"SERUM FERRITIN LEVELS IN NEWBORNS AT 3 MONTHS OF LIFE AFTER INTACT UMBILICAL CORD MILKING VERSUS DELAYED CORD CLAMPING" – RANDOMIZED CONTROLLED TRIAL"

BY

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"SERUM FERRITIN LEVELS IN NEWBORNS AT 3 MONTHS OF LIFE AFTER INTACT UMBILICAL CORD MILKING VERSUS DELAYED CORD CLAMPING" – PROSPECTIVE RANDOMIZED CONTROLLED TRIAL ,** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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LIST OF ABBREVIATIONS USED

UCM	Umbilical Cord Milking
I -UCM	Intact umbilical cord milking
DCC	Delayed Cord Clamping
WHO	World Health Organization
AAP	American Academy of Pediatrics
NFHS	National family health survey
RBC	Red Blood Cell
Hb	Haemoglobin
PCV	Hematocrit
ACOG	American College of Obstetricians and Gynecologists
RCOG	The Royal College of Obstetricians and Gynaecologists
SUMAN	Surakshit Matritva Aashwasan
LMP	Last menstrual period
SNOSE	serially numbered opaque sealed envelope
SpO2	Oxygen saturation
CLIA	chemiluminescence immunoassay

ABSTRACT

"SERUM FERRITIN LEVELS IN NEWBORNS AT 3 MONTHS OF LIFE AFTER INTACT UMBILICAL CORD MILKING VERSUS DELAYED CORD CLAMPING" - RANDOMIZED CONTROLLED TRIAL

INTRODUCTION: The major public health concern worldwide, especially in developing countries like India is Anemia in infancy, which later results in impairment of brain development and maturation, growth retardation, poor cognitive, motor and social- emotional development. According to WHO in 2021 prevalence of anemia was seen in more than half of the population in the Southeast Asia region, and about 50% of cases were due to iron deficiency (WHO; 2021)**.** From the analysis of the National family health survey (NFHS) -5 data, a study found that the anemia prevalence among children in India is 75.2% at 6-8 months, 78.7% at 9-11 months.

The timing of umbilical cord clamping has a profound effect on blood volume and in turn on the total iron content of newborn. We can minimize perinatal iron deficiency by either of the two placental transfusion interventions which we currently followed like delaying umbilical cord clamping [Passive transfusion] or by Intact umbilical cord milking (UCM) which results in rapid flow of blood from the placenta to baby [Rapid transfusion].

OBJECTIVES:

- **PRIMARY:** To evaluate the levels of Serum ferritin in late preterm and term newborns after intact umbilical cord milking versus delayed cord clamping at 3rd month of life.

- **SECONDARY:**

A) Impact of delayed cord clamping versus intact umbilical cord milking on hemoglobin (Hb), hematocrit at 3-5 days of life and at the 3rd month of life.

B) APGAR score at one and five minutes.

C) Total serum bilirubin, direct serum bilirubin at 3-5days of life and development of jaundice requiring phototherapy or exchange transfuse ion in both groups, development of polycythemia (hematocrit>65%) and hemodynamic parameters like Capillary refilling time, Respiratory rate, Heart rate and Oxygen saturation at 3-5 days

METHODS: Randomized controlled trial conducted between January 2021 to December 2021 in KLE'S Dr. Prabhakar Kore Hospital. After obtaining ethical clearance from our institution and informed written consent from parents all mothers who fulfil the inclusion criteria were enrolled in the study. The enrolled subjects were randomized into 2 groups

Group A: Newborns delivered after intact umbilical cord milking.

Group B: Newborns delivered after delayed cord clamping.

- SAMPLING PROCEDURE- Computer generated chart was used.
- RANDOMIZATION- SNOSE method

After delivery of the baby, the baby was placed on the maternal abdomen when delivered via vaginal route and placed over the mother's thigh in cesarean section. In Intact umbilical cord milking group, an umbilical cord was milked 5 times usually within 20 seconds towards a neonate in 25cm length from the umbilical stump which is still attached to the placenta by a delivering obstetrician at the speed of 10cm/second with 2-second interval to allow blood refilling from placenta and then

clamped 2-3cms from the umbilical stump. In DCC group, the umbilical cord was clamped 2-3cms from the umbilical stump by the delivering obstetrician at 60-180seconds after delivering the neonate.

MEASUREMENT OF OUTCOMES:

- Hemodynamic parameters like Pulse rate, Respiratory rate, Capillary refilling time, and Oxygen saturation (SpO2) were measured Hemoglobin (Cyanmethemoglobin method), Hematocrit (cell-free counter) and Serum bilirubin levels were collected at birth and at 3-5 days of life.
- Serum ferritin (CLIA method: chemiluminescence immunoassay) measured.

RESULTS AND ANALYSIS

During the study period, there were 3410 deliveries. Of which 249 mothers were eligible. Rest all were not eligible for various reasons like gestational age of less than 34 weeks (655) and trained persons not available for intervention (2506).

Out of eligible mothers, 59 mothers were excluded. A total of 190 mothers were enrolled and they were randomized into two arms; Delayed cord clamping(n=95) and umbilical cord milking group(n=95). Of these 190 mothers who were enrolled, the intervention was done only for 180 babies i.e delayed cord clamping (n= 92) and umbilical milking (n= 88). Ten newborns were not intervened because of cord abnormalities (n= 4) or requirement of active resuscitation (n = 6). Follow-up done in total 108 babies. Of which 63 babies underwent delayed cord clamping and 45 babies received umbilical cord milking. Serum ferritin levels measured at 3 months of life showed comparable results i.e mean ferritin levels in DCC group is 258.07 and in

UCM group is 248.44. Mean difference is 9.63, and there is no significant difference in serum ferritin levels at 3 months of life (p- value 0.72).

CONCLUSION:

- Umbilical cord milking and delayed cord clamping resulted in comparable levels of hemoglobin and hematocrit at 3-5 days of life and ferritin levels at 3 months of life, implying that a similar amount of placental transfusion occurs in both the groups with no increased risk of polycythemia or neonatal hyperbilirubinemia requiring exchange transfusion in either groups.
- We conclude that Umbilical cord milking is the better option to prevent anemia in infancy in cases where active resuscitation is needed and delayed cord clamping cannot be done due to undue limitation.

Keywords:

Placental transfusion, Umbilical cord milking, Delayed cord clamping, Anemia in infancy

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INTRODUCTION

The major public health concern worldwide, especially in developing countries like India is Anemia in infancy, which later results in impairment of brain development and maturation, growth retardation, poor cognitive, motor and social-emotional development.

Infants' growth and development are likely affected by hemoglobin levels at birth and iron storage levels in the first few months of life. (1)

During the initial few years of life, the process of neurodevelopment both anatomically and physiologically will be at peaks. This process will be influenced by genetic, biological and environmental factors.

Maternal and fetal iron status is linked to many neuro - cognitive and mental health disorders in the offspring based on the timing of iron deficiency in the pregnancy. (2)

The chronic irreversible effects of iron deficiency anemia highlight the importance of prevention rather than treatment especially during infancy and early childhood. (3) Hence interventions that enhance the total body's iron stores at birth will directly affect the outcomes of neonates and infants. (4)

In utero, due to exposure of the fetus to a hypoxic environment as fetal aortic oxygen saturation is about 45%, erythropoietin production is at peak and red blood cell (RBC) production is rapid. After birth, based on the gestational age, a physiological nadir in hemoglobin levels is seen due to suppression of erythropoietin production in a relative hyperoxia environment between 8-12 weeks. (5)

The placenta is an organic connection between the fetus and the uterine wall which helps in the physiological exchange of blood between the fetus and mother. The umbilical cord is a connecting tube-like structure that acts as a lifeline between the fetus and the mother. It contains umbilical arteries and umbilical vein which carry blood from the infant to the placenta and from the placenta to the infant respectively.

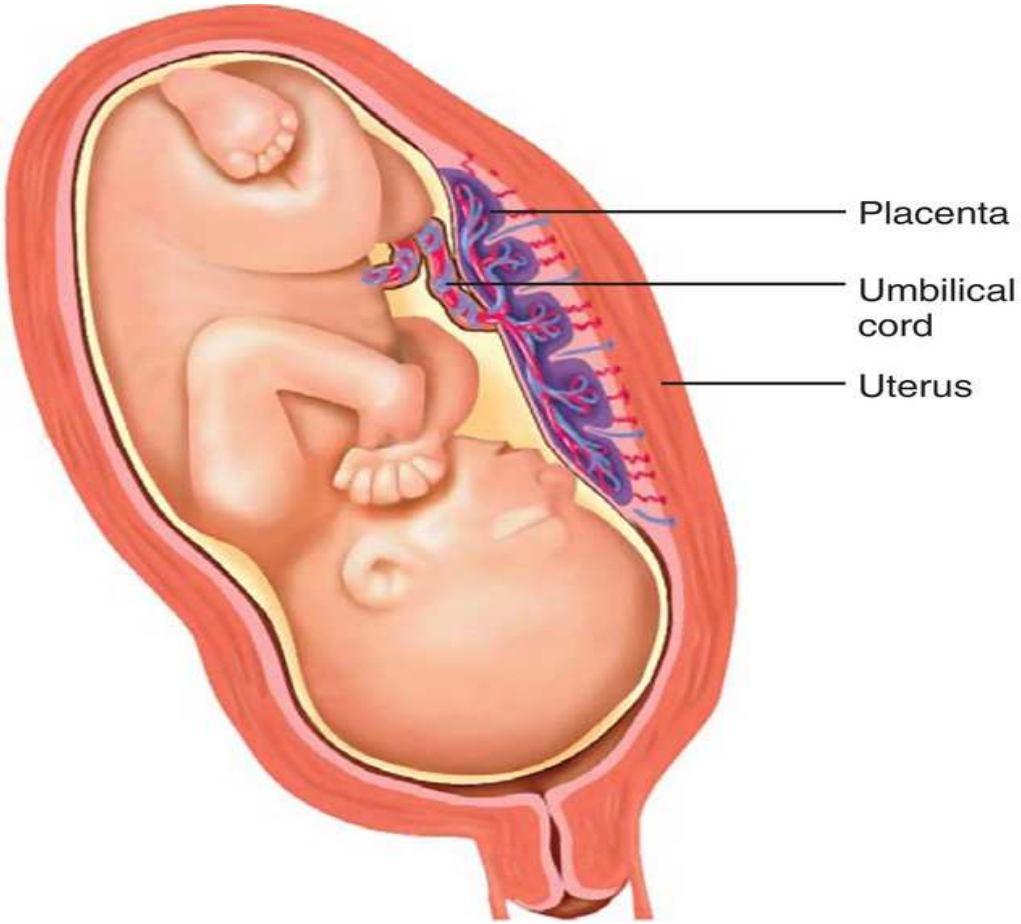

Figure 1. Showing placenta and umbilical cord connection between baby and the mother.

The redistribution of blood between the placenta and the infant at birth is known as “Placental transfusion”. (6) Timing of umbilical cord clamping has a profound effect on blood volume and in turn on the total iron content of a newborn. (7)

Iron stores in infancy are affected by the placental transfusion as in term neonates approximately 80ml of blood flow occurs from the placenta to newborn during the first minute of delivery and reaches 100ml within 3 minutes. (8) This phenomenon additionally supplies

40 – 50 mg/kg of iron to term neonates which can prevent anemia during infancy. (9)

Perinatal iron deficiency is a major concern as about 30% of reproductive-age women are anemic worldwide and this in turn affects myelination, neurotransmitter synthesis and brain programming of fetus, neonates and infants. (10)

We can minimize perinatal iron deficiency by either of the interventions which we currently followed like delaying umbilical cord clamping [Passive transfusion] or by Intact umbilical cord milking (UCM) which results in the rapid flow of blood from the placenta to baby [Rapid transfusion] (8)

Delayed umbilical cord clamping (DCC) is a method of umbilical cord clamping from 60 to 180 seconds after delivery leading to the passive transfer of blood from the placenta to the baby. Umbilical cord milking (UCM) is an active transfer method in which blood from a cord is squeezed towards the newborn. (11)

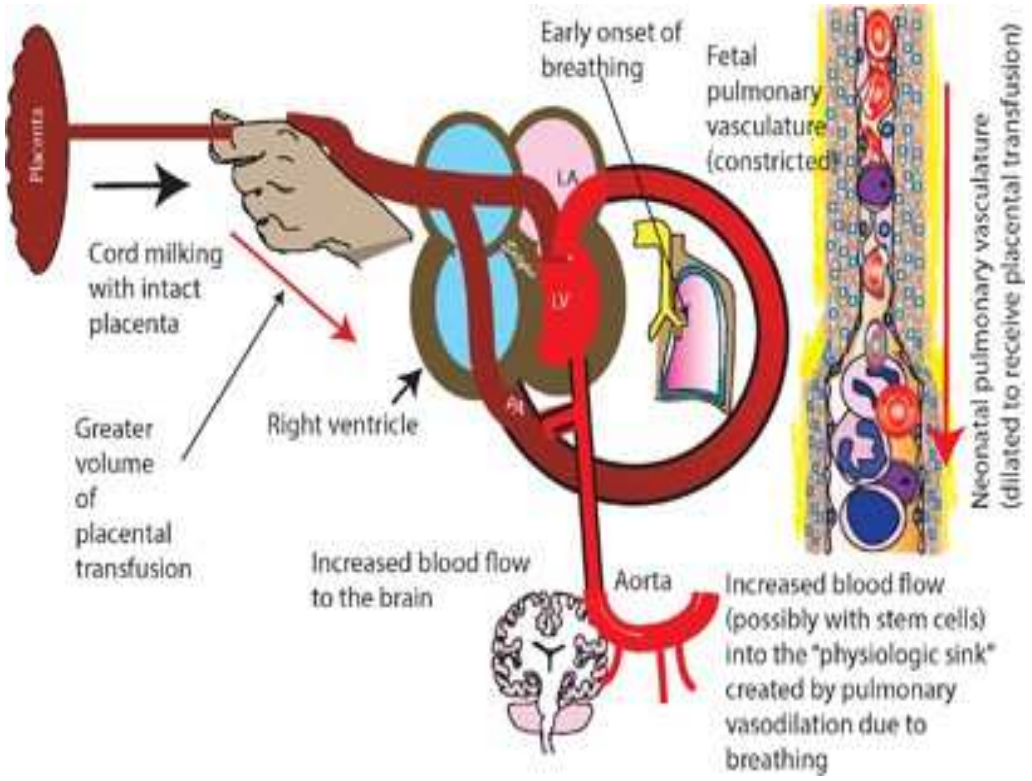

Figure 2 – Intact- Umbilical cord milking (I -UCM)

DCC may not be feasible in few clinical situations such as non- reassuring fetal status and maternal hemodynamic vulnerability. In contrast to DCC, the Intact UCM arm provides the benefit of placental transfusion without delaying resuscitation and is a time-saving method of placental transfusion. (12)

World Health Organization (WHO) and American Academy of Pediatrics (AAP) recommends "Delay in cord clamping for 60-180 seconds for all neonates not requiring resuscitation, with simultaneous initiation of essential neonatal care for improving maternal and baby health" (13)

Even though DCC has shown beneficial efforts in both term and preterm and is recommended by many international organizations including WHO, it is not widely practiced. The main reason of not practicing clinically may be difficulty in

implementation and umbilical cord milking showing similar benefits and neonatal outcomes on iron stores at various intervals of postnatal life.

Currently, there is limited data in the literature, especially in the Indian scenario on which modality provides the best outcome in infants to prevent the depletion of iron stores.

The objective of our study is to know the better method of umbilical cord clamping by evaluating the effects of intact umbilical cord milking versus delayed cord clamping on serum ferritin levels at 3 months of age.

OBJECTIVES OF THE STUDY

PRIMARY OBJECTIVE:

- To evaluate the levels of Serum ferritin in late preterm and term newborns after intact umbilical cord milking versus delayed cord clamping at 3rd month of life.

SECONDARY OBJECTIVE:

- Impact of delayed cord clamping versus intact umbilical cord milking on hemoglobin (Hb), hematocrit at 3-5 days of life and at the 3rd month of life.
- APGAR score at one and five minutes.
- Total serum bilirubin, direct serum bilirubin at 3-5days of life and development of jaundice requiring phototherapy or exchange transfusion in both groups, development of polycythemia (hematocrit>65%) and hemodynamic parameters like Capillary refilling time, Respiratory rate, Heart rate and Oxygen saturation at 3-5 days

REVIEW OF LITERATURE

GLOBAL STATISTICS ON THE PREVALENCE OF ANEMIA

About 1.62 billion population are affected by Anemia with 9% prevalence in developed countries and 43% prevalence in developing countries. (14)

The most vulnerable age groups for anemia are children and reproductive-aged women.

Globally prevalence of anemia under the age of five years is 47%. (15)

It is estimated globally that 273 million under 5 years of age group were anemic in 2011, and about ~50% of those cases were attributable to iron deficiency. (16)

ANEMIA IN INFANCY IN INDIA, A PERSPECTIVE

According to WHO in 2021 prevalence of anemia was seen in more than half of the population in Southeast Asia region, and about 50% of cases were due to iron deficiency (WHO; 2021)

From the analysis of National family health survey (NFHS) -5 data, a study found that the anemia prevalence among children in India is 75.2% at 6-8 months, 78.7% at 9-11 months then decreases to 53.4% at 48-59 months. (17)

As per recent NFHS 5, the percentage of Anemic children under five years of age group has increased to 67.1% compared to NFHS - 4 and 2 out of 3 children under-five in India are anemic.

The cause of this difference in the prevalence of anemia from the 6th to 12th months might be an increase in iron requirements with an increase in body weight as the infant's weight triples by end of the first year.

Table 1 - Prevalence of anemia in India as per age groups (6 – 17 months of age); 2019-2021 (NFHS-5)

	6-8 months	9-11 months	12-17 months
Mild(10.0 – 10.9 g/dl)	29	27.5	26.3
Moderate(7.0 – 9.9g/dl)	44.2	48.1	50.3
Severe(<7.0g/dl)	2	3.1	3.5
Any anemia	75.2	78.7	80.0

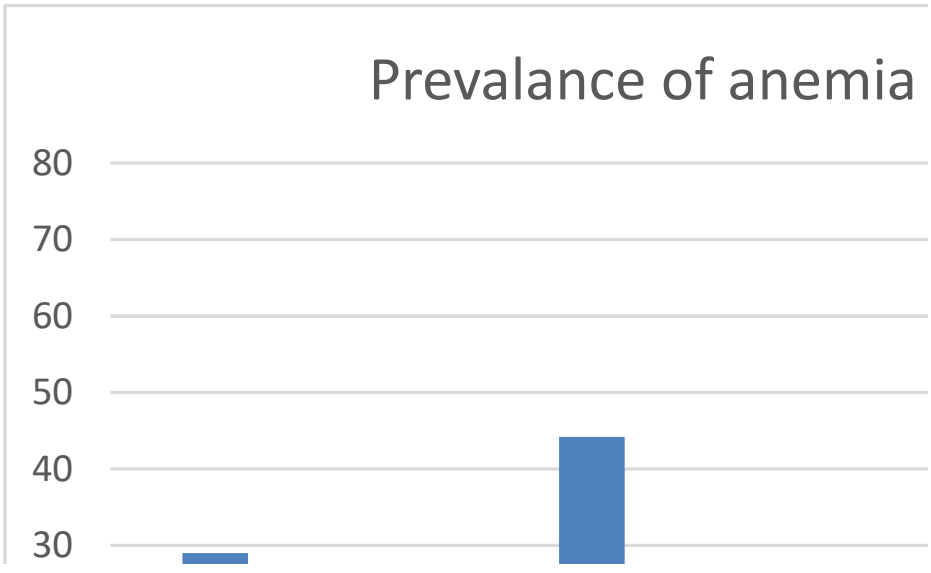

Figure 3. Bar chart showing data of prevalence of anemia in 6-8 months

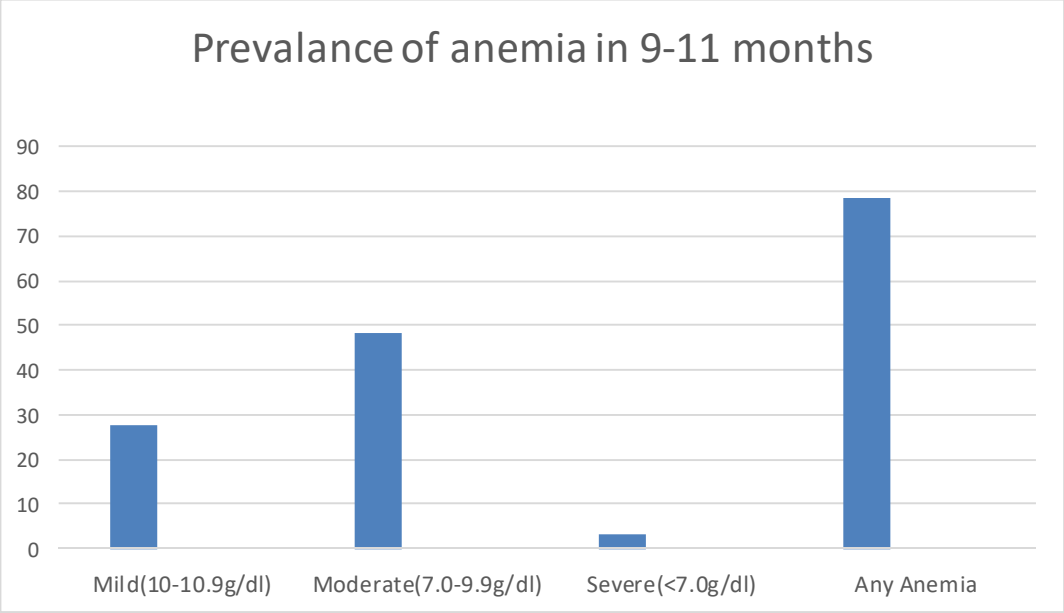

Figure 4. Bar chart showing data of prevalence of anemia in 9-11 months

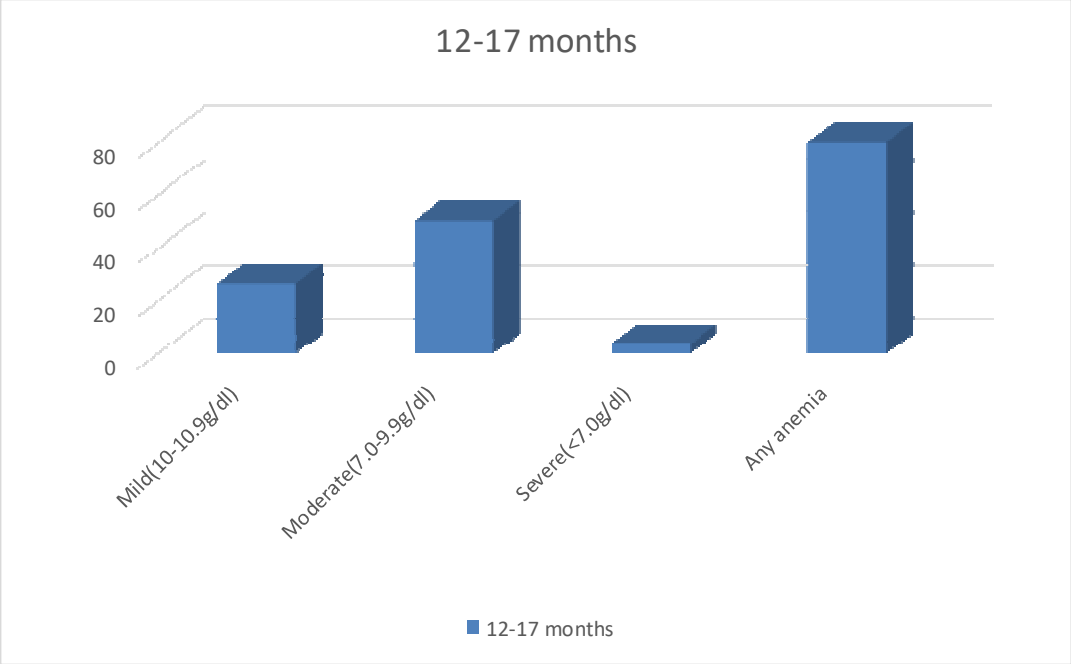

Figure 5. Bar chart showing data of prevalence of anemia in 12-17 months

Table 2: Prevalence of anemia in India based on sex among 6 to 59 months of age; 2019-21 (NHFS-5)

Anemia	Male	Female
Mild	29.0	29.5
Moderate	36.0	35.6
Severe	2.2	2.0
Total	67.2	67.0

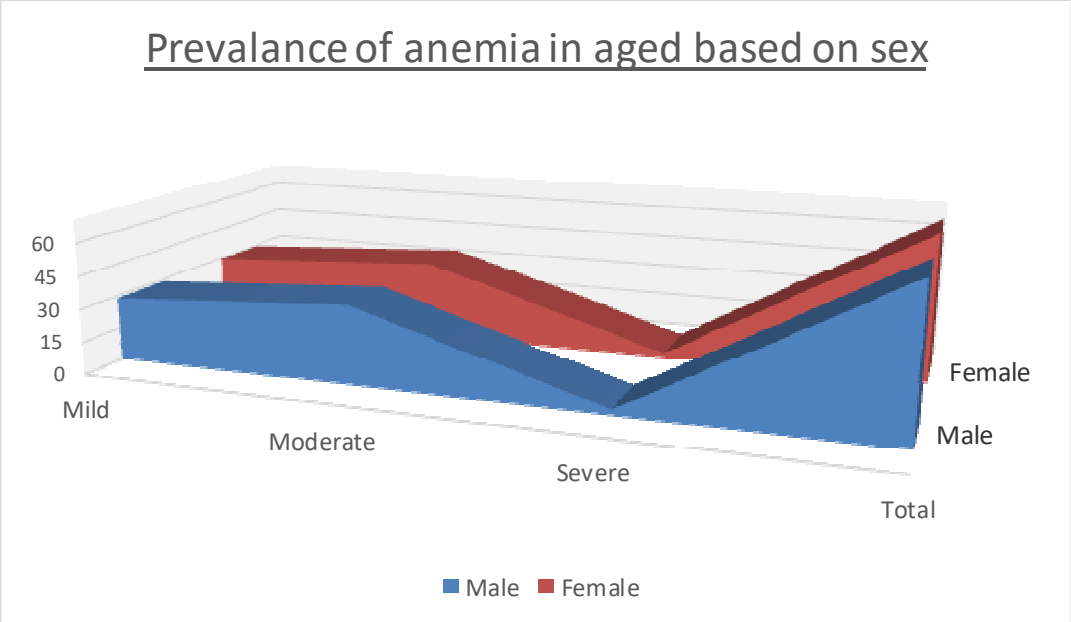

Figure 6. Graphical representation of data on prevalence of anemia based on sex

HEMATOLOGIC PHYSIOLOGY OF FETUS AND NEWBORN

Transport of iron from mother to fetus occurs mostly during the third trimester of pregnancy. (18) In utero due to exposure to a relatively hypoxic environment, fetal erythropoietin levels will be high and RBC production is rapid hence hemoglobin levels will be at peak immediately after birth. and later in the hyperoxia state hemoglobin levels will fall drastically and RBC production by day 7 is less than one-tenth the level in utero. Hemoglobin levels will drop from approximately 17gm/dl at birth to around 11.2gm/dl in the first 8 to 12 weeks of life (19) and this nadir is referred to as "physiological anemia of infancy".

Hemoglobin nadir in preterm babies is lower than in term infants because of variation in stimulus for erythropoietin production i.e., in term infants' erythropoietin is produced at hemoglobin levels of 10 – 11gm/dl but in preterm infants erythropoietin is produced at hemoglobin levels of 7-9gm/dl.

After 2 months of life, Hemoglobin levels increase slightly and usually reach about 11.8gm/dl by 4 to 6 months of life. (20) Redistribution of total body iron is the main cause of changes in hemoglobin concentration during the first few months of infancy. The estimated total body iron concentration is about 260mg of which is Hemoglobin (70%), 24% is in the form of Ferritin and myoglobin, rest 6% is in the form of Iron-containing enzymes. (21)

After 3 months of life, total body iron concentration will remain almost constant but the distribution of iron concentration changes markedly and ferritin stores will be reduced to about 12%. (21) After weaning due to exogenous sources of iron, total body concentration increases to around 420mg. (22)

Table 3. Haemoglobin (Hb) changes in Infancy (5)

Age in weeks	Hb in Term babies	Hb levels in preterm babies-weight 1.2-2.5kg	Hb levels in preterm babies- weight <1.2kg
0	17.0	16.4	16.0
1	18.8	16.0	14.8
3	15.9	13.5	13.4
6	12.7	10.7	9.4
10	11.4	9.8	8.5
20	12.0	10.4	9.0
50	12.0	11.5	12.0

Table 4. Hemoglobin (Hb) levels at nadir and the time of nadir (5)

Maturity of baby at birth	Hb level at Nadir	Time of Nadir(week)
Term babies	9.5-11.0	6-12 weeks
Preterm babies with weight between 1.2 – 2.5 kg	8.0-10.0	5-10 weeks
Preterm babies with weight <1.2kg	6.5-9.0	4-8 weeks

FACTORS INFLUENCING IRON STATUS AT BIRTH

"Maternal iron status, gestational age, birth weight of neonates are major factors which influence iron status of a newborn" (23) Studies showed that maternal anemia during pregnancy is a strong determinant for anemia during infancy. (24-27)

Studies showed that birth weight and iron stores at birth are directly proportional (28)

An increase in liver size during the last 8 weeks of gestation, might be the cause of increased total iron concentration in term gestation. (23)

IRON STORES DEPLETE DURING INFANCY

Exclusively breastfeeding during the first 6 months of life is the optimal feeding practice worldwide. Breastmilk alone meeting all the developmental needs of an infant during the first 6 months of life is controversial. (29)

The amount of iron absorbed from breast milk is almost the same as the total amount of iron lost and the maximum loss through the sloughing of epithelial cells in the gastrointestinal tract. (30)

Due to relatively more rapid rate of growth in preterm babies and low iron stores with higher requirement of iron for RBC production these infants are at higher risk of anemia in infancy and this exaggeration of normal physiological anemia is termed as "Anemia of prematurity". (23)

EFFECTS OF ANEMIA ON INFANCY

Normal brain development requires a crucial nutrient named Iron. (31) Iron is required for neurogenesis and differentiation of brain cells. (32,33,34)

In the fetus, iron stores are more utilized for RBC production, remaining stores will be taken by other tissues like the brain, (31) hence brain development will be impaired if iron stores are deficit. Correlating with poor cognitive, motor, and/or social and emotional development later age. (31)

STRATEGIES TO PREVENT IRON DEFICIENCY DURING INFANCY (35)

1. Iron supplementation for all pregnant women
2. Delayed cord clamping
3. Early initiation of breastfeeding
4. To continue breastfeeding exclusively for 6 months

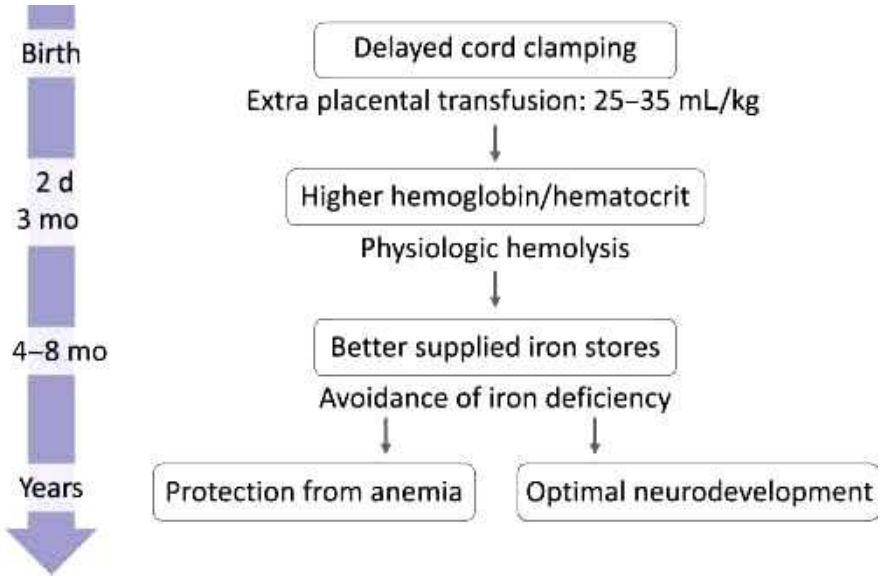

Figure 7 – showing strategy of preventing iron deficiency by delayed cord clamping

UMBILICAL CORD

A channel between the fetus and the placenta, plays a major role in both physiological and genetical. Contains umbilical vessels which normally include two umbilical arteries and one umbilical vein. The umbilical vein carries oxygenated blood rich in nutrients from the placenta to the fetus and the umbilical arteries carries deoxygenated blood from the fetus to the placenta.

Umbilical cord forms by the 5th week of intrauterine life and replaces yolksac; a source of nutrients for the fetus. The blood flow through the umbilical cord is approximately 35ml/min at 20 weeks, and 240ml/ min at 40 weeks of gestation. (36)

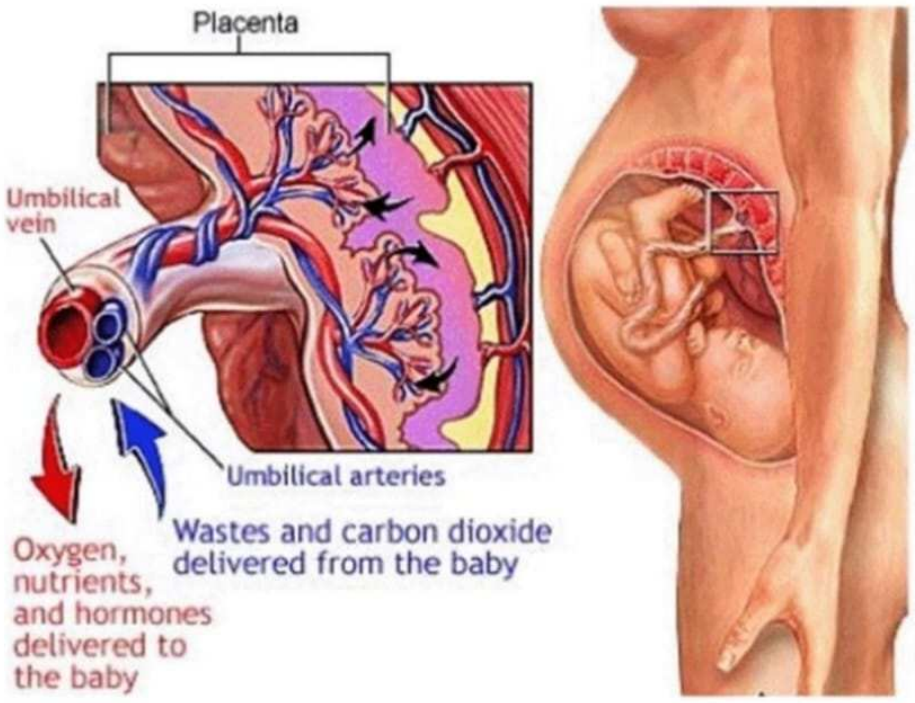

Figure 8. Showing umbilical cord with umbilical arteries and vein

PLACENTAL BLOOD TRANSFUSION

An additional amount of blood volume transferred from the placenta to the neonate at birth through the umbilical vein is defined as placental transfusion. (37)

In utero, the total fetoplacental circulation is approximately 110-115ml/kg of the fetal body weight with about 70ml/kg of the body weight in fetus and 45ml/kg of the body weight in the placenta (38). The average amount of placental transfusion was about 81ml (range 50 to 163ml) or approximately 25ml/kg (range 16 to 45ml/kg) and placental transfusion contributes to 20% of the infant's blood volume at birth. (39)

Placental transfusion is mainly affected by the following factors (37)

1. Timing of cord clamping

2. Placement of newborn immediately post-delivery and gravity

3. Uterine contractions following delivery

4. Rate of placental transfusion

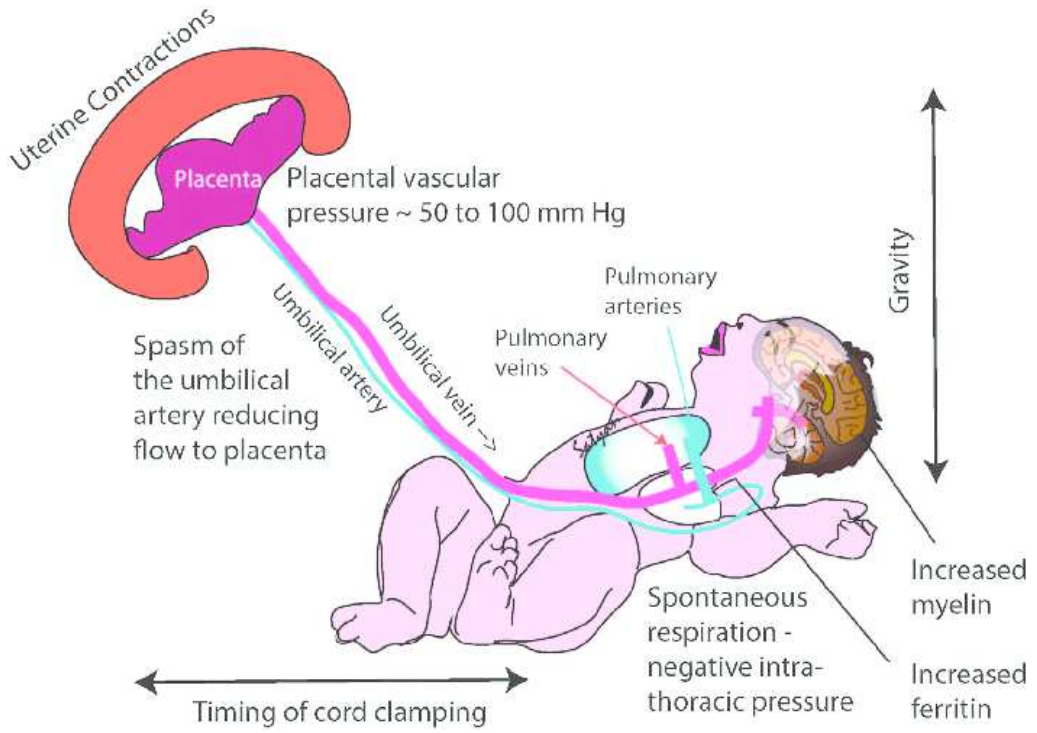

Figure 9– showing factors influencing the placental transfusion.

TIMING OF CORD CLAMPING

The timing of cord clamping can have a significant role in the amount of blood transfer between the placenta and the neonate and hence in turn will affect the total body iron content of newborns.

For a brief period of time after birth there will be circulation occurring between the infant and the placenta through the umbilical vein and arteries. A study conducted by Yao and Lind, 1974 by measuring the residual blood volume in the placenta after clamping the umbilical vessels at various time intervals, showed that blood flows through the umbilical arteries during initial 20 – 25 seconds and after 45 seconds blood transfusion is insignificant. (40) In the umbilical vein, blood continues to flow from the placenta to infant for up to 3 minutes after delivery. (22)

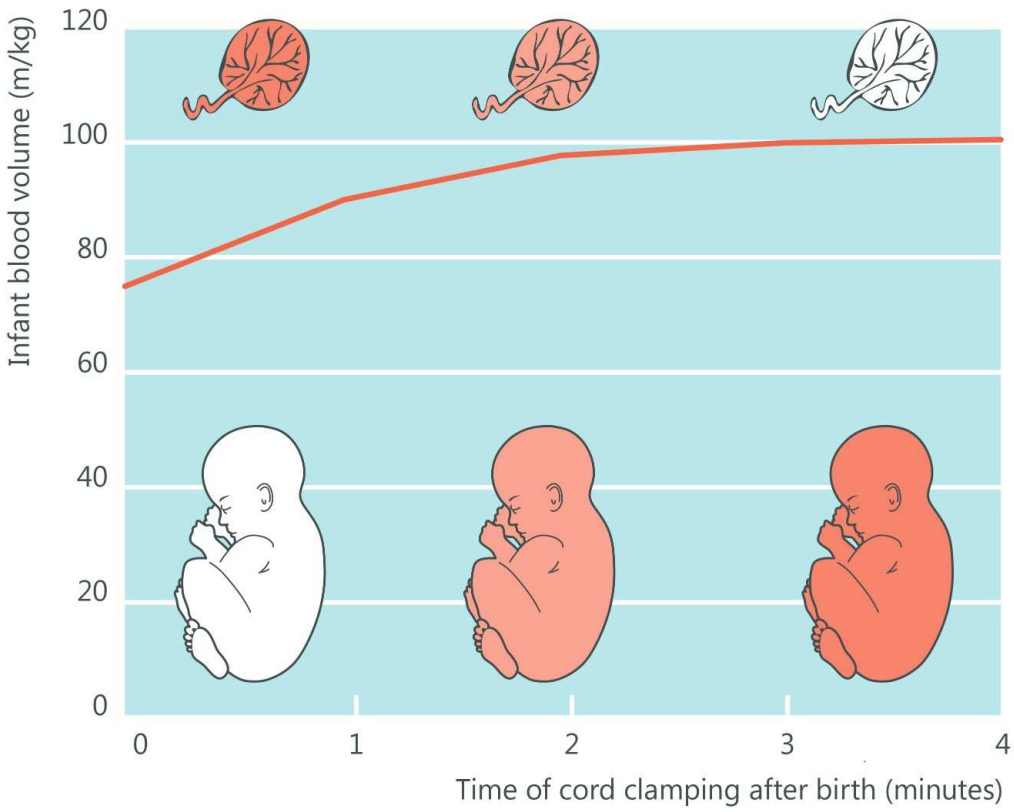

Figure 10. Timing of cord clamping and the amount of placental blood transfusion

PLACEMENT OF NEWBORN IMMEDIATELY POST DELIVERY

One of the studies showing that gravity also plays an important role in placental transfusion i.e. if the infant is held sufficiently high enough above the mother's uterus (500-600mm) placental transfusion can be prevented by stopping blood flow through the umbilical vein and which also allows back flow through the umbilical arteries. (40)

Figure 11: Showing placement of newborn immediately after delivery

RATE OF PLACENTAL TRANSFUSION

The rate of placental transfusion is rapid initially and then slows down in a stepwise fashion, with about 25% of the blood volume getting transferred in the first 15-30 seconds after uterine contraction of birth, 50-78% will get transferred by 60 seconds and the remaining volume of blood will be transferred by 3 min. (41)

Placental transfusion provides an additional 15- 40 ml/kg birth weight of blood from the placenta to the infant. Hence delay in cord clamping will maximize the blood volume with an additional increase of total volume by about 30 - 40% in a term neonate. (22,42)

In contrast, if the umbilical cord is clamped immediately, placental transfusion will be stopped abruptly and about 20ml/kg of infant whole blood volume and 25mg/kg of iron will remain in the placenta. (41,43)

Approximately 30% more blood will be received by a newborn who receives placental transfusion at delivery when compared to immediate cord clamping. Hence, the timing of the cord clamp will significantly affect the volume of placental transfusion. Total placental blood volume will not be transferred to the newborn, some residual volume will be retained in the placenta which probably prevents volume overload. (38)

UTERINE CONTRACTIONS

Uterine contractions which naturally occur again between 1 and 3 minutes after the birth contraction, might be the reason for the last step of the passive transfusion of blood from the placenta to the baby. (45)

DELAYED CORD CLAMPING IN TERM NEONATES

Studies conducted on healthy-term infants stated that delayed cord clamping increases hemoglobin levels and iron stores up to six months of life and hence decreases the risk of iron deficiency and early onset of anemia in the neonatal period. (46-49) These studies also accessed both the risks and benefits associated with delayed cord clamping. A recent Cochrane review showed that delayed cord clamping group babies developed significantly high bilirubin values requiring phototherapy than early cord clamping group. Increased blood volume in newborns after delayed cord clamping might be the potential cause of jaundice. (47)

In contrast, studies done by Mathew et al, and Rabe et al found that there is no significant difference in mean serum bilirubin values, development of neonatal hyperbilirubinemia, or requirement for phototherapy or exchange transfusion after DCC. (48,49)

Andersson et al (46) conducted a large study evaluating neonatal outcomes after delayed cord clamping versus early cord clamping and concluded that DCC does not increase the risk of neonatal hyperbilirubinemia, no significant differences in Apgar scores at five minutes, incidence of Neonatal intensive care unit admissions, or respiratory distress in comparisons of Early versus delayed cord clamping in healthy term newborns. (46-49)

Delayed cord clamping leads to significantly higher mean bilirubin values but no increase in the requirement of phototherapy. (50) Hence these studies have shown that delayed cord clamping decreases neonatal anemia and prevents depletion of iron stores in early infancy which has the positive effect of growth and development during infancy. So on the basis of these studies delayed cord clamping can be followed universally in healthy-term infants. Mercer et al suggested that at four months of life ferritin levels and brain myelination were better in DCC group infants. (51)

DELAYED CORD CLAMPING IN PRETERM INFANTS

Universal acceptance of following delayed cord clamping in preterm infants is still controversial because studies conducted so far in a preterm group gives contradictory conclusions. As this group is more vulnerable even many clinicians are reluctant for delayed cord clamping as this may interfere with the resuscitation of the newborn. A study conducted on the basis of responses to the questionnaires by members of the American College of Obstetricians and Gynecologists (ACOG) stated that “even after proven evidence of benefits of delayed cord clamping only 9.3% of obstetricians shows adherence of following delayed cord clamping” and many

obstetricians are on the conclusion that no significant impact seen on neonatal outcomes on the basis of different point intervals of umbilical cord clamping. (52,53)

Reasons for not following DCC universally are difficulty in practicing and being unaware of scientific evidence. (53)

Several studies have shown that delaying cord clamping by more than 30 seconds has advantages in preterm infants like necessity and demand for RBC transfusions have come down, intraventricular bleed risk decreased and the risk of late-onset sepsis. (49,53,54,55) These studies stated that no significant changes were noted in Apgar scores, neonatal outcome parameters like respiratory distress, or hyperbilirubinemia requiring treatment. (49,53)

Anup et al stated that in infants born less than 32 weeks of gestation, there is no statistically significant difference between in rate of outcomes of death in delayed cord clamping versus umbilical cord milking, but statistically significant high rates of severe intraventricular hemorrhage seen in umbilical cord milking group. (56)

UMBILICAL CORD MILKING IN TERM INFANTS

Umbilical cord milking is a process in which the cord is milked several times which increases the placental transfusion from the placenta toward the baby. It has also shown similar beneficial effects as that of delayed clamping and this process doesn't interfere with active resuscitation if required for the baby. (12,57)

Katheria et al suggested that umbilical cord milking is a feasible and safer placental transfusion method in newborns requiring active resuscitation and newborn with acidosis. (58) Another study from Katheria et al showed that UCM had higher language and cognitive scores when compared with DCC group infants. (59)

A study done by Girish et al stated that no statistical differences were seen in resuscitative efforts and short-term outcomes in either of the groups who received umbilical cord clamping or early cord clamping in neonates who are depressed at birth. (60) Literature data suggests that umbilical cord milking will be quick and effective enough to provide placental transfusion to depressed infants. (61)

Erickson et al (2011) stated that umbilical cord milking results in placental transfusion in term infants at the time of cesarean section with higher hematocrit at 36-48 hours of life. (62) Amit Upadhayay et al (2013) study showed that Umbilical cord milking is a safe procedure and improved Hemoglobin, iron status at 6 weeks of life among term and near-term neonates. (63)

The most recent systematic review conducted by Kazuma et al (2021) stated that- “UCM might be as beneficial as DCC in term infants”. (64)

UMBILICAL CORD MILKING IN PRETERM INFANTS

Hosono &amp demonstrated that UCM resulted in high blood pressure values and high urine output during the initial 12 hours of life, a shorter period for the need for assisted ventilation and less need for blood transfusion. (65,66)

In a study done by Rabe et al, UCM and DCC resulted in similar outcomes in terms of haemoglobin, need for transfusions, intraventricular haemorrhage, sepsis, necrotizing enterocolitis, and death. (67)

Anup et al concluded that UCM is a more efficient technique to improve blood volume after cesarean section in preterm infants. (68) One of the studies showed that UCM may decrease the risk of IVH and improve neurodevelopmental outcomes compared to DCC in preterm babies. (69)

In contrast, A study conducted by Haribalakrishna et al concluded that UCM significantly increased the risk of severe intraventricular hemorrhage in preterms, especially at lower gestational ages but when compared with immediate cord clamping group frequency of RBC transfusions are less but clinical outcomes are not significantly improved. (70)

Bimlesh et al did a study on moderate to late preterm infants shown that iron stores at 6 weeks of life are higher in the umbilical cord milking group but a higher incidence of phototherapy requirement seen in the milking group (33% compared to 9% in the early cord clamping group) (71)

One of the studies done in an Indian setup concluded that in term infants no statistically significant differences were seen in ferritin levels and in growth parameters at 1 year of age (72)

Jaiswal et al stated that ferritin levels at 6 weeks of life are significantly higher in group receiving milking with delayed cord clamping than only milking or only delayed cord clamping. (73,74)

DELAYED CORD CLAMPING VERSUS UMBILICAL CORD MILKING

Rabe et al who compared these two techniques of DCC and UCM concluded that in preterm infants milking the cord four times achieved a similar amount of placenta-fetal blood transfusion compared with delaying clamping the cord for 30 seconds. (67)

Currently, only a few studies comparing these two procedures in term neonates exist. The most recent studies done by Zanardo et al 2021 and Mangla et al 2020 (AIIMS, New Delhi) state that hematocrit values are on the higher side at 48hours of life following intact umbilical cord milking than by delayed cord

clamping. (75,76) Hence Umbilical cord milking may be a promising alternative for enhancing placental transfusion in preterm and term infants.

JUSTIFICATION

Various health organizations including national and international like WHO, ACOG, RCOG (The Royal College of Obstetricians and Gynaecologists) recommends Delayed cord clamping. “In November 2019, Ministry of Health and Family Welfare, Government of India launched a new health program called **SUMAN** -Surakshit Matritva Aashwasan and issued an advisory on physiological cord clamping, which refers to deferring the cord clamping till natural delivery of placenta”.

Universal application of DCC is limited at the practice level even after many evidences based on clear guidelines. (53) Reasons may be due to concerns about the risk of hypothermia, delay in routine care or delay in initiation of resuscitation when needed, resulting in failure to adopt this recommendation.

Another alternative procedure that may improve placental transfusion is umbilical cord milking (UCM). Studies based on evidence show that UCM improves placental transfusion in both preterm and term infants when compared to immediate cord clamping and Its safety has been demonstrated in many randomized controlled trials. UCM is a simple but effective method that can have similar results as DCC and can be accomplished within few seconds.

The purpose of this study is to assess the effects of intact umbilical cord milking versus delayed cord clamping on hematological parameters at 3-5 days and 3 months of life

MATERIALS & METHODS

Source of data: Study is conducted on newborns delivered in KLE Dr. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, Belgaum.

Method of collection of data

Study design: Randomized, single – center, open – label, parallel – group, performed over 1-year span at KLE'S Dr. Prabhakar Kore Hospital and MRC, Belagavi.

Study Duration: One year (January 2021 to December 2021)

Study place: KLE Dr. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, Belgaum.

INCLUSION CRITERIA

1) Newborns delivered after intact umbilical cord milking or delayed cord clamping
2) Gestational age of 34 0/7 weeks to 41 6/7 weeks
3) Does not require active resuscitation at birth
4) Maternal Hb >9.9mg/dl
5) Parental written informed consent given.

EXCLUSION CRITERIA:

1) Multiple gestation
2) Cord abnormalities (cord prolapse, true knots)
3) Rh sensitized mothers
4) Antenatal scan showing any major congenital anomalies of fetus
5) Abnormal placentation
6) Mothers with smoking habits
7) Bleeding disorders in mothers

SAMPLE SIZE:

Sample size formula:

The minimum sample size formula based on mean and standard deviation is

$$n = \frac{(z_\alpha + z_\beta)^2 (s_1^2 + s_2^2)}{(\bar{X}_1 - \bar{X}_2)^2}$$

Where z_α is linked with the level of significance and z_β is linked with the power of the test. For 5% level of the significance z_α =1.96 and z_β =0.84 for 80% power of the test.

Ref:

X_1 is the mean of the first group (15.14) and X_2 is the mean of the second group (14.71)

s_1 is the standard deviation of the first group (0.80) and s_2 is the standard deviation of the second group (0.78).

With these values the sample size obtained is 108 total size i.e 54 in each group.

METHODOLOGY:

PROTOCOL:

After obtaining ethical clearance from our institution and informed written consent from parents all mothers who fulfil the inclusion criteria were enrolled for the study.

The enrolled subjects were randomized into 2 groups after confirming the period of gestation either by mother's Last menstrual period (LMP) or by Dating ultrasound scan done on 1st trimester.

Group A: Newborns delivered after intact umbilical cord milking.

Group B: Newborns delivered after delayed cord clamping.

SAMPLING PROCEDURE-

Computer generated chart was used.

RANDOMIZATION-

It was done at the time of delivery in labour room by keeping the group written slip in serially numbered opaque sealed envelope (SNOSE method).

All obstetricians were previously instructed on how to carry out both the techniques in both vaginal and cesarean section deliveries.

- After delivery of the baby, the baby will be placed on the mother's abdomen when delivered via vaginal route and over mother’s thigh in cesarean section. In Intact umbilical cord milking group, an umbilical cord was milked for 5 times usually within 20 seconds towards a neonate in 25cm length from umbilical stump which is still attached to the placenta by a delivering obstetrician at the speed of 10cm/second with 2-second interval to allow blood refilling from placenta and then clamped 2-3cms from the umbilical stump.

Figure 12: Umbilical cord before milking procedure

Figure 13: Umbilical cord after milking procedure

- In DCC group, the umbilical cord was clamped 2-3cms from umbilical stump by the delivering obstetrician at 60-180seconds after delivering the neonate.

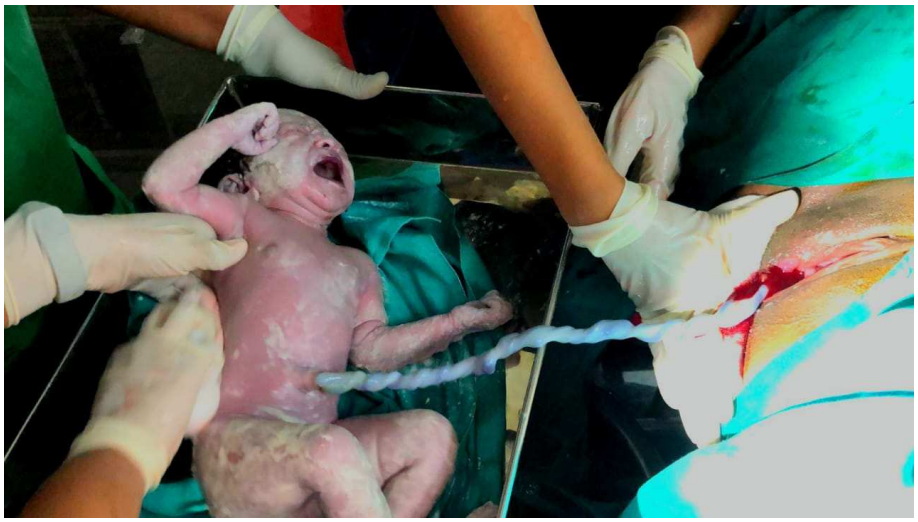

Figure 14: Delayed cord clamping

- Neonates from both the groups were kept by mother side and undergo routine standard management except for babies requiring NICU care.
- Breast feeding for all neonates started as per standard guidelines.
- In both groups, the data was recorded by Pediatric Postgraduate residents posted in labor room and blinded for outcome of the study.

MEASUREMENT OF OUTCOMES:

- Hemodynamic parameters like Pulse rate, Respiratory rate, Capillary refilling time, Oxygen saturation (SpO2) were measured at birth and at 3-5 days of life.
- Blood samples for Hemoglobin (Cyanmethemoglobin method), Hematocrit (cell free counter) and Serum bilirubin levels were collected from newborn at 3-5days of life
- If serum bilirubin level was within the phototherapy range, phototherapy was initiated. (According to the AAP bilirubin nomogram/ Bhutani chart.)
- Hematocrit >65% is considered as polycythemia
- Follow up done at 3rd month of life. Growth parameters like Weight, Length and Head circumference were measured. Hemoglobin, Hematocrit, Serum ferritin (by CLIA method : chemiluminescence immunoassay) measured.

Figure 15: Serum ferritin Kit

- Normal serum ferritin levels at 3rd month of life are 50-200 ng/ml.

- The weight of the baby measured by a Weighing machine and charted on WHO growth chart

Figure 16: Weighing machine

- Length- measured using Infantometer and charted on WHO growth chart

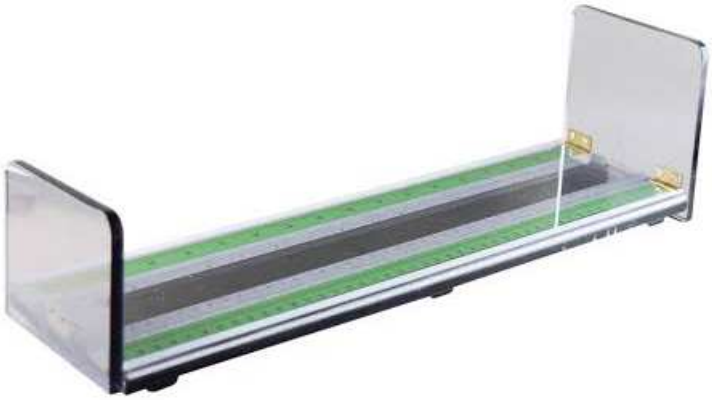

Figure 17: Infantometer

- Head circumference- measured using a measuring tape by placing it over the occipital protuberance at the back and just over the supraorbital ridge and the glabella in front and plotted on WHO growth chart.

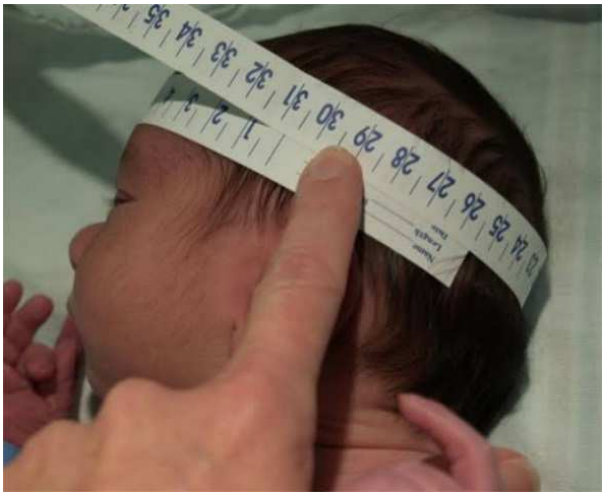

Figure 18: picture showing - measuring head circumference

DATA COLLECTION:

Mother and newborn data were recorded and entered in predesigned study proforma. Post intervention hematological parameters were collected and entered in master chart and all the data were uploaded into the Microsoft excel sheet at regular intervals. Till 3 months of life none of the newborns were received iron supplementations. Follow up was done at 3months of age, and serum hemoglobin, hematocrit and ferritin levels were estimated and recorded in data collection sheet and excel sheet.

STATISTICAL ANALYSIS:

- The study is focused on comparison of two groups.
- For the continuous quantitative variables mean and standard deviation calculated.
- The inter group continuous variables were compared using suitable tools of statistics like unpaired student's t test.
- Two quantitative variables, within a group, were compared using student's paired t test.
- The categorical data were expressed in terms of rates, ratios and percentage.
- The association between the outcome, clinical and demographic characteristics was tested using Chi-square test or Fisher's exact test.
- Discrete variables are represented by median. Nonparametric tests were used for comparing discrete variables.

Suitable graphs were used to depict the comparison. For all the tests the value of p less than 5% (0.05) was considered significant.

RESULTS AND ANALYSIS

During the study period, there were 3410 deliveries. Of which 249 mothers were eligible. Rest all were not eligible for various reasons like gestational age less than 34 weeks (655) and trained persons not available for intervention (2506).

Out of eligible mothers, 59 mothers were excluded. Reasons for exclusion are multiple gestations, cord abnormalities (cord prolapse, true knots), Rh negative mother, an antenatal scan showing any major congenital anomalies of the fetus, abnormal placentation, mothers with smoking habits, bleeding disorders in mothers

A total of 190 mothers were enrolled and they were randomized into two arms; Delayed cord clamping(n=95) and umbilical cord milking group(n=95). Of these 190 mothers who were enrolled, an intervention was done only for 180 babies i.e. delayed cord clamping (n= 92) and umbilical milking (n= 88). Ten newborns were not intervened because of cord abnormalities (n= 4) or the requirement of active resuscitation (n = 6)

Follow-up done in total 108 babies. Of which 63 babies underwent delayed cord clamping and 45 babies received umbilical cord milking.

CONSORT

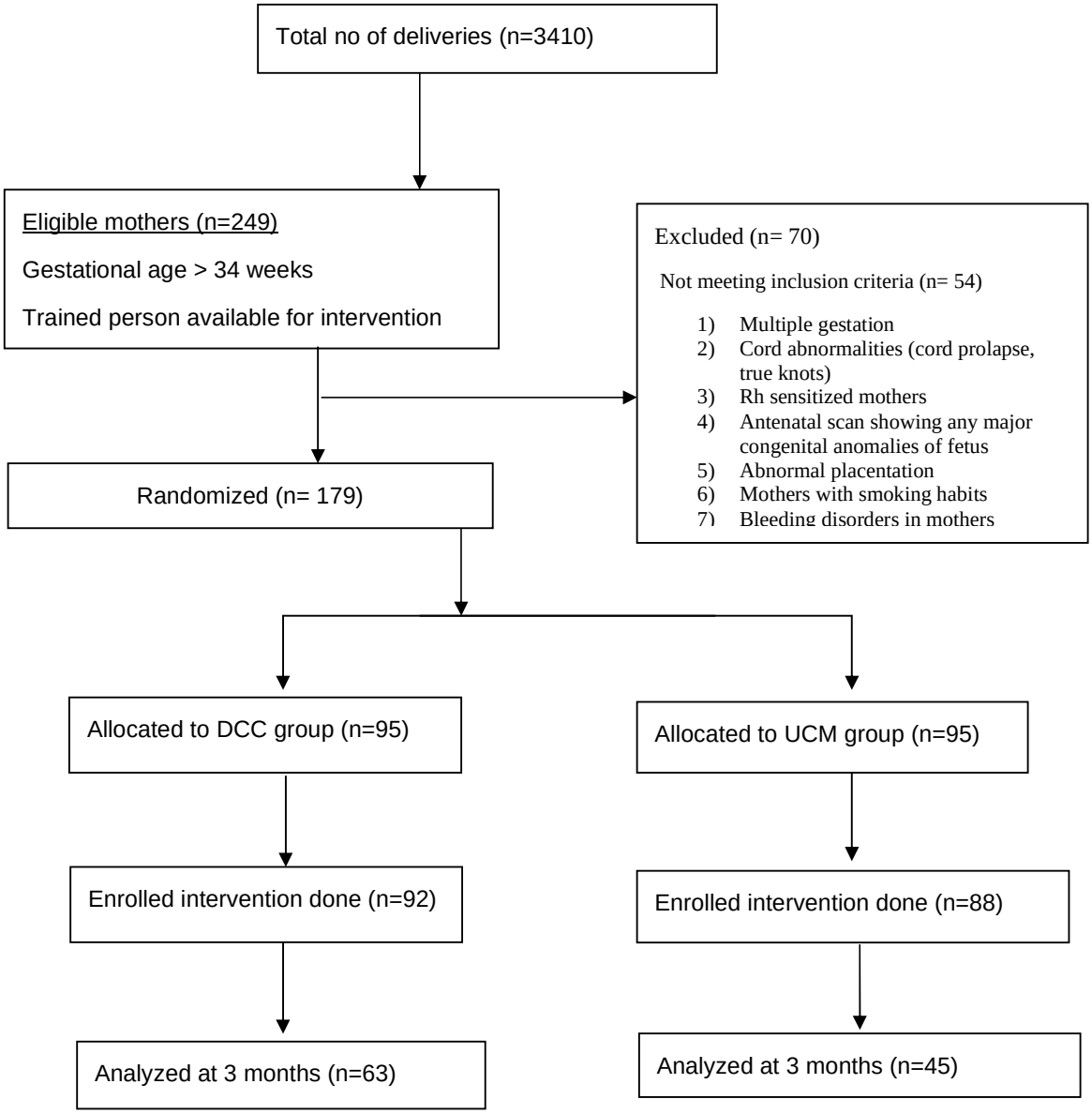

Figure 19: Study algorithm

MATERNAL HEMOGLOBIN LEVELS

Table 5: Comparison of two groups (DCC and UCM) with mother Hb (%) by independent t test

	GROUP	N	Mean	SD	T Test	P Value
MOTHER HB	DCC	92	12.12	1.09	0.57	0.56
	UCM	88	12.03	1.05		

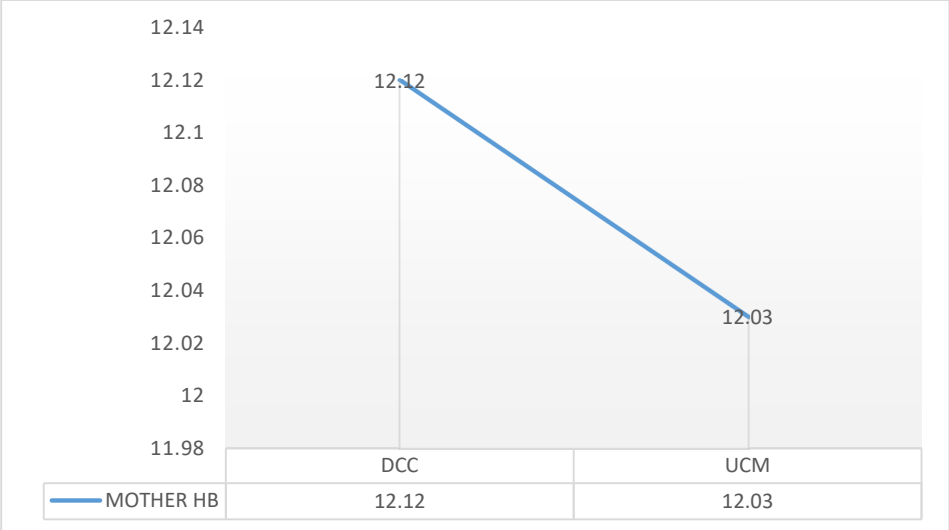

Figure 20: Comparison of two groups (DCC and UCM) with mother Hemoglobin (%)

All the mothers who were enrolled are non-anemic (Hb more than 11g/dl). Mean Hb in mothers of DCC group was 12.2 g/dl and that of UCM group was 12.03 with mean difference of 0.17, which was statistically and clinically not significant (p value 0.56)

IRON SUPPLEMENTATION DURING PREGNANCY

Table 6: showing number of mothers received iron supplementation during pregnancy

			GROUP		Total
			DCC	UCM	
IRON SUPLEMENTATION	Yes	n	2	1	3
		%	2.2%	1.1%	1.7%
	No	n	90	87	177
		%	97.8%	98.9%	98.3%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

Chi-Square: 0.29, P Value: 0.51, Statistically not significant

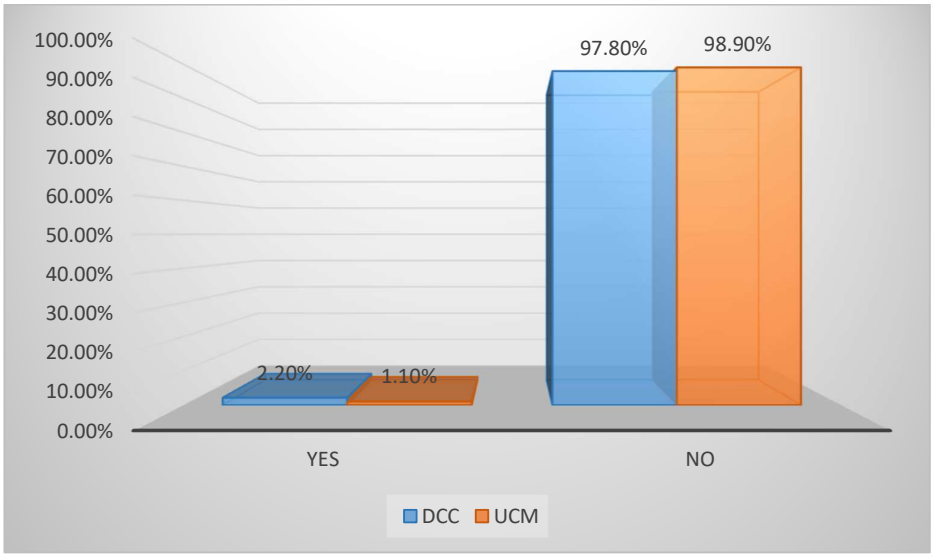

Figure 21: showing number of mothers received iron supplementation during pregnancy

MODE OF DELIVERY

Table 7: showing mode of deliveries in both groups

			GROUP		Total
			DCC	UCM	
MODE OF DELIVERY	Elective LSCS	n	12	15	27
		%	13.0%	17.0%	15.0%
	Emergency LSCS	n	18	46	64
		%	19.6%	52.3%	35.6%
	NVD	n	62	27	89
		%	67.4%	30.7%	49.4%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

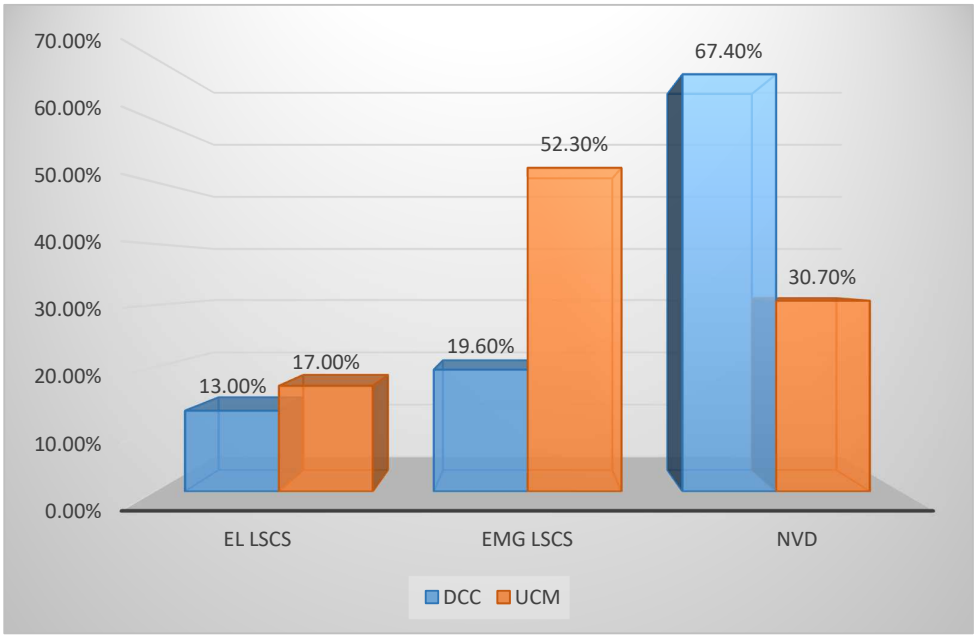

Figure 22: showing mode of deliveries in both groups

GENDER DISTRIBUTION IN BOTH GROUPS:

Table 8: Comparison of DCC and UCM with gender

			GROUP		
			DCC	UCM	Total
SEX	Male	n	48	49	97
		%	52.2%	55.7%	53.9%
	Female	n	44	39	83
		%	47.8%	44.3%	46.1%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

Chi-Square: 0.22, P Value: 0.37, Statistically not significant

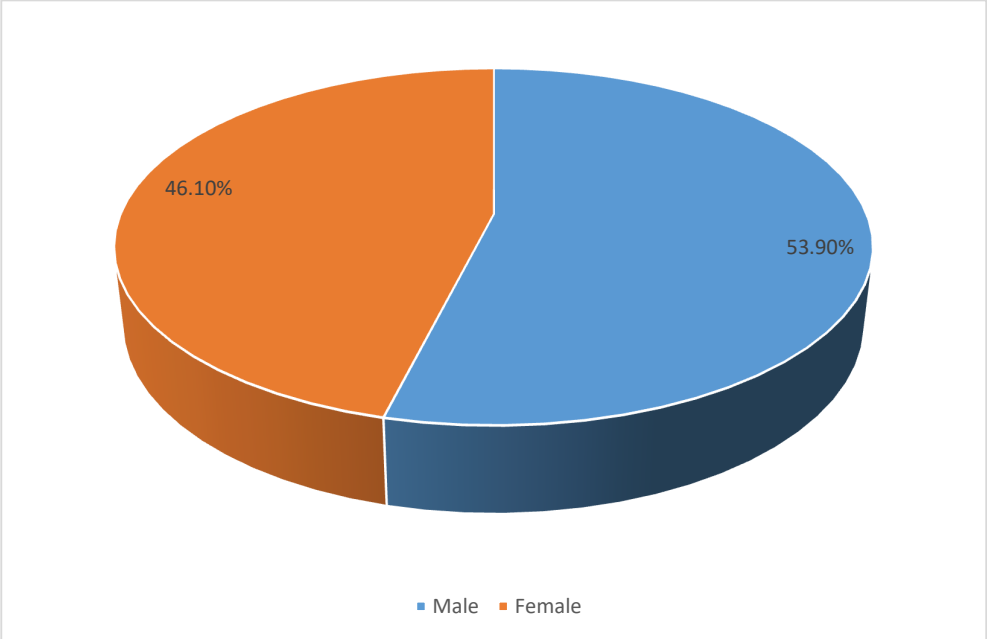

Figure 23: Gender distribution in our study

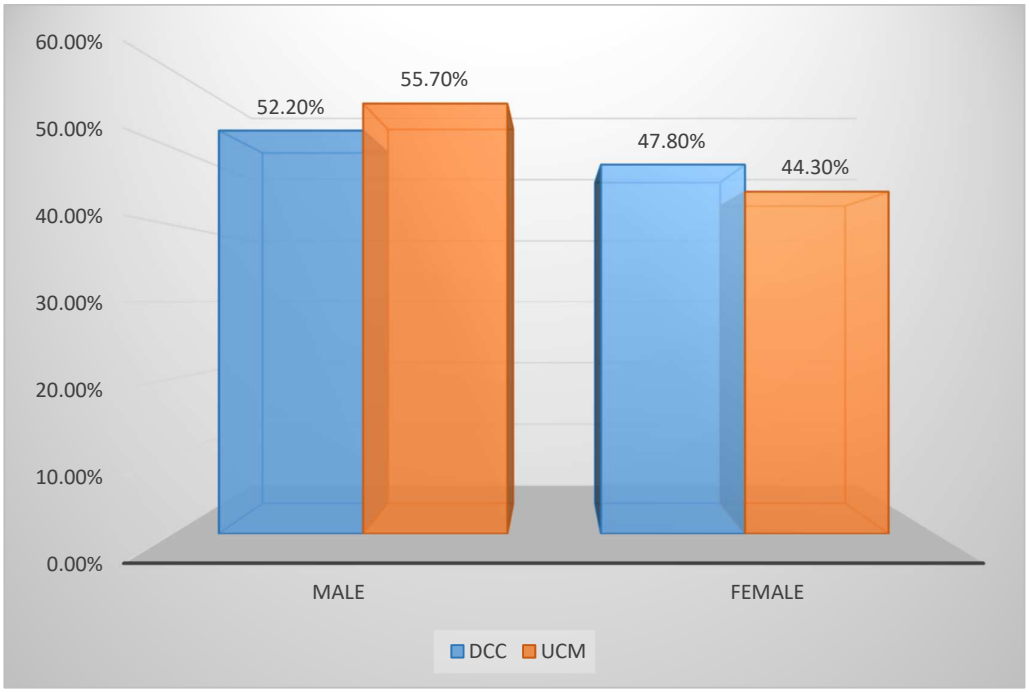

Figure 24: Comparison of DCC and UCM with gender

In our study out of 180 newborns, 97 were boys (48 in DCC group and 49 in UCM group) and 83 were girls (44 in DCC group and 39 in UCM group) as shown in above table and figure. There was no significant statistical difference among the two groups with respect to gender (p value 0.37).

COMPARISON OF GESTATIONAL AGE

Table 9: Comparison of DCC and UCM based on gestational age

			GROUP		Total
			DCC	UCM	
Gestational age	34-36.5 weeks (late preterm)	n	6	6	12
		%	6.5%	6.8%	6.7%
	37-41.5 weeks (term)	n	86	82	168
		%	93.5%	93.2%	93.3%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

Chi-Square: 0.006, P Value: 0.58, Statistically not significant

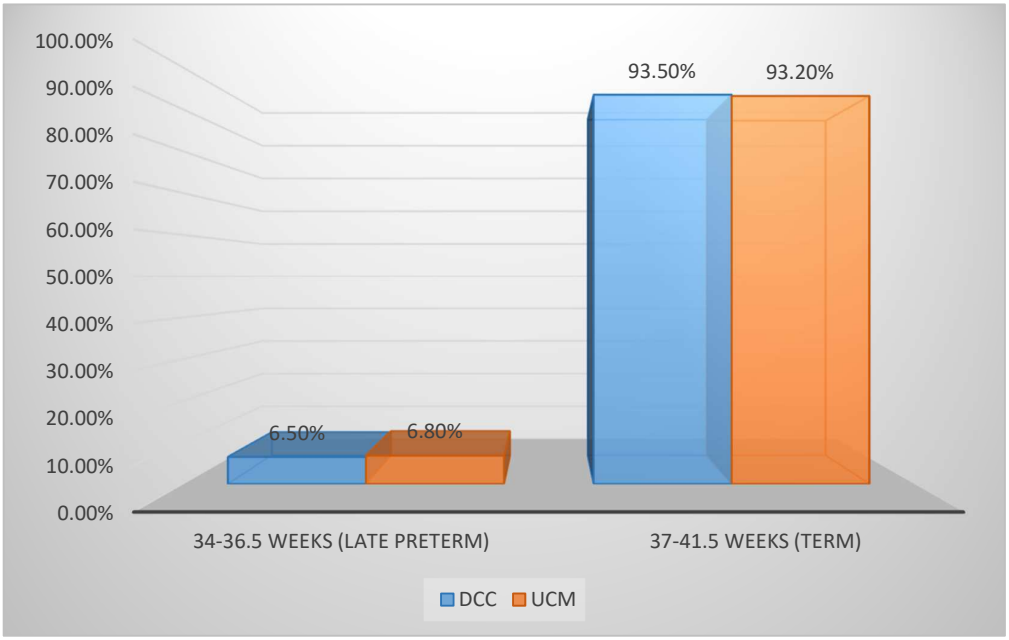

Figure 25: Comparison of DCC and UCM based on gestational age

COMPARISION OF WEIGHT:

Table 10: Comparison of Birth weights and weight at 3 months of life between DCC and UCM by independent t test

	GROUP	N	Mean	SD	T Test	P Value
B WEIGHT	DCC	92	2810.32	328.29	-1.77	0.58
	UCM	88	2869.09	433.25		
WEIGHT AT 3 MONTHS	DCC	63	4851.71	611.68	-1.12	0.26
	UCM	45	4980.22	534.91		

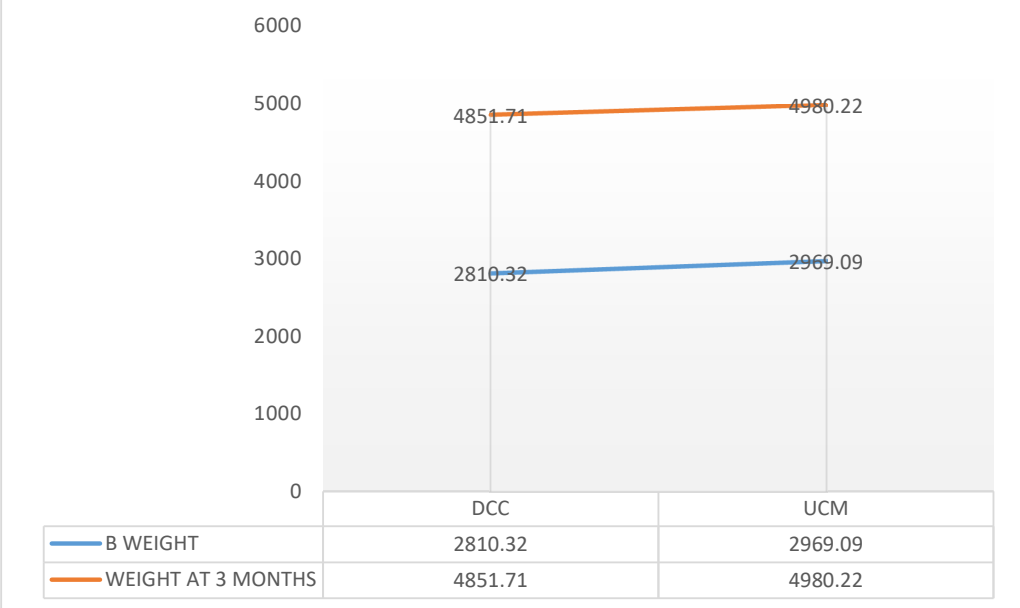

Figure 26: Comparison of weights at birth and at 3 months of life between both groups

Mean birth weight in DCC group was 2.81kg and in UCM group was 2.869 kg with mean difference of 0.159, it is statistically not significant. (p value – 0.58)

APGAR SCORES

Table 11: Comparison of APGAR scores in UCM and DCC groups at first minute

			GROUP		Total
			DCC	UCM	
APGAR 1	6	n	0	3	3
		%	0.0%	3.4%	1.7%
	7	n	84	75	159
		%	91.3%	85.2%	88.3%
	8	n	7	10	17
		%	7.6%	11.4%	9.4%
	9	n	1	0	1
		%	1.1%	0.0%	0.6%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

Chi-Square: 4.95, P Value: 0.17, Statistically not significant

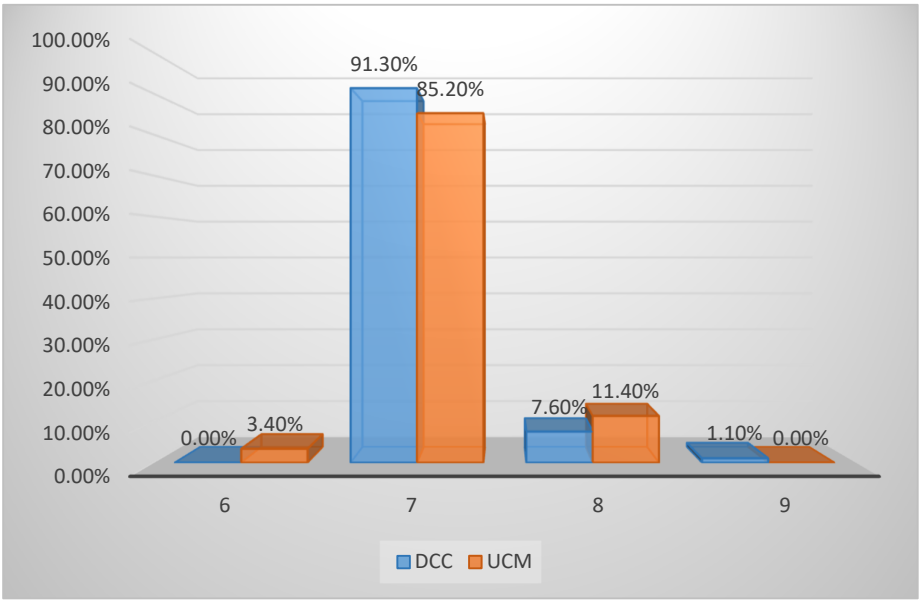

Figure 27: Comparison of APGAR scores in UCM and DCC groups at 1st minute

Table 12: Comparison of APGAR scores in UCM and DCC groups at 5 minutes of life

			GROUP		Total
			DCC	UCM	
APGAR 5	8	n	8	12	20
		%	8.7%	13.6%	11.1%
	9	n	80	74	154
		%	87.0%	84.1%	85.6%
	10	n	4	2	6
		%	4.3%	2.3%	3.3%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

Chi-Square: 1.61, P Value: 0.44, Statistically not significant

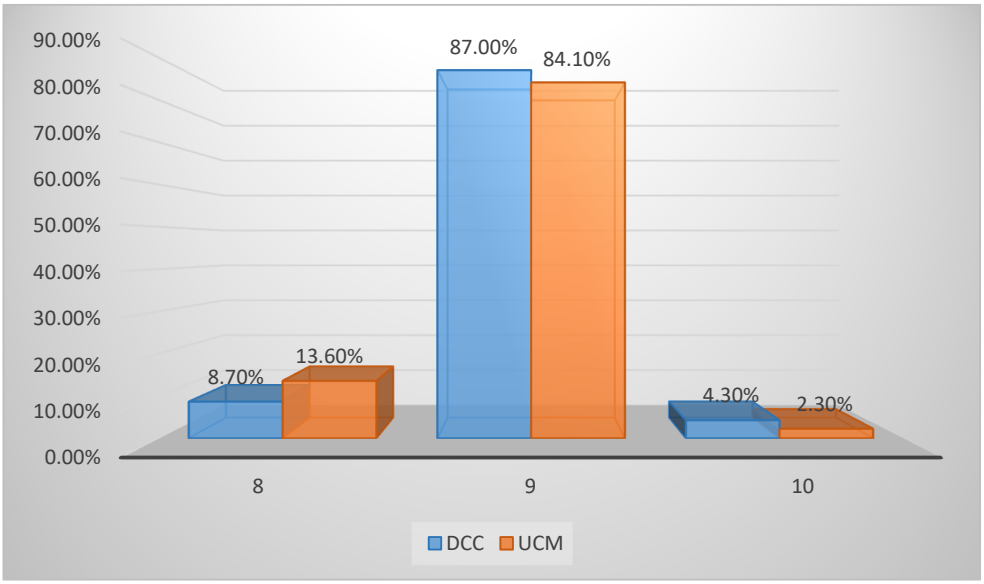

Figure 28: Comparison of APGAR scores in UCM and DCC groups at 5 minutes of life

HEMATOLOGICAL PARAMETERS AT 3–5 DAYS OF LIFE:

Hemoglobin levels at 3-5 days of life

Table 13: Comparison of Hemoglobin (Hb) DCC and UCM at 3-5 days of life

			GROUP		Total
			DCC	UCM	
Hb(%) at 3 – 5 days of life	12.1-13	n	2	1	3
		%	2.2%	1.1%	1.7%
	13.1-14	n	5	3	8
		%	5.4%	3.4%	4.4%
	14.1-15	n	8	6	14
		%	8.7%	6.8%	7.8%
	15.1-16	n	9	5	14
		%	9.8%	5.7%	7.8%
	16.1-17	n	18	19	37
		%	19.6%	21.6%	20.6%
	17.1-18	n	15	23	38
		%	16.3%	26.1%	21.1%
	18.1-19	n	13	14	27
		%	14.1%	15.9%	15.0%
	19.1-20	n	7	5	12
		%	7.6%	5.7%	6.7%
	20.1-21	n	3	2	5
		%	3.3%	2.3%	2.8%
	21.1-22	n	7	5	12
		%	7.6%	5.7%	6.7%
	22.1-23	n	1	2	3
		%	1.1%	2.3%	1.7%
	23.1-24	n	2	1	3
		%	2.2%	1.1%	1.7%
	24.1-25	n	2	1	3
		%	2.2%	1.1%	1.7%
	25.1-26	n	0	1	1
		%	0.0%	1.1%	0.6%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

Chi-Square: 6.79, P Value: 0.91, Statistically not significant

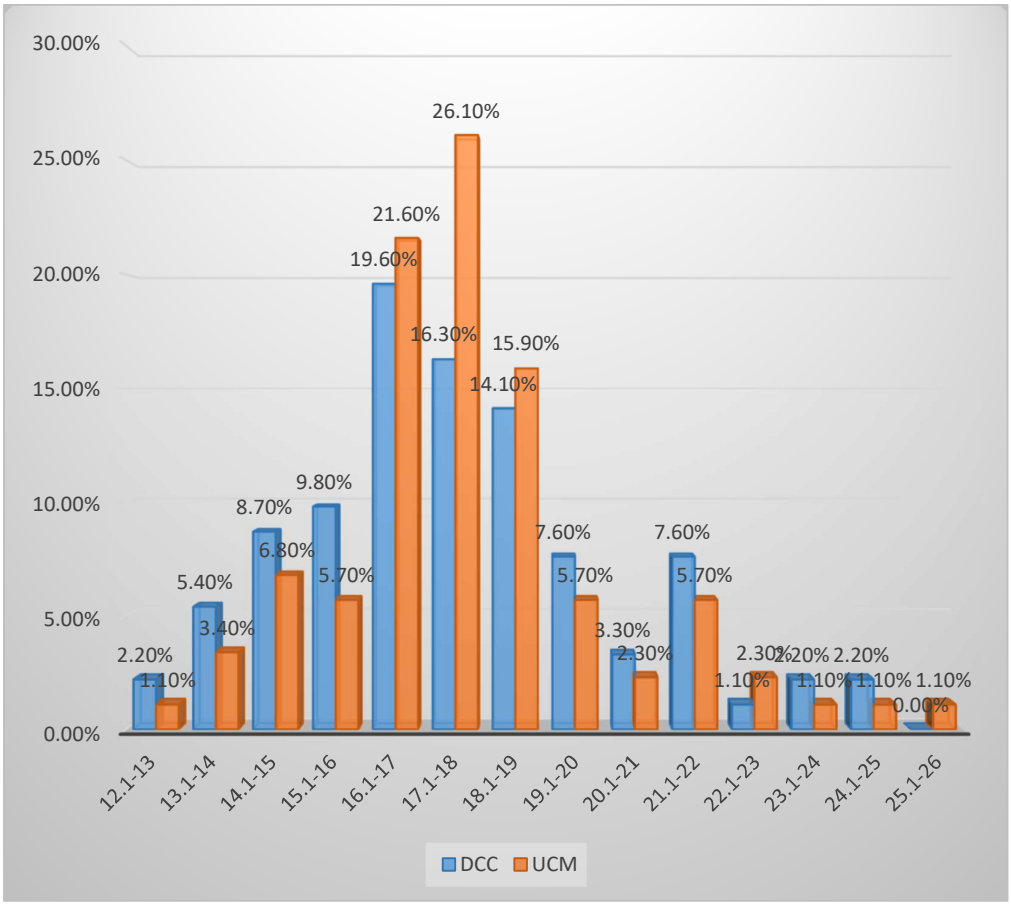

Figure 29: Comparison of Hemoglobin (Hb) DCC and UCM at 3-5 days of life

Number of babies (and percentage of babies) in both groups with variable degree hemoglobin values at 3-5 days of life. There is no clinical and statistical significance between these two study groups.

MEAN SERUM BILIRUBIN AT 3-5 DAYS OF LIFE IN BOTH GROUPS

Table 14: Comparison of two groups (DCC and UCM) with Total Bilirubin at 3-5 days and Direct Bilirubin at 3-5 days by independent t test

	GROUP	N	Mean	SD	T Test	P Value
Total Bilirubin At 3–5 days	DCC	92	11.17	3.80	-0.08	0.93
	UCM	88	11.21	3.31		
Direct Bilirubin AT 3–5 days	DCC	92	0.39	0.19	-0.81	0.41
	UCM	88	0.42	0.22		

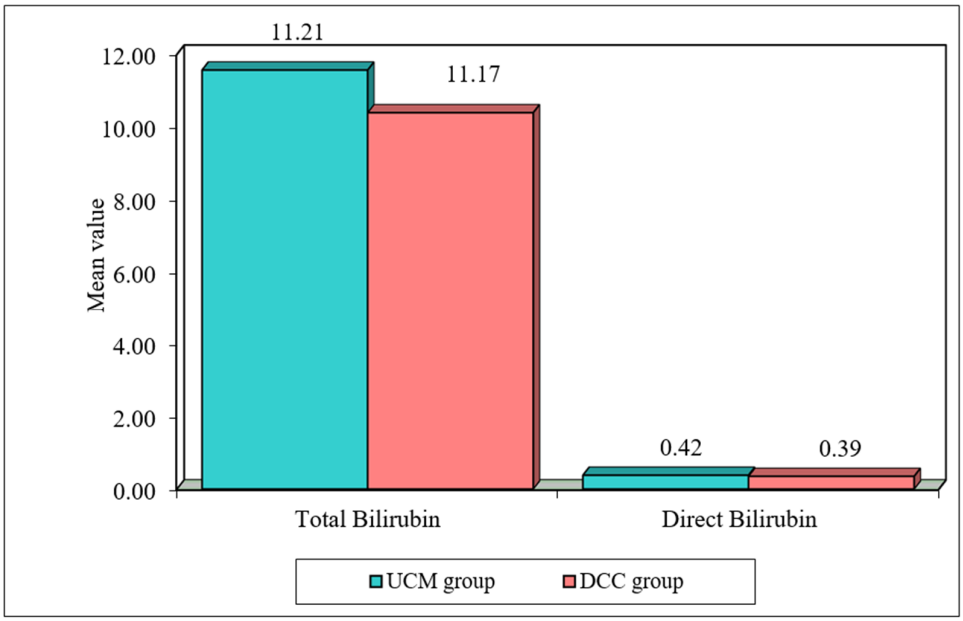

Figure 30: Comparison of two groups (DCC and UCM) with Total Bilirubin at 3-5 days and Direct Bilirubin at 3-5 days

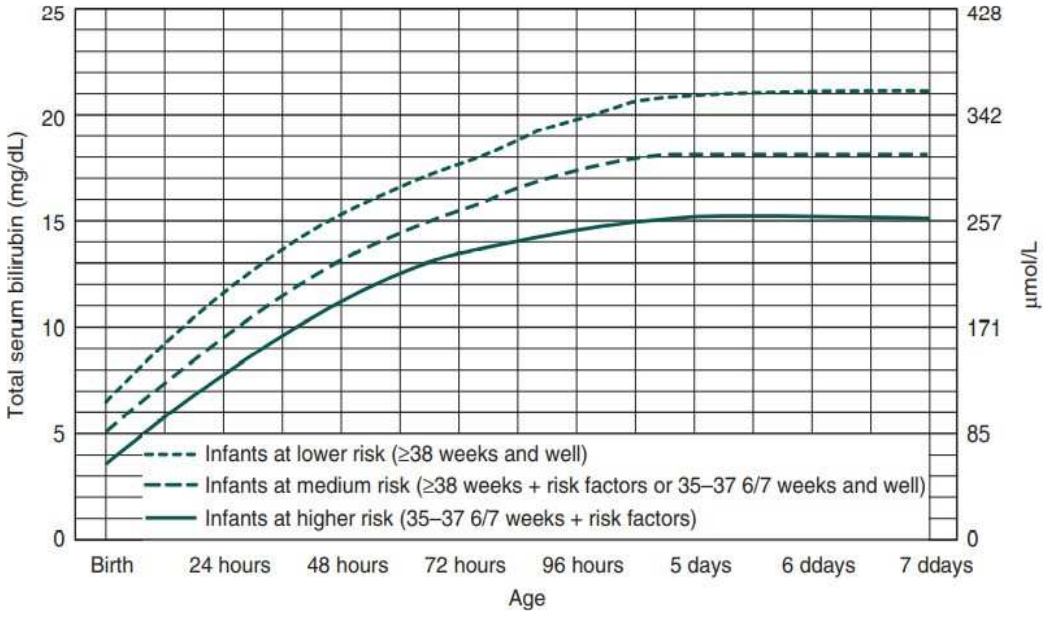

Figure 31: Bhutani nomogram showing phototherapy thresholds (Recommended by AAP, 2004)

`As per the above threshold levels for the requirement of phototherapy, for term infants with low-risk phototherapy range TSB level is more than 15mg/dl. Hence Both UCM and DCC are safer methods of placental transfusion and doesn't have a statistically significant risk of hyperbilirubinemia. Statistically significant value is clinically not significant between both the groups.

Table 15: Number of newborns with peak bilirubin levels who were admitted to NICU for phototherapy in both groups.

			GROUP		Total
			DCC	UCM	
PHOTOTHERAPY	Yes	n	11	7	18
		%	10.8%	8.0%	10%
	No	n	81	81	162
		%	89.2%	92.0%	90%
Total		n	92	88	180
		%	100.0%	100.0%	100.0%

Chi-Square: 2.30, P Value: 0.09, Statistically not significant

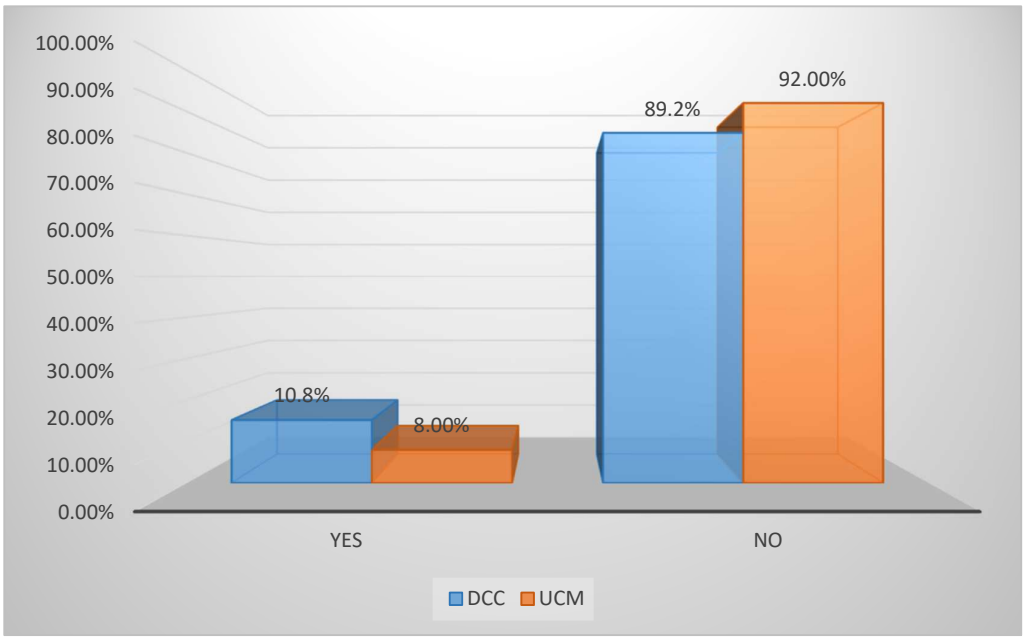

Figure 32: Showing the percentage of babies with hyperbilirubinemia in phototherapy range in both groups.

Table 16: showing newborns received Exchange transfusion

	DCC group (n=92)	UCM group(n=88)
Need of exchange transfusion	0	0

Total 18 neonates received phototherapy. Of which 11 babies received DCC and 7 babies received UCM at birth, these babies were in the phototherapy range between 48 hours to 72 hours of life and underwent phototherapy for 12 to 24 hours of duration.

The remaining 162 babies (90%) had no significant hyperbilirubinemia of phototherapy range. It is clinically and statistically not significant (p-value 0.09). None of the babies in either group required an exchange transfusion.

Table 17: Showing days of life when phototherapy required in UCM and DCC groups

			GROUP		Total
			DCC	UCM	
DAY OF PHOTOTHERAPY	2 days	n	3	3	6
		%	27.2%	42.9%	33.3%
	3 days	n	8	4	12
		%	72.8%	57.1%	66.6%
Total		n	11	7	18
		%	100.0%	100.0%	100.0%

Chi-Square: 0.65, P Value: 0.37, Statistically not significant

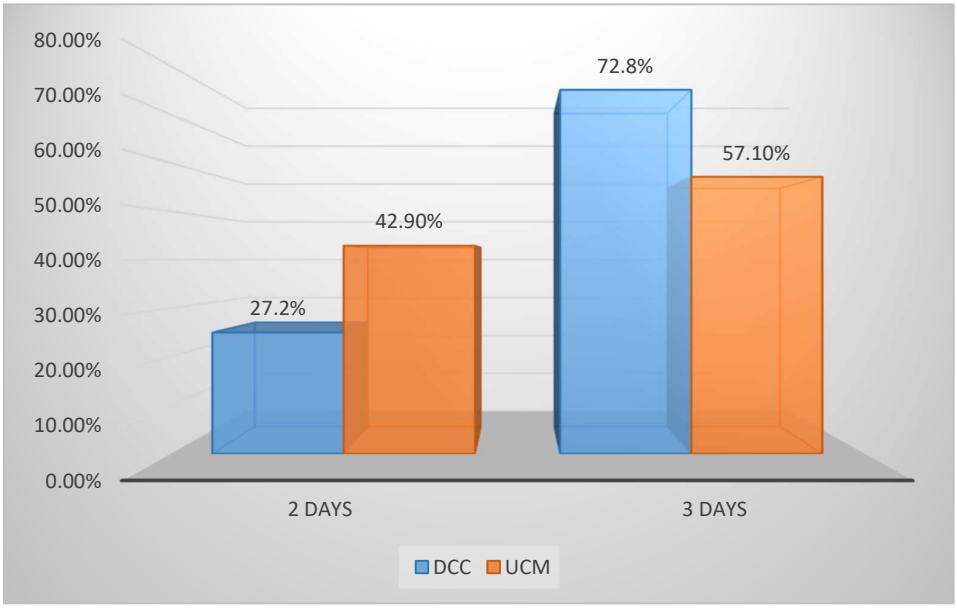

Figure 33: Showing days of life when phototherapy required in UCM and DCC groups

Table 18: Peak Mean Total Bilirubin level (Among the babies admitted for phototherapy)

Peak serum bilirubin (mg/dl)	DCC group (n=14)	UCM group (n=7)	Mean difference	p value
	15.7	16.6	-0.9	0.94

Peak mean serum total bilirubin among the babies admitted for phototherapy in DCC group is 15.7 and in UCM group is 16.6 with mean difference of 0.9. Peak mean serum bilirubin levels did not differ significantly between the two groups (p value-0.94).

HEMATOLOGICAL PARAMETERS AT 3 MONTHS OF LIFE:

Hemoglobin levels at 3 months of life

Table 19: Comparison of Hb (%) values at 3 months of life between DCC and UCM

			GROUP		Total
			DCC	UCM	
HB AT 3 MONTHS	8.1-9	n	0	2	2
		%	0.0%	4.5%	1.9%
	9.1-10	n	5	5	10
		%	7.8%	11.4%	9.3%
	10.1-11	n	22	13	35
		%	35.9%	29.5%	33.3%
	11.1-12	n	16	13	29
		%	25.0%	29.5%	26.9%
	12.1-13	n	13	8	21
		%	20.3%	15.9%	18.5%
	13.1-14	n	5	3	8
		%	7.8%	6.8%	7.4%
	14.1-15	n	2	1	3
		%	3.1%	2.3%	2.8%
Total		n	63	45	108
		%	100.0%	100.0%	100.0%

Chi-Square: 4.16, P Value: 0.65, Statistically not significant

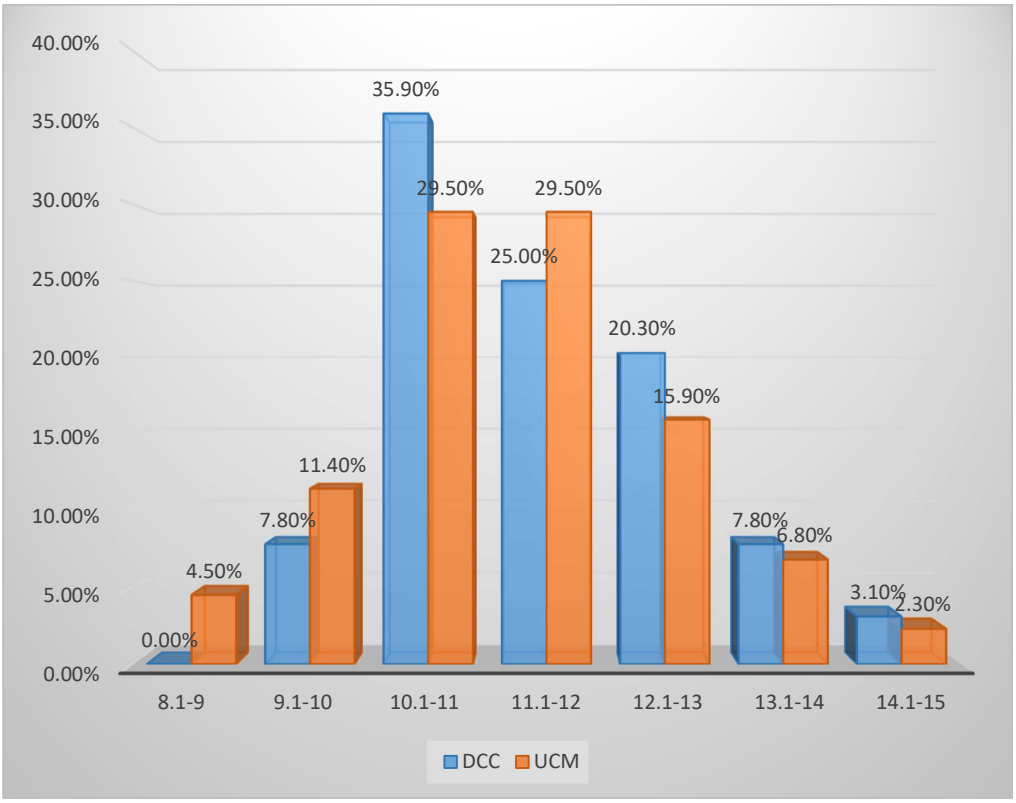

Figure 34: Comparison of Hb (%) values at 3 months of life between DCC and UCM

During comparison of number of infants with variable degrees of Hb levels between 2 study groups at 3 months of follow-up, most of the infants have Hb between 10.1 to 13.0 mg/dl in both the groups. And there is no statistical difference between the groups (P Value: 0.65).

Ferritin levels at 3 months of follow up:

Table 20: Comparison of two groups with Ferritin levels at 3 months of life by independent t test

	GROUP	N	Mean	SD	T Test	P Value
FERRITIN AT 3 MONTHS	DCC	63	258.0722	133.70935	0.35	0.72
	UCM	45	248.4423	141.74276		

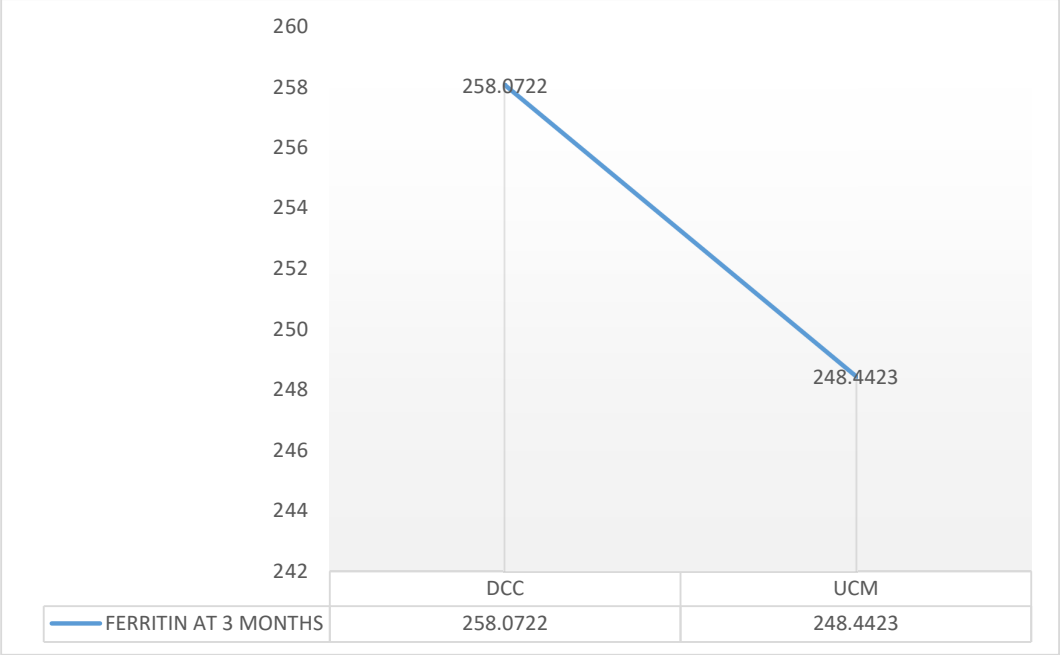

Figure 35: Comparison of two groups with Ferritin levels at 3 months of life by independent t test

The mean ferritin levels in the DCC group is 258.07 and in the UCM group is 248.44. Mean difference is 9.63, there is no significant difference in serum ferritin

levels at 3 months of life (p- value 0.72).

COMPARISON BETWEEN BOTH GROUPS AT BIRTH AND AT 3 MONTHS OF LIFE

Table 21: Comparison of two groups (DCC and UCM) with Hb at 3-5 days of life and at 3 months of life by independent t-test

	GROUP	N	Mean	SD	T Test	P Value
HB AT 3-5 DAYS	DCC	92	17.62	2.61	0.38	0.69
	UCM	88	17.77	2.43		
HB AT 3 MONTHS	DCC	63	11.58	1.19	0.93	0.35
	UCM	45	11.36	1.27		

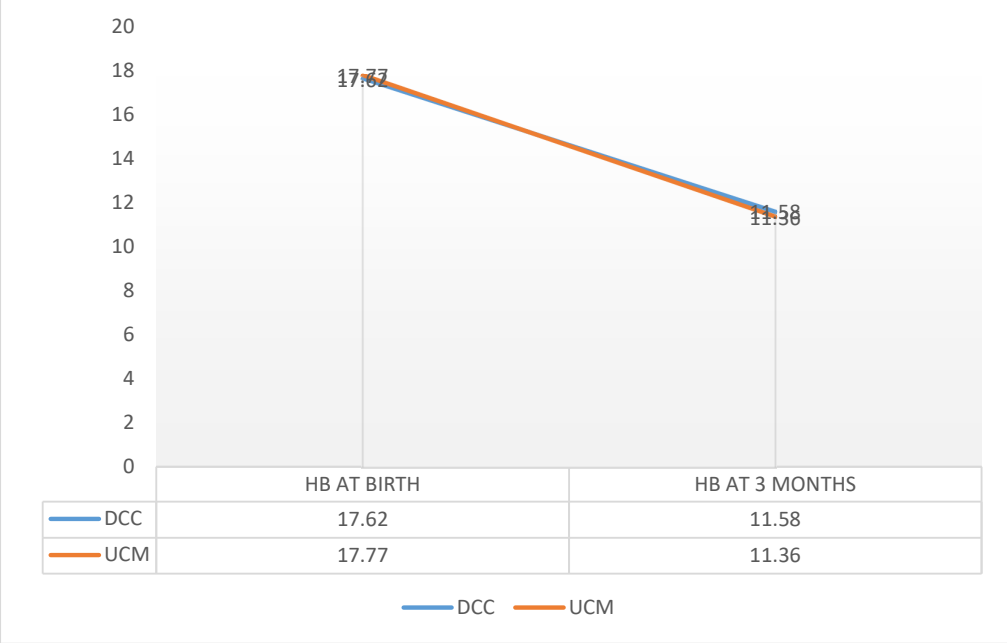

Figure 36: Hb levels at 3-5 days of life and at 3 months of life in DCC group and UCM group

At 3-5 days of life, the mean Hb in the DCC group is 17.62 and in the UCM group is 17.77 with a mean difference of 0.15 and a p-value is 0.69, it is statistically not significant. In 3rd month of life, the mean Hb in the DCC group is 11.58 and in the UCM group is 11.36 with a mean difference of 0.22 and p-value is 0.35, it is also statistically not significant. We also noticed a fall in hemoglobin levels at 3 months of age in both groups, which corresponds to physiological anemia of infancy.

Table 22: Comparison of two groups (DCC and UCM) with PCV (Hematocrit) at 3-5days of life and at 3 months of life by independent t test

	GROUP	N	Mean	SD	T Test	P Value
PCV AT 3-5 DAYS	DCC	92	60.37	5.84	1.95	0.050
	UCM	88	54.35	7.29		
PCV AT 3 MONTHS	DCC	63	39.59	10.70	1.10	0.27
	UCM	45	37.50	8.02		

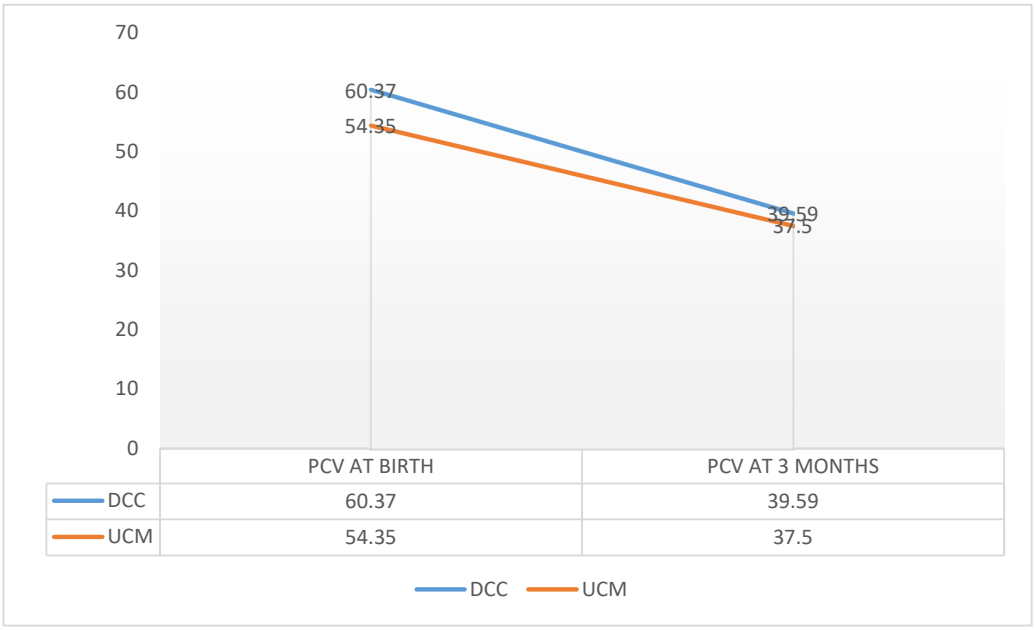

Figure 37: Comparison of two groups (DCC and UCM) with PCV at 3-5 days and at 3 months of age

At 3-5 days of life Mean PCV (hematocrit value) in DCC is 60.37 and UCM is 54.35, mean difference is 6.02. PCV is comparatively more in the DCC group than in the UCM group. There is a Statistical difference between the two groups (p-value 0.050). However clinically it is not significant as hematocrit is not as high to cause polycythemia. But it may be beneficial as it increases the oxygen-carrying capacity

At 3 months of life Mean PCV (hematocrit value) in DCC is 39.59 and UCM is 37.50, mean difference is 2.09. No Statistical difference between the two groups.

Table 23: Comparison of DCC and UCM based on Hb at 3-5 days of life and 3 months of life with ferritin levels at 3 months of life based on gestational age

GROUP		Gestational age (weeks)	N	Mean	SD	T Test	P Value
DCC	Hb at 3-5 days of life	34-36.5	6	18.41	1.94	0.76	0.44
		37-41.5	86	17.56	2.65		
	Hb at 3 months	34-36.5	3	11.90	1.38	0.46	0.64
		37-41.5	60	11.57	1.19		
	FERRITIN AT 3 MONTHS	34-36.5	3	221.66	72.27	-0.48	0.63
		37-41.5	60	259.86	136.11		
UCM	Hb at 3-5 days of life	34-36.5	6	18.16	2.99	0.41	0.68
		37-41.5	82	17.74	2.40		
	Hb at 3 months	34-36.5	1	11.50	.	0.13	0.89
		37-41.5	44	11.36	1.27		
	FERRITIN AT 3 MONTHS	34-36.5	1	126.10	.	0.84	0.40
		37-41.5	44	248.44	141.74		

DISCUSSION

Our study included late preterm and term neonates. We compared the effects of Umbilical cord milking with Delayed cord clamping on hematological parameters at 3-5 days of life and at 3 months of age and conclude that Iron stores (ferritin levels) are almost similar in both groups. Life-threatening risk factors like polycythemia, severe neonatal jaundice requiring exchange transfusion or prolonged phototherapy are not seen with both the placental transfusion techniques.

We observed that Umbilical cord milking in preterm and term neonates is safer and as effective as delayed cord clamping in increasing the hemoglobin level and iron stores in the body.

The accurate method for measuring placental transfusion is red cell mass and circulating blood volume which requires labeling red blood cells with biotin. This method is complex and time-consuming and also not feasible to do on a large scale newborns. Hence to assess the effectiveness of placental transfusion Hb and hematocrit is preferable.

The terms early cord clamping and immediate cord clamping are used interchangeably which refers to clamping of the cord within 30seconds after birth without milking the cord. In 2013 Amit Upadhyay et al concluded that mean Hb at 6 weeks of life was higher in the milking group(11.9gm/dl) when compared with immediate cord clamping (10.8gm/dl). In 2008 Hosono et al also stated that the Hb value at birth in extreme preterm was higher in the milking arm when compared to immediate cord clamping. As several previous studies stated that immediate cord clamping has no beneficial role over delayed cord clamping and umbilical cord milking and deprives the newborn of receiving extra blood through placental

transfusion, we have not included this method for comparing the other two placental transfusion techniques.

Our study has two groups, one group of newborns who received delayed cord clamping, whereas the other group of newborns received umbilical cord milking.

In our study, neonates allocated to the umbilical cord milking (UCM) group had hemoglobin values of 17.77 g/dl and 11.36 g/dl compared with the delayed clamping group of 17.62 g/dl and 11.58 g/dl at 3-5 days and at 3 months of life respectively.

This indicates that a similar amount of placental transfusion was received in both groups and this directly states that the procedure of milking the umbilical cord for 5 times transfers the same amount of placental blood as delayed cord clamping.

In our study hemoglobin and hematocrit values were almost the same in both the study groups at birth and at 3 months of life, this indicates that either of the intervention (DCC/UCM) is essential and mandatory for the prevention of anemia in infancy.

Our milking technique is similar to the method used by Rabe et al (67) and our results are slightly similar to the same study(67) which showed that umbilical cord milking for 4 times in very low birth weight newborns led to a similar amount of placento-fetal blood transfusion compared with delayed cord clamping. Amit Upadhayay et al(63) also used the same speed 10cm/sec for umbilical cord milking but milking is done only thrice and done after clamping and cutting the umbilical cord.

Table 24: Characteristics of the varies Umbilical cord milking studies.

Study	Population	UCM Characteristic		Control condition
		No. of times	Speed	
Our study	**12 preterm 168 term infants**	**5**	**10cm/sec**	**DCC**
Rabe et al.2011[67]	58 preterm (GA 24 – 32 weeks)	4	10cm/sec	DCC
Upadhyay et al.2013[63]	200 infants (GA more than 34 weeks 6days)	3	10cm/sec	DCC
Hosono et al[65]	40preterm (GA 24- 28 weeks)	3	10cm/sec	ICC
Erickson-Owens[62] Mercer[51]	24 full term infants	5	Not reported	ICC

Various characteristics of other UCM studies were compared with our study (table 24)

Table 25: Comparing the Hb levels at 48 hours with UCM with other studies

Intervention	**Our study**	Panburana et al. 2020[8]	Jaiswal et al.2015[73]	Yadav et al.2015[74]
UCM	**17.77**	17	16.2	16.5
DCC	**17.62**	16.9	15.8	16.2

In our study we observed mean Hb levels at 48 hours is in line with other studies. We found higher Hb levels in both study groups at 48 hours of life compared to other studies (Table 25).

Table 26. Comparison between levels of Haematocrit (PCV) and Serum bilirubin levels in various studies.

Study	Mean PCV at 48 hours		Mean Serum bilirubin at 48 hours		No. of Polycythaemi a cases		No. cases which needed of phototherapy	
	UCM	DCC	UCM	DCC	UCM	DCC	UCM	DCC
Our study	**54.35**	**60.37**	**11.21**	**11.17**	**0**	**0**	**7**	**14**
Jaiswal et al.2015[73]	48.51	48	5.84	6	0	0	6	11
Panburana et al.2020[8]	50.6	50.3	10.4	9.9	1	0	8	13
Mangla et al.2020[76]	57.7	55.9	Not reported	Not reported	0	2	1	1
Yadav et al.2015[74]	49.9	48.8	7.2	7.2	0	0	4	4
Upadhya et al.2013[63]	41.7	36.8	7.2	6.5	0	0	0	0

In our study, 18 newborns (10 percent) required phototherapy, 11 babies in DCC, and 7 babies in UCM had hyperbilirubinemia that required phototherapy, which was greater than in prior studies. But none of the groups had life-threatening risk factors like polycythemia, and severe neonatal jaundice requiring exchange transfusion.

Table 27. Comparing Serum ferritin levels during follow-up with other studies

Study	Serum ferritin levels		Remarks
	DCC	ICC	
Our study	**258.07ng/ml**	**Not done**	**Follow up done at 3 months of life, comparison done between DCC and UCM in late preterm and term neonates.**
Andersson et al.2011[46]	117 mcg/L	81 mcg/L	follow-up done at4months. Full term neonates were included for study.
Chopra et al.2018[77]	86ng/ml	50.5ng/ml	Follow-up at 3months, GA >35 weeks included
Bora et al.2015[4]	113.9ng/ml	43.9ng/ml	Follow up done at 6 months. And comparison was done between UCM and ICC. Ferritin level of 113.9ng/ml in UCM group.

Table 28. Comparing Serum ferritin levels during follow-up with other studies

Study	Serum ferritin levels		Remarks
	DCC	UCM	
Our study	**258.07ng/ml**	**248.44ng/ml**	**Follow up done at 3 months of life, comparison done between DCC and UCM in late preterm and term neonates.**
Vashistha et al[78]	131.64	133.53	follow-up done at 6 weeks. Full term neonates were included for study.
Jaiswal et al[73]	142.7	134	Follow-up at 6 weeks, GA >35 weeks included

In terms of serum ferritin levels, our findings showed that at 3 months of life, serum ferritin levels were comparable between the delayed cord clamping group (258.07 ng/ml) and the milking group (248.44 ng/ml). Serum ferritin levels in the DCC group were slightly more than the UCM group, with a mean difference of 9.63ng/ml but it is statistically not significant (p-value 0.72).

In a study done by Chaparro et al 2006(23) Ferritin levels at six months of life were higher in the delayed cord clamping group than in the early cord clamping group with a mean difference - 11.80 ug/L.

The strengths of our study were:

- Study design- Randomized control trial with an appropriate sample size and follow-up at 3 months of life is the main strength of our study.
- UCM (umbilical cord milking) and DCC (delayed cord clamping) technique were standardized by conscientious demonstrations to all the postgraduates who attends the labor room calls for deliveries and sections.
- Assessing iron status by Serum ferritin levels
- We have a record of all possible clinical side effects which may affect the baby because of increased placental transfusion like neonatal jaundice, and polycythemia.

The limitations of our study were

- We have not recorded the actual amount of blood transfused in each newborn. Currently no direct, accurate, and simple method for measuring blood volume is available. The available method was too expensive for our setup.

- To minimize frequent blood samplings, we assessed the serum bilirubin level only single time between 3 – 5 days of life.

- We have examined all the babies clinically to rule out sepsis rather than lab tests, such as CRP at follow up. Few babies who may have subclinical sepsis may have had falsely elevated serum ferritin levels.

CONCLUSION

(1) Umbilical cord milking and delayed cord clamping resulted in comparable levels of hemoglobin and hematocrit at 3-5 days of life and ferritin levels at 3 months of life, implying that similar amount of placental transfusion occurs in both the groups

(2) Umbilical cord milking resulted in similar iron stores when compared to that of delayed cord clamping, which signifies that UCM is as effective as DCC to prevent anemia in infancy.

(3) Umbilical cord milking is as effective as delayed cord clamping in achieving higher hemoglobin and ferritin levels at 3 months of life in late preterm and term infants delivered by both cesarean and vaginal deliveries.

(4) There is no increased risk of polycythemia or neonatal hyperbilirubinemia requiring exchangetransfusion by umbilical cord milking method, which implies that UCM doesn't produce significant hemodynamic disturbances resulting in deleterious effects on the baby.

(5) We conclude that Umbilical cord milking is a better option to prevent anemia in infancy in cases where active resuscitation is needed and delayed cord clamping cannot be done due to undue limitation as it is faster, time-saving with equal benefits as DCC

IMPLICATION FOR PRACTICE

- Umbilical cord milking may be safely practiced in situations where delayed umbilical cord clamping is practically difficult like in cases where active resuscitation is needed for babies.
- As DCC has been recommended universally as standard care of placental transfusion by WHO, AAP, and ACOG, if DCC is not an option, we recommend using UCM in Preterm and term neonates

IMPLICATION FOR FURTHER RESEARCH

- Further studies with longer follow-up till 8-12 months are encouraged to give strength for concluding whether the initial advantage in hemoglobin and ferritin is sustained later in infancy.
- Further large sample-sized studies with neurodevelopment as the main outcome may be considered

SUMMARY

This study entitled "**Serum ferritin levels in newborns at 3 months of life after intact Umbilical cord milking versus Delayed cord clamping" - Randomized controlled trial"** was done to compare two different techniques of placental transfusion (DCC and UCM) with respect to the amount of blood transfusion during birth and their effect of other hematological parameters like hemoglobin and iron stores at 3 months follow-up.

During the study period, the total number of eligible mothers are 229 and randomized 190 mothers. The intervention was done in 180 mothers. A total of 108 cases completed the full study including 3 months of follow-up. The comparison was done between the two groups and described in the results and analysis.

Findings inferred from our study are: Umbilical cord milking is safer and as effective as delayed cord clamping in achieving high hemoglobin and ferritin levels in both cesarean and vaginal deliveries among preterm and term infants. Both methods of placental transfusion transfer almost equal amounts of blood hence hemoglobin and ferritin levels of both groups are almost similar. There is no significant risk of polycythemia or neonatal hyperbilirubinemia requiring exchange transfusion by umbilical cord milking method. Hence UCM can be safely practiced at institutional levels. National and international health organizations need to provide guidelines for practicing umbilical cord milking instead of immediate cord clamping when delayed cord clamping is not feasible or contraindicated. This prevents deprivation of the newborn from their potential extra placental blood which is rich in red blood cells and stem cells and can effectively prevent anemia in infancy.

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ANNEXURE – I

INFORMED CONSENT FORM

K.L.E ACADEMY OF HIGHER EDUCATION AND RESEARCH

"SERUM FERRITIN LEVELS IN NEWBORNS AT 3 MONTHS OF LIFE AFTER INTACT UMBILICAL CORD MILKING VERSUS DELAYED CORD CLAMPING" – RANDOMIZED CONTROLLED TRIAL

Principal Investigator: - **REG NO: BM120008**

Co-investigator:- Dr. _____

PURPOSE OF THE STUDY:

Umbilical cord clamping and cutting is one the most unique parts of birth process. It prevents excessive blood loss in the mother and allows the newborns to be taken away from mother to revive. However the timing for cord clamping and cutting is uncertain and with different timing approaches there are various benefits and risks. These procedures help in increased transfer of blood from mothers to the neonates and increases the iron stores in the newborn in first few months of life. The purpose of this study is to study the effects of serum ferritin in newborns after intact umbilical cord milking versus delayed cord clamping.

VOLUNTARY PARTICIPATION

Your child's participation in the 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, Belagavi.

Potential Risks and Benefits:

There are no risks involved except some pain, and blood collection at the site of blood drawing.

To know the best method of placental transfusion to reduce Perinatal Iron deficiency.

PRIVACY AND CONFIDENTIALITY

The only people who will know that you are a research participant are members 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.

Use of photography/Identifying details: Any photograph or identification details will be disclosed only with your permission.

QUERIES

If you have any queries, you may contact **REG NO: BM120008** PG Student Department of Pediatrics, JNMC.

If you have any queries regarding or rights or research participation you may contact: -

Dr. _____ DEPARTMENT OF PAEDIATRICS JNMC.

In the event of an emergency, you can contact KLE'S Dr. Prabhakar Kore Hospital.

Dr. HARSHA HEGDE, Chairperson, JNMC, IEC and Scientist D, ICMR, National institute of traditional medicine, Belagavi – 9480422500

STATEMENT OF CONSENT

I hereby voluntarily agree for my child's participation in this study and I agree for collecting blood samples of my child twice. I understand that even 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 or thumbprint of person providing consent

Date

Signature or thumbprint of witness/ parents

Date

Signature of person obtaining consent

Date

Person requesting consent, please check applicable boxes:

- ☐ **Consent obtained (for adult responders)**
- ☐ **Assent (for minor respondents)**
- ☐ **Consent from authorized person of minor respondent**

ANNEXURE- II

PROFORMA

SCREENING FORM

Screening number: ☐☐☐ IP number: ☐☐☐☐☐☐☐☐☐☐

Date of screening(dd-mm-yyyy): ☐☐ ☐☐ ☐☐☐☐

First name: ☐☐☐☐☐☐☐☐☐☐

Middle name: ☐☐☐☐☐☐☐☐☐☐

Last name: ☐☐☐☐☐☐☐☐☐☐

Husband's name: ☐☐☐☐☐☐☐☐☐☐

Age (years): ☐☐

Address: H.no. - ☐☐☐

Street - ☐☐☐☐☐☐☐☐☐☐☐☐

Taluka- ☐☐☐☐☐☐☐☐☐☐

Dist- ☐☐☐☐☐☐☐☐☐☐

Phone no: ☐☐☐☐☐☐☐☐☐☐

Eligibility:

YES – 1 NO – 2

1.Singleton Live Gestation.

.

2.Gestational age (34 to 41 6/7 weeks) of pregnancy

3.Maternal Hb:>9.9mg/dl.

4.Multiple gestation

5.History of smoking

6.Known case of bleeding disorders

7.Cord abnormalities

8.Abnormal placentation

9.Rh sensitized pregnancy

10.Congenital anomalies of fetus.

11.Babies requiring active resuscitation.

Is she eligible?
If eligible, consent to be taken.

Consent:

a. Does the woman assent to participate?
b. Has the study consent form been signed?

If Consent given,

Enrollment done:

If enrolled, Randomization done.
Was the woman randomized?
If not randomized indicate the reason:

1. Withdrawal from the study ☐
2. Other ☐

Date of Randomization: ☐☐ ☐☐ ☐☐☐☐
(dd-mm-yyyy)
Participant number: ☐☐☐☐☐☐☐
(See sealed envelope)
Batch:

Investigator's name:

Signature:

DATA COLLECTION PROFORMA

"Serum ferritin levels in newborns at 3 months of life after intact umbilical cord milking versus delayed cord clamping" - A Prospective Randomized Controlled Trial

Screening Id: ☐☐☐☐ Enrollment number: ☐☐☐

Date of admission (dd-mm-yyyy): ☐☐ ☐☐ ☐☐☐☐

Date of discharge (dd-mm-yyyy) ☐☐ ☐☐ ☐☐☐☐

YES – 1 NO – 2

Scans:

a. Dating scan is done ☐

b. Anomaly scan done ☐

Any anomalies noted ☐

c. Growth scan done ☐

H/O Parenteral iron correction in the current pregnancy: ☐

Obstetric history:

Married Life (years): ☐☐

Consanguinity: ☐ (YES - 1, NO - 2)

If YES, Degree of consanguinity: ☐☐☐☐☐☐

Obstetric score:

Gravida- ☐☐ Para- ☐☐ Live- ☐☐ Abortion- ☐☐

Last menstrual period (dd-mm-yyyy): ☐ ☐☐ ☐☐ ☐☐

Expected date of delivery (dd-mm-yyyy): ☐☐ ☐☐ ☐☐☐☐

USG EDD (dd-mm-yyyy): ☐☐ ☐☐ ☐☐☐☐

Period of gestation (weeks/ days)

If according to LMP : ☐☐ ☐☐

According to C.EDD : ☐☐ ☐☐

Provisional diagnosis:

Investigations-

Date (dd-mm-yyyy): ☐☐ ☐☐ ☐☐☐☐

Hemoglobin (g/dl). ☐☐

Packed cell volume (%): ☐☐

Blood Group: ☐ ☐☐☐☐☐☐☐☐

HIV : ☐ (Non- reactive – 1, Reactive – 2)

HbsAg : ☐ (Non- reactive – 1, Reactive – 2)

VDRL : ☐ (Negative – 1, Positive – 2)

LABOUR ROOM DETAILS: (YES-1, NO-2)

a. Place: OT ☐ Labour room ☐

b. Mode of delivery: Vaginal : ☐ Caesarean : ☐

Emergency : ☐ Elective : ☐

c. Liquor: Clear : ☐ meconium stained : ☐

d. Baby extraction: easy : ☐ difficult : ☐

e. Baby cried immediately after birth : ☐

f. Cord clamping method:

- Intact umbilical cord milking:

  Milking done how many times: ☐ times

- Delayed cord clamping:

  Time period : ☐ seconds

NEONATAL PROFORMA

DATE OF BIRTH (dd/mm/yr) | | | | | | | | |

SEX | MALE | FEMALE |

APGAR SCORE

APGAR	0	1	2	1 MIN	5 MIN
HEART RATE	ABSENT	<100	>100		
RESPIRATION	ABSENT	WEAK CRY	GOOD		
MUSCLE TONE	LIMP	SOME FLEXION	CRYING		
REFLEX IRRITABILITY	NO RESPONSE	GRIMANCE	ACTIVE MOVEMENTS		
SKIN COLOUR	BLUE / PALE	ACROCYANOTIC	COMPLETELY PINK		
TOTAL SCORE					

VITALS AT BIRTH

HEART RATE | | | |

RESPIRATORY RATE | | | |

SpO2 | | | |

CRT | | |

TEMPERATURE | | | |

ANTHROPOMETRY

BIRTH WEIGHT (in grams) | | | | |

LENGTH (in cms) | | |

HEAD CIRCUMFERENCE (in cms) | |

HEAD TO TOE-

FACE

EYES

EARS

ORAL CAVITY

NECK

CHEST

ABDOMEN

EXTREMITIS

SKIN

SYSTEMIC EXAMINATION-

- CVS
- RS
- P/A
- CNS

(At 3rd -5th Day of Life)

VITALS

HEART RATE			
RESPIRATORY RATE			
SpO2			
CRT			
TEMPERATURE			

FEEDING	WELL	NO

RESPIRATORY DISTRESS	YES	NO
JITTIRINESS	YES	NO
JAUNDICE	YES	N0
TREATMENT GIVEN	PHOTOTHERAPY	
	EXCHANGE TRANSFUSION	

INVESTIGATIONS

HEMOGLOBIN	
HEMATOCRIT	
TOTAL BILIRUBIN	
DIRECT BILIRUBIN	

AT 3RD MONTH OF LIFE

GENERAL PHYSICAL EXAMINATION

- HEART RATE
- RESPIRATORY RATE
- SpO2
- CAPILLARY REFILLING TIME

ANTHROPOMETRY

- WEIGHT
- LENGTH
- HEAD CIRCUMFERENCE

INVESTIGATIONS

HEMOGLOBIN	
HEMATOCRIT	
SERUM FERRITIN	

1) History of fever during initial 3 months of life

YES	NO

2) Any history of mother been COVID Positive

YES	NO

ANNEXURE III- KEY TO MASTERCHART

UCM	-	Umbilical cord milking
DCC	-	Delayed cord clamping
HB	-	Hemoglobin
PCV	-	Hematocrit
TB	-	Total bilirubin
DB	-	Direct bilirubin
EL LSCS	-	Elective lower segment cesarean section
Emg LSCS	-	Emergency lower segment cesarean section

ENROLL NO	GROUP	GESTATION	MOTHER HB	IRON SUPLEMENTATION	MODE OF DELIVERY	SEX	B WEIGHT	APGAR 1	APGAR 5	HB	PCV	TB	DB	PHOTOTHERAPY	DAY OF PHOTOTHERAPY	WEIGHT AT 3 MONTHS	HB	PCV	FERRITIN
1	DCC	37.3	12.5	NO	EL LSCS	MALE	3200	7	9	18.7	55	9.94	0.31	NO		5800	11	19.1	493
2	UCM	38.3	14	NO	EL LSCS	FEMALE	3100	8	9	21.6	60.2	11.18	0.18	NO					
3	DCC	39.3	15	NO	NVD	MALE	3300	9	10	18.7	55.8	13.49	0.44	NO		6050	12.5	32.4	378.1
4	DCC	38.3	12	NO	Emg LSCS	MALE	2300	8	9	20	57.4	8.42	0.19	NO					
5	UCM	40.4	12.9	NO	NVD	MALE	3500	8	10	18.7	53.4	12.03	0.63	NO					
6	DCC	38.6	10.9	NO	EL LSCS	MALE	3000	8	9	18.3	50.4	10.16	0.46	NO		5600	12	31.9	90.77
7	UCM	37.5	10	NO	EL LSCS	FEMALE	3200	8	9	17.5	49.5	8.59	0.4	NO					
8	UCM	41.1	10.5	NO	NVD	FEMALE	3340	8	9	17.3	50.2	6.5	0.8	NO					
9	UCM	37.4	12.5	NO	NVD	FEMALE	2250	8	9	18.3	58.2	12.51	0.24	NO					
10	DCC	35.1	10.4	NO	NVD	FEMALE	3200	7	9	21.5	73.5	13.5	0.33	NO					
11	DCC	38.4	10.8	NO	NVD	MALE	3200	8	10	19.1	61.9	12.82	0.75	NO		6050	10	20.5	127.1
12	UCM	38.2	12.9	NO	Emg LSCS	MALE	2800	7	8	17.5	50.3	11.2	0.5	NO					
13	UCM	38.2	10.8	NO	EL LSCS	FEMALE	2890	7	9	18.7	61.7	11.4	0.2	NO					
14	DCC	35.3	15.4	NO	Emg LSCS	FEMALE	2800	8	10	17.5	53.5	11.76	0.13	NO					
15	UCM	39.3	12.9	NO	Emg LSCS	FEMALE	3000	8	10	22.9	66	6.1	0.06	NO					
16	DCC	37.6	13.7	NO	EL LSCS	MALE	3150	8	10	20	66.2	9.31	0.18	NO					
17	DCC	39.5	13.7	yes	EL LSCS	FEMALE	2800	7	9	19.1	59	9.66	0.1	NO					
18	UCM	39	12	NO	Emg LSCS	FEMALE	2900	7	9	18.3	60.8	11.59	0.27	NO					
19	UCM	38.1	10.8	yes	NVD	MALE	2800	7	9	21.7	63.2	6.55	0.19	NO		4800	11.5	32.5	246.6
20	UCM	36.4	10	NO	Emg LSCS	FEMALE	2500	7	8	23.3	64.2	11.55	0.36	NO					
21	UCM	36.3	11.6	NO	Emg LSCS	FEMALE	2750	7	9	20	59.6	6.06	0.85	NO					
22	UCM	38.5	11.6	NO	Emg LSCS	FEMALE	3500	7	9	20.4	65.5	9.94	0.31	NO					
23	UCM	39.6	12	NO	NVD	MALE	2800	8	9	17.9	64.8	15.08	0.25	NO					
24	UCM	38.3	12.5	NO	NVD	MALE	3200	7	8	18.2	52.4	10	0.8	NO					
25	DCC	39.3	12.1	NO	Emg LSCS	MALE	3000	7	9	15	58.1	15.96	0.5	NO					
26	DCC	39.3	11.2	NO	NVD	FEMALE	3000	7	8	15.8	60.6	8.34	0.29	NO					
27	DCC	37.6	12	NO	EL LSCS	MALE	3100	7	8	17.5	69.2	12.02	0.25	NO					
28	DCC	39.3	12.5	NO	NVD	MALE	3400	7	9	17.9	48.6	9.6	0.21	NO					
29	UCM	38.2	12.9	NO	Emg LSCS	MALE	3100	7	9	15.8	53.6	10.23	0.57	NO		5800	12.5	31.4	131.9
30	DCC	40	12	NO	Emg LSCS	FEMALE	2900	8	9	21.6	52.8	14.31	0.54	NO		5400	11.8	48	115.3
31	DCC	40.1	12.2	NO	NVD	MALE	3100	7	9	22	68.5	14	0.2	NO		5650	10.8	31.9	121.6
32	UCM	37.5	13	NO	Emg LSCS	FEMALE	2500	8	9	18.7	42.7	10.34	0.39	NO					
33	UCM	38.6	11	NO	NVD	MALE	3300	7	9	20	54.8	15.9	0.56	NO					
34	DCC	40.4	12	NO	NVD	MALE	2900	7	9	20.8	59.7	10.11	0.42	NO					
35	UCM	40	12	NO	El LSCS	MALE	3800	7	8	18.7	44.6	10.44	0.47	NO		6000	9.8	28	80.3
36	DCC	38.1	10	NO	NVD	FEMALE	2600	7	9	21.6	41.9	12.31	0.5	NO					
37	DCC	40.2	12	NO	NVD	MALE	2800	7	9	17.1	56	11.71	0.58	NO		4900	13.5	58.2	134
38	UCM	40.2	14.1	NO	NVD	FEMALE	2600	8	9	20	64.1	11.07	0.36	NO		5800	13	50.5	148.3
39	DCC	39.5	12	NO	NVD	MALE	2700	7	9	21.6	52.7	5.53	0.38	NO		4750	11	31.1	407.5
40	UCM	39.6	13	NO	NVD	FEMALE	3000	7	9	21.6	57.5	10.38	0.67	NO					
41	UCM	39.5	12	NO	NVD	MALE	3400	7	9	20	64.6	13.89	0.32	NO					
42	DCC	13	13	NO	NVD	MALE	2800	7	8	19.1	57.6	8.19	0.39	NO					
43	DCC	40	13	NO	NVD	FEMALE	2200	7	9	21.7	55.4	7.32	0.24	NO		4200	12	30.6	54.35
44	UCM	38.2	12	NO	El LSCS	MALE	3000	8	9	22.9	68.5	9.9	0.63	NO		5400	10.9	29.9	215
45	DCC	39.4	12	NO	NVD	FEMALE	2750	7	9	22	64.8	13.82	0.41	NO		5460	10.5	31	402.5
46	DCC	40.1	12	NO	NVD	FEMALE	3000	7	8	23.3	70.5	7.2	0.29	NO		5400	11	37.7	105
47	UCM	39	12	NO	El LSCS	MALE	3600	7	9	18.3	56.5	12.25	0.45	NO		5600	12	53.5	506.1
48	UCM	39.3	13	NO	Emg LSCS	MALE	3400	7	8	22	62.4	10.76	0.43	NO		5600	11	37.9	119
49	DCC	40.3	12	NO	NVD	MALE	3100	7	9	22.9	55	10.66	0.53	NO		5200	10	30.7	235
50	DCC	39.4	10.5	NO	NVD	MALE	2900	7	9	25	56.7	16.3	0.44	yes		4200	12	37.9	559.9
51	UCM	41	10	NO	Emg LSCS	FEMALE	3800	7	9	18	52.7	13.4	0.48	NO		5600	13	50	479.6
52	UCM	39	13	NO	El LSCS	MALE	3800	7	9	24.6	48.9	14.9	0.48	NO		5600	10	34.6	246.9
53	UCM	40.1	14	NO	El LSCS	MALE	3200	7	9	21.6	46.9	11.92	0.29	NO		5400	10	31.6	446.9
54	DCC	39	12	NO	NVD	FEMALE	3000	8	9	24.1	53.1	12.8	0.23	NO		5200	11	36.1	490
55	UCM	37.2	12	NO	Emg LSCS	MALE	2500	7	9	25.8	52.5	10.47	0.57	NO		4800	12	50.3	491.1
56	DCC	39.1	12	NO	El LSCS	FEMALE	2600	7	9	23.3	61.2	16.1	0.51	NO					
57	DCC	38.6	14	NO	NVD	MALE	2500	7	9	16.6	52.7	6.53	0.29	NO		4400	12	50.7	108
58	UCM	37.6	13	NO	El LSCS	MALE	2500	7	9	20	57.5	10.95	0.25	NO		4600	9	18.8	52.5
59	DCC	39.1	13	NO	El LSCS	MALE	3000	7	9	16.2	44.5	10.08	0.41	NO		5400	13	54	422.8
60	UCM	39	10	NO	Emg LSCS	MALE	2900	7	9	20.4	57.7	5.45	0.55	NO		4600	11	33	119.2
61	DCC	39.5	12	yes	Emg LSCS	FEMALE	3100	7	9	15.4	575	10.57	0.35	NO		5200	10	41	117.7
62	UCM	40.4	12	NO	Emg LSCS	MALE	2700	7	9	18.7	47.2	7.57	0.54	NO		5000	10.5	41.7	111.3
63	DCC	37.5	13	NO	NVD	MALE	2500	7	9	20	59.8	15.22	0.26	yes	3	4300	10.5	38.1	250.6
64	UCM	38.4	10	NO	Emg LSCS	FEMALE	2900	7	9	15.5	41.7	11.67	0.13	NO		4800	11.5	35.2	177.4
65	DCC	39.5	13	NO	Emg LSCS	FEMALE	2800	7	9	20.4	57.7	5.45	0.55	NO		4800	11	38.4	162.6
66	UCM	38.1	12	NO	El LSCS	MALE	3300	7	9	17	51	10.47	0.47	NO		5500	11.5	38.9	242.9
67	DCC	38.2	11	NO	NVD	FEMALE	3200	7	9	16.8	52.8	14.73	0.46	yes	3	5400	12	38.9	158.6
68	DCC	40	13	NO	NVD	MALE	3200	7	9	16	46.6	8.47	0.41	NO		5100	11	35.5	114
69	UCM	38.5	12	NO	Emg LSCS	MALE	3300	7	9	18.5	60	12.46	0.49	NO					
70	UCM	39.1	11.5	NO	Emg LSCS	FEMALE	3450	7	9	16.5	57.6	9.91	0.35	NO		5900	10	31.2	249.7
71	DCC	35.6	12.3	NO	NVD	MALE	2900	7	8	15.6	47.2	8.5	0.36	NO		4700	11.2	29.5	142.4
72	DCC	40.4	12	NO	Emg LSCS	MALE	3600	7	9	15.5	48	10.62	0.4	NO		5600	10	35.4	131.4
73	UCM	40	12.4	NO	NVD	MALE	3100	7	9	16.8	52.1	15.17	0.35	NO					
74	DCC	38	13	NO	NVD	MALE	2500	7	9	18.5	54	7.16	0.1	NO		4120	13	68.3	232.7
75	DCC	38.4	11	NO	NVD	FEMALE	2500	7	9	17	44.6	5.79	0.15	NO					
76	DCC	40.2	12.1	NO	NVD	MALE	3200	7	9	15	50.4	10.9	0.26	NO		6050	11	34.8	315.3
77	DCC	40.3	12	NO	NVD	FEMALE	2500	7	9	16.5	55.4	11	0.3	NO		4800	14.5	69.1	247
78	DCC	39.3	13.2	NO	NVD	MALE	3000	7	9	20.6	70.6	11.19	0.81	NO		6250	14	64.8	484.7

79	DCC	40.4	10.6	NO	NVD	MALE	2700	7	9	14.5	51.7	13.7	0.4	NO		4820	14.2	68.7	282.6
80	UCM	39.3	12	NO	NVD	FEMALE	3000	7	9	16.3	54.9	8.7	1.3	NO		5200	13.5	50.4	401.9
81	DCC	39.3	10	NO	NVD	FEMALE	2400	7	9	18.1	64	11.8	1.1	NO		4850	10.5	39.5	172.5
82	UCM	39	11	NO	EL LSCSMALE	MALE	3700	7	9	17.5	63.9	4.5	0.13	NO		5600	11	28.6	150.1
83	DCC	39	11	NO	NVD	FEMALE	3600	7	9	17.1	57.9	7.24	1	NO		5300	10.2	35.8	371.4
84	DCC	39.4	12	NO	NVD	FEMALE	2800	7	9	17.5	58.3	9.7	0.38	NO		4800	13	46.2	352.9
85	UCM	39	13	NO	Emg LSCS	FEMALE	3100	7	9	16.1	49.3	13.3	0.36	NO		5200	13.8	46.2	352.9
86	DCC	39.3	12	NO	NVD	FEMALE	2700	7	9	15.5	51	8.66	0.86	NO		4700	12.9	46.4	400.8
87	DCC	38	11	NO	NVD	FEMALE	2500	7	8	16.2	48.9	1.34	0.55	NO		4600	13.2	46.4	446.5
88	UCM	39.4	13	NO	NVD	MALE	2800	7	9	18	54.1	9.8	0.21	NO		4800	14.5	46.8	596.2
89	UCM	40	11	NO	Emg LSCS	FEMALE	3000	7	9	17	56.1	15.8	0.48	NO		5200	13.6	46.2	329.6
90	DCC	38.6	13	NO	NVD	MALE	2600	7	9	15.2	50.2	7.29	0.321	NO		4600	12.6	49	438.7
91	UCM	39.5	10	NO	Emg LSCS	FEMALE	3200	7	9	16.8	57.2	9.7	0.31	NO					
92	DCC	38.2	13	NO	Emg LSCS	FEMALE	2800	7	9	17.2	57.2	9.8	0.29	NO		4900	14	58.8	270
93	UCM	39	12	NO	NVD	FEMALE	2200	7	8	18.5	60.5	1.58	0.5	NO					
94	UCM	40.3	11	NO	EL LSCSMALE	MALE	2800	7	9	16.2	52.2	9.1	0.26	NO					
95	DCC	38	13	NO	Emg LSCS	FEMALE	2200	7	9	18.5	61.8	15.3	0.4	NO					
96	UCM	37.5	12	NO	Emg LSCS	MALE	3000	7	9	15.1	42.6	15.4	0.59	NO					
97	DCC	38.2	11	NO	NVD	MALE	2600	7	9	17	52.1	10.08	0.23	NO					
98	DCC	39.4	13	NO	EL LSCSMALE	MALE	2400	7	9	18.6	63.1	14.9	0.26	NO					
99	UCM	37	12	NO	El LSCS	FEMALE	2400	7	9	17.9	58	12.12	0.46	NO					
100	UCM	38.3	13	NO	Emg LSCS	FEMALE	2200	7	9	18	62.9	11.09	0.37	NO					
101	UCM	36.1	11	NO	Emg LSCS	FEMALE	2000	7	9	16.2	43	11.7	1.4	NO					
102	DCC	38.3	13	NO	Emg LSCS	MALE	2900	7	9	16.8	52	8.45	0.481	NO					
103	UCM	36.4	13	NO	Emg LSCS	FEMALE	2600	7	9	15.1	35.9	11.89	0.55	NO					
104	UCM	39.4	11	NO	NVD	MALE	2900	7	9	14.1	35.9	9.83	0.44	NO					
105	DCC	39.6	13	NO	Emg LSCS	MALE	2900	7	9	14.5	36	10.4	0.5	NO					
106	UCM	38.4	11	NO	Emg LSCS	MALE	3100	7	9	17.4	55.6	8.71	0.7	NO		5200	11	34.1	183.7
107	DCC	37.1	10	NO	NVD	FEMALE	2400	7	9	18	58	11.2	0.5	NO					
108	UCM	39.6	12	NO	NVD	MALE	2800	7	9	15.3	41.1	8.59	0.74	NO					
109	DCC	39.4	14	NO	NVD	MALE	3700	7	9	16.8	56.2	9.48	0.131	NO		5060	12.4	37.1	152.9
110	UCM	39	12	NO	EL LSCS	FEMALE	2500	7	9	17.5	61.1	9.87	0.42	NO		4600	11.2	34.9	230.1
111	DCC	40.5	13	NO	Emg LSCS	MALE	3300	7	9	14.2	33.4	8.23	0.33	NO		5300	12.5	39.8	144.7
112	UCM	37.5	13	NO	Emg LSCS	FEMALE	2600	7	9	16.1	44.3	9.4	0.32	NO		4600	11.2	34	272.7
113	UCM	38.1	12	NO	Emg LSCS	FEMALE	2400	7	9	17.6	56.5	8.4	0.32	NO					
114	UCM	37.3	11	NO	Emg LSCS	FEMALE	3800	7	9	17.4	53.6	16.8	0.77	yes	3				
115	DCC	38	12	NO	NVD	MALE	2700	7	9	14.6	30.2	9.13	0.2	NO		4700	11.3	36.1	431.7
116	DCC	36.4	13	NO	NVD	MALE	2400	7	9	18.6	59.9	7.46	0.36	NO		4400	11	34.3	283.9
117	DCC	39.4	12	NO	NVD	MALE	2700	7	9	17.5	51.8	8.22	0.39	NO					
118	DCC	36.5	13	NO	NVD	MALE	2800	7	9	18.2	52.5	21.01	0.65	yes	3	4800	13.5	53.4	238.7
119	UCM	35.2	14	NO	Emg LSCS	MALE	2800	7	9	17.2	56.9	17.28	0.2	yes	2				
120	DCC	37	13	NO	NVD	MALE	2800	7	9	16.8	56.2	9.26	0.35	NO		4900	13	43.4	316.8
121	DCC	41	11	NO	NVD	FEMALE	2700	7	9	18.6	63.7	12.2	0.25	NO					
122	UCM	38.6	12	NO	NVD	MALE	2800	7	9	14.9	39.8	1.83	0.45	NO		4900	11	35.4	173.3
123	DCC	39.1	12	NO	NVD	FEMALE	2500	7	9	18	63.3	14.9	0.44	YES	3	4200	12.3	42.5	315.6
124	UCM	41.2	13	NO	EMGLSCS	MALE	3100	7	9	16.5	51.3	14.6	0.48	NO		4400	12.5	40.7	585.4
125	DCC	40.5	12	NO	EMGLSCS	FEMALE	2600	7	9	17.4	48.5	12.74	0.19	NO					
126	DCC	38.4	13	NO	NVD	MALE	2800	7	9	17.5	57.6	9.38	0.22	NO		4700	11.5	37.4	287.9
127	UCM	39.1	12	NO	EMGLSCS	MALE	3400	7	9	17.5	60.3	8.6	0.42	NO		4900	11.5	36.7	475.9
128	UCM	39.2	13	NO	EMGLSCS	MALE	3000	7	9	12.8	45.9	3.5	0.3	NO		4280	10.3	36.8	60.7
129	DCC	37.6	10	NO	NVD	FEMALE	2300	7	9	12.4	30.4	18.3	0.22	YES	2	4000	10.5	37.8	438.5
130	DCC	38.1	11.2	NO	EMGLSCS	FEMALE	3100	7	9	16.1	54.7	16.4	0.56	YES					
131	DCC	38.1	12	NO	EMGLSCS	FEMALE	2400	7	9	16.3	58.5	18.3	0.42	yes	3	3800	12.2	36.7	434.1
132	UCM	39.2	11.6	NO	EMGLSCS	MALE	2600	7	8	17.4	58	12.8	0.56	NO					
133	UCM	39.6	12.5	NO	EMGLSCS	MALE	2500	7	9	17.5	54.2	12.9	0.34	NO		4250	11.1	33.1	257.1
134	UCM	40.4	12	NO	EMGLSCS	FEMALE	3300	7	8	18.6	55.2	10.45	0.2	NO					
135	DCC	39.3	13	NO	NVD	FEMALE	3100	7	9	18.2	57.5	17.5	0.5	YES	3	4300	10.2	32.9	68.7
136	DCC	39.1	12	NO	NVD	MALE	2900	7	8	13.4	48	1.45	0.12	NO		4800	10.2	31.8	316
137	DCC	38.1	11	NO	NVD	FEMALE	2400	7	9	18.2	52.4	14.9	0.3	NO		4200	11.6	37.4	436.5
138	DCC	39.2	13	NO	NVD	FEMALE	2900	7	9	16.8	56.1	17	0.5	YES	3	4200	11.2	34.4	242.9
139	UCM	39.4	12.6	NO	EMGLSCS	MALE	4000	7	9	17.2	56.4	11.4	0.09	NO					
140	UCM	39.5	12	NO	NVD	MALE	2800	7	9	16.2	49.2	8.7	0.1	NO		5000	11.2	36.5	362.3
141	DCC	39	11	NO	NVD	FEMALE	2300	7	9	17.4	63.5	6.9	0.3	NO		4000	12.4	43.4	182.3
142	DCC	38.1	13.6	NO	NVD	MALE	2600	7	9	16.7	54.5	16.5	0.5	YES	2				
143	UCM	39.1	13	NO	EMGLSCS	FEMALE	3100	7	9	16.5	50.7	9.5	0.4	NO					
144	UCM	38.5	13	NO	EMGLSCS	MALE	3800	7	9	18.5	62	15.4	0.6	YES	2	5200	10.2	31.2	248.4
145	DCC	37.4	13	NO	EL LSCS	MALE	3200	7	9	18.2	49.7	10.8	0.4	NO		5000	10.8	33.9	251.8
146	DCC	40	12	NO	NVD	MALE	3100	7	9	14.5	48.2	12.1	0.4	NO					
147	UCM	36.6	10.6	NO	EL LSCS	FEMALE	2200	7	9	17.5	55.8	12.2	0.4	NO		3800	11.5	37.3	126.1
148	DCC	37.5	10	NO	EL LSCS	FEMALE	2700	7	9	16.5	56.6	9.6	0.4	NO		4500	10.1	27.5	97.7
149	UCM	36.4	14	NO	NVD	MALE	3500	7	9	17.2	57.3	12	0.1	NO					
150	UCM	39.3	10.4	NO	EMGLSCS	FEMALE	3300	7	9	17.8	60.2	14.9	0.2	NO		4700	12.2	39.4	142.8
151	DCC	40	13	NO	NVD	FEMALE	3100	7	9	16.8	52.5	18.5	0.4	YES	3	4700	11.2	34.8	163.3
152	UCM	39.1	12	NO	NVD	MALE	3100	7	9	13.8	42.7	8.9	0.3	NO					
153	DCC	37.5	10	NO	EL LSCS	FEMALE	2700	7	9	17.2	56.6	9.6	0.44	NO					
154	DCC	38.1	12.4	NO	NVD	MALE	2000	7	9	19.2	60.2	13.9	0.4	NO		3200	11.2	36	459.4
155	UCM	39.4	12.1	NO	NVD	FEMALE	2300	7	9	14.5	48	13.7	0.52	NO		4200	10.3	44.4	213.9
156	UCM	38.3	12.6	NO	EMG LSCS	FEMALE	2900	7	9	16.5	54.3	13.36	0.37	NO		4600	10.2	41	221.1
157	DCC	37.5	11.6	NO	NVD	FEMALE	2800	7	9	16.5	41	16.65	0.67	YES	3	4700	9.4	20.1	223.9
158	DCC	39.6	12.4	NO	NVD	FEMALE	2600	7	8	16.2	50.2	11.5	0.2	NO					
159	UCM	37.5	11.2	NO	EMG LSCS	MALE	3000	7	9	14.5	52.6	9.58	0.39	NO		5400	9.5	21.2	246.6
160	UCM	38.2	13	NO	EMG LSCS	MALE	2500	7	9	16.8	54.8	12.84	0.22	NO		4800	10.8	44	203.9
161	DCC	39.3	11.3	NO	NVD	FEMALE	2700	7	9	15.5	63.5	13.2	0.49	NO		4900	11.2	43.4	182.3
162	DCC	40.1	12	NO	NVD	FEMALE	2500	7	9	14	48.5	6.6	0.19	NO		4800	12.5	47	189.2
163	UCM	38.1	10	NO	EMG LSCS	MALE	2900	7	9	14.5	52.4	16	0.22	YES	3	4700	8.9	21.1	244.1
164	UCM	40.2	14	NO	NVD	FEMALE	2600	7	8	16.4	52	11.2	0.2	NO					
165	UCM	37.6	12	NO	EMG LSCS	MALE	2500	7	9	17.5	60.4	12.97	0.43	NO		4500	12.8	46	222.6
166	UCM	40	12.3	NO	NVD	MALE	2400	7	98	17.4	67.6	18.4	0.3	NO					

167	UCM	40	12.4	NO	NVD	MALE	3000	7	9	16.2	40.5	17.3	0.6	YES	3				
168	DCC	38	12	NO	EL LSCS	MALE	2700	7	9	13.5	43.7	1.5	0.5	NO		4400	10.5	32.1	285.5
169	UCM	37.4	13	NO	Emg LSCS	FEMALE	2700	7	9	14	52	12.5	0.54	NO		4100	11.2	42	74.7
170	DCC	38.5	10	NO	Emg LSCS	FEMALE	2400	7	9	14	63.4	10.2	0.49	NO		4000	11	36.5	77.1
171	DCC	39.5	13	NO	NVD	FEMALE	2900	7	9	14.6	49.5	11.6	1.1	NO		4500	10.4	34	76.4
172	DCC	38.3	12	NO	NVD	FEMALE	3200	7	9	16	54	10.9	0.38	NO		5400	11	34	73.5
173	UCM	39.1	13	NO	NVD	MALE	3700	7	9	17	60	18.04	0.31	yes	3	5200	11	35.1	142.2
174	DCC	39.3	12	NO	Emg LSCS	MALE	2800	7	9	18	64.7	17.06	0.46	yes	2	5700	11.2	37	266.4
175	DCC	40	12	NO	NVD	MALE	2750	7	9	13	46.5	12	0.2	NO		4800	11.5	36.6	268.5
176	UCM	39.4	10.2	NO	Emg LSCS	FEMALE	3000	6	8	16.8	52.5	15.4	0.31	yes	2				
177	UCM	38.4	11.4	NO	Emg LSCS	FEMALE	3100	7	9	15	60.3	10.6	0.4	NO		4200	12	38.5	268.8
178	DCC	38.1	12.8	NO	Emg LSCS	MALE	2600	7	9	14	60	11.9	0.44	NO		4000	10.9	36.6	273.1
179	UCM	40.2	12	NO	NVD	MALE	2700	6	8	18.3	58.2	12	0.67	NO					
180	UCM	40	12	NO	NVD	MALE	2900	6	8	13.5	48	12.4	0.24	NO		4800	12.8	39.5	79.7