"EFFECT OF HUMAN MILK FORTIFICATION WITH A HUMAN MILK FORTIFIER ON SHORT TERM GROWTH OF PRETERM LOW BIRTHWEIGHT INFANTS- A RANDOMIZED CONTROLLED TRIAL AT A TERTIARY HEALTH CARE CENTRE".

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LIST OF ABBREVIATIONS USED

AAP	American Academy of Paediatrics
AGA	Appropriate for Gestational Age
BDNF	Brain-Derived Neurotrophic Factor
CI	Confidence Interval
CLABSI	Central Line Associated Blood Stream Infection
CNTF	ciliary neurotrophic factor
DHM	Donor Human Milk
EDD	Expected Date of Delivery
EGF	Epidermal Growth Factor
EMBA	European Milk Bank Association
ESPGHAN	European Society of Pediatric Gastroenterology Hepatology and Nutrition
ELBW	Extremely Low Birth Weight.
GDNF	Glial Cell-Line Derived Neurotrophic Factor
HB-EGF	Heparin-binding growth factor
HM	Human Milk
HMF	Human Milk Fortifiers
IGF	Insulin-like Growth Factor
IL	Interleukin
IFN	Interferon
KMC	Kangaroo Mother Care
Kcal	Kilocalorie
g	gram
GA	Gestational Age
G-CSF	Granulocyte Colony-Stimulating Factor
Kg	Kilogram
LBW	Low Birth Weight
LDL	Low Density Lipoprotein
LGA	Large for Gestational Age
LMP	Last Menstrual Period

LoS	Late onset Sepsis
MALT	Mucosa Associated Lymphoid Tissue
MAMPs	Microbial Associated Molecular Patterns
MD	Mean Difference
MFG-E8	Milk fat globule-EGF factor 8 protein
mg	milligram
μg	microgram
MMF	Mothers Milk Fortifiers
MoM	Mother's Own Milk
NBW	Normal Birth Weight
NICE	National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NEC	Necrotizing Enterocolitis
NFHS	National Family Health Survey
NNS	Non-Nutritive Sucking
pIgR	Polymeric Immunoglobulin Receptors
P	P-value
PRRs	Pathogen Recognition Receptors
PUFA	Poly Unsaturated Fatty Acid
RR	Relative Risk
SGA	Small for Gestational Age
sIgA	Secretory IgA
IU	International unit
TGF	Transforming Growth Factor
TNF	Tumor Necrosis Factor
TPN	Total Parenteral Nutrition
VEGF	Vascular Endothelial Growth Factor
VLBW	Very Low Birth Weight
WHO	World Health Organisation

ABSTRACT

"EFFECT OF HUMAN MILK FORTIFICATION WITH A HUMAN MILK FORTIFIER ON SHORT TERM GROWTH OF PRETERM LOW BIRTHWEIGHT INFANTS- A RANDOMIZED CONTROLLED TRIAL AT A TERTIARY HEALTH CARE CENTRE"

Background and objectives

The global incidence of preterm delivery and low birth weight range from 5-18% and 15.5%, respectively.[1,3] In India, 13% of newborns are born preterm (3.5 million out of 27 million), and 18% of Indian children are born with low birth weight. [13] For proper catch-up growth and improved neurodevelopmental outcomes, preterm very low birth weight neonates require additional calories, protein, and minerals. [15] The objective of this study is to see how human milk fortification with human milk fortifiers affects preterm low birth weight infants' short-term growth.

Methodology

The present study is a hospital-based randomised control trial and was carried out in neonatal intensive care unit (NICU) of Dr. Prabhakar Kore Hospital and Medical Research Centre, attached to Jawaharlal Nehru Medical College, Belagavi under the department of paediatrics from April 2021 to August 2021. A total of 111 preterm newborns with a birth weight of less than 1800g and more than/equal to 1000g were admitted in NICU attached to the hospital during this period, 41 amongst them were excluded as they were not fulfilling the criteria for the enrollment in the study. 70 preterm newborns (gestational age based on New Ballard score), with a birth weight of less than 1800g and more than/equal to 1000g tolerating feeds of at least 80ml/kg/day for three days were enrolled and randomized into the study and control group based on computer-generated random number sequence. Study

group received fortified human milk. Fortification was done using Lactodex HMF sachet, 1 gram of which was added to every 25ml of human milk. The Control group received un-supplemented human milk during the study period. Total 6 newborns dropped out from the trial during this period, 2 of which were from study group and 4 in the control group. So, a total of 64 subjects (33 in cases and 31 in the control group) were analysed for the results.

Results

In present study, the mean weight gain in the study group from the time of birth, enrollment, and discharge was considerably higher compared to control group. There was a significant improvement in weight gain from the time of enrollment of preterm low birth infants receiving human milk fortified with an HMF sachet (used as fortifier) when compared to those fed on unfortified human milk (mean difference of 2.22g/kg/day, p-value 0.001). Improved weight gain from the time of enrollment was observed in the fortification group even on subgroup analysis, but the weight gain for AGA (mean difference of 1.59g/kg/day, p-value 0.12) and 1000-1250 grams (mean difference of 0.69g/kg/day and p-value of 0.78) weight category was statistically insignificant. The head circumference growth and increase in length were not significantly different between the two groups. There were no major concerns with respect to feed intolerance, sepsis and NEC with the use of human milk fortifier sachet as a fortifier in this study.

Conclusion

Our study found that fortification benefits were more marked for premature newborns with birth weight of more than 1250 grams and SGA babies when compared to preterm $\leq$1250 gram and AGA babies, respectively. Hence, routine fortification can be recommended for

SGA newborns and those weighing more than 1250g because the cost remains a key stumbling block in the usage of fortifiers for LBW babies.

Key words :Preterm, low birth weight, HMF sachet, short-term growth.

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INTRODUCTION

The rate of preterm birth in 184 nations ranges from 5 to 18 per cent; in India, 13 per cent (3.5 million babies out of 27 million) of newborns are premature, and the rate of premature birth in Belagavi is 8.6 per cent. [1,2]

Any live birth before 37 completed weeks of gestation is regarded as preterm birth. Breast milk is the optimum nutritional normative standard for the nutrition of newborns; hence it is especially important for premature babies. Human milk not only provides appropriate nourishment, but also protects against some of the most common NICU problems, such as infections, NEC (necrotizing enterocolitis), BPD (bronchopulmonary dysplasia), and RLF (retrolental fibroplasia).[3,4]

Low birth weight newborn is defined by WHO as newborns weighing less than 2500 grams, very low birth weight is less than 1500 grams, and extremely low birth weight is defined as birth weight of less than 1000 grams.[5] Small for gestational age, preterm, or a combination of factors might result in low birth weight. Low birth weight affects 15 to 20% of all babies born around the world. Every year, around 1.1 million babies die as a result of prematurity-related problems. [6]

Many preterm babies are unable to feed directly at the breast at first due to their immaturity and accompanying issues. Mothers of premature babies require competent assistance in such conditions to express enough milk to feed their babies, usually via gavage, and to sustain their milk production until the baby is able to feed directly at the breast. From the first day of life, a stable very low birth weight preterm newborn can be started on enteral feeding. Daily feed volumes should be gradually increased by 30-40mL/kg for those preterm babies who are stable and those less than

1000 grams infants with no evidence of feed intolerance. According to some studies, LBW babies who received only unfortified mother's milk were seen to suffer from osteopenia and also had deficiency of minerals and vitamins such as iron, zinc, vitamin A and vitamin D. [7] Breast milk in standard amounts (~150-180 mL/kg/day) does not offer enough protein (3.5 to 4.5 g/kg/d) or energy (110 to 135 kcal/kg/d) required to meet the needs of premature newborns.[8]

Early growth restriction, infection, and delayed development are common in LBW infants.[9] Improving the care of preterm low birth weight neonates can greatly reduce death and morbidity. Optimal nutrition throughout the neonatal period is crucial for neonates' immediate and long-term health and well-being.[10]

An infant's nutritional needs vary depending on their gestational age. When given at normal amounts, unfortified human milk, despite being the best source of nutrition, does not offer adequate nutrients for VLBW infants, resulting in slow growth and a risk of neurocognitive impairment.[11] There have been studies that show that adding extra nutrients to human breast milk, usually in the form of a powder or liquid "multi-nutrient fortifier," can assist to reduce this deficiency. When combined with expressed breast milk, human milk fortifiers can provide a nutritional boost of 10-20%. [8] According to a study, the effects of fortification were greater in SGA than in AGA. [12] Another recent study in south India showed no significant differences in growth indicators like weight gain and head circumference between neonates who received supplemented human milk and those who did not.[11]

So, this study intends to assess the nutritional advantage on short-term growth with fortification of human milk given to premature low birth weight babies versus those preterm LBW babies who received unfortified human milk.

OBJECTIVES

The objective of this study was;

- To assess the effect of human milk fortification with a human milk fortifier on short term growth of preterm low birth weight infants.

REVIEW OF LITERATURE

Preterm birth rates in 184 nations range from 5 to 18 percent; in India, 13 percent (3.5 million kids out of 27 million) are born prematurely, and the rate of premature birth in Belagavi is 8.6 percent. [1,2] According to data from the National Family Health Survey (NFHS-4) conducted in 2015–16, 18% of Indian children are born with low birth weight. [13]

Small for gestational age, preterm, or premature newborns with VLBW (very low birth weight) who are exclusively given unfortified human milk can have nutrient deficiencies as a result of inadequate protein and mineral content in expressed human milk, resulting in extra-uterine growth restriction, this corresponds to poor neurodevelopmental outcomes between the ages of 18 and 24 months.[9,14] For appropriate catch-up growth and improved neurodevelopmental outcomes, VLBW newborns require extra calories, protein, and minerals, and expressed human milk fortification has been recommended as the standard of treatment.[15] However, there is concern that multi-nutrient fortifiers may lead to adverse events such as feed intolerance and necrotizing enterocolitis. combination of factors might result in low birth weight.[8]

Background

After examining all cohort studies to date, the WHO committee for recommendations on optimal feeding of low birth weight infants recommends breastfeeding or mother's own expressed milk [MoM] as the best option. There has been no further research on this topic since then, and due to the overwhelming evidence, it is unlikely that any more trials/studies will be done to corroborate or refute this result.[3]

For enteral feeding of the LBW neonate, maternal breast milk remains the preferred option. Observational data and meta-analyses of studies comparing cohorts of newborns fed breast milk to formula reveals that breastfeeding offers several advantages, including a lower risk of illness and higher cognitive development scores. Although own mother's milk feeding has been linked to delayed length and weight growth in newborns compared to formula fed neonates, the difference isn't significant at 18 months.[3]

Nutrient deficiencies like iron deficiency, zinc deficiency, osteopenia, vitamin A and vitamin D inadequacy have all been observed in neonates fed only on un-supplemented own mother's milk.[3]

The European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) highly recommends the use of own mother's milk for low-birth-weight infants, but also stresses the need for additional nutrients, with an emphasis on preterm infants' dietary requirements.[4]

According to a Cochrane review and meta-analysis that looked at growth, necrotizing enterocolitis, all-causes of death, and neurodevelopmental impairment, formula-fed neonates had higher in-hospital rates of weight gain, linear growth, and head growth (moderate-quality evidence). However, it was linked to a much-increased risk of necrotizing enterocolitis (moderate-quality evidence). There was no evidence that either intervention had an impact on long-term development or growth.[5] Given our country's low socioeconomic status and poor sanitary conditions, using formula milk should be approached with caution, as sepsis is already prevalent in the majority of India's neonatal care units. Another stumbling block is the high cost of formula feeding, which usually results in over-dilution and poor nutrition.[6]

In contexts where donor milk is accessible, only pasteurised donor human milk from a donor human milk bank is advised for feeding if own mother's milk is not available. Furthermore, appropriate fortification is required for feeding premature and low-birth-weight neonates. Formula milk should be considered if donor human milk is not available.[6]

According to a study conducted by Gathwala et al, human milk fortification delivers larger amounts of proteins and calcium essential for preterm newborns sustained growth. Premature neonates that are only fed unfortified human milk have been associated to slower growth and nutritional deficiencies during and after hospitalisation.[10]

There was no significant difference in growth parameters like weight gain and head circumference between neonates who received fortified versus those who received unfortified milk in a recent study in south India by Adhisivam et al to look for the influence of fortification of pasteurized human donor milk.[11]

Mukhopadhyay et al study of the effects of fortification of human milk on the early growth of preterm newborns with VLBW, found that small for gestational age (SGA) babies had more significant effect than the appropriate for gestational age (AGA) babies.[12]

Breast milk fortification can be started for LBW babies whose birth weight is less than 1800 grams and who are receiving at least 50-80 ml/kg/day of enteral feeds, according to the latest National Neonatology Forum-India guidelines. In resource-constrained settings, fortification may be initiated only for infants who do not gain weight while receiving sufficient amount of breast milk.[6]

In a recent Cochrane systematic review, 18 randomized trials including 1456 infants were identified. Individual trials had a weak methodology and were often small. According to these meta-analyses, multi-nutrient breast milk fortification boosts in-hospital growth rates by a mean daily weight gain of 1.76 g/kg (low to moderate certainty).[8]

PRETERM AND LOW BIRTH WEIGHT CLASSIFICATION: -

Neonates should be categorized according to their gestational age (GA), as this has a stronger association with outcomes than a birth weight-based classification. If the infant is small for gestational age (SGA) or large for gestational age (LGA), the birth weight becomes crucial. Classification of neonates based on gestational age: - [16]

- Calculation of gestational age using obstetric data: -
    a. Last Menstrual Period (LMP)

       Knowing the first day of last menstruation can help to figure out EDD (Expected Date of Delivery). The EDD is 280 days from the start of the LMP, according to the convention. This method ignores cycle length irregularities, incorrect LMP recall, and ovulation timing variability.[17]

    b. By Ultrasonography

       Ante-natal sonography can also be used to determine the gestational age and expected delivery date, as well as the state of the child's intrauterine growth. It is most accurate if done during the first trimester.[16]

TRIMESTER	ACCURATE WITHIN (days)
1ST	7
2ND	14
3RD	21

Figure 1. Accuracy of antenatal Ultrasonography in determining gestational age[16]

c. Modified Dubowitz (Ballard) examination for new-borns [16]

- The modified Ballard score has limitations, particularly when the neuromuscular component is used in sick new-borns.
- It has two components.
  i. Neuromuscular maturity.
  ii. Physical maturity.

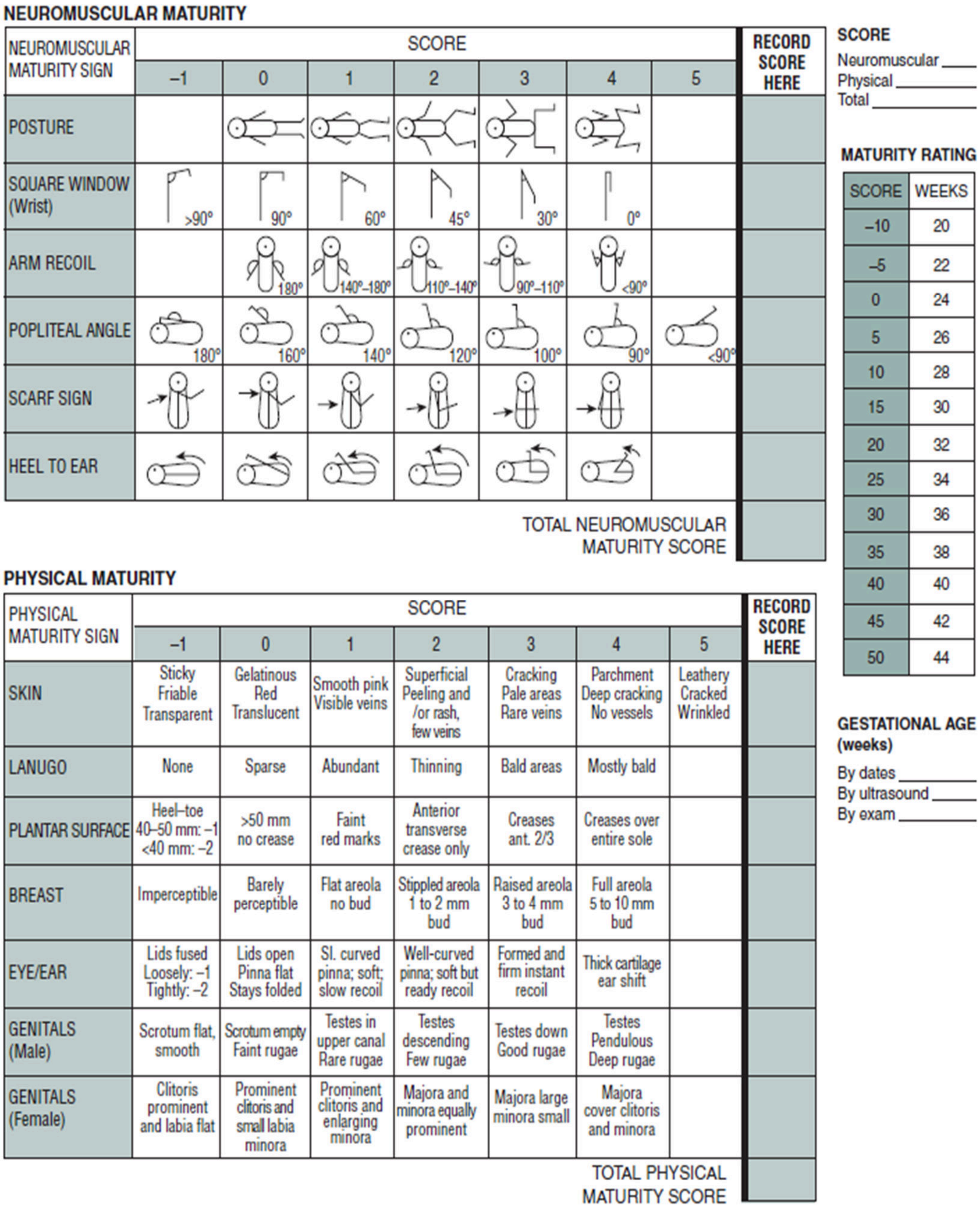


Figure 2. Updated Ballard scale for gestational age assessment.[16]

The Ballard Score has been updated to accommodate extremely preterm babies.[16]

- **Classification of neonates (Based on gestational age): -** [16]

    a. Preterm neonates are those born before the 37th week of pregnancy (258 days).

    Preterm neonates include the following:

Subgroups	Weeks	Days
Extremely preterm	<28	195
Early preterm infants	<34	237
Late preterm	Between 34 0/7 and 36 6/7	238 to 258

Figure 3. Classification of neonates (Based on gestational age)[16]

b. Term neonates are those born between the 37th and 42nd week of pregnancy (259 to 293 days).

Classification of neonates based on birth weight: - [16]

1. Normal birth weight (NBW): 2,500 - 4,000 grams.
2. Low birth weight (LBW): less than 2,500 grams.

Subgroup	Weight
Very low birth weight (VLBW)	<1500g
Extremely low birth weight (ELBW)	<1000g

Figure 4. Classification of neonates based on birth weight [16]

CHALLENGES IN NUTRITION OF PRETERM NEONATES

It is difficult to address the nutritional needs of preterm new-borns because of the particular mix of challenges they face.

- Gut microbiota

Preterm neonates have an immature immune system and gastrointestinal tract, making it difficult to meet their nutritional needs. Preterm newborns gut flora differs from mature babies due to a unique combination of circumstances, including the environment during their stay in the intensive care unit (NICU) and the clinical course, treatment protocol, and feeding methods. The infant's growth may be hampered by this variance in gut flora makeup, which may influence energy demand, production, and storage.[18]

The gut microbiota of a full-term infant born vaginally and exclusively breastfed is commonly considered to be the gold standard.[26] Low oxygen levels early after birth, in general, offer an ideal environment for the growth of facultative anaerobes such as bacteria from the genera Enterobacter, staphylococcus, enterococcus, and streptococcus.[20,21]

Residual oxygen permits facultative anaerobes to flourish in the gut of newborns, resulting in a lower redox potential that allows obligate anaerobes to survive.[20] The gut flora is also influenced by gestational age; preterm newborns have a limited diversity of microorganisms and take longer to colonise than term babies.[22]Furthermore, they reduced the number of commensals while the number of potentially dangerous microorganisms is increased.[21] In comparison to term newborns, preterm infants' guts are dominated by Klebsiella, Enterococcus, Enterobacter, and Escherichia.[23]

At birth, the immune system is not fully functional. It matures along with gut microbiota through time from a largely Th2 immune response to a much more balanced immune response that includes both Th1 and Th2.[24]The mucosa associated lymphoid tissue (MALT) in the gut is the major location of interaction between commensal bacteria and other environmental antigens with the immune system.[25]Microbes and their products interact with the host through pathogen recognition receptors (PRRs) that recognise microbial associated molecular patterns (MAMPs) or G-protein coupled receptors.[25]

Breastfeeding has a very important role in the development of the immune system.[26] It contains immunological components, most notably immunoglobulins like IgA, which aid in immune system development.[26] It also contains nonspecific factors that include enzymes and proteins with antimicrobial properties, such as inhibiting bacterial development by destabilizing the proteoglycan layer by enzymatic action or restricting bacterial growth by lactoferrin, which removes iron required for bacterial growth.[26]Some components provide protection by preventing adherence of bacteria to the mucosal surface of the gastrointestinal tract.[26]The differences in metabolites between human milk and formula milk, particularly carbohydrate and fatty acids, are important determinants for gut microbiota.[27]

Premature birth has an impact on the immune system's development as well as the composition of breast milk, which includes an immunological component.[35] Preterm milk has more secretory immunoglobulin, which provides more protection against harmful organisms while compensating for the underdeveloped immune system.[28]

The immune system is also involved in the development of NEC (necrotising enterocolitis), a disease marked by an elevated inflammatory response.[24]

The gut's functional and structural development is critical for the start of milk feeding since it impacts nutrient absorption and digestive efficiency. The presence of digestive enzymes (such as maltase, peptidase, lactase, and sucrase) can be found as early as the 8th week of pregnancy, and their activity increases with gestational age, peaking by the 40th week. The consumption of milk improves the maturation of gastrointestinal function. [29]

So, nutrition and prematurity affect the maturation of the gastrointestinal tract, gut microbiota, and its immune system. These closely related processes along with prematurity affect the new-borns growth and development.[18]

- Maturation of feeding skills in preterm infants.[30]

In the case of premature infants, establishing breastfeeding and sustaining milk production during the transition phase presents unique challenges. To begin breastfeeding infants must be haemodynamically and physiologically stable.[31]

- Initial feeding methods in LBW neonates based on gestational age.[30]
    a. **< 28 weeks**: Because sucking efforts and gut propulsive movement do not develop adequately in these infants, intravenous fluid should be administered to them at the beginning.[30]
    b. **28-31 weeks:** Although sucking spurts occur, there is no coordination between suck, swallow and breathe, so we should start feeding through a feeding tube with a spoon feed / paladai trials on occasion.[30]
    c. **32-34 weeks:** These neonates have a more mature sucking pattern, with better breathing and swallowing coordination, which improves as they grow. These newborns should be introduced to spoon / paladai feeding as soon as possible.[30]
    d. **>34 weeks:** Because these neonates have a mature sucking habit and improved coordination between breathing and swallowing, breastfeeding could be the first feeding option.[30]

In the establishment of breastfeeding for premature extremely LBW babies, enough caloric and nutritional supply, as well as an ideal volume of milk supply, pose unique challenges (VLBW). Mothers of these infants are required to express milk until the infants are physiologically stable, which is sometimes challenging owing to prolonged hospital stays and separation from the infants. The condition of the infants may cause stress in the mother, resulting in decreased milk production.[31]

The development of newborns sucking is aided by non-nutritive sucking (NNS). Kangaroo mother care (KMC), in which infants are placed in direct skin-to-skin contact with their mothers, aids in milk production, neonatal growth, and breastfeeding duration.[31]

NUTRITION OF PRETERM LOW BIRTH WEIGHT NEONATE

- Human milk

    a. Composition [27,32]

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

Figure 5. Composition of Human milk [27,32]

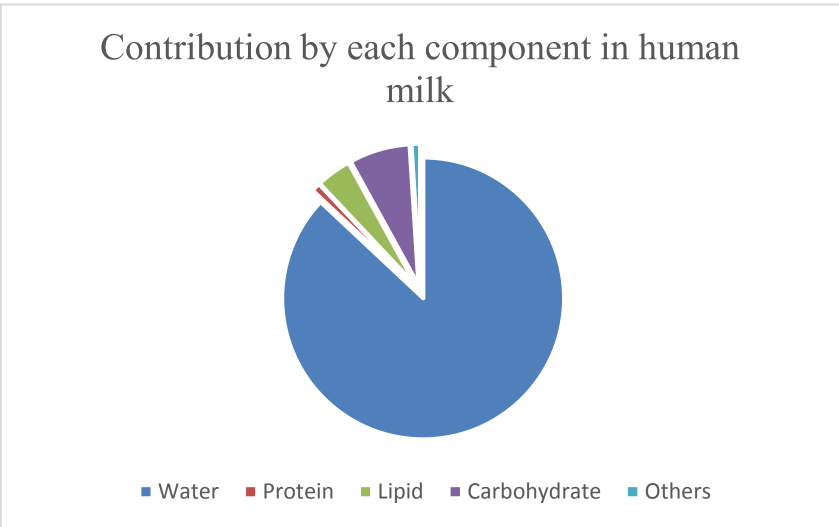


Figure 6. Composition of Human milk (graph) [27,32]

- **Carbohydrate: -** Lactose, a disaccharide, is the most common carbohydrate and the principal source of energy in colostrum, but it is fairly low and gradually increases with time. Although they are not easily digested, oligosaccharides are abundant and perform key roles as probiotic (facilitating commensal organism growth), defence (Fucose), and brain development (sialic acid).[27]
- **Bioactive molecules: -** Bioactive dietary components are substances that "change biological processes or substrates, hence influencing body function or condition, and thereby health".[45] The mammary epithelium synthesises 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 transport. For example, Antigen-specific B lymphocytes migrate to the mammary gland in breastfeeding mothers, sIgA is transported into the duct lumen by polymeric immunoglobulin receptors (pIgR).[33] Thematic changes in the proteins that make up milk at different phases of lactation, as well as variations between term and preterm milks, have been revealed using proteomic analysis.[34] When donor milk is required, research suggest that it should be matched to the infant's developmental stage wherever possible, however this is sometimes challenging in practise.[33,34]

  Important bioactive factors present in human milk.[33]

  - Cells such as macrophages, stem cells.
  - Immunoglobulins including, IgG, IgM, sIgA.
  - Cytokines such as TGF-β, IL-6, IL-7, IL-8, IL-10, IFN-γ, TNF-α.

- Chemokines (e.g., G-CSF, Macrophage Migratory Inhibitory Factors)
- Growth factors (e.g., EGF, VEGF, IGF, Erythropoietin).
- Hormones (e.g., Somatostatin, Calcitonin)
- Anti-microbial (e.g., Lactoferrin, Lactadherin/MFG E8)
- Metabolic hormones (e.g., Adiponectin, leptin, Ghrelin)

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.[33]In the newborn gut, EGF has a number of protective mechanisms in place, including:

- reversing TNF-induced changes in intestinal and hepatic tight junction proteins.[33]
- suppression of programmed cell death.[33]

Heparin-binding growth factor (HB-EGF) is the primary growth factor responsible for the resolution of damage caused by hypoxia, reperfusion injury, haemorrhagic shock / injury during resuscitation, and necrotizing enterocolitis. Furthermore, EGF levels in preterm milk are greater than in mature milk.[33]

The enteral 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.

Peristalsis, a function that is often compromised in the preterm gut, can be improved by BDNF. GDNF from breast milk improves neuron survival and growth. Human milk contains BDNF, GDNF, and a similar protein called ciliary neurotrophic factor (CNTF) which may be detected up to 90 days after delivery.[33]

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 damage.[33]

Leptins play an important role in the postnatal growth of infants by regulating energy conversion and infant BMI, as well as hunger regulation.[33,35]

- Human milk contains PUFAs such as Eicosapentaenoic acid and docosahexaenoic acid, which play a key role in brain development.[32]

Human milk differs from that of other mammals in that it has a higher proportion of PUFA (Poly Unsaturated Fatty Acid) and a smaller amount of protein. Vitamins and essential minerals such as Calcium, Magnesium, Phosphorus, Sodium, and Potassium are also present.[32]

b. Preterm versus Term milk

- Protein, salt, fat, and free amino acid levels are initially higher in preterm milk, but they eventually decrease.[27]
- Mineral content, including trace elements, is similar in term and preterm milk, with the exception of calcium, which is significantly

lower in preterm milk and appears to remain constant over time, whereas zinc and copper are relatively higher in preterm milk when compared to term milk and gradually decrease over time.[27,36]

- In the case of preterm babies, the increase in lactose content of milk throughout the first few weeks is more substantial.[27]
- No difference in the level of leptin between term and preterm milk.[35]
- In term, lipoprotein lipase activity is higher, although bile salt stimulated lipase activity is equivalent in preterm and term.[27]

c. Benefits of human milk [27]

- Decreased incidence of LoS (late onset sepsis).
- Reduced incidence of Necrotising enterocolitis (secretory immunoglobulins, lipases, lysozymes, growth factors, and oligosaccharides all play a role in protective mechanism).
- Reduced incidence of retinopathy of prematurity.
- Reduced incidence of hospital re-admission and
- Better neurodevelopment.
- Decreased long-term risk of metabolic syndrome and lower LDL (low density lipoprotein levels) compared to those received formula feeding.

d. Potential benefits to preterm infants (studies have shown mixed results) [27]

- Feed tolerance.
- Time to reach full feed and
- Protection against allergic disease.

Challenges in providing human milk to premature

- Intolerance to high fluid volumes. [27]
- For babies in neonatal intensive care units, mothers should use a breast pump regularly after delivery to enhance milk output and make sure that they empty the breast each time. [27]
- When compared to term babies, preterm infants have a higher nutritional need per kg (kilogram). [27]

Enteral intake recommendations for preterm:[16]

Nutrient	Enteral Intake Recommendations for Preterm Infants.
Protein	3.5–4.5 g/kg/day
Carbohydrate	10-14 g/kg/day
Fat	5-7 g/kg/day
Vitamin A	400-1,500 IU/kg/day
Vitamin D	200-400 IU/day
Vitamin E	2.2-12 IU/kg/day
Vitamin K	4.4-28 µg/kg/day
Calcium	100–220 mg/kg/day
Magnesium	7.9–15 mg/kg/day
Iron	2–4 mg/kg/day
Zinc	1,000–3,000 µg/kg/day

Figure 7. Enteral intake recommendations for preterm[16]

- The caloric requirements of new-borns are approximately 110–135 kcal/kg per day.[4]To ensure appropriate growth, preterm babies often require more energy.[37]

- Low birth weight and preterm new-borns require more energy to maintain their temperature and gas exchange.[27]

Low-Birth-Weight Neonates' Energy Requirements:[16]

	Average Estimation (kcal/kg/day)
Energy expended	40–60
Resting metabolic rate	40–50
Activity	0–5
Thermoregulation	0–5
Synthesis	15
Energy stored	20-30
Energy excreted	15

Figure 8. Low-Birth-Weight Neonates' Energy Requirements[16]

e. Challenges with donor milk

- Donor milk is more frequently supplied by mothers of term newborns, and this milk contains less protein, bioactive compounds, and fat than preterm milk.[27]
- There is a likelihood of infectious agent transmission. A uniform strategy for donor screening and pasteurization of milk aids in limiting the spread of this disease.[27]
- Pasteurization causes a considerable reduction in secretory immunoglobulin (IgA), enzymes, water-soluble vitamins, PUFA (polyunsaturated fatty acid), IGF, and other growth factors.[27]

f. Fetal origin of adult disease / developmental origin of adult disease.

- The physiological system that develops during the neonatal period tends to persist. Barker's concept demonstrated a link between prenatal development and metabolic problems in adults.[38]

- **Total Parenteral Nutrition**

In the treatment of premature infants, total parenteral nutrition (TPN) is commonly used. The National Institute for Health and Care Excellence (NICE) recently released guidelines for best practices in neonatal parenteral nutrition. However, healthcare-associated sepsis, TPN expertise and infrastructure, monitoring facilities, and cost remain important barriers to widespread TPN adoption in resource-constrained settings. Preterm neonates, with a birth weight of less than 1500 grams and/or gestation of less than 32 weeks, are at risk of subsequent growth failure. Preterm neonatal growth failure is linked to long-term malnutrition and poor neurodevelopmental outcomes. TPN should be started in all neonates ≤31 wk, according to NICE guidelines. However, because gram-negative sepsis and sepsis-related death are more common in developing countries, TPN should be administered in units based on resources. In our country, the availability and affordability of standardized TPN bags are a serious concern. In the Indian setting, due to the greater incidence of Central Line Associated Blood Stream Infection (CLABSI) and the high cost of TPN, it can be safely withdrawn after the neonate can tolerate at least 100 mL/kg/d oral feeds.[39]

- **Role of human milk fortification: -**
    a. It provides the benefits of mother's own milk as well as the potential to fill nutritional shortages, such as proteins, calories, sodium, calcium, phosphorus, and iron, as well as vitamins A and D. [16]
    b. Preterm HM has a higher protein, salt, chloride, and magnesium content than term milk. The levels of these nutrients, however, remain below preterm requirements, and the greater concentration only lasts for about the first 21 days of lactation.[16]
    c. Human Milk Fortifiers [HMF] available in India:[16]
        - Bovine-based HMF: - Lactodex-HMF and PreNAN HMF and
        - Donor HM-based: - Neolact MMF and Neolact MMF plus.
    d. When bovine milk–based HMF is added to HM, the energy, protein, vitamin, and mineral content rises to levels that are more suitable for premature newborns. The fortifier, which is based on donor HM, boosts energy, protein, and mineral intake. However, because the vitamin content of the meal does not rise much with the use of this product, a daily multivitamin and iron supplement is usually given.[16]
- **Guidelines for the use of human milk fortifiers: -**
  a. WHO Optimal feeding of LBW in low- and middle-income countries 2011: -
    - In cases where very low birth weight neonates are on mother's own milk or donor human milk, human milk fortification should not be done on a regular basis.[3]
    - In cases where neonates do not gain weight despite adequate nutrition, the use of human milk fortifiers (ideally human milk-based) is recommended.[3]

b. ESPGHAN recommendations (2010)

- Promoted the use of human milk for premature babies as a standard practice.[5]
- In addition, nutrient fortification to be done as needed to meet daily protein and calorie requirements.[5]

c. American academy of paediatrics (AAP), ESPGHAN, Milan Consensus statement in 2015.[7]

- Suggested human milk fortification for all preterm babies with birth weight <1800 g.

d. EMBA Working Group 2019: - [9]

- Individualised fortification should be done for neonates weighing less than 1800g once the feed volume reaches 50-80ml/kg/day.

e. National neonatology forum- feeding of low-birth-weight neonates (2020) weak recommendation based on low to moderate quality evidence:[6]

- For preterm less than 1800g: - Once infants are receiving at least 50-80ml/kg/day of feed, fortification can be initiated.
- In resource-constrained settings, Fortification can only be practiced for newborns who do not gain enough weight despite adequate nutrition.

In terms of fortification timing, a recent systematic analysis comparing early and delayed fortification found no differences in anthropometry, incidence of sepsis or NEC between the two groups. To summarise, current evidence is limited and does not suggest the ideal time to commence fortification.[40]

There is limited efficacy data on human milk-based fortifiers, and their high costs preclude their implementation in resource-constrained settings. The OptiMoM study[41], is the first trial to compare the efficacy of fortifier based on human milk to those based on bovine milk in the absence of formula milk. There was no difference in feeding tolerance, postnatal development, or morbidity even when NEC grade 2 was taken into account (4.7 vs. 4.9 percent). In another study[42], the fortifiers based on human milk were never directly compared to those based on bovine milk, and many of the babies who developed NEC on the bovine fortifier were those who also had bovine formula, despite the fact that this study showed a significant reduction in NEC rates from 16 to 6% and cost-effectiveness.

There was low quality evidence in a recent Cochrane systematic review [8] meta-analysis indicating fortification increases the risk of NEC in preterm new-borns, with an usual relative risk (RR) of 1.57 (95 percent CI 0.76–3.23).

NUTRITIONAL ASSESSMENT

The periodic nutritional assessment determines whether or not neonatal nutrient and energy requirements for optimum growth are satisfied. [43]

- **Growth parameters**[43]

  Weight, head circumference, and length are all factors to consider. Serial measurements of these variables assist in determining whether or not the growth is appropriate.

  - Weight: A minimum predicted increment of 15 gram/kg/day should be assessed every day. Once the baby has reached a weight of 2.0 kg, the daily weight gain objective should be 20 to 30 grams per day.
  - Length: Weekly assessments should be carried out, with a minimum expected weekly increment of 1 cm.

  - Head circumference: weekly assessment should be carried out, with a minimum expected increment of 1 cm every week.

- **Biochemical assessment** [43]

  Serial biochemical parameter evaluations can also be used to monitor the nutritional status of preterm infants:

  - Protein status: urea nitrogen and serum albumin levels are monitored.
  - Electrolytes: In newborns who are given diuretics, have limited intakes, or have slow growth, serum sodium, chloride, and bicarbonate levels are measured.
  - Anaemia: reticulocyte count and the level of haemoglobin are measured to determine the severity of the condition.

The goal of this study is to compare the nutritional benefits of fortified human milk for preterm low birth weight babies to preterm LBW babies who received unfortified human milk in terms of short-term growth.

METHODOLOGY

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

Study Design:

The study design is randomized controlled trial.

Study duration:

This study was conducted from April 2021 to August 2021.

Source of data

Preterm weighing less than 1800g but more or equal to 1000g admitted in the Neonatal Intensive Care Unit and Post-natal wards under Department of Paediatrics, KLES Dr Prabhakar Kore hospital and Medical Research Centre, Belagavi.

Sample size

A total of 70 new-borns were divided into two groups of 35 each as cases and controls who fulfilled the selection criteria were enrolled.

Sampling procedure

Based on previous study target sample size was calculated for a power of 90% with Alpha error of 5% for a 2-tailed test.[17]

n = Size per group

$\mathbf{Z_X}$ = The standard normal deviate for a one or two side study.

SD_1 = Standard deviation study group.

SD_2 = Standard deviation control group.

α = alpha error. (In this study 5%)

β = In this study 90%.

υ_1 = mean of study group.

υ_2 = mean of control group.

$$\mathbf{N} = (Z_{1-\alpha/2} + Z_{1-\beta})\ [(SD_1)^2 + (SD_2)^2]\ /\ \upsilon_1 - \upsilon_2$$
$$= (1.96 + 1.28)^2\ (1.6^2 + 2^2)\ /\ 16.6 - 14.2$$
$$\sim 29 \text{ in each group.}$$

Target sample size calculated was 29 for each group, accounting for 20% follow up loss sample size was taken as 35 in each group.

Selection criteria: -

<u>Inclusion criteria</u>

a) Preterm babies (Gestational age based on New Ballard score).

b) Birth weight less than 1800g and more than/equal to 1000g tolerating feeds of at least 80ml/kg/day for 3 days.

<u>Exclusion criteria</u>

a) Birth asphyxia.

Any one of the following:

1. Apgar of less than or equal to 5 at 10 min, or

2. Cord blood pH less than or equal to 7.00 or

3. Base deficit more than or equal to 16 mmol/L

b) Congenital malformation / anomalies.

c) Weight less than 1000g.

Ethical clearance

Prior to the commencement, the ethical clearance was obtained from Ethical and Research committee, Jawaharlal Nehru Medical College, Belagavi.

CTRI registration

Prior to the commencement, CTRI registration number was obtained after registering for the study.

(Registration number: CTRI/2021/04/032907).

Informed consent

Children fulfilling the selection criteria were selected and their legal guardian were briefed about the nature of the study in their local language and written informed consent was obtained.

Method of data collection

The babies who met the inclusion requirements were recruited in the study and were randomised into study and control groups based on a computer-generated sequence once they readily accepted feeds of at least 80ml/kg/day for three days (expressed human milk).

At the time of recruitment, baseline data were collected, including socio-demographic information, mother's antenatal details, mode of delivery, need for resuscitation, APGAR score, post-natal need for CPAP, ventilator or oxygen supplementation, any blood component or antibiotic given, day of initiation of feed, and clinical condition of the infant.

Intervention:

Those in the study group got fortified human milk, whereas those in the control group got unfortified human milk. Lactodex HMF sachet was used to fortify expressed breast milk; 1 gram of HMF sachet was used to fortify 25ml of expressed breast milk.

Composition of 1g lactodex HMF sachet.

- Protein (0.27g)
- Total fat (0.04g)
- Carbohydrate (0.49g)
- Vitamins (vitamin C, E, A, B6, B2, B1, D3, B12, K)
- Calcium (15.8mg).
- Zinc (40mg) and other minerals (chloride, iron, copper, manganese, potassium, Phosphorus)

After that, the new-born's anthropometric measures were obtained during their hospital stay and after 28 days of life as follows:

1. During hospital stay/ till the child achieve weight of 2000g
    - Daily weight measurement and
    - Weekly head circumference and length measurement.
2. On follow up (after 4 completed weeks of life)
    - Measurement of weight, head circumference and length.

Equipment's used

1. Weighing was done with an Essae-BS-250 electronic weighing scale.
2. Infantometer was used to measure the length of new-born's and
3. Head circumference was measured using non stretchable tape.

The study excluded children with congenital abnormalities, such as congenital heart disease, and those who were not on enteral feeds by 14 days of life. Neonates who required oxygen therapy for more than 10 days or who required ventilatory assistance for more than 7 days were also excluded.

If the new born in the control arm does not gain weight of at least 10 gram/kg/day after 4 weeks of life, milk fortification was initiated.

Outcome variables: -

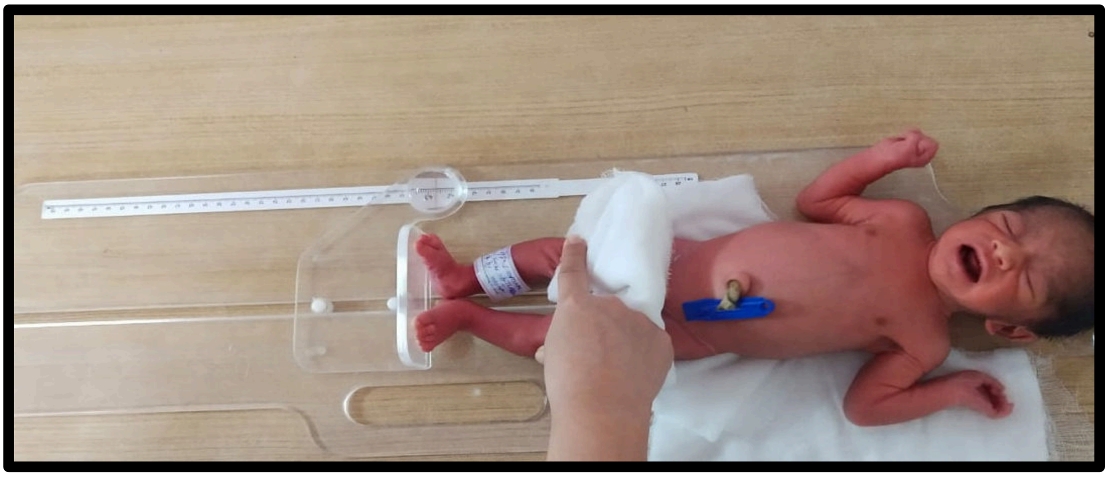
Following outcomes were measured: -

1. Weight gain in g/kg/day from day of enrollment till four completed weeks of life (birth weight as base).
2. Head circumference increase in cm/week from day of enrollment till four completed weeks of life.
3. Length increase in cm/week from day of enrollment till four completed weeks of life.

Statistical analysis

The study's statistical analysis done as follows: Data were entered in MS-Excel and analysed in SPSS V25. Descriptive statistics were represented with percentages, mean with SD or Median with IQR depends on the nature of the data. The Shapiro wilk test was applied to find normality. Chi-square test, Fisher Exact test, ANOVA Kruskal Wallis, Mann-Whitney U test and independent t-test were calculated. $P<0.05$ was considered as statistically significant.

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 non stretchable tape.

RESULTS

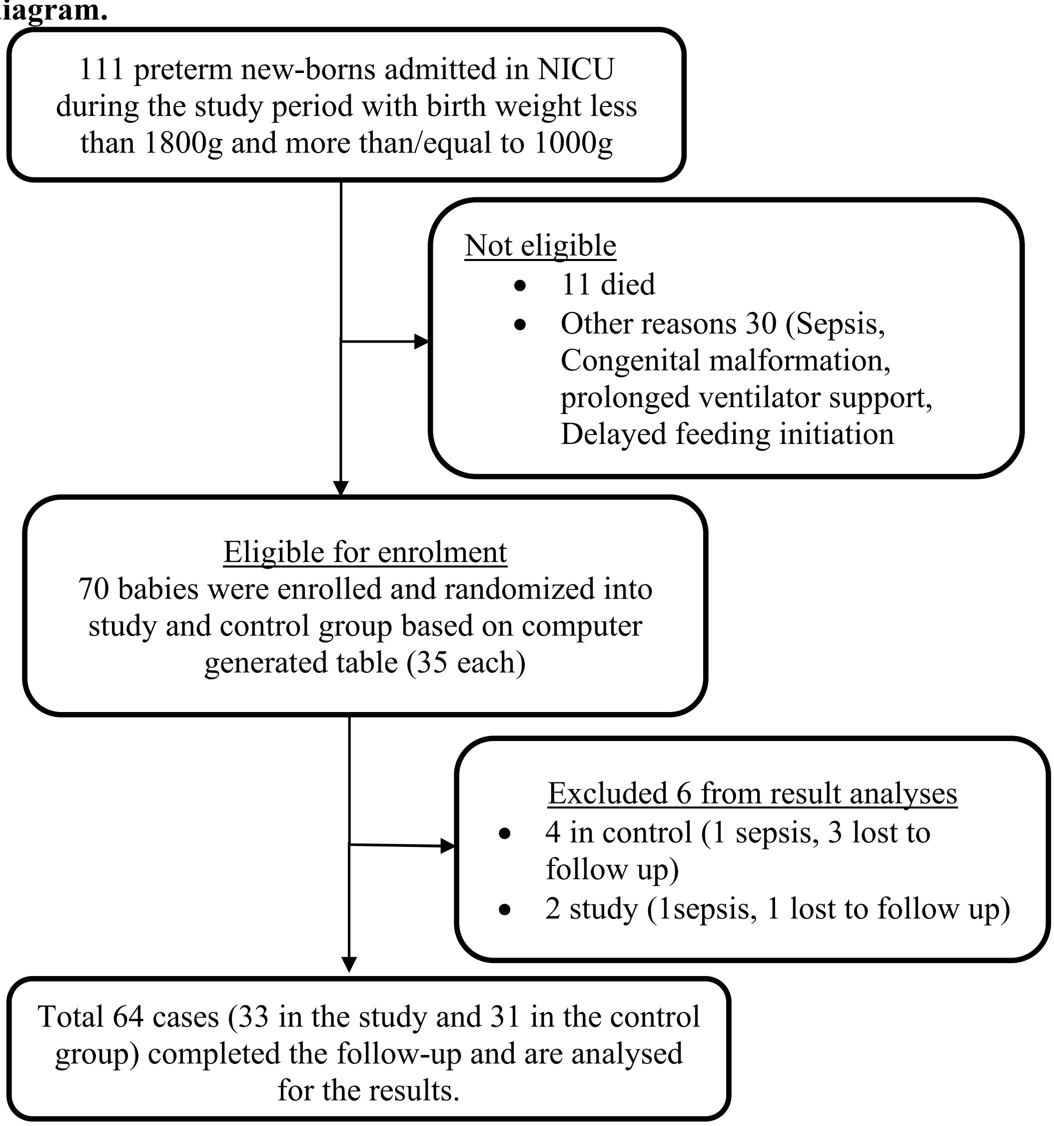
This is hospital based randomised control trial was done in the Neonatal Intensive Care Unit under the Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, attached to Jawaharlal Nehru Medical College, Belagavi from April 2021 to August 2021. A total of 70 preterm new-borns were enrolled and randomized into the study and control group based on the computer-generated sequence, 35 in each group. Neonates were divided into two groups as below

- **Study:** 35 new-borns admitted in Neonatal Intensive Care Unit under Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, meeting the criteria were enrolled in the study group after randomization. This group received fortified human milk. The fortification was done using Lactodex HMF, 1 gram was added to every 25ml of milk.
- **Control:** 35 new-borns admitted in Neonatal Intensive Care Unit under Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, meeting the criteria were enrolled in the control group after randomization. This group received un-supplemented human milk during study period.
- In the present study overall 6 cases were excluded after enrollment from result analysis. 1(2.86%) in either group developed sepsis, 1(2.86%) in case and 3(8.57%) in control lost to follow up.

  MS-Excel was used to enter data, and SPSS V25 was used to analyze it. Depending on the nature of the data, descriptive statistics were represented as percentages, mean with SD, or median with IQR. To determine normalcy, the Shapiro Wilk test was used. The following tests were performed: Chi-square test, Fisher Exact test, ANOVA Kruskal Wallis, Mann-Whitney U test, and independent t-test. It was considered that $P < 0.05$ was statistically significant.

The data was analysed and the final results were tabulated as below.

Study flow diagram.

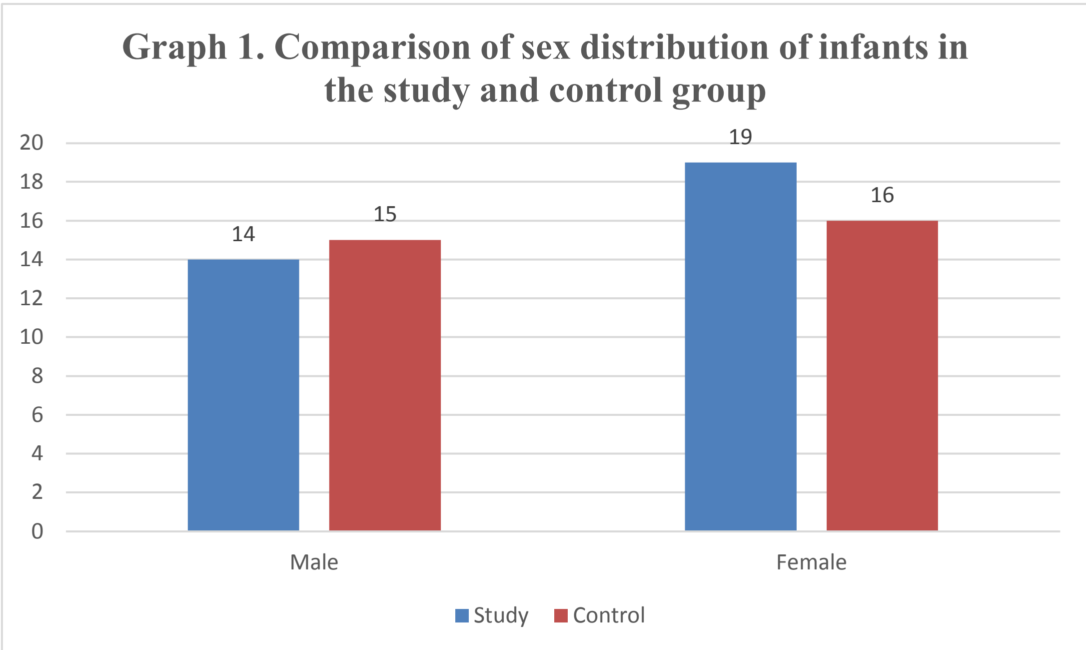


Cases Excluded from result analyses after enrollment	Study (n=35)		Control (n=35)	
	Count	%	Count	%
Sepsis	1	2.86%	1	2.86%
Lost to follow up	1	2.86%	3	8.57%

Figure 9. Cases excluded from result analysis after enrolment.

Table 1. Comparison of sex distribution of infants in the study and control group

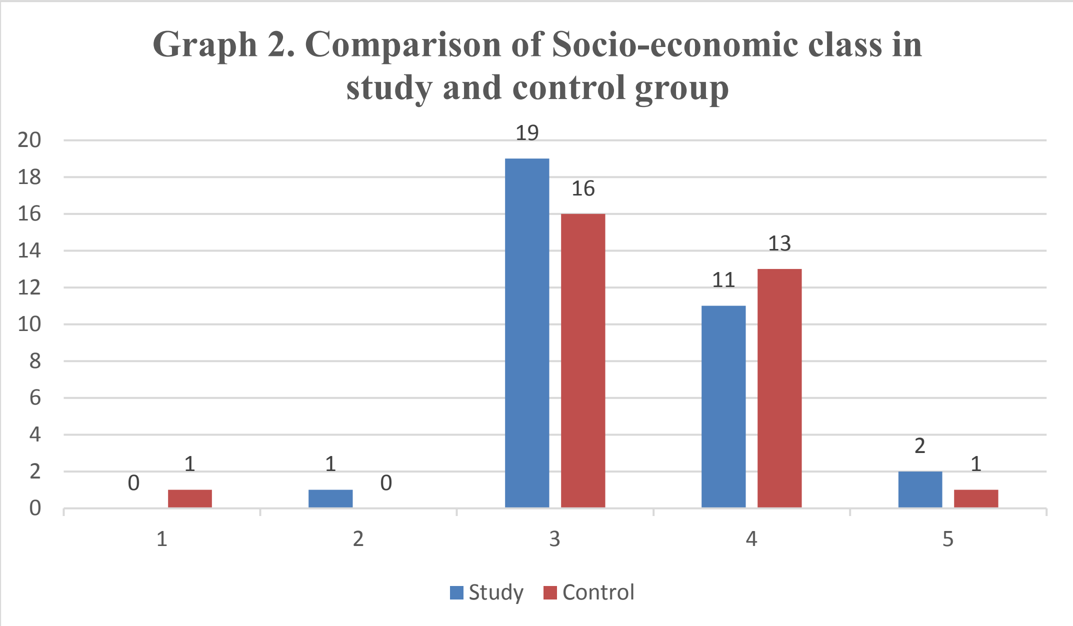
Sex of the child	Study		Control	
	Count	%	Count	%
Male	14	42.4%	15	48.4%
Female	19	57.6%	16	51.6%
Total	33	100.0%	31	100.0%
P=0.8				



In the present study majority of subjects were female in study (57.6%) as well as control (51.6%) group. In comparison the distribution of male was less in study group (42.4%) as well as control group (48.4%) though the difference was not statistically significant (p=0.8).

Table 2. Comparison of Socio-economic class in study and control group.

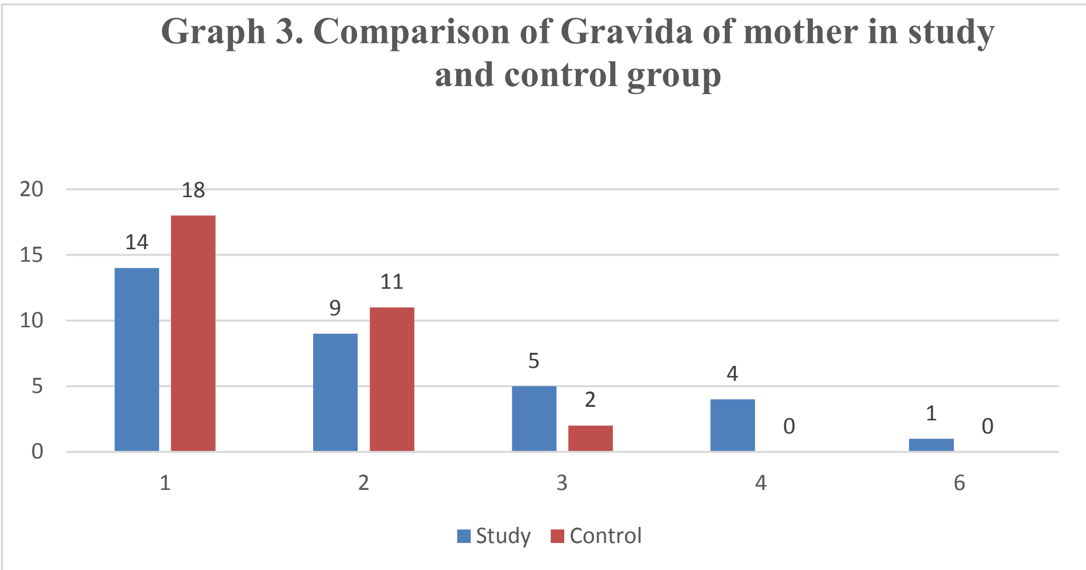
Socio-Economic	Study		Control	
	Count	%	Count	%
1	0	0%	1	3.2%
2	1	3.0%	0	0%
3	19	57.6%	16	51.6%
4	11	33.3%	13	41.9%
5	2	6.1%	1	3.2%
Total	33	100.0%	31	100.0%
P=0.61				



With regards to socioeconomic status, 19 (57.6%) in the study and 16 (51.6%) in control belonged to class 3, followed by 11 (33.3%) in the study and 13 (41.9%) in the control group belonged to class 4, only a few participants on either side belonged to class 1, 2 and 5 according to revised modified B.G. Prasad's socioeconomic classification. So, the two groups were statistically comparable.

Table 3. Comparison of Gravida of mother in study and control group

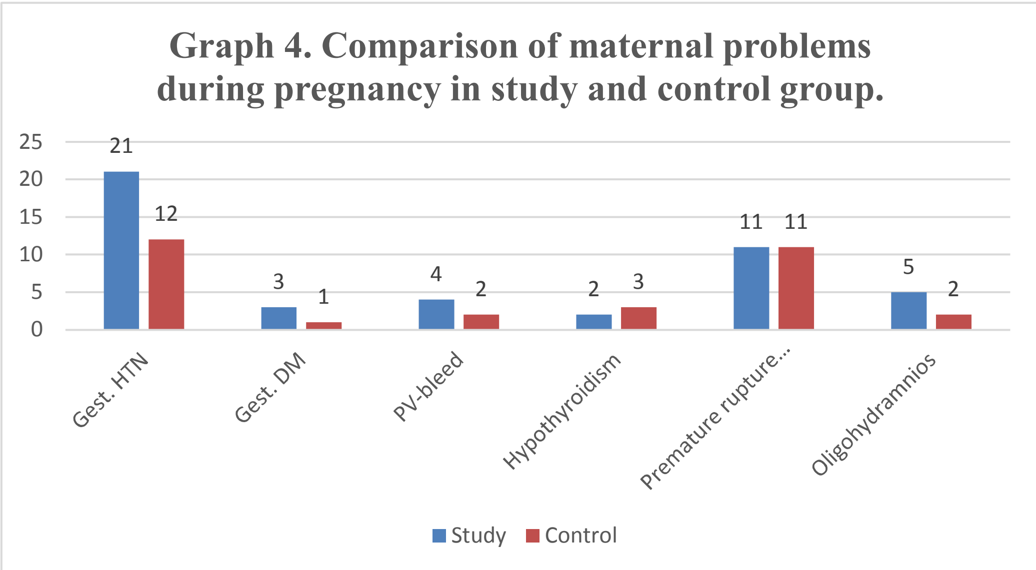
Gravida	Study		Control	
	Count	%	Count	%
1	14	42.4%	18	58.0%
2	9	27.3%	11	35.5%
3	5	15.2%	2	6.5%
4	4	12.1%	0	0%
6	1	3.0%	0	0%
Total	33	100.0%	31	100.0%
P=0.14				



The majority of mothers in study (14) as well as control group (18) were primigravida, followed by 19 mothers in study and 13 in control group who had gravida of at least 2 (p=0.14). The groups were comparable as the difference between the two were not statistically significant.

Table 4. Comparison of maternal complications during pregnancy in study and control group.

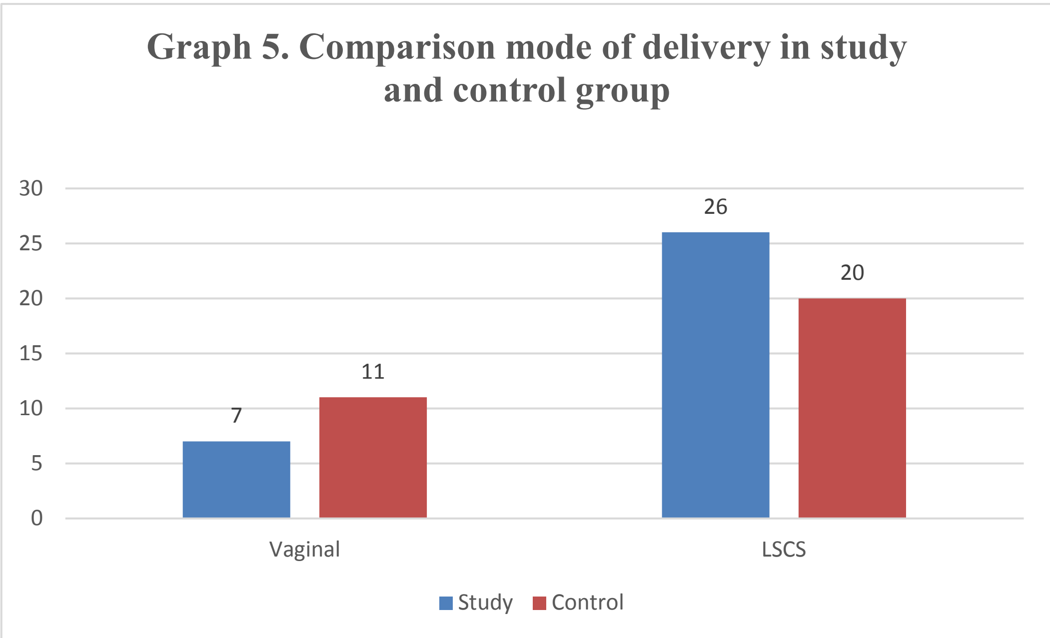
	Study (n=33)		Control (n=31)		p-value
	Count	%	Count	%	
Gest. HTN	21	63.6%	12	38.7%	0.08
Gestational diabetes mellitus	3	9.1%	1	3.2%	0.61
PV-bleed	4	12.1%	2	6.5%	0.67
Hypothyroid	2	6.1%	3	9.7%	0.67
PROM	11	33.3%	11	35.5%	1
Oligohydramnios	5	15.2%	2	6.5%	0.43



The majority of the mothers in study 21 (63.6%) and 12 (38.7%) in the control group had a history of gestational hypertension during pregnancy (p= 0.14), followed by 11 in the study and 11 in control had a history of premature rupture of membrane (p=1), few mothers in either group had a history of gestational diabetes mellitus, per vaginal bleed, hypothyroidism. The groups were comparable as the difference between the two were not statistically significant.

Table 5. Comparison mode of delivery in study and control group

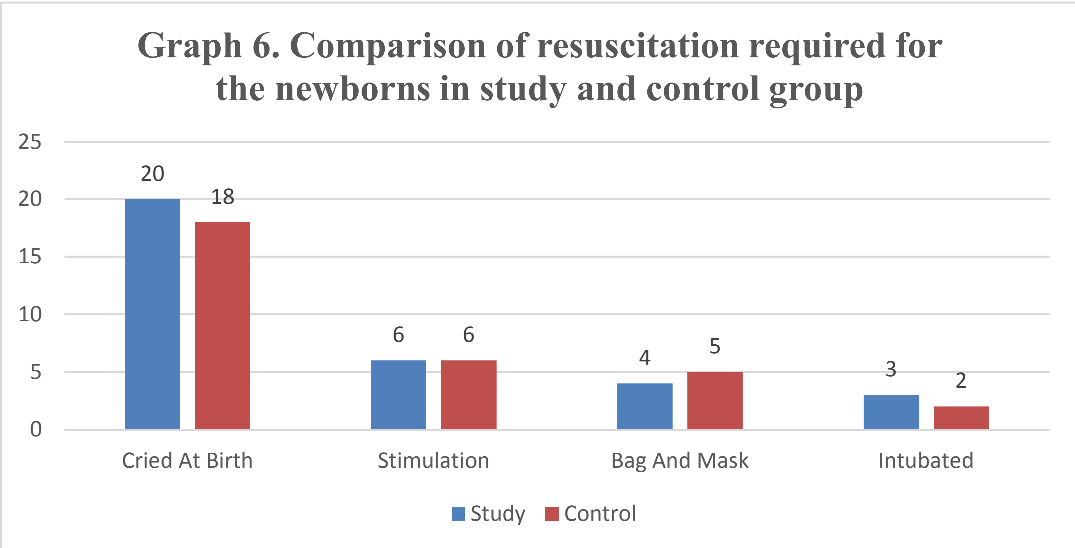
Method of Delivery	Study		Control	
	Count	%	Count	%
Vaginal	7	21.2%	11	35.5%
LSCS	26	78.8%	20	61.3%
Total	33	100.0%	31	100.0%
P=0.27				



The majority of mothers in either group, 26 (78.8%) in the study and 20(61.3%) in the control group, had LSCS. Most of them were emergency LSCS. 7(21.2%) mothers in the study and 11(35.5%) in the control group gave birth to the baby vaginally (P=0.27). The difference between study and control were statistically insignificant and hence were comparable.

Table 6. Comparison of resuscitation required for the new-borns in study and control group

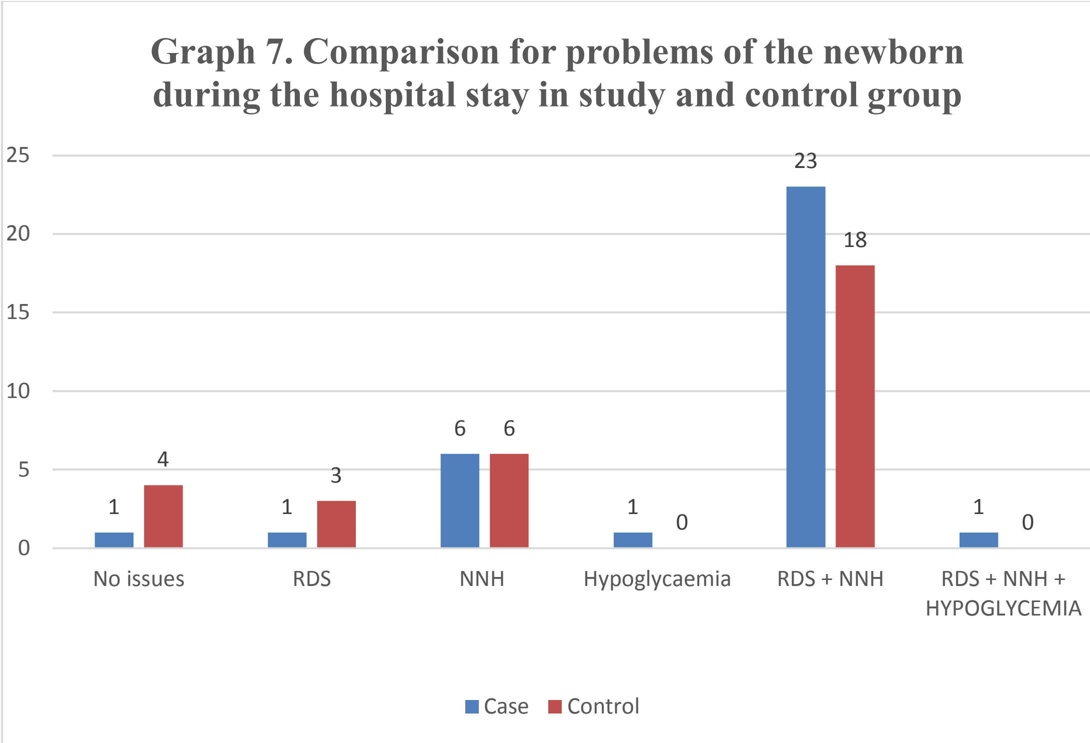
Resuscitation	Study		Control	
	Count	%	Count	%
Cried At Birth	20	60.6%	18	58.1%
Stimulation	6	18.2%	6	19.4%
Bag And Mask	4	12.1%	5	16.1%
Intubated	3	9.1%	2	6.5%
Total	33	100.0%	31	100.0%
P=0.95				



13 babies in either group required resuscitation, 6(18.2%) in the study and 6(19.4%) in control required stimulation, 4(12.1%) new-borns in the study and 5(16.1%) in the control group required bag and mask ventilation, 3(9.1%) new-borns in the study needed intubation during resuscitation versus 2(6.5%) in the control group(p=0.95). However, the differences observed were not statistically significant and hence the groups were comparable.

Table 7. Comparison for problems of the new-born during the hospital stay in study and control group

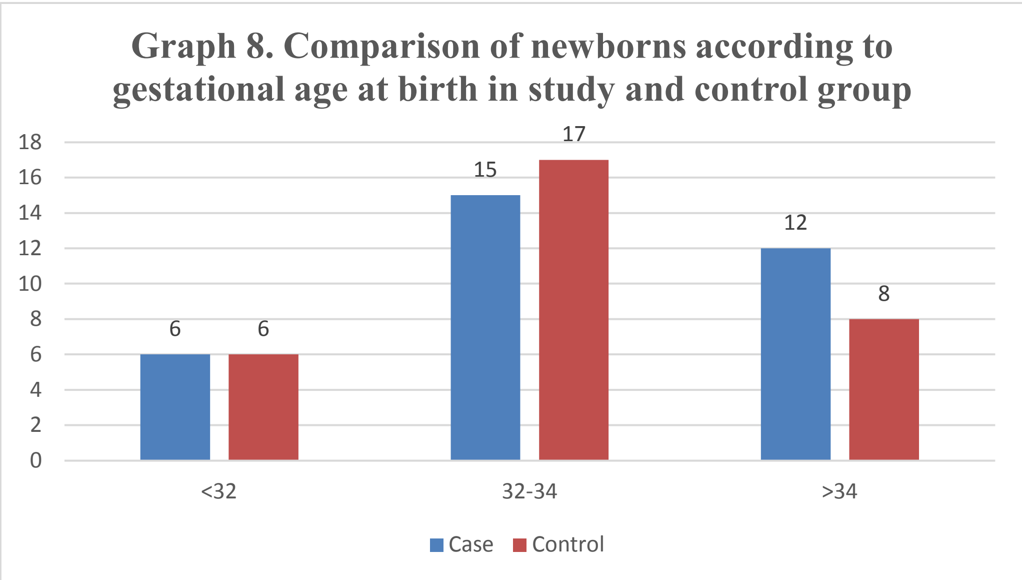
Problems	Case		Control	
	Count	%	Count	%
No issues	1	3.0%	4	12.9%
Problems	32	97.0%	27	87.1%
RDS (respiratory distress syndrome)	1	3.0%	3	9.7%
NNH (neonatal hyperbilirubinemia)	6	18.2%	6	19.4%
Hypoglycaemia	1	3.0%	0	0%
RDS + NNH	23	69.7%	18	58.1%
RDS + NNH + Hypoglycaemia	1	3.0%	0	0%
NEC	0	0%	0	0%
Feed intolerance	0	0%	0	0%
Total	33	100.0%	31	100.0%
P=0.37				



The majority of neonates 23 (69.7%) in the study and 18(58.1%) in control, had respiratory distress syndrome with hyperbilirubinemia during their NICU stay. Followed by 6 newborns in either group who had only hyperbilirubinemia, few infants had hypoglycaemia and respiratory distress syndrome alone (P=0.37). However, the differences observed were not statistically insignificant. Hence the groups were comparable.

Table 8. Comparison of newborns according to gestational age at birth in study and control group

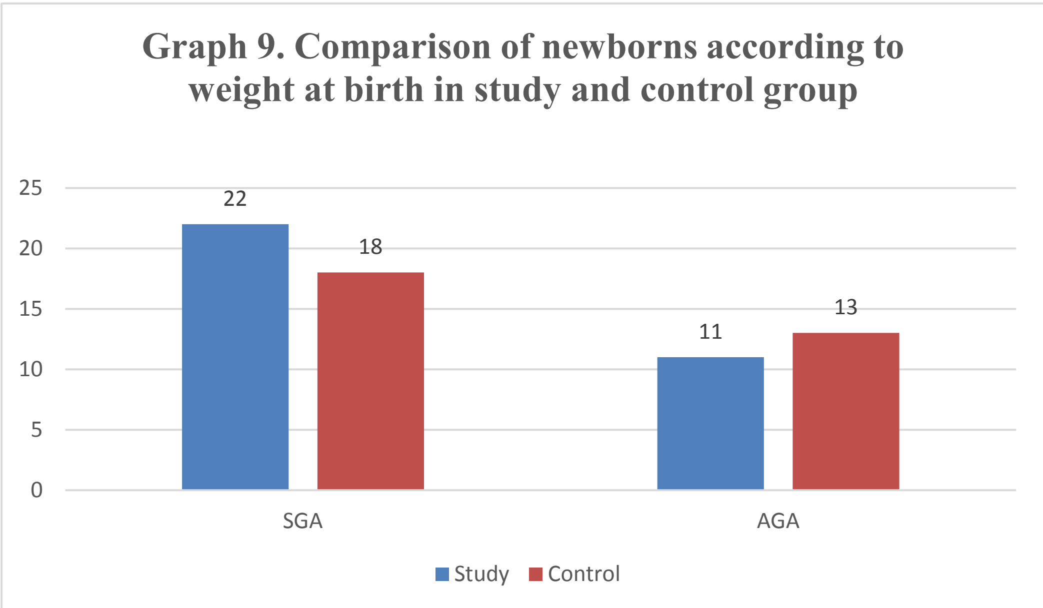
Gestational age	Case		Control	
	Count	%	Count	%
<32	6	18.2%	6	19.4%
32-34	15	45.5%	17	54.8%
>34	12	36.4%	8	25.8%
Total	33	100.0%	31	100.0%
P=0.65				



In this study, 6 new-borns in the study and 6 in the control group were less than 32 of gestation, 15(45.5%) new-borns in cases and 17(54.8%) in the control group were between 32-34 wk gestational age, 12 in cases and 8 in the control group were more than 34 weeks of gestation. (p=0.65) So, the difference between cases and control were statistically not significant, and they were comparable.

Table 9. Comparison of new-borns according to weight at birth in study and control group

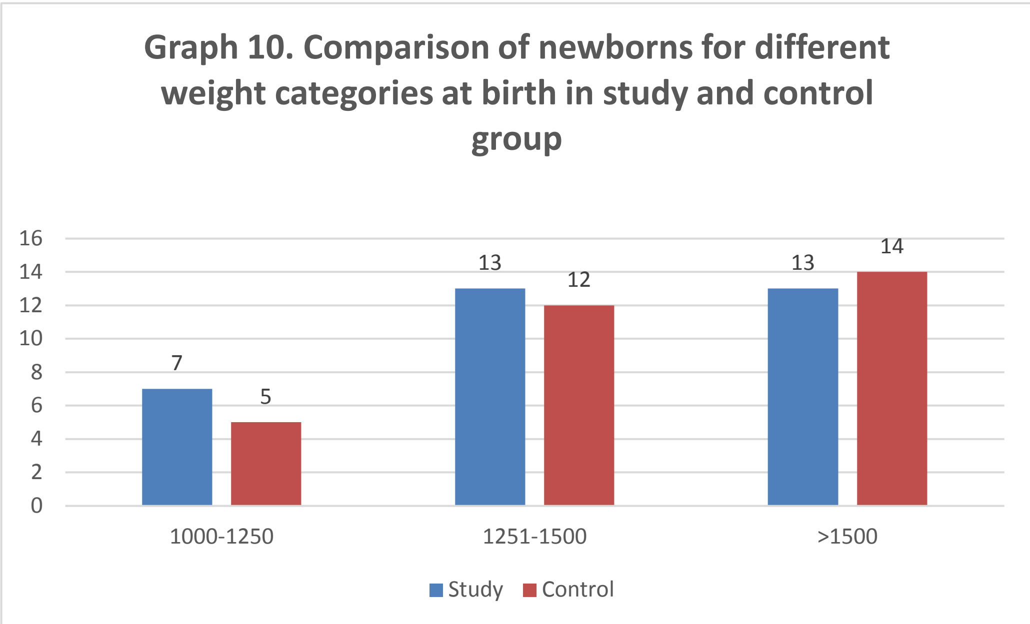
weight (SGA/AGA/LGA)	Study		Control	
	Count	%	Count	%
SGA	22	66.7%	18	58.1%
AGA	11	33.3%	13	41.9%
Total	33	100.0%	31	100.0%
P=0.61				



The majority of the newborns, 22(66.7%) in the study and 18(58.1%) in the control group were (SGA) Small for Gestational Age, (P=0.67). The differences between the two groups were statistically insignificant, and hence they were comparable.

Table 10. Comparison of new-borns for different weight categories at birth in study and control group

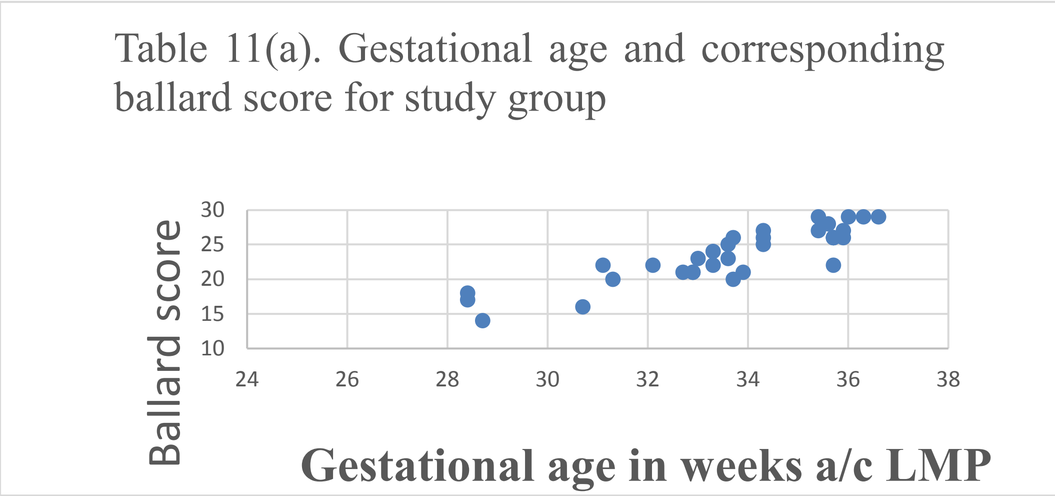
Weight	Study		Control	
	Count	%	Count	%
1000-1250	7	21.2%	5	16.1%
1251-1500	13	39.4%	12	38.7%
>1500	13	39.4%	14	45.2%
Total	33	100.0%	31	100.0%
P=0.84				

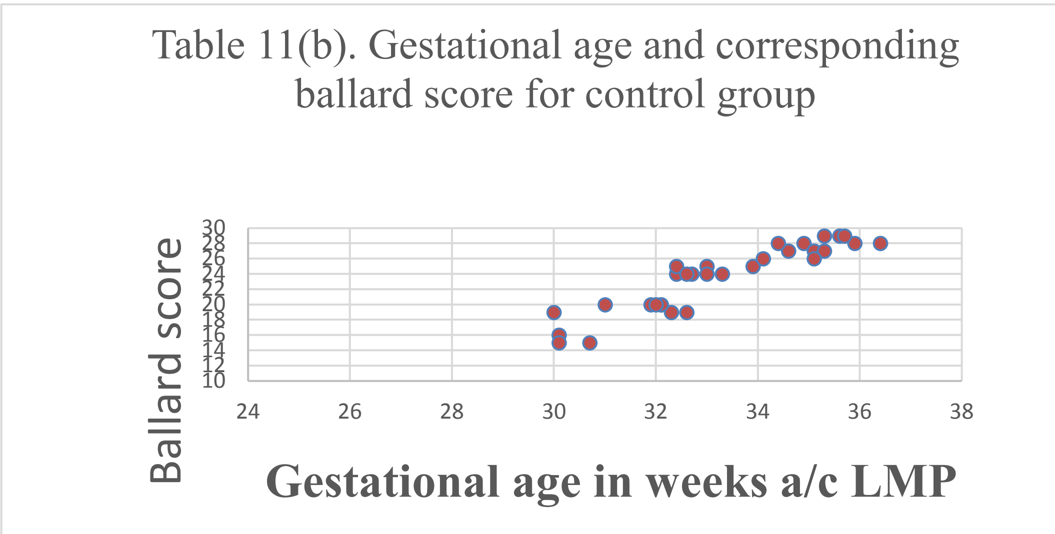


In this study, 13(39.4%) in the study and 14(45.25%) in control belong to >1500g weight category, followed by 13(39.4%) in the study versus 12(38.7%) in the control group were in 1251-1500g weight category, 7(21.2%) new-borns in the study and 5(16.1%) in control were in 1000-1250g weight category (P=0.84). Hence, the two groups were comparable as the differences were not statistically significant.

Table 11. Comparison based on Ballard score in study and control group

Variable	Group	Minimum	Maximum	Mean	SD	Median	IQR	P-value
BALLARD SCORE	Study	14	29	23.58	3.95	24.00	6	0.96
	Control	15	29	23.55	4.29	24.00	7	

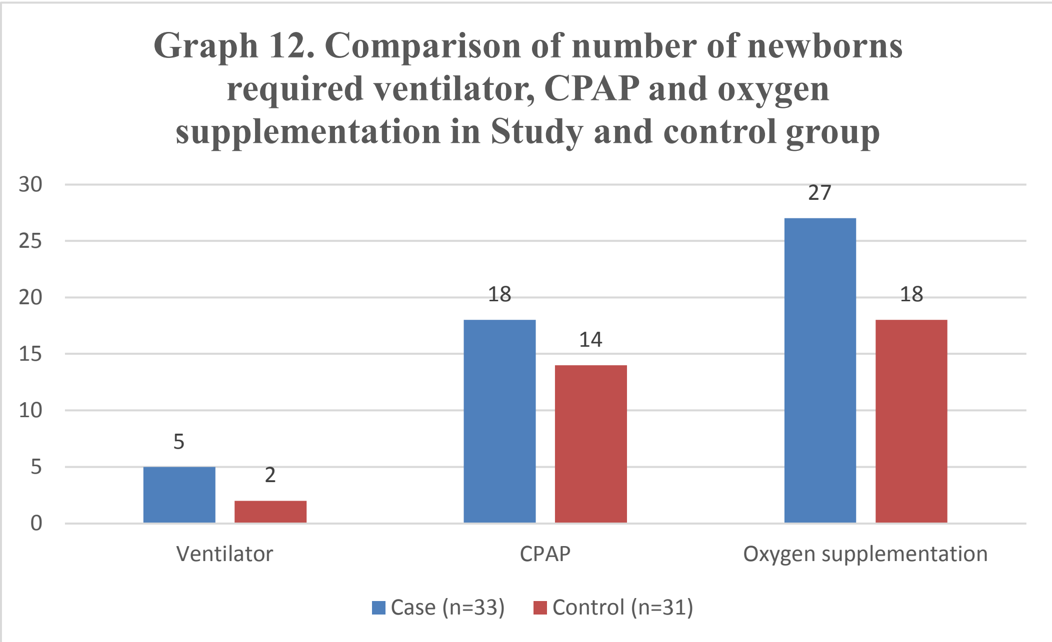




In this study, the mean Ballard score is 23.58 for study and 23.55 for the control group (P-value of 0.96 and a mean difference of 0.03). Hence, they were comparable.

Table 12 Comparison of number of new-borns required ventilator, CPAP and oxygen supplementation in study and control group.

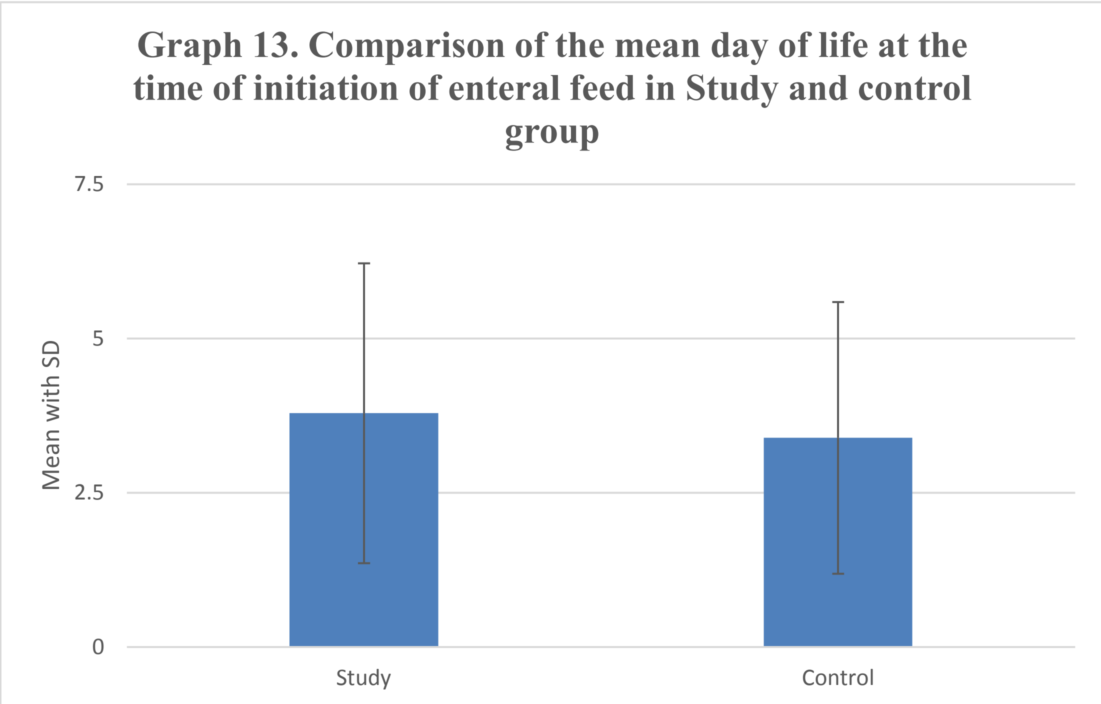
Support required	Study (n=33)		Control (n=31)		p-value
	Count	%	Count	%	
Ventilator	5	15.2%	2	6.5%	0.43
CPAP	18	54.5%	14	45.2%	0.62
Oxygen supplementation	27	81.8%	18	58%	0.06



In the present study majority of newborns, 27(81.8%) in the study and 18(58.1%) in the control group, required oxygen supplementation (P=0.06). Followed by 18(54.5%) in the study and 14(45.2%) in the control group, needed CPAP support (P=0.62) and few of them in either group required ventilator support. The groups were comparable as the difference between the two were not statistically significant.

Table 13. Comparison of the mean day of life at the time of initiation of enteral feed in Study and control group.

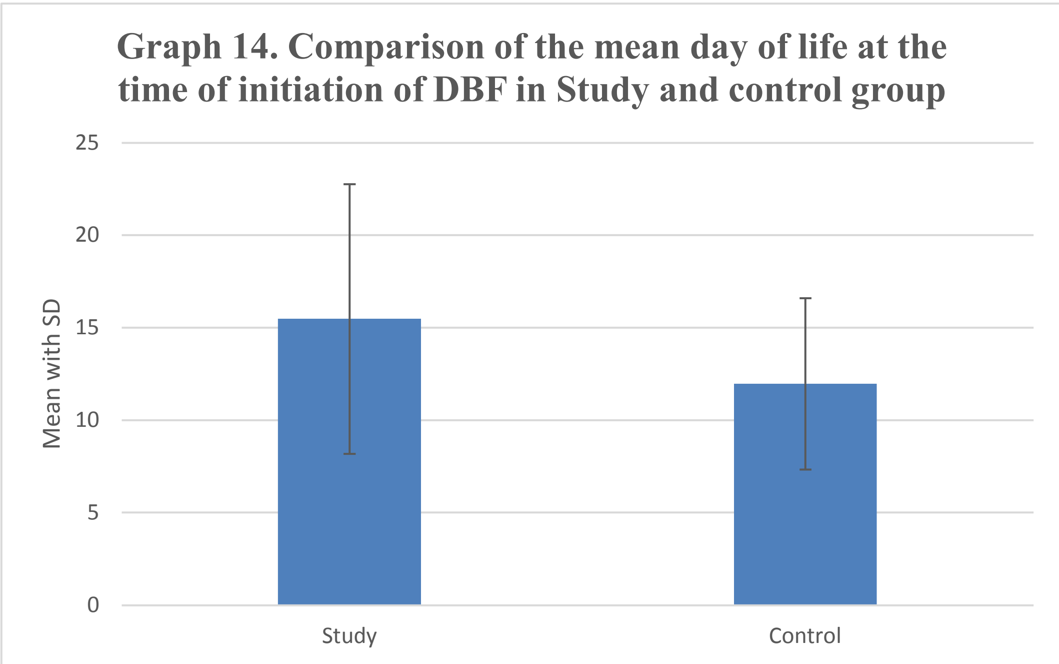
Variable	Group	Minimum	Maximum	Mean	SD	Median	IQR	P-value
Day of life at the initiation of feed	Study	1	10	3.79	2.43	3.00	3	0.58
	Control	1	8	3.39	2.20	3.00	4	



In the present study mean day of life at the time of enteral feed initiation was 3.79 for the study and 3.39 for the control group (mean difference of 0.40 and P-value of 0.58). The groups were comparable as the difference between the two was not statistically significant.

Table 14. Comparison of the mean day of life at the time of initiation of DBF in Study and control group

Variable	Group	Minimum	Maximum	Mean	SD	Median	IQR	P-value
Initiation of DBF (day of life)	Study	3	31	15.48	7.29	13.00	9	0.05
	Control	5	22	11.97	4.64	12.00	7	



In the present study mean day of life at the time of initiation of direct breastfeeding was 15.48 for the study and 11.48 for the control (mean difference of 3.51 and P-value of 0.05). This difference between the two is statistically not significant.

Table 15. Comparison of anthropometric parameters at birth in study and control group at birth.

Variable	Group	Minimum	Maximum	Mean	SD	Median	IQR	P-value
Length (in cm) at birth	Study	36.5	48.0	41.86	2.35	42.00	3.0	0.8
	Control	36.3	45.0	41.55	2.54	41.50	4.0	
Head circumference at birth (in cm)	Study	26.0	33.7	29.68	1.74	29.50	2.7	0.5
	Control	25.5	33.0	29.32	1.81	30.00	3.0	
weight (in gram) at birth	Study	1060	1790	1442.42	219.50	1400.00	300	0.46
	Control	1030	1790	1480.97	211.57	1420.00	400	

when we compared the study and control groups for length (mean difference of 0.31, p=0.8), head circumference (mean difference of 0.36, p=0.5) and weight at birth (mean difference of 38.5, p=0.46) we did not find any statistically significant difference. Hence, they were comparable.

Table 16. Comparison of overall increase in anthropometric parameters in study and control group between birth and follow up at the end of the trial.

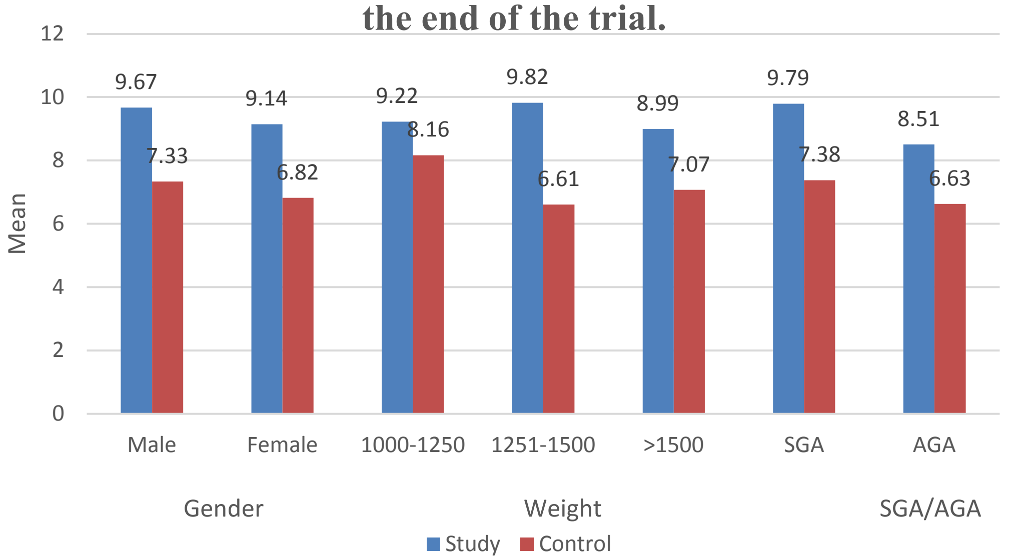
Variable	Group	Minimum	Maximum	Mean	SD	Median	IQR	P-value
weight gain (g/kg/day) from birth	Study	2.00	13.25	9.37	2.54	9.67	3.61	<0.001
	Control	3.50	12.36	7.07	1.90	7.20	3.11	
Increase in head circumference (cm/wk) from birth	Study	0.50	0.93	0.73	0.10	0.70	0.15	0.75
	Control	0.50	0.84	0.72	0.08	0.72	0.14	
Increase in length(cm/wk) from birth	Study	0.57	1.38	1.09	0.20	1.12	0.16	0.34
	Control	0.81	1.30	1.07	0.14	1.10	0.27	

When we compared anthropometric parameters for gain from birth to the end of the study, significant weight gain was observed in the study group (mean difference of 2.2g, p <0.001). The increase in head circumference (mean difference of 0.01, p=0.75) and length (mean difference of 0.02, p=0.34) during this period were statistically not significant.

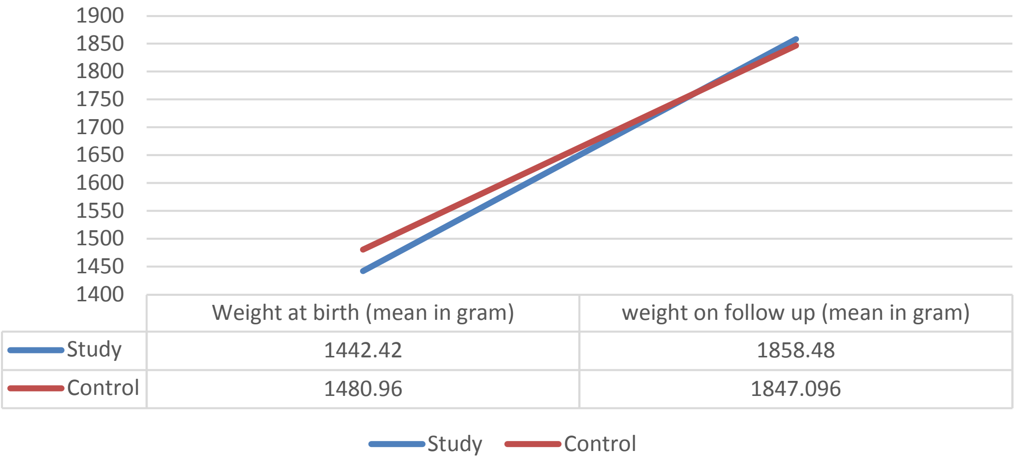
Table 17. Comparison of weight gain in study and control sub-groups between birth and follow up at the end of the trial.

Variable	Gender	Study		Control		P-value
		Mean	SD	Mean	SD	
Weight gain (g/kg/day) from birth.	Male	9.67	2.42	7.33	2.20	0.01
	Female	9.14	2.66	6.82	1.59	0.05
Weight gain (g/kg/day) from birth	1000-1250	9.22	3.90	8.16	2.96	0.62
	1251-1500	9.82	2.36	6.61	1.88	0.001
	>1500	8.99	1.91	7.07	1.40	0.006
weight gain (g/kg/day) from birth	SGA	9.79	2.74	7.38	1.28	0.001
	AGA	8.51	1.92	6.63	2.51	0.06

Graph 15a. Comparison of weight gain in study and control sub-groups between birth and follow up at the end of the trial.



Graph 15b. Comparison of weight gain in study and control between birth and follow up at the end of the trial (line diagram)



	Weight at birth (mean in gram)	weight on follow up (mean in gram)
Study	1442.42	1858.48
Control	1480.96	1847.096

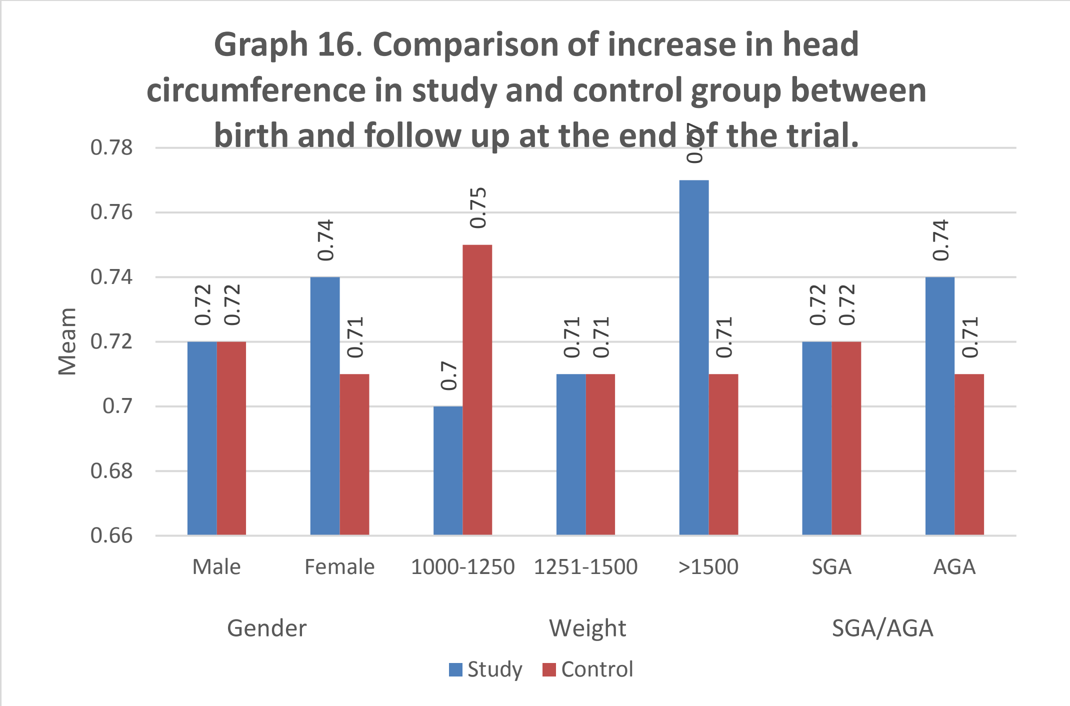
The weight gain from birth in the study group was significantly better for males (mean difference of 2.34g/kg/day, p-value 0.01) when compared to females (mean difference of 2.32g/kg/day, p-value 0.05), for whom the gain was not statistically significant.

When we compared weight gain from birth in different birth weight subgroups, we observed that there was statistically significant weight gain for >1500g (mean difference of 1.88g/kg/day, p=0.006) and 1251 -1500g (mean difference of 3.21g/kg/day and p-value of 0.001) weight category, although it was not statistically significant for 1000-1250g (mean difference of 1.06g/kg/day and p-value of 0.62).

Similarly, when we compared AGA and SGA, we observed that there was significant weight gain from birth for SGA babies (mean difference of 2.31g/kg/day, p=0.001), but it was not statistically significant for AGA (mean difference of 1.88g/kg/day and p-value of 0.06).

Table 18. Comparison of increase in head circumference in study and control group between birth and follow up at the end of the trial.

Variable	Weight	Study		Control		P-value
		Mean	SD	Mean	SD	
Increase in head circumference (cm/wk) from birth.	Male	0.72	0.09	0.72	0.09	0.98
	Female	0.74	0.11	0.71	0.07	0.51
Increase in head circumference (cm/wk) form birth	1000-1250	0.70	0.12	0.75	0.08	0.41
	1251-1500	0.71	0.10	0.71	0.09	0.98
	>1500	0.77	0.10	0.71	0.07	0.11
Increase in head circumference (cm/wk) from birth	SGA	0.72	0.10	0.72	0.06	0.98
	AGA	0.74	0.11	0.71	0.10	0.51

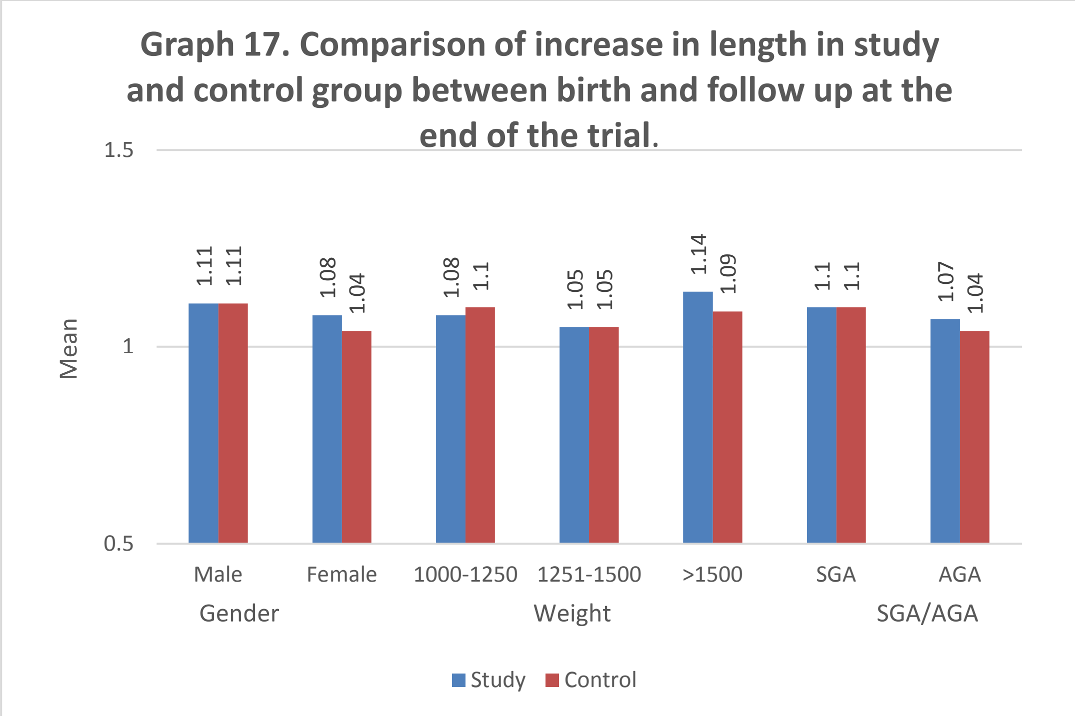


When we compared the increase in head circumference from birth to the end of the study, we did not observe any statistically significant difference between the groups (p=0.75)

Similarly, when the increase in head circumference from birth was compared for the gender of the newborns, weight at birth and SGA vs AGA newborns, there was no significant difference between the subgroups.

Table 19. Comparison of increase in length in study and control group between birth and follow up at the end of the trial.

Variable	Weight	Study		Control		p-value
		Mean	SD	Mean	SD	
Increase in length (cm/wk) from birth.	Male	1.11	0.19	1.11	0.13	0.94
	Female	1.08	0.21	1.04	0.15	0.61
Increase in length(cm/wk)	1000-1250	1.08	0.22	1.10	0.15	0.86
	1251-1500	1.05	0.26	1.05	0.14	0.99
	>1500	1.14	0.09	1.09	0.15	0.30
Increase in length(cm/wk) from birth	SGA	1.10	0.19	1.10	0.14	0.99
	AGA	1.07	0.22	1.04	0.14	0.65



When we compared the increase in length from birth to the end of the study, we did not observe any statistically significant difference between the groups. ($p=0.34$).

Similarly, when the increase in length from birth was compared for the gender of the newborns, weight at birth and SGA vs AGA newborns, there was no significant difference between the subgroups.

Table 20. Comparison of duration of hospital stay and day of life at the time enrollment between study and control group.

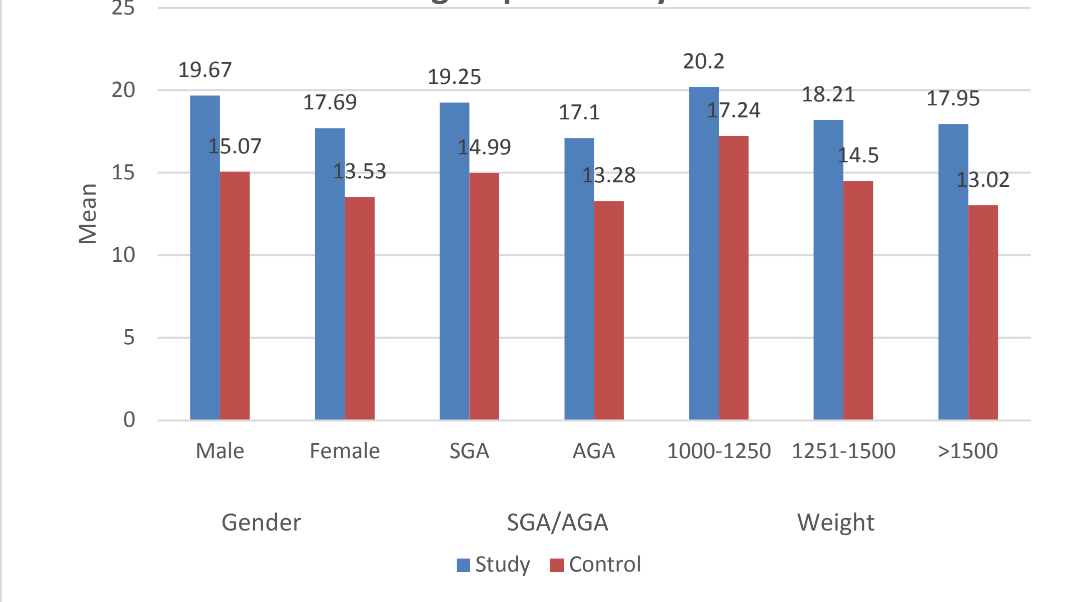
Variable	Study		Control		P-value
	Mean	SD	Mean	SD	
Duration of stay in hospital	13.79	3.71	12.32	4.29	0.15
Day of life at enrollment day	9.39	2.55	9.16	2.25	0.7

The mean duration of hospital stay was 13.79 in the study group versus 12.32 for the control group (mean difference of 1.47 with a P-value of 0.15). The mean day of life at the time of enrollment in the study was 9.39 and 9.16 in the control group (mean difference of 0.23 with P-value of 0.7). These differences were not statistically significant.

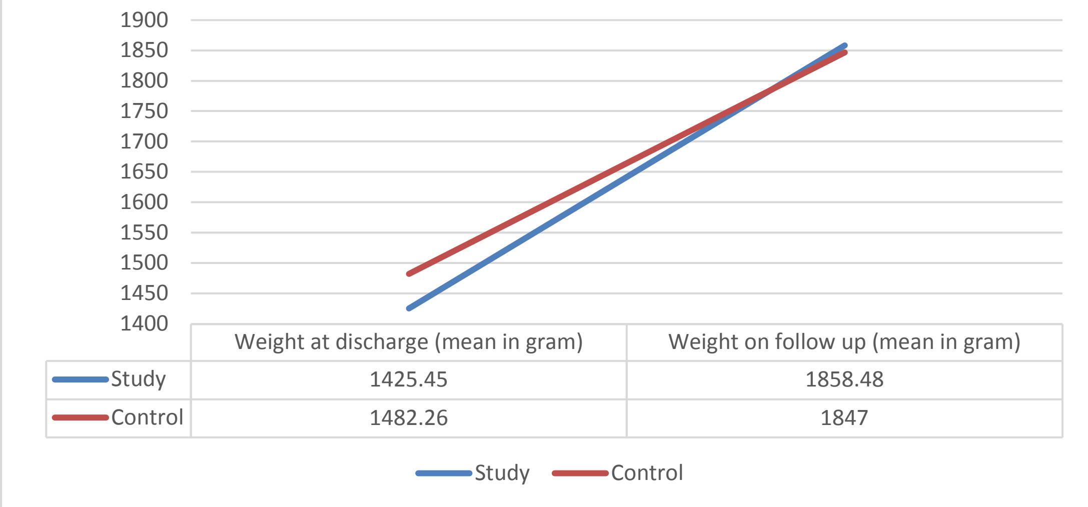
Table 21. Comparison of weight gain between discharge and follow up at the end of the trial in the different subgroups of study and control.

Variable	Study		Control		P-value
	Mean	SD	Mean	SD	
Weight gain(g/kg/day) between discharge and follow up	18.53	4.45	14.27	3.78	<0.001

Variable	Gender	Study		Control		
		Mean	SD	Mean	SD	
Weight gain in g/kg/day between discharge and follow up	Male	19.67	4.05	15.07	3.81	0.004
	Female	17.69	4.64	13.53	3.71	0.007
Weight gain in g/kg/day between discharge and follow up	SGA	19.25	4.75	14.99	2.68	0.002
	AGA	17.10	3.54	13.28	4.87	0.04
Weight gain in g/kg/day between discharge and follow up	1000-1250	20.20	7.11	17.24	5.76	0.46
	1251-1500	18.21	4.60	14.50	2.78	0.02
	>1500	17.95	2.05	13.02	3.33	<0.001



Graph 18a. Comparison of weight gain between discharge and follow up at the end of the trial in the different subgroups of study and control



Graph 18b. Comparison of weight gain between discharge and follow up at the end of the trial in the study and control.

The weight gain from discharge to the end of the trial was statistically significant in the study group when compared with the control group (mean difference of 4.26g/kg/day, p-value 0.001).

The weight gain from discharge to end of the study was significantly better for males (mean difference of 4.50g/kg/day, p-value 0.004) as well as females (mean difference of 4.36g/kg/day, p-value 0.007) in the study group when compared to control group.

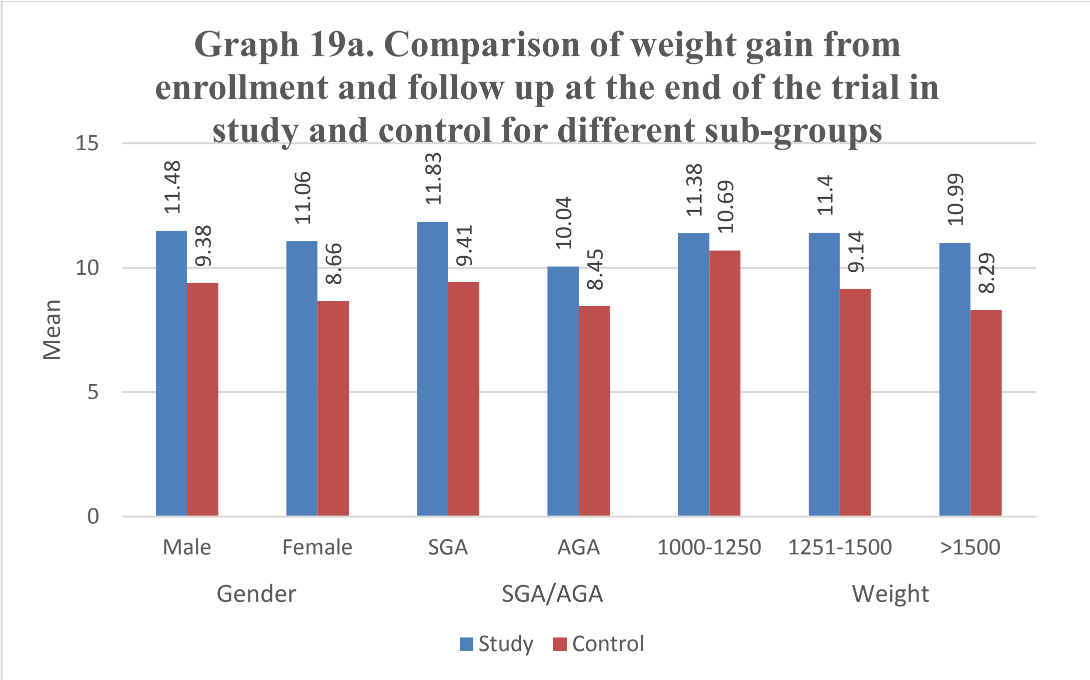
When we compared weight gain from discharge in different birth weight subgroups, we observed that there was statistically significant weight gain for >1500g (mean difference of 4.93g/kg/day and p-value of <0.001) and 1251 -1500g (mean difference of 3.71g/kg/day and p-value of 0.02) weight category, although it was not statistically significant for 1000-1250g ((mean difference of 2.96g/kg/day and p-value of 0.46).

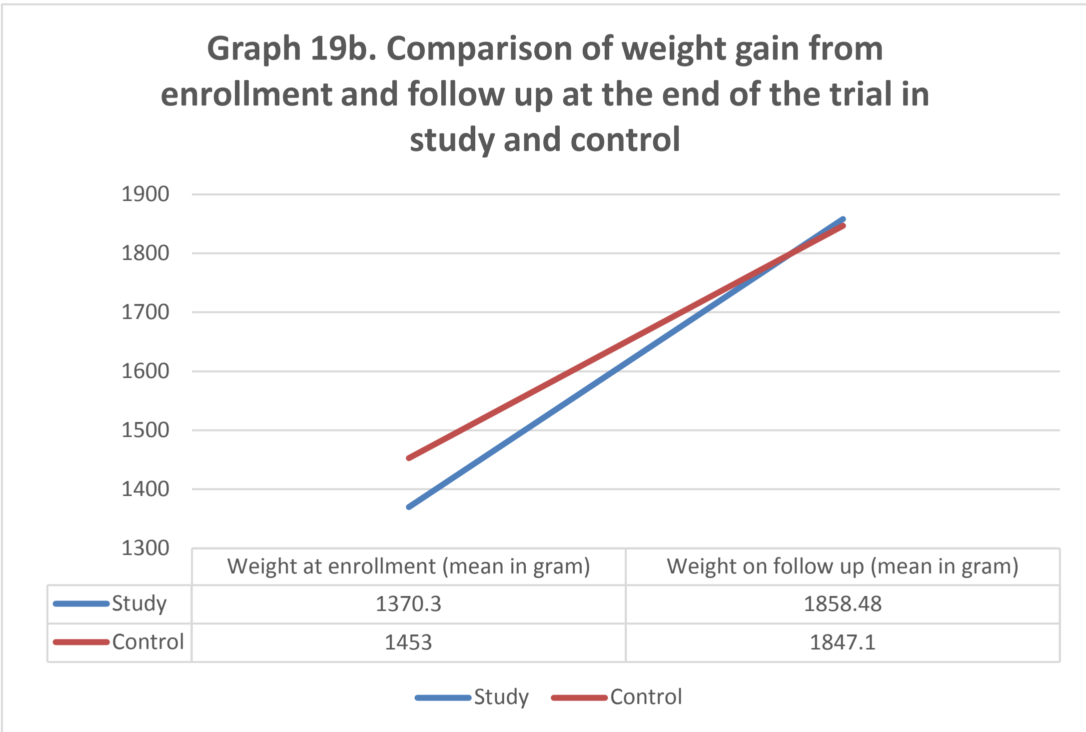
Similarly, when we compared AGA and SGA newborns of both the groups, statistically significant weight gain was observed for SGA (mean difference of 4.26/kg/day, p-value 0.002) but not for AGA (mean difference of 3.82g/kg/day, p-value 0.04).

Table 22. Comparison of weight gain from enrollment and follow up at the end of the trial in study and control for different sub-groups.

Variable	Study		Control		P-value
	Mean	SD	Mean	SD	
Weight gain(g/kg/day) from day of enrollment in study	11.23	2.47	9.01	2.60	0.001

Variable	Gender	Study		Control		P-value
		Mean	SD	Mean	SD	
Weight gain from enrollment in g/kg/day	Male	11.48	2.48	9.38	2.65	0.04
	Female	11.06	2.52	8.66	2.59	0.009
Weight gain from enrollment in g/kg/day	SGA	11.83	2.64	9.41	2.32	0.004
	AGA	10.04	1.61	8.45	2.94	0.12
Weight gain from enrollment in g/kg/day	1000-1250	11.38	3.89	10.69	4.51	0.78
	1251-1500	11.40	2.16	9.14	2.07	0.01
	>1500	10.99	1.99	8.29	2.01	0.002





The weight gain from enrollment to the end of the trial was statistically significant in the study group when compared with the control group (mean difference of 2.22g/kg/day, p-value 0.001).

The weight gain from enrollment to end of the study was significantly better for males (mean difference of 2.10g/kg/day, p-value 0.04) as well as females (mean difference of 2.40g/kg/day, p-value 0.009).

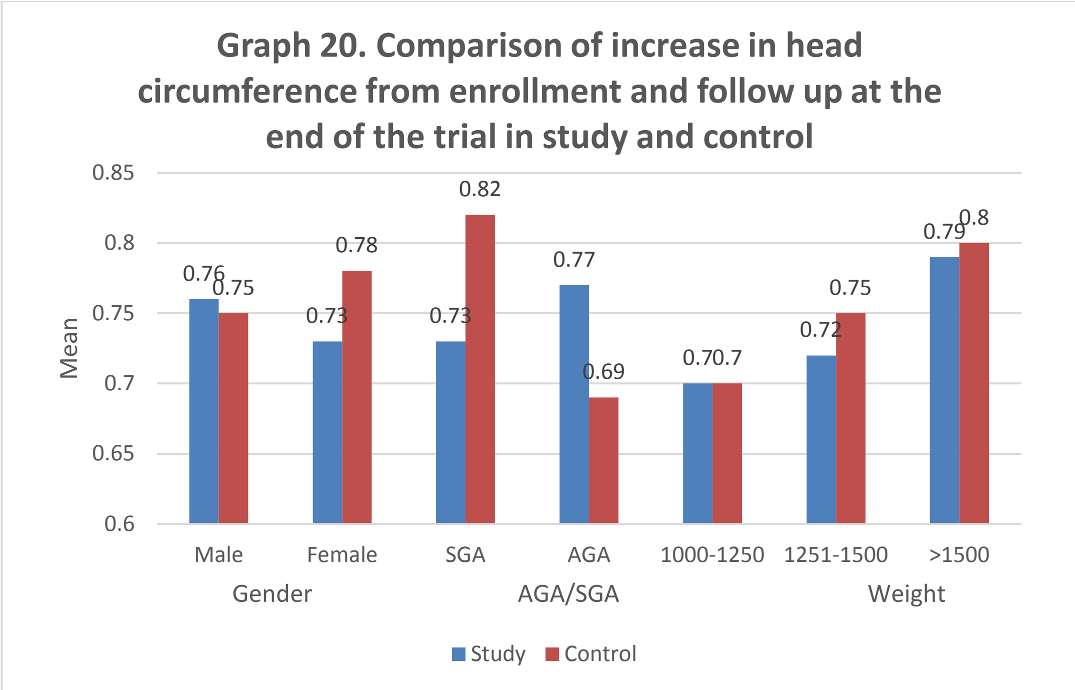
When we compared weight gain from enrollment in different birth weight subgroups, we observed that there was statistically significant weight gain for >1500g (mean difference of 2.7g/kg/day and p-value of 0.01) and 1251 -1500g (mean difference of 2.36g/kg/day and p-value of 0.01) weight category, although it was not statistically significant for 1000-1250g (mean difference of 0.69g/kg/day and p-value of 0.78).

Similarly, when we compared AGA and SGA newborns of both the groups, statistically significant weight gain was observed for SGA (mean difference of 2.42g/kg/day, p-value 0.004) but not for AGA (mean difference of 1.59g/kg/day, p-value 0.12).

Table 23. Comparison of increase in head circumference from enrollment and follow up at the end of the trial in study and control.

Variable	Study		Control		P-value
	Mean	SD	Mean	SD	
Increase in HC from enrollment in cm/week	0.74	0.17	0.76	0.17	0.69

Variable	Gender	Study		Control		P-value
		Mean	SD	Mean	SD	
Increase in HC from enrollment in cm/wk	Male	0.76	0.11	0.75	0.17	0.73
	Female	0.73	0.21	0.78	0.17	0.47
Increase in HC from enrollment in cm/wk	SGA	0.73	0.15	0.82	0.15	0.08
	AGA	0.77	0.21	0.69	0.17	0.28
Increase in HC from enrollment in cm/wk	1000-1250	0.70	0.18	0.70	0.14	0.96
	1251-1500	0.72	0.15	0.75	0.19	0.72
	>1500	0.79	0.19	0.80	0.16	0.91



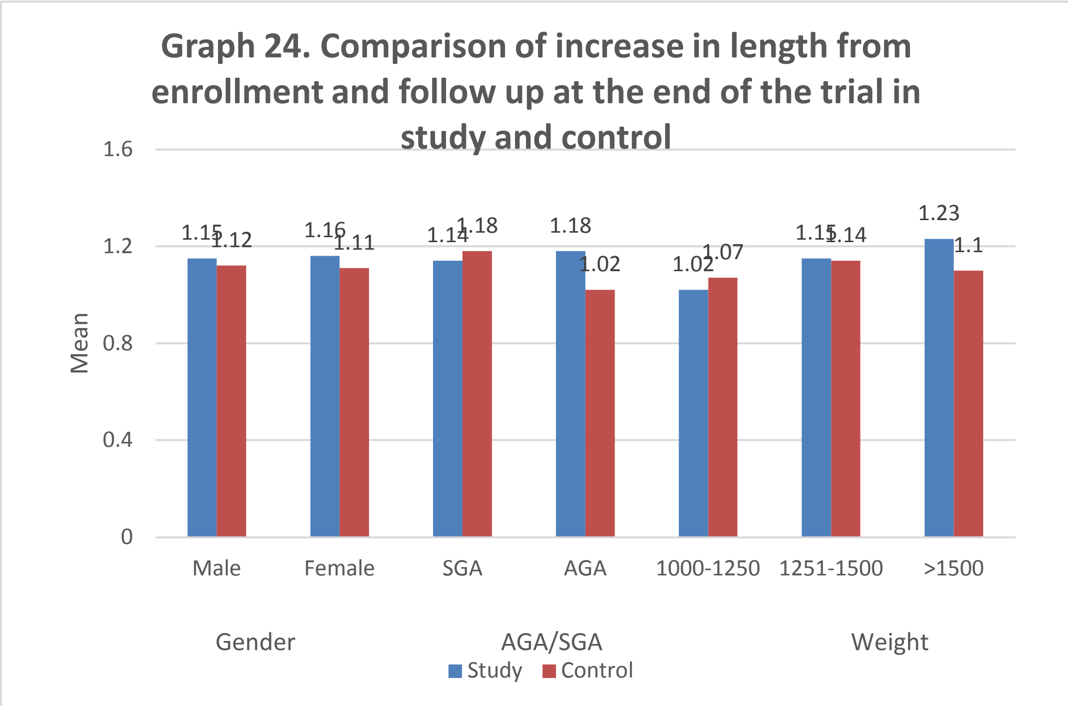
When we compared the increase in head circumference from the time of enrollment to the end of the study, we did not observe any statistically significant difference between the groups. **(p=0.69)**

Similarly, when the increase in head circumference was compared for the gender of the newborns, weight at birth and SGA vs AGA newborns, there was no significant difference between the subgroups.

Table 24. Comparison of increase in length from enrollment and follow up at the end of the trial in study and control.

Variable	Study		Control		P-value
	Mean	SD	Mean	SD	
Increase in Length from day of enrollment in cm/wk	1.16	0.26	1.11	0.22	0.45

Variable	Gender	Study		Control		P-value
		Mean	SD	Mean	SD	
Increase in Length from enrollment in cm/wk	Male	1.15	0.26	1.12	0.21	0.73
	Female	1.16	0.26	1.11	0.23	0.50
Increase in Length from enrolment in cm/wk	SGA	1.14	0.25	1.18	0.20	0.64
	AGA	1.18	0.29	1.02	0.21	0.13
Increase in Length from enrolment in cm/wk	1000-1250	1.02	0.24	1.07	0.20	0.71
	1251-1500	1.15	0.33	1.14	0.20	0.88
	>1500	1.23	0.16	1.10	0.24	0.12



When we compared the increase in length from the time of enrollment to the end of the study, we did not observe any statistically significant difference between the groups. **(p=0.73)**

Similarly, when the increase in length was compared for the gender of the newborns, weight at birth and SGA vs AGA newborns, there was no significant difference between the subgroups.

DISCUSSION

Preterm very low birth weight new-borns require additional calories, minerals, and protein for appropriate catch-up growth and improved neurodevelopmental outcomes.[15] Preterm birth rates in 184 nations range from 5 to 18 per cent; in India, 13 per cent (3.5 million kids out of 27 million) are born prematurely.[1,2] According to the National Family Health Survey (NFHS-4) data from 2015–16, 18% of Indian children are born with low birth weight.[13]

The World Health Organization (WHO) defines low birth weight (LBW) as a weight of less than 2.5 kg at birth. The global prevalence of LBW is 15.5 per cent, resulting in nearly 20 million LBW neonates being born each year, with 96.5 per cent of them being born in developing countries.[3] Premature infants with very low birth weight (VLBW) who are exclusively given unfortified human milk can have nutrient deficiencies as a result of inadequate protein and mineral content in expressed human milk, resulting in extra-uterine growth restriction, which translates to poorer neurodevelopmental outcomes and cognitive skills between the ages of 18 and 24 months.[9,14] The treatment standard has been suggested to be fortification of expressed human milk.[15] However, multi-nutrient fortifiers have been associated with negative side effects such as feed intolerance and necrotizing enterocolitis.[8]

The third trimester of pregnancy is the most crucial phase for human brain growth and development, and these processes of developmental occur in the NICU environment for preterm VLBW neonates. According to recent research, it is not only the quantity of growth that matters but also the quality of growth. Improved neurodevelopment has been linked to improvements in linear growth and fat-free body mass in VLBW preterm newborns. Thus, strengthening premature newborns nutritional care has become a top priority for improving neurodevelopmental outcomes. To address the high requirements of preterm LBW newborns, human milk should be supplemented with nutrients short in supply, particularly calcium, phosphate, and protein.[9]

Nutrient deficiencies such as iron deficiency, zinc deficiency, osteopenia, vitamin A and vitamin D inadequacy have all been observed in neonates fed only on un-supplemented own mother's milk.[3] Those neonates who had accumulated mineral deficiencies had a greater risk of metabolic bone disease and poor skeletal growth than those born at term, but the long-term impacts on bone mass and health are unknown.[44] Moreover, there is concern that prenatal and early childhood growth restriction may have long-term metabolic and cardiovascular implications.[45]

Breast milk alone, on the other hand, may not be sufficient to supply the nutritional needs of growing premature infants. Feeding preterm new-borns expressed breast milk enriched with protein, non-protein energy (carbohydrate or fat), minerals, and other micronutrients, may boost nutrient acquisition and growth (gain in weight, length, and head circumference). Higher nutritional intake at this vital phase may be especially significant for infants who are unable to consume large amounts of milk, who have delayed growth, or who have persistent nutritional and metabolic needs.[4]

WHO – Guidelines for optimal feeding of low birth weight infants in low and middle income countries (2011)

When compared to formula, feeding mother's own milk had significant benefits in terms of mortality (18 percent reduction), serious infections or NEC (60 percent reduction), and mental development scores (5.2 points higher). In one study, the only evident drawback was a reduction in length after 9 months. Given the minimal costs of implementing mother's own milk feeding, the demonstrated advantages were definitely worth the cost. WHO also recommended that infants with LBW, especially those with VLBW, who were unable to get their mother's milk should be given donor human milk. Standard infant formula should be fed only to those LBW new-borns, including VLBW, who cannot be fed the mother's own milk or donor human milk. If VLBW infants who cannot be fed their mother's milk or donor human milk fail to gain weight after appropriate feeding with conventional infant formula, they should be given preterm infant formula. There was no indication of any substantial benefits or harms linked with giving fortified human milk in terms of mortality, NEC, mental development scores, or anthropometric status. Hence, for resource-limited settings it was recommended that those VLBW new-borns who were fed their mother's milk or donor human milk do not require the addition of a bovine milk-based human-milk fortifier. Human-milk fortifiers, ideally those based on human milk, should be administered to VLBW infants who do not gain weight despite appropriate breast milk intake (weak evidence). [3]

ESPGHAN 2010

Recommended addition of addition nutrients to human milk for feeding preterm infants as those VLBW who were given un-supplemented human milk developed multi-nutrient deficiency. It also recommends calorie intake of at least 110 kcal/kg/day and protein of 3.5gram per kg per day. So, it recommends human milk fortification for all the neonates with birth weight of less than 1800 grams. [4]

EMBA 2019

They recommend individualised human milk fortification for preterm neonates with birth weight of less than 1800 gram once they reach feed volume of at least 50-80ml/kg/day.[9]

NNF 2020

Breast milk fortification with multi-nutrients can begin in premature LBW new-borns weighing less than 1800 grams and getting oral feeds of at least 50 to 80 mL per kg/day. Fortification may be started solely for newborns who do not gain enough weight despite appropriate breast milk intake in resource-constrained settings (weak recommendation). There were also ethical and financial issues to consider.[6]

Furthermore, researchers have expressed worry that rapid weight gain at this critical stage could be connected to changes in fat distribution and "programmed" metabolic consequences, thereby increasing long-term risks of insulin resistance and hypertension.[46]

Despite the fact that HM fortification is widely used in NICUs around the world, there is still a lot of inconsistency, variability, and even doubt about it. Considering multi-nutrient fortification of human breast milk has the potential to alter crucial preterm baby outcomes, this study aims to find an association between fortification of human milk and early growth of preterm low birth weight children.

The present study is a hospital-based randomised control trial and was carried out in neonatal intensive care unit (NICU) under the department of paediatrics, Dr. Prabhakar Kore Hospital and Medical Research Centre, attached to Jawaharlal Nehru Medical College, Belagavi from April 2021 to August 2021. A total of 111 preterm newborns with a birth weight of less than 1800g and more than/equal to 1000g were admitted in NICU attached to the hospital during this period, 41 amongst them were excluded as they were not fulfilling the criteria for the enrollment in the study. 70 preterm newborns (Gestational age based on New Ballard score), with a birth weight of less than 1800g and more than/equal to 1000g tolerating feeds of at least 80ml/kg/day for three days were enrolled and randomized into the study and control group based on computer-generated random number sequence. Study group received fortified human milk. Fortification was done using Lactodex HMF sachet, 1 gram of which was added to every 25ml of human milk. The Control group received un-supplemented human milk during the study period. Total 6 newborns dropped out from the trial during this period, 2 of which were from study group and 4 in the control group. So, a total of 64 subjects (33 in cases and 31 in the control group) were analysed for the results.

In the present study it was observed that gender distribution for both the groups were similar. Majority (51-60%) of subjects in both the groups were female (p-value of 0.8). This finding was comparable to that of Lucas et al (1996), to assess the effect of human milk fortification on developmental outcomes in preterm newborns, and included 53 per cent of females in both groups.[47] G. Gathwala et al. (2012), in their study to assess the effect of fortification of breast milk on the growth of preterm infants had 56.7% males in the study and 46.7% in the control group.[10] Similarly, Gupta et al (2018), in their study to determine the effect of fortification of human milk with an infant formula powder versus unfortified human milk on the growth of preterm very low birth weight infants, had the higher distribution of males in fortification as well as standard care arm (53.3 % versus 52.1%).[48]

In our study it was observed that the socio-economic distribution for both the groups were similar. Majority (50-60%) of mothers in both the groups belonged to socioeconomic class 3, followed by those from class 4 according to revised BG Prasad's classification.

The majority of the mothers in the study (42.4%), as well as the control group (58.0%), were primigravida (p=0.14). Though there was a difference between the groups, it was not statistically significant.

In the present study, it was observed that the distribution of antenatal complications among the two groups was comparable. The majority of mothers in both the groups had gestational hypertension (p-value of 0.08), followed by premature rupture of membranes (p-value of 1). Some of the mothers also had gestational diabetes mellitus (p-value 0.61), hypothyroidism (p-value 0.67), per vaginal bleed (p-value 0.67) in both the groups. Similar observations were made by Adhisivam et al. (2019), in their study to estimate the effect of fortification of donor milk on the incidence of NEC among preterm, where the majority of mothers (p-value of 1) in either group had gestational hypertension, followed by premature rupture of membrane (20% mothers in the study versus 17.5% in control arm). [8]

The number of babies requiring resuscitation in the current study was comparable (p=0.95) between the two groups (in both groups, 13 newborns required resuscitation). The majority of them cried after stimulation (6 in each group), but 4 newborns in the study and 5 in the control group required bag and mask ventilation, and only 3 newborns in the study vs 2 in the control group required intubation during resuscitation.

The distribution of children across different weight sub-categories was similar in our study (P=0.84). The majority of participants were in the >1500g birth weight category (39.4 percent in the study and 45.25 percent in the control group), followed by the 1251-1500g weight category (39.4 percent in the study and 38.7 percent in the control group) and the 1000-1250g weight category (21.2 percent new-borns in study and 16.1 percent in control). In their study, Gupta et al (2018) compared the effects of fortification of human milk with an infant formula powder versus unfortified human milk on the growth of preterm very low birth weight infants, the infants were divided into two groups: those weighing less than 1250 g and those weighing more than 1250 g. 42.7 per cent of the newborns in the study and 47.9% of the babies in the control group weighed less than 1250 grams.[48]

In the current study, the distribution of SGA and AGA babies was comparable between the two groups (p=0.61). The majority of newborns in the study and control groups (66.7 per cent and 58.1 per cent, respectively) were small for gestational age (SGA). In a study by Mukhopadhyay et al. (2007), 45 per cent of those in the study and 34.7 per cent of those in the control arm were SGA.[12]

Anthropometric parameters at birth

In our study, the study and control groups had similar mean weight, head circumferences, and birth lengths, with minor differences that were statistically insignificant.

In present study, mean weight at birth in the study and control groups was 1442.42g and 1480.97g respectively (p-value of 0.46), the mean head circumference at birth was 29.68cm and 29.32cm respectively (p-value of 0.5), and the mean length at birth in the study and control groups was 41.86cm and 41.55cm respectively (p-value of 0.46).

Gathwala et al. (2012) in their study to assess the effect of fortification of breast milk on the growth of preterm infants, observed that the mean birth weight was 1670g and 1658g in the study group and control group respectively (p-value of more than 0.05), mean head circumference was 29.28 cm in the study group as well as control group (p-value of more than 0.05) and, mean length at birth was 42.26cm and 41.20cm in the study group and control group respectively (p-value of more than 0.05). The comparison between the groups showed subtle differences which were statistically not significant. [10]

OUTCOME OF HUMAN MILK FORTIFICATION

In our study, the mean weight gain in the study group from the time of birth, enrollment, and discharge was considerably higher when compared to the control group at the end of the trial (follow-up after 4 weeks of life). This suggests that the study participants gained weight during their hospital stay and after being discharged from the hospital.

1. **Weight gain.**

   a. **From birth to end of the study.**

In the present study, there was a significant improvement in weight gain from birth for premature infants with low birth weight receiving human milk fortified with HMF sachet when compared to those receiving unfortified human milk (mean difference of 2.20g/kg/day, p-value <0.001). Significant improvement in weight gain from birth was seen in the study group even when sub-group analysis based on sex, weight at birth, and AGA Vs SGA newborns.

Significant improvement in weight gain was observed from birth in the study group males (mean difference of 2.34g/kg/day, p-value 0.01) when compared to control males. Though weight gain from birth was not statistically significant for study group females (mean difference of 2.32g/kg/day, p-value 0.05). When we compared weight gain from birth in different birth weight subgroups, we observed that there was statistically significant weight gain for >1500g (mean difference of 1.88g/kg/day, p=0.006) and 1251-1500g (mean difference of 3.21g/kg/day and p-value of 0.001) weight category, although it was not statistically significant for 1000-1250g (mean difference of 1.06g/kg/day and p-value of 0.62). Similarly, when we compared AGA and SGA, we observed that there was significant weight gain from birth for SGA babies (mean difference of 2.31g/kg/day, p=0.001), although it was not statistically significant for AGA (mean difference of 1.88g/kg/day and p-value of 0.06).

b. From discharge to the end of study.

The weight gain from discharge to the end of the study was statistically significant in the study group when compared with the control group (mean difference of 4.26g/kg/day, p-value 0.001). Even sub-group analysis based on sex, birth weight, and AGA/SGA, showed better weight gain in the study group.

Males (mean difference of 4.50g/kg/day, p-value 0.004), as well as girls (mean difference of 4.36g/kg/day, p-value 0.007) in the study group, gained significantly more weight from the time of discharge to end of the study when compared to those in the control group. Similarly, when we compared AGA and SGA newborns of both the groups, statistically significant weight gain was observed for SGA (mean difference of 4.26/kg/day, p-value 0.002) but not for AGA (mean difference of 3.82g/kg/day, p-value 0.04). When we compared weight gain from discharge in different birth weight subgroups, we observed that there was statistically significant weight gain for >1500g (mean difference of 4.93g/kg/day and p-value of <0.001) and 1251 -1500g (mean difference of 3.71g/kg/day and p-value of 0.02) weight category, although it was not statistically significant for 1000-1250g ((mean difference of 2.96g/kg/day and p-value of 0.46).

c. From enrollment to end of the study.

In the current study, feeding HMF sachet-fortified human milk to premature infants with low birth weight resulted in a substantial increase in weight gain from enrollment as compared to feeding unfortified human milk (mean difference of 2.22g/kg/day, p-value 0.001). Even when sub-group analysis based on sex, birth weight, and AGA Vs SGA was performed, improved weight gain was seen in the study group neonates.

Both males (mean difference of 2.10g/kg/day, p-value 0.04) and female newborns (mean difference of 2.40g/kg/day, p-value 0.009) in the study group gained significantly more weight from the time of enrollment through the end of the trial when compared to those in the control group. Similarly, when we compared AGA and SGA newborns of both the groups, statistically significant weight gain was observed for SGA (mean difference of 2.42g/kg/day, p-value 0.004) but not for AGA (mean difference of 1.59g/kg/day, p-value 0.12). When we compared weight gain from the time of enrollment in different birth weight subgroups, we observed that there was statistically significant weight gain for >1500g (mean difference of 2.7g/kg/day and p-value of 0.01) and 1251 -1500g (mean difference of 2.36g/kg/day and p-value of 0.01) weight category, although it was not statistically significant for 1000-1250g (mean difference of 0.69g/kg/day and p-value of 0.78).

In the recent Cochrane review (2020), including 18 studies with 1456 premature infants, significantly improved weight gain (mean difference of 1·76g/kg/day, 95% CI of 1.33 to 2·22) was observed with the fortification of human milk as compared to unfortified human milk. The findings of this meta-analysis were similar to those of our study.[5]

Another study done by Mukhopadhyay et al. (2007), was a randomised control trial where they studied the effect of human milk fortification using HMF sachet. It was observed that the study group had significantly better weight gain (p=0.001). However, as in our study, no significant weight gain was observed in this study in the AGA subgroup (p-value of 0.12). [12]

Another study by Gupta et al. 2018, a randomised control trial where they compared effect of fortification using infant formula powder against unfortified human milk on the growth of premature infants, significant weight gain was observed in the study group compared to control arm (mean difference of 1.98, p-value of less than 0.001). Though infant formula powder was used in this study the results were comparable to that of our study. [47]

In the study by Nicholl et al. (1999), to study Changes in growth and metabolism in very low birth weight infants fed with fortified breast milk, significant weight gain was observed in those who received fortified milk arm ($p < 0.001$).[49]

2. Head circumference

a. From birth to end of the study

In the present study, there was no significant improvement in head circumference gain from birth for premature infants with low birth weight receiving human milk fortified with HMF sachet when compared to those receiving un-supplemented human milk (mean difference of 0.01cm/week and p-value of 0.75). Even on sub-group analysis based on sex, weight at birth and AGA/SGA increase in head circumference was not significantly different between the two groups.

b. From enrollment to end of trial

In the present study when we compared premature infants with low birth weight who received HMF sachet fortified human milk to those who received unfortified human milk, there was no significant improvement in head circumference gain from enrollment to the end of the study (mean difference of -0.02 and p-value of 0.69). Similarly, when the increase in head circumference from the time of enrollment for subgroups based on gender, birth weight, AGA and SGA, were compared there was no significant difference between the study and control group.

In the Cochrane review and meta-analyses (2020), it was observed that there was a modest increase in mean head growth (0.08cm/wk) after multi-nutrient fortification.[5] These findings were similar to our study.

In another study by Mukhopadhyay et al. (2007) to study the effect of human milk fortification using HMF sachet, in contrast to our study, significant improvement in head circumference (p-value of 0.017) was observed in this study.[12]

In another study by Gupta et al. 2018 where formula milk powder was used for fortification, there was no discernible increase in head size (p=0.12).[48]

In the study by Lucas et al. (1996) it was observed that the control group had better short-term head circumference gain during the study period although the difference between the two groups were statistically insignificant. These findings were similar to findings observed in some of the sub-groups of our study. [47]

3. Length

a. From birth to end of trial

When we compared premature infants with low birth weight who received HMF sachet fortified human milk to those who received unfortified human milk, there was statistically no significant improvement in length gain from birth (mean difference of 0.01cm/week and p-value of 0.75). Similarly, when the increase in length from the time of birth for subgroups based on gender, birth weight, AGA and SGA, were compared there was no significant difference between the subgroups.

b. From enrollment to end of trial

In the present study, there was no significant improvement in the length gain from the time of enrollment till the end of the study for premature babies with low birth weight receiving human milk fortified with HMF sachet when compared to those receiving unfortified human milk (mean difference of 0.05cm/wk and p-value of 0.45). Similarly, when the increase in length from the time of enrollment for subgroups based on gender, birth weight, AGA and SGA, were compared there was no significant difference between the subgroups.

In the Cochrane review and meta-analyses (2020), it was observed that the multi-nutrient fortification of human milk for preterm resulted a modest increase in mean length gain (0.12cm/wk). [5] These findings were in agreement with our study.

In the study by Mukhopadhyay et al. (2007) to study the effect of human milk fortification using HMF sachet, in contrast to our study, significant improvement in head circumference was observed after fortification of milk in study group.[12]

Significant linear growth (p=0.02) was observed in the study by Gupta et al. 2018 where formula milk powder was used for fortification. [48]

In the study done by Lucas et al. (1996) it was observed that the control group had better short-term length gain during the study period although the difference between the two groups were statistically insignificant. These findings were similar to findings observed in some of the sub-groups in the current study. [47]

The study done by Lucas et al. also showed that there were no significant changes in growth or development indices between the study and control groups either Nine or Eighteen months of age. [47]

In this study, the beneficial effect of fortification of human milk on early breastfeeding initiation and length of hospital stay was not observed. The study group had a mean day of life of 15.48, whereas the control group had a mean day of life of 11.48 (mean difference of -3.51 and P-value of 0.05), albeit the difference was not statistically significant. In our study, the average length of stay in the study group was 13.79 days compared to 12.32 days in the control group (mean difference of -1.47 with a P-value of 0.15).

In the observational research by Montjaux-Regis et al. (2011), preterm babies who received their own mothers' milk gained weight faster than those fed donor milk using routine fortification (p-value 0.016), although no difference was observed in linear development.[50]

In our study, there no case of NEC was observed in either group after enrollment in the study. This finding was consistent with the observation of Gupta et al. (2018), where there was only one case of NEC in the fortification arm.[48] Similarly, no baby developed NEC after randomisation in the study done by Mukhopadhyay et al. (2007).[12] In the current study, only one case of sepsis in either group was seen after enrollment. This was in line with the findings of Gupta et al., who found no significant difference in the incidence of sepsis in the study group when compared to the control group.[48]

To summarise in the present study, there was a significant improvement in weight gain from the time of enrollment of preterm low birth infants receiving human milk fortified with an HMF sachet (used as fortifier) when compared to those fed on unfortified human milk (mean difference of 2.22g/kg/day, p-value 0.001). Improved weight gain from the time of enrollment was observed in the fortification group even on subgroup analysis, but the weight gain for AGA (mean difference of 2.59g/kg/day, p-value 0.12) and 1000-1250 gram (mean difference of 0.59g/kg/day and p-value of 0.78) weight category was statistically insignificant. The head circumference growth and increase in length were not significantly different between the two groups. There were no major concerns with respect to feed intolerance, sepsis and NEC with the use of human milk fortifier sachet as a fortifier in this study.

However, despite the benefits of human milk fortification seen in preterm low birth weight neonates, our study did have certain limitations viz.

1. Small sample size.
2. Short term follows up.

Further studies with large sample size & longer follow up are required to study the long-term effect on weight gain and linear growth. Further studies are also required to study the effect of human milk fortification on metabolic parameters e.g., Calcium, Phosphorus, Vitamin D3 etc. Neurodevelopment and immunological factors need further evaluation and studies to explain this aspect of fortification of human milk.

CONCLUSION

This study was conducted to assess the effectiveness of human milk fortification on the early growth of preterm low birth weight infants. The study population included preterm neonates weighing between 1000 to 1800 grams. They were divided into two groups of 35 each based on a computer-generated random number sequence.

- Socio-demographic features including socio-economic class and parity were comparable between the groups.
- Gestational age, birth weight, head circumference and length were comparable at birth in the two groups.
- Human milk fortification promotes weight gain during early infancy:
  In this study, it was observed that fortification of expressed breast milk using HMF sachets help in improved weight gain of preterm LBW babies during early infancy. It was also observed that these benefits were more marked for babies more than 1250 grams and SGA babies when compared to $\leq$1250 gram and AGA babies respectively.
- The use of human milk fortifier did not result in any substantial improvements in head circumference or length of preterm LBW newborns.
- Fortification of human milk did not have any effect on early breastfeeding initiation or duration of hospital stay.
- Human milk fortification was not associated with an increased risk of necrotising enterocolitis and feed intolerance in our study.

Although the fortification of human milk for preterm babies is now routine practice in most neonatal units, our study found that fortification benefits were more marked for premature newborns with birth weight of more than 1250 grams and SGA babies when compared to preterm ≤1250 gram and AGA babies, respectively. Hence, routine fortification can be recommended for SGA newborns and those weighing more than 1250g because the cost remains a key stumbling block in the usage of fortifiers for LBW babies.

SUMMARY

Preterm low birth weight neonates who are exclusively given un-supplemented mothers milk can develop nutrient deficiencies as a result of inadequate protein and mineral content in expressed human milk, leading to extra-uterine growth retardation and poor neurodevelopmental outcomes later on. [9,14] In our country, 13% of babies are born prematurely and 18% are born with low birth weight.[1,13]

Human milk fortification using multi-nutrient fortifiers is an effective way to meet an infant need for additional nutrients and protein during early infancy.[15] However, there are concerns regarding the effect and adverse events linked to its use.[8]

The infants whose parents were willing to participate in the study and fulfilling the selection criteria were enrolled after taking written valid consent. After enrollment, 70 newborns were randomised into study and control groups based on computer-generated random number sequences. At the time of recruitment baseline data were collected, including socio-demographic information, antenatal history, events during delivery, post-natal care and complication. Those in the study group received fortified human milk, whereas those in the control group received un-supplemented mothers' milk. Lactodex HMF sachet was used to fortify expressed breast milk; 1 gram of HMF sachet was used to fortify 25ml of expressed breast milk. These babies were then monitored for weight gain, increase in head circumference and length during their stay in hospital and on follow up after 4 weeks of life.

In the present study, it was observed that there was a significant gain of weight from birth in the study group when compared to the control group (mean difference of

2.2g/kg/day with a p-value of less than 0.001). Similarly, when we compared weight gain between discharge to end of the trial (mean difference of 4.26g/kg/day with ap-value of less than 0.001) and from enrollment to end of the trial (mean difference of 2.22g/kg/day with a p-value of 0.001), statistically significant weight gain was observed for the study group. Although when compared to the control group, there was no significant increase in weight gain in the 1000-1250 grams weight category and AGA newborns in the study group of the trial.

In this study, it was observed that gain in head circumference (mean difference of -0.02cm with a p-value of 0.069) and length (mean difference of 0.05cm with a p-value of 0.45) during the study period was statistically not significant.

The anticipated benefits like early initiation of breastfeeding and decreased duration of hospital stay were not seen in our study. There was no case of necrotising enterocolitis in either group after enrollment in the study.

Even though the sample size of our study was small, the present trial shows that there is a significant benefit of human milk fortification in the early growth of neonates. Hence, we recommend that routine use of human milk fortification using should be practised for preterm low birth weight babies, primarily SGA babies more than 1250 grams. The results of this study pave the way for larger clinical trials in our country.

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ANNEXURE I

CONSENT FOR PARTICIPATION IN RESEARCH

“Effect of human milk fortification with a human milk fortifier on short term growth of preterm low birth weight infants- A randomized controlled trial at a tertiary care centre”

Principal Investigator: -

You have been asked to involve your child in the said study to be conducted at neonatal care unit under department of paediatrics KLE University’s Dr Prabhakar kore charitable hospital, Belagavi, PG student in department of paediatrics at Jawaharlal Nehru medical college, Belgaum.

Purpose of the study

Participation of your child will help us to know the effect of human milk fortification on short term growth of preterm low birth weight neonates and will help to rationalise it’s use, in this study babies in will be divided in two groups and depending upon that they will be they will be given supplementation. 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.

Risk And Benefits

There are no significant risks involved. If any complications during the procedure, then baby will be treated with best of our knowledge. There is no compensation or payment for such medical treatment.

If you attain any complication during the period, you may contact PG in Pediatrics and Principal and Professor, Department of Pediatrics.

During the course of the study, you will be informed of any significant new findings such as changes in the risks or benefits resulting from participation in the research.

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.

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 Hospital & Medical Research Center, Belgaum.

Queries

If you have any queries, you may contact, Post Graduate Student Department of Paediatrics JNMC, Belagavi-590010.

If you have any queries regarding or rights or research participation you may contact: Principal, Professor Department Of Paediatrics, JNMC, Belagavi-590010.

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

Statement 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: -

[Study / control] [Subject no.: -]

PATIENT PARTICULAR'S: -

Name: __________ Sex: DoB: -

I.P no. ____________________ DoA: DoD: -

Address:

____

Mb. /Phone No: ___________________________ MBG: _________|_________________

I	II	III	IV	V

Social class: [Modified prasad's social classification 2020]

LMP: ____/____/ 20____ EDD: ____/____/ 20____

GA: - ______wk______d (a/c LMP) , __________ wk (a/c New Ballard score)

HISTORY: -

ANTE-NATAL

Gest. HTN	Y / N
GDM	Y / N
Hypothyroid	Y / N
Fever	Y / N
Cardio-vascular disease	Y / N
PV bleed /APH	Y / N
PROM	Y / N
Oligo-hydramnios	Y / N
Polyhydramnios	Y / N
UTI	Y / N

ANOMALY SCAN: -

OTHERS: -

NATAL

Mode of delivery: - NVD/Assisted vaginal/ LSCS

Indication-

Resuscitation: -

Cried at birth	
Stimulation	
Bag and mask [if required dur^{n}]	
Intubated	

Apgar at

1min ____ / 10

5min ____ / 10

Cong. Anomalies: - Y / N

POST-NATAL

	Y/N	Duration
ventilator		
CPAP		
Oxygen		

Sepsis: - Y / N

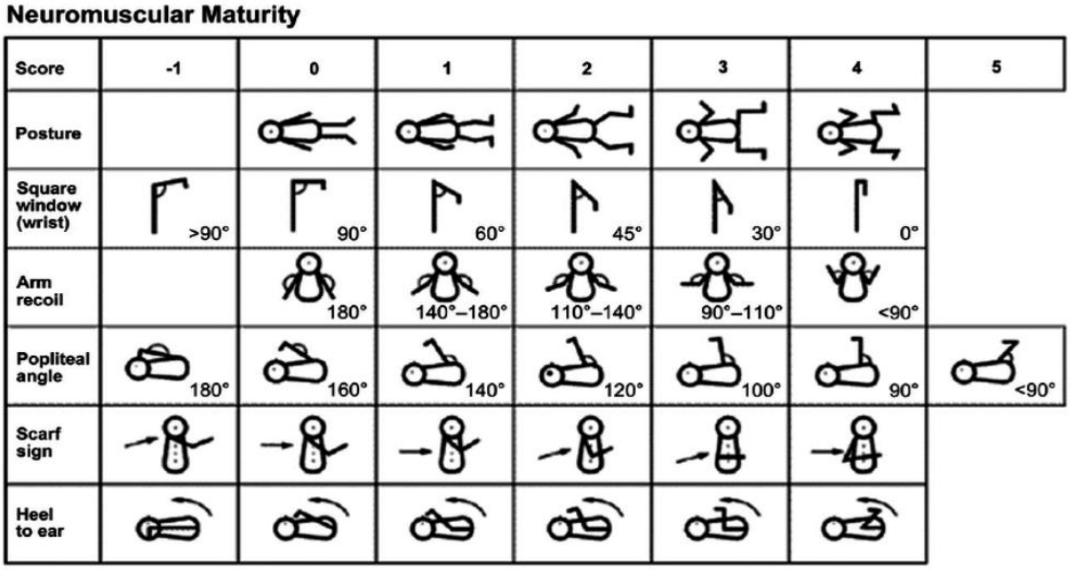
Received any

Blood components: - Y / N

Antibiotics: - Y / N

Day of life at the time of initiation of feeds**: -**

New Ballard score: - **[Total score :-]**



Physical Maturity

	-1	0	1	2	3	4	5
Skin	Sticky, friable, transparent	Gelatinous, red, translucent	Smooth, pink; visible veins	Superficial peeling and/or rash; few veins	Cracking, pale areas; rare veins	Parchment, deep cracking; no vessels	Leathery, cracked wrinkled
Lanugo	None	Sparse	Abundant	Thinning	Bald areas	Mostly bald	
Plantar surface	Heel-toe 40-50 mm: –1 <40 mm: –2	>50 mm, no crease	Faint red marks	Anterior transverse crease only	Creases anterior ⅔	Creases over entire sole	
Breast	Imperceptible	Barely perceptible	Flat areola, no bud	Stippled areola, 1–2 mm bud	Raised areola, 3–4 mm bud	Full areola, 5–10 mm bud	
Eye/Ear	Lids fused loosely: –1 tightly: –2	Lids open; pinna flat; stays folded	Slightly curved pinna; soft; slow recoil	Well curved pinna; soft but ready recoil	Formed and firm, instant recoil	Thick cartilage, ear stiff	
Genitals (male)	Scrotum flat, smooth	Scrotum empty, faint rugae	Testes in upper canal, rare rugae	Testes descending, few rugae	Testes down, good rugae	Testes pendulous, deep rugae	
Genitals (female)	Clitoris prominent, labia flat	Clitoris prominent, small labia minora	Clitoris prominent, enlarging minora	Majora and minora equally prominent	Majora large, minora small	Majora cover clitoris and minora	

Maturity Rating

Score	Weeks
-10	20
-5	22
0	24
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44

Intervention: -

Fortified Human milk(No. of days)	**Mother's own milk**(No. of days)	**Donor Milk** (No. of days)	**Day of life at time of DBF Initiation: -**

DATA COLLECTION: -

DoL	**I.V fluids/EBM/DHM/DBF/HMF**	**PROBLEMS**			
		Feed intolerance	NEC	Sepsis	Any Other
1	**I.V fluids/EBM/DHM/DBF/HMF**				
2	**I.V fluids/EBM/DHM/DBF/HMF**				
3	**I.V fluids/EBM/DHM/DBF/HMF**				
4	**I.V fluids/EBM/DHM/DBF/HMF**				
5	**I.V fluids/EBM/DHM/DBF/HMF**				
6	**I.V fluids/EBM/DHM/DBF/HMF**				
7	**I.V fluids/EBM/DHM/DBF/HMF**				
8	**I.V fluids/EBM/DHM/DBF/HMF**				
9	**I.V fluids/EBM/DHM/DBF/HMF**				
10	**I.V fluids/EBM/DHM/DBF/HMF**				
11	**I.V fluids/EBM/DHM/DBF/HMF**				
12	**I.V fluids/EBM/DHM/DBF/HMF**				
13	**I.V fluids/EBM/DHM/DBF/HMF**				
14	**I.V fluids/EBM/DHM/DBF/HMF**				
15	**I.V fluids/EBM/DHM/DBF/HMF**				
16	**I.V fluids/EBM/DHM/DBF/HMF**				
17	**I.V fluids/EBM/DHM/DBF/HMF**				
18	**I.V fluids/EBM/DHM/DBF/HMF**				
19	**I.V fluids/EBM/DHM/DBF/HMF**				
20	**I.V fluids/EBM/DHM/DBF/HMF**				
21	**I.V fluids/EBM/DHM/DBF/HMF**				
22	**I.V fluids/EBM/DHM/DBF/HMF**				
23	**I.V fluids/EBM/DHM/DBF/HMF**				
24	**I.V fluids/EBM/DHM/DBF/HMF**				
25	**I.V fluids/EBM/DHM/DBF/HMF**				
26	**I.V fluids/EBM/DHM/DBF/HMF**				
27	**I.V fluids/EBM/DHM/DBF/HMF**				
28	**I.V fluids/EBM/DHM/DBF/HMF**				

Age	**Length (in cm)**	**Head circumference (in cm)**	**Weight [in gram]**
Day 1			
1 week			
2 weeks			
3 weeks			
4 weeks/ F/u [days]			
Enrollment			

S.no.	**Description**	
1.	Birth weight (_________gm)	SGA / AGA / LGA
2.	Head circumference (________cm at birth)	Micro / Normal / Macro
3.	If SGA Ponderal index: -	>2.5 2-2.5 <2.0

PROBLEMS:

NEC	Y / N
FEED INTOLERANCE	Y / N
SEPSIS	Y / N

OUTCOME: - (from enrollment)

S.no.	Description	Result
1.	Weight gain (in g/kg/day):	
2.	Head circumference (increase in cm/wk.):	
3.	Length (increase in cm/wk.):	

ANNEXURE III

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 (GoI)

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/ 415. **Date: 29/08/2020**

To,
BM0119008
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 **"EFFECT OF HUMAN MILK FORTIFICATION WITH A HUMAN MILK FORTIFIER ON SHORT TERM GROWTH OF PRETERM LOW BIRTH WEIGHT INFANTS – A RANDOMIZED CONTROLLED TRIAL AT A TERTIARY HEALTH CARE CENTRE"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE IV

KEY TO MASTERCHART

1. Group number-Study: 1, Control:2.
2. Socio-economic class - CLASS 1,2,3,4,5 represented by same number respectively.
3. Gestational hypertension -present: 1, absent: 2
4. GDM-present: 1, absent: 2
5. Hypothyroidism-present: 1, absent: 2
6. Fever -present: 1, absent: 2
7. Cardio vascular disease-present: 1, absent: 2
8. PV-bleed / APH-present: 1, absent: 2
9. PPROM-present: 1, absent: 2
10. Oligo-hydramnios -present: 1, absent: 2
11. Poly-hydramnios-present: 1, absent: 2
12. UTI-present: 1, absent: 2
13. Method of delivery: - VAGINAL: - 1, LSCS: - 3
14. Resuscitation
    a. Cried at birth: - 1
    b. Stimulation: - 2
    c. Bag and mask: - 3
    d. Intubated: - 4
15. Gender male: - 1, female: - 2
16. Problems

a. O for no issues

b. 1 for RDS (respiratory distress syndrome)

c. 2 for NNH (neonatal hyperbilirubinemia)

d. 3 for NEC

e. 4 for Hypoglycaemia

f. 5 for RDS + NNH

g. 6 for RDS + NNH + HYPOGLYCEMIA all are present

h. 7 for infection

i. 8 feed intolerance

17. Length (in cm)

18. Head circumference (in cm)

19. Weight (in gram)

20. Weight

a. SGA: - 1

b. AGA: - 2

c. LGA: - 3

21. Head circumference

a. Micro: - 1

b. Normal: - 2

c. Macro: - 3

22. Ponderal index

a. >2.5 by 1,

b. Between 2 – 2.5 by 2,

c. < 2 by 3

23. Weight gain (g/kg/day) after 4 wk

24. Increase in head circumference (cm/wk) after 4 wk

25. Increase in length(cm/wk) after 4 wk

26. Cm for centimetre

27. Kg for kilogram

28. Wk for week

ANNEXURE V
MASTER CHART

Serial Number	Group number	In Patient number	GROUP	SOCIO-ECONOMIC CLASS	GRAVIDA	PARITY	GEST. HTN	GDM	HYPOTHYROIDISM	FEVER	CARDIO-VASCULAR DISEASE	PV-BLEED / APH	PPROM	OLIGO-HYDRAMNIOS	POLY-HYDRAMNIOS	UTI	CONGENITAL ANOMALY	MODE OF DELIVERY	GESTATION (WKS A/C LMP)	BALLARD SCORE	RESUSCITATION	APGAR (AT 1 MIN)	APGAR (AT 5 MIN)	Sex of the child	VENTILATOR (no. of days)	CPAP (no. of days)	Oxygen supplementation (no. of days)	Day of life at the time of initiation of feeds	FORTIFIED HUMAN MILK	MOTHER'S OWN MILK	DBF initiation (day of life)	PROBLEMS	Length (in cm)	Head circumference (in cm)	weight at birth (in gram)	weight (SGA/AGA/LGA)	Head circumference (micro/ normal/ macro)	ponderal index	weight gain between birth and follow up after 4 wk of life(g/kg/day)	Increase in head circumference between birth and follow up after 4 wk of life (cm/wk)	Increase in length between birth and follow up after 4 wk of life(cm/wk)	Duration of stay in hospital	Day of life at enrollment day	weight at discharge (in grams)	weight at enrollment (in grams)	weight on follow up (in grams)	wt gain in g/kg/day between discharge and follow up	weight gain from enrollment in g/kd/day	Increase in HC from enrollment in cm/wk	Increase in Length from enrollment in cm/wk
1	1	1022830	1	3	1	0	1	2	2	2	2	2	2	2	2	2	2	3	33.7	20	1	6	8	2	0	0	0	1	23	28	21	2	36.5	27	1060	1	2	2	2	0.5	0.63	14	6	1020	1000	1120	6.74	4.05	0.51	0.64
2	2	1023618	1	3	1	0	2	2	2	2	2	2	2	2	2	2	2	1	31.1	22	1	5	7	2	0	0	2	3	14	26	3	5	40	27	1480	2	1	2	7.6	0.62	0.75	11	7	1440	1400	1800	14.31	9.66	0.67	1
3	3	1024114	1	3	2	1	1	2	2	2	2	2	2	1	2	2	2	3	33.0	23	1	5	8	2	0	0	0	1	22	23	12	2	40	28	1400	2	2	2	6.2	0.67	0.67	12	7	1400	1320	1680	10	8.04	0.7	0.7
4	4	1024350	1	4	2	1	1	2	2	2	2	2	2	2	2	2	2	3	32.9	21	1	6	9	1	0	0	3	4	19	25	24	5	41	29.5	1600	2	2	2	7.66	0.79	1.02	13	10	1550	1500	1980	14.94	9.68	1	1
5	5	1025664	1	3	3	2	1	2	2	2	2	2	2	2	2	2	2	3	36.0	29	2	6	8	1	0	0	7	5	15	24	12	5	41	28	1400	1	1	2	5.8	0.62	0.57	14	9	1400	1300	1630	10.27	7.86	0.7	0.8
6	6	1025818	1	3	1	0	1	1	2	2	2	2	2	1	2	2	2	3	35.4	29	1	6	8	2	0	0	2	2	22	25	9	5	39	29	1300	1	1	2	12.8	0.62	1.1	15	7	1360	1260	1800	22.57	13.85	0.46	0.77
7	7	1026074	1	3	1	0	2	2	2	2	2	2	2	2	2	2	2	3	35.6	28	1	5	8	1	0	2	2	3	18	26	14	5	43	31	1790	1	2	2	11.28	0.88	1.1	10	7	1770	1710	2450	17.27	12.92	0.84	1.12
8	8	1026587	1	4	1	0	1	2	2	2	2	2	2	2	2	2	2	3	35.9	26	1	7	7	2	0	0	0	1	15	28	9	2	44	32	1780	2	2	2	7.14	0.93	0.93	9	7	1630	1680	2160	15.68	9.64	1	1.17
9	9	1027134	1	5	4	2	1	2	2	2	2	2	1	2	2	2	2	3	35.9	27	1	5	7	2	0	0	1	2	15	27	9	5	43	30.5	1300	1	2	3	10.58	0.68	1.12	9	7	1220	1220	1740	17.4	12.5	0.7	1.15
10	10	1027112	1	3	6	2	1	2	2	2	2	2	2	2	2	2	2	1	34.3	25	1	5	7	2	0	0	0	2	15	27	11	2	41	29	1250	1	2	3	8.8	0.81	1.28	8	7	1180	1210	1580	14.55	9.87	1.07	1.37
11	11	1027930	1	4	3	0	1	2	2	2	2	2	2	1	2	2	2	3	36.6	29	1	7	8	2	0	2	1	4	17	24	13	5	48	32.5	1560	1	2	3	8.97	0.81	1.28	15	9	1530	1460	1980	19.24	11.12	0.94	1.5
12	12	1029787	1	4	3	2	1	2	2	2	2	1	2	2	2	2	2	3	32.9	21	2	4	7	2	0	3	1	5	16	22	15	5	41	30.5	1200	1	2	3	12.9	0.7	1.13	18	11	1280	1140	1680	25.65	14.52	0.6	0.95
13	13	1028752	1	3	1	0	2	2	2	2	2	2	2	1	2	2	2	3	35.7	26	1	5	8	1	0	0	0	1	19	28	21	4	43	30.5	1350	1	2	3	12.59	0.75	1.25	11	7	1320	1260	1860	21.06	14.82	0.77	1.22
14	14	1029454	1	3	4	2	2	2	2	2	2	1	2	2	2	2	2	3	33.7	26	3	3	8	1	0	2	1	3	16	25	12	5	46	32	1780	1	2	3	10.39	0.67	1.13	11	10	1670	1690	2380	19.95	12.51	0.84	1.34
15	15	1030299	1	2	2	1	2	2	2	2	2	2	1	2	2	2	2	3	35.7	22	2	6	8	2	0	0	2	3	17	26	15	5	42.5	28	1500	2	2	3	8.9	0.84	1.1	12	8	1440	1410	1890	17.65	11.04	1	1.34
16	16	1031065	1	4	4	1	1	1	2	2	2	2	1	2	2	2	2	1	35.4	27	2	5	8	2	0	0	0	1	18	28	8	2	43	33.7	1700	1	2	2	8.3	0.92	1.21	12	7	1620	1600	2140	18	10.96	0.96	1.28
17	17	1031844	1	3	2	1	1	2	2	2	2	2	2	2	2	2	2	3	34.3	26	1	6	8	2	0	3	2	4	15	25	15	1	44	31	1520	1	2	3	9.8	0.79	1.1	12	11	1400	1390	1980	20.09	12.53	0.74	1.3
18	18	1034638	1	3	4	1	2	1	2	2	2	2	1	1	2	2	2	3	28.7	14	3	5	7	1	0	5	3	7	16	22	31	5	38	26	1100	2	2	2	11.07	0.88	1.31	23	13	1270	1070	1490	22.23	11.94	0.74	1.22
19	19	1035013	1	3	1	0	1	2	2	2	2	2	1	2	2	2	2	3	35.7	26	3	5	7	2	2	3	2	8	15	21	19	5	45	31.5	1360	1	2	3	7.81	0.61	0.91	23	14	1500	1260	1700	16.34	10.12	0.55	0.98
20	20	1035056	1	5	2	1	1	2	2	2	2	2	1	2	2	2	2	3	33.6	23	2	4	7	2	0	0	3	2	18	27	11	5	40	28.4	1720	2	2	1	8.9	0.68	1.12	12	7	1670	1640	2180	16.48	10.47	0.31	1.16
21	21	1042210	1	3	1	0	2	2	1	1	2	2	1	2	2	2	2	3	34.3	27	1	5	7	2	0	2	1	3	17	26	12	5	40.5	31.2	1400	1	2	2	9.95	0.83	1.23	13	9	1370	1310	1790	20	12.25	0.89	1.33
22	22	1036871	1	3	1	0	1	2	2	2	2	2	2	2	2	2	2	3	36.3	29	1	6	8	2	0	0	1	1	19	28	5	0	43	30	1700	1	1	2	12.33	0.67	1.24	7	6	1590	1590	2350	18.63	14.43	0.7	1.4
23	23	1047758	1	4	3	2	1	2	2	2	2	2	2	2	2	2	2	3	35.7	26	1	7	8	2	0	0	1	1	16	24	9	2	44	31	1400	1	2	3	11.58	0.82	1.33	12	8	1370	1300	1870	21.01	14.04	0.54	1.5
24	24	1051283	1	3	1	0	1	2	2	2	2	2	2	2	2	2	2	1	33.6	25	3	4	8	1	0	5	3	8	15	21	18	5	41	29.5	1090	1	2	3	6.88	0.63	1.07	15	12	1130	1240	1580	25.81	10.07	0.59	0.93
25	25	1051369	1	3	1	0	1	2	2	2	2	2	1	2	2	2	2	3	33.3	22	1	5	7	1	0	3	2	4	20	25	13	5	41	29	1600	2	2	2	7.8	0.75	1.2	17	9	1590	1500	1950	20.46	10.05	0.85	1.4
26	26	1051368	1	3	1	0	1	2	2	2	2	2	1	2	2	2	2	3	33.3	24	1	5	7	1	3	5	3	10	14	18	26	5	40	28.5	1780	2	2	1	5.95	0.68	1.18	17	15	1790	1760	2100	15.84	6.83	0.65	0.97
27	27	1051621	1	4	1	0	1	2	2	2	2	2	2	2	2	2	2	1	32.7	21	4	5	7	1	2	1	1	5	15	23	15	6	42	29	1360	1	2	3	10.67	0.79	1.08	15	12	1360	1320	1810	20.69	11.63	0.74	1
28	28	1053559	1	4	3	2	1	2	2	2	2	2	2	2	2	2	2	1	32.1	22	2	5	8	1	0	2	1	4	17	25	13	5	43	31	1300	2	2	3	11.18	0.57	1.38	15	10	1270	1250	1680	19.72	10.67	0.9	1.84
29	29	1053901	1	4	2	1	2	2	2	2	2	1	2	2	2	2	2	3	28.4	17	4	4	6	1	1	2	2	5	19	24	31	5	40	28	1200	1	2	3	9.67	0.65	1.06	15	11	1150	1050	1560	21.36	13.71	0.67	1.12
30	30	1053902	1	4	2	1	2	2	2	2	2	1	2	2	2	2	2	3	28.4	18	4	4	6	1	1	2	2	4	19	25	31	5	39	28.2	1120	1	2	3	13.25	0.7	1.06	15	10	1130	1040	1580	25.12	15.56	0.74	0.9
31	31	1054793	1	4	2	1	2	2	2	2	2	2	1	2	2	2	2	1	31.3	20	1	5	7	1	0	2	1	3	20	26	13	5	44	30	1600	2	2	3	11.2	0.7	1.16	14	10	1620	1540	2120	20.84	12.5	0.7	1.22
32	32	1057480	1	3	2	1	2	2	1	2	2	2	2	2	2	2	2	3	33.9	21	1	5	7	2	0	4	2	7	16	22	15	5	43	30	1580	1	2	3	7.12	0.7	1.09	17	13	1560	1470	1940	16.04	9.3	0.82	1.18
33	33	1058955	1	3	1	0	2	2	2	2	2	2	1	2	2	2	2	3	30.7	16	1	5	7	2	0	4	3	8	15	21	26	5	42	29	1320	1	2	3	12	0.77	1.21	19	14	1440	1330	1780	25.76	11.76	0.8	1.4
34	1	1023506	2	4	1	0	2	2	2	2	2	2	1	2	2	2	2	1	32.3	19	1	6	9	2	0	4	3	5	0	23	18	5	40	26.5	1150	1	2	3	4.66	0.63	0.88	11	11	1110	1110	1300	9.72	5.91	0.62	1.03
35	2	1023640	2	3	2	1	2	2	2	2	2	2	2	2	2	2	2	1	31.0	20	1	6	9	1	0	0	6	6	0	23	13	5	40.5	28	1420	2	2	2	4.53	0.5	0.81	16	10	1410	1365	1600	11.16	5.92	0.39	0.9
36	3	1024073	2	3	1	0	2	2	2	2	2	2	2	2	2	2	2	1	32.7	24	2	6	8	2	0	0	5	5	0	23	12	5	42.5	28	1600	2	2	2	4.91	0.67	0.87	14	11	1710	1670	1820	4.92	3.35	0.83	0.83
37	4	1024151	2	4	1	0	2	2	2	2	2	2	1	2	2	2	2	3	34.9	28	1	6	9	1	0	0	0	1	0	25	11	2	45	31	1720	1	2	3	8.7	0.63	0.92	14	7	1740	1620	2120	15.79	10.39	1	1
38	5	1024551	2	3	2	1	2	2	2	2	2	1	2	2	2	2	2	1	30.7	15	3	7	10	2	0	0	0	1	0	28	22	2	42	28	1520	2	2	2	5.91	0.62	0.9	9	7	1430	1420	1780	11.52	8.17	0.48	0.8
39	6	1025434	2	3	1	0	2	2	2	2	2	2	1	2	2	2	2	1	33.0	25	1	6	7	1	0	0	0	1	0	28	7	2	44	31	1710	2	2	2	4.3	0.61	0.95	15	7	1780	1720	2020	10.8	6.27	0.5	0.67
40	7	1027156	2	4	3	2	2	2	2	2	2	2	2	2	2	2	2	3	35.1	27	1	6	9	2	0	0	0	1	0	28	6	0	41.5	30	1400	1	2	3	8.61	0.72	0.84	8	7	1340	1340	1750	13.95	10.1	0.8	0.8
41	8	1027789	2	5	1	0	1	2	1	2	2	2	1	1	2	2	2	3	34.6	27	2	5	8	1	0	2	1	4	0	22	14	5	41	30.3	1300	1	2	3	6.1	0.7	1.16	13	11	1230	1190	1530	14.43	9.02	0.63	1.05
42	9	1027920	2	3	1	0	1	2	2	2	2	2	2	2	2	2	2	3	35.9	28	2	5	8	2	0	0	2	2	0	27	8	5	45	30.5	1350	1	2	3	6.6	0.68	1.05	12	7	1430	1430	1810	14.82	9.09	1.03	1.32
43	10	1029444	2	4	2	1	1	2	2	2	2	2	2	2	2	2	2	1	36.4	28	1	5	8	2	0	0	0	1	0	28	5	0	44.9	33	1620	1	2	3	7.2	0.81	0.93	5	5	1750	1750	2190	10.87	9.06	0.98	1.12
44	11	1029866	2	3	1	0	1	2	1	2	2	2	1	2	2	2	2	3	32.4	24	1	6	8	1	0	0	0	1	0	28	15	0	42	31	1780	2	2	2	8.25	0.77	1.1	6	7	1690	1680	2250	12.11	10.01	0.84	1.12
45	12	1030651	2	3	1	0	1	2	2	2	2	2	1	2	2	2	2	3	30.0	19	1	6	7	2	0	4	2	6	0	23	18	5	37.5	27	1410	2	2	1	4.8	0.79	0.9	18	12	1390	1310	1620	12.55	7.1	0.74	0.93

46	13	1032198	2	4	1	0	1	2	1	2	2	2	1	2	2	2	2	3	35.6	29	2	5	8	1	0	0	2	2	0	27	9	5	44	31	1520	1	2	3	7.74	0.72	1.17	11	8	1600	1770	2070	16.28	6.58	0.8	1.28
47	14	1032746	2	3	1	0	2	2	2	2	2	2	2	2	2	2	2	1	32.4	25	1	6	7	2	0	0	0	1	0	28	6	2	43	29	1700	2	2	2	8.3	0.84	1.2	6	6	1630	1630	2110	12.28	9.74	0.92	1.37
48	15	1034219	2	3	2	1	1	2	2	2	2	2	2	2	2	2	2	1	33.9	25	1	6	8	1	0	0	2	3	0	24	11	1	43	31	1300	1	2	3	8.8	0.8	1.2	11	9	1230	1190	1630	17.1	11.68	0.88	1.23
49	16	1036402	2	4	2	1	2	2	2	2	2	2	2	1	2	2	2	3	32.6	19	2	6	8	2	0	4	2	5	0	22	16	5	36.3	25.5	1400	2	2	1	5	0.75	1.1	18	11	1430	1310	1620	10.44	7.15	0.63	1.05
50	17	1036681	2	3	1	0	2	2	2	2	2	2	1	2	2	2	2	3	35.3	27	1	6	8	2	0	0	0	1	0	24	8	2	44	31	1680	1	2	3	7.14	0.77	1.3	10	7	1580	1560	2040	13.7	9.53	0.8	1.4
51	18	1037083	2	4	2	1	1	2	2	2	2	2	2	2	2	2	2	3	30.1	16	3	5	7	2	0	3	3	7	0	22	22	1	37	26.5	1030	1	2	2	8.5	0.72	1.03	21	13	1140	1110	1310	15.01	6.07	0.52	0.96
52	19	1037937	2	4	1	0	2	2	2	2	2	2	1	2	2	2	2	3	32.6	24	3	4	6	1	0	3	2	6	0	21	14	5	41	29	1700	2	2	2	7.02	0.75	1.11	16	11	1640	1550	2070	16.87	9.87	0.81	1.02
53	20	1044323	2	3	1	0	1	2	2	2	2	2	2	2	2	2	2	3	35.1	26	1	8	9	1	0	0	0	1	0	23	7	0	43	30	1760	1	2	2	8.15	0.7	1.2	9	7	1820	1880	2270	11.12	6.93	0.9	1.4
54	21	1044521	2	3	2	1	2	2	2	2	2	2	1	2	2	2	2	3	34.4	28	3	3	7	1	0	1	0	3	0	26	9	5	43	31	1790	1	2	2	7.85	0.77	1.23	9	9	1730	1780	2210	13.41	8.29	0.77	1.26
55	22	1047628	2	1	1	0	2	2	2	2	2	2	2	2	2	2	2	3	35.7	29	1	5	8	1	0	0	0	1	0	28	7	2	39	30.5	1240	1	2	2	6.13	0.82	1.17	9	7	1640	1630	2060	16.13	11.56	0.77	1.07
56	23	1048983	2	3	1	0	2	2	2	2	2	2	2	2	2	2	2	1	31.9	20	1	7	8	1	0	0	0	5	0	24	13	5	39.5	31	1500	2	2	2	8.6	0.84	1.28	13	10	1440	1390	1890	17.65	11.12	0.91	1.16
57	24	1051191	2	3	2	1	2	1	2	2	2	2	2	2	2	2	2	1	33.0	24	1	7	10	2	0	2	2	3	0	25	10	1	40.7	29	1230	1	2	3	9.13	0.79	1.28	9	9	1150	1120	1650	20.33	14.86	0.88	1.4
58	25	1052675	2	4	1	0	1	2	2	2	2	2	2	2	2	2	2	1	30.1	15	3	6	7	1	0	5	2	6	0	22	19	5	37	26	1200	2	2	2	12.36	0.79	1.13	14	11	1150	1100	1660	25	15.06	0.7	0.88
59	26	1052568	2	3	1	0	2	2	2	2	2	2	2	2	2	2	2	3	32.1	20	4	5	8	1	2	2	3	5	0	22	13	5	39.5	27.9	1360	2	2	2	3.5	0.62	1.08	13	11	1270	1250	1500	10.57	6.34	0.55	1.25
60	27	1052567	2	3	1	0	2	2	2	2	2	2	2	2	2	2	2	3	32.1	20	4	6	8	2	2	3	3	6	0	23	13	5	40.7	28.2	1410	2	2	2	8.7	0.65	1.09	13	12	1360	1340	1740	16.85	9.79	0.66	1.32
61	28	1053918	2	4	2	1	1	2	2	2	2	2	2	2	2	2	2	3	33.3	24	1	4	8	2	0	2	0	3	0	24	12	5	40	28.5	1380	1	2	2	6.2	0.65	1.01	14	10	1350	1270	1700	16.91	10.75	1	1.18
62	29	1056313	2	4	2	1	2	2	2	2	2	2	1	2	2	2	2	3	34.1	26	2	6	9	2	0	5	2	8	0	19	15	5	45	30.2	1780	1	2	3	5.39	0.68	1.05	24	14	1830	1680	2120	18.11	7.5	0.63	0.89
63	30	1057986	2	4	2	0	2	2	2	2	2	1	2	2	2	2	2	3	32.0	20	1	5	7	1	0	2	1	3	0	26	11	5	41.5	29	1300	1	2	3	7.88	0.77	1.11	12	9	1330	1280	1720	17.65	11.68	0.77	1.47
64	31	1059034	2	4	3	1	1	2	2	2	2	2	2	2	2	2	2	3	35.3	29	1	6	8	2	0	0	3	2	0	24	7	5	44.8	30.2	1650	1	2	3	8.11	0.65	1.26	9	8	1620	1600	2100	14.55	10.45	0.97	1.34