the authorized representative/ parent: _____

Date: _____

Name: _____

Relation to the Subject: _____

Signature of the witness: _____

Date: _____

Name: _____

Signature of investigator: _____

Date: _____

Name: _____

ANNEXURE II – PROFORMA

SCREENING PORFORMA

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

--	--	--	--	--	--	--	--	--	--	--	--

INCLUSION CRITERIA

1. Baby less than 36 completed week. Yes No
2. 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

3. STABLE Yes No
- on ventilator support, Yes No
 - on parenteral nutrition Yes No
4. Living within 50-100 km of KLE ,Hospital Yes No
5. Requiring DONOR MILK- Yes No
- If yes , indication

I. Mother not alive	YES	NO
II. Mother is not well/ not physically healthy for breast feeding	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 study consent form been signed Yes No

If Consent given , Enrollment done Yes No

Mother ID

Baby ID

If Enrolled ,randomization done Yes No

Date of randomization

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------	----------------------

Participant number-

NAME OF THE INVESTIGATOR-

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

SIGNATURE-

PROFORMA

To study the effect of Pooled Pasteurized Donor Human Milk v/s UnPooled Pasteurized Preterm Donor Human Milk on the short term growth of Preterm infants in NICU at KLE HOSPITAL,BELAGAVI –A randomized controlled trial.

Participant Id - Screening Id- **I. SOCIO-DEMOGRAPHIC DATA**

- (I –Receiving preterm milk)
- (II- Receiving pooled milk)

Date of admission - 1. Mother`s Name: 2. Mother`s education: Illiterate Primary Secondary Graduate
Post Graduate 3. Mother`s Occupation: Home maker Employed Self employed 4. Father`s Name: 5. Father`s education: Illiterate Primary Secondary Graduate
Post Graduate

6. Marrital History:

Years of marital life - Consanguinous Non- Consanguinous 9. General information given by: Mother Father 10. Written informed consent Yes No **II. Maternal history**11. Age of mother at the time of delivery - a. Gravida: Primi Multi - G P L A b. Antenatal Visits: Done Not done c. Antenatal USG scan: Done Not done

12. Antenatal risk factors for preterm birth

 Yes No

a. Elderly primi >30 years	<input type="checkbox"/>	<input type="checkbox"/>
b. Short statured <140 cm	<input type="checkbox"/>	<input type="checkbox"/>
c. Preeclampsia and eclampsia	<input type="checkbox"/>	<input type="checkbox"/>
d. Anemia	<input type="checkbox"/>	<input type="checkbox"/>
e. Gestational diabetes mellitus	<input type="checkbox"/>	<input type="checkbox"/>
f. Previous stillbirth, IUD	<input type="checkbox"/>	<input type="checkbox"/>
g. Previous cesarean section	<input type="checkbox"/>	<input type="checkbox"/>
h. Grand multipara	<input type="checkbox"/>	<input type="checkbox"/>
i. PPROM	<input type="checkbox"/>	<input type="checkbox"/>
j. . IUGR	<input type="checkbox"/>	<input type="checkbox"/>
k. Twins	<input type="checkbox"/>	<input type="checkbox"/>
l. placenta previa/placenta abruption	<input type="checkbox"/>	<input type="checkbox"/>
m. Rh isoimmunization	<input type="checkbox"/>	<input type="checkbox"/>
n. congenital malformations	<input type="checkbox"/>	<input type="checkbox"/>

III. Birth history1. Mode of Delivery: PTNVD FTNVD LSCS ◆ Previous LSCS ◆ Malposition ◆ Multiple pregnancy ◆ Pre-eclampsia ◆ PPROM OR PROM ◆ Cephalo –pelvic disproportion ◆ Failed Induction of Labor 2. Date of delivery - 3. Time of delivery- : AM PM 4. Gestational age in weeks: <28 28-34 34-36 36+7 days

5. APGAR score at five minutes

3. Systemic examination
 - a. Cardiovascular system-
 - b. Respiratory system-
 - c. Per abdomen-
 - d. Central nervous system-

4. Feeding history

Type	Days of life																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
MOM																												
DHM Preterm Preterm plus Term																												
Formula feeds																												
Amount (ml/day)																												
Method RTF																												
SF/Paladay																												
Direct																												

5. Date of starting DHM:
6. Total duration of DHM given: days
7. Total amount of DHM given : ml
8. Type of DHM given – Pooled preterm donor milk Unpooled preterm donor milk
9. Hospital stay
 - a. NICU: days

10. Anthropometry at discharge

	Measured	Expected
Weight (kg)		
Reading 1		
Reading 2		
Reading 3		
Mean		

11.	Length (cm) Reading 1 Reading 2 Reading 3 Mean		
	Head circumference (cm) Reading 1 Reading 2 Reading 3 Mean		
	Mid arm circumference (cm) Reading 1 Reading 2 Reading 3 Mean		

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

12. PDHM Yes No

a. If yes, amount: ml/day

Baby`s follow up at 2 weeks

Feeding patterns:

		If yes: estimate no of time baby is fed		c. If yes: who decided that your baby should be fed this?			d. If yes, how was this fed to your baby?		
		Yes	No	Myself	Doctor	Family member	Cup/ Spoon/ Palladi	Bottle with nipple	N G tube
1	Breastmilk (if yes, ask 2-5)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	Breastmilk directly from your breast	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	Breastmilk expressed from your breast	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	Breastmilk from another mother (Donor Human Milk)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5	Infant Formula	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6	Tinned, powdered or fresh animal milk (cow, goat, etc.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

ANTHROPOMETRY

	Measured	Expected
Weight (kg)		
Reading 1		
Reading 2		
Reading 3		
Mean		
Length (cm)		
Reading 1		
Reading 2		
Reading 3		
Mean		
Head circumference (cm)		
Reading 1		
Reading 2		
Reading 3		
Mean		
Mid arm circumference (cm)		
Reading 1		
Reading 2		
Reading 3		
Mean		

Follow up at 4 weeks**Feeding patterns:**

		Yes No		If yes: estimate no of times baby is fed	c. If yes: who decided that your baby should be fed this?			d. If yes, how was this fed to your baby?		
					Myself	Doctor	Family member	Cup/ Spoon/ Palladi	Bottle with nipple	NG tube
1	Breastmilk (if yes, ask 2-5)	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	Breastmilk directly from your breast	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	Breastmilk expressed from your breast	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	Breastmilk from another mother (Donor Human Milk)	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5	Infant Formula	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6	Tinned, powdered or fresh animal milk (cow, goat, etc.)	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

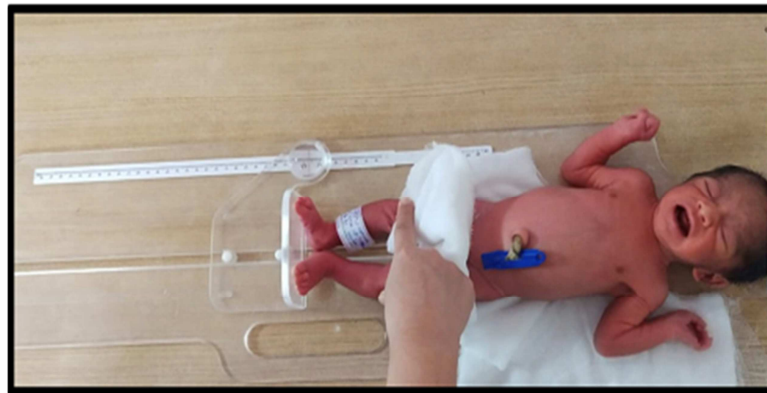
ANTHROPOMETRY:

	Measured	Expected
Weight (kg)		
Reading 1		
Reading 2		
Reading 3		
Mean		
Length (cm)		
Reading 1		
Reading 2		
Reading 3		
Mean		
Head circumference (cm)		
Reading 1		
Reading 2		
Reading 3		
Mean		
Mid arm circumference (cm)		
Reading 1		
Reading 2		
Reading 3		
Mean		

ANNEXURE III – 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

ANNEXURE-IV KEY TO MASTER CHART

Sex

F - Females

M - Males

Mother`s & Fathers` education

1 - Illiterate

2 - Primary

3 - Secondary

4 - Graduate

5 - Post graduate

Occupation

1 - Homemaker

2 - Employed

3 - Self employed

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
- NVD - Preterm vaginal delivery

Gravida status

- 1 - Primigravida
- 2 and above - Multigravida

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 fit
- 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
- PDHM - Pasteurized donor human milk
- RTF - Ryles tube feeding
- SF/Paladai - Spoon feed

Anthropometric Parameters

- Wt. - Weight
- Lt. - Length
- H.C - Head circumference
- MUAC - Mid Upper Arm Circumference

