THE EFFECT OF TIMING OF UMBILICAL CORD CLAMPING ON NEWBORN ANEMIA: IMPLICATIONS FOR CLINICAL PRACTICE IN THE PERUVIAN AMAZON

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Abstract

Delayed umbilical cord clamping has been shown to decrease the risk of infant anemia. A pre-post study design was used to determine the effectiveness of a two-component intervention (workshop and directive) in delaying cord clamping. Women were recruited from the labour room of Hospital Iquitos in Iquitos Peru pre (N=112) and post intervention (N=112). Maternal hemoglobin was assessed before delivery; time-to-cord clamping was recorded at delivery and infant hemoglobin was measured at four months of age. Time-to-clamp increased significantly by 113 seconds following the intervention ($\beta_{adjusted}$ = 113.2 seconds, 95% CI: 96.6, 129.9). Maternal anemia was identified as an effect modifier on the relationship between time-to-clamp and infant anemia. In infants born to anemic women, the odds ratio of developing anemia is 0.59 for every one minute delay in cord clamping. These findings add to the mounting support for delayed cord clamping as a means to decrease infant anemia.

Résumé

Des études ont démontré que retarder la coupure du cordon ombilical pourrait réduire les risques d'anémie infantile. Un plan d'étude pré/post a été utilisé pour déterminer l'efficacité d'une intervention visant à retarder la coupure du cordon. Des femmes ont été recrutées à l'Hôpital Iquitos (Pérou) pré- (N=112) et post-intervention (N=112). Le statut hématologique des mères a été quantifié, le moment où le cordon est coupée a été chronométré et le niveau d'hémoglobine des enfants à été mesuré. La coupure du cordon a été retardée de manière significative de 113 secondes suite à l'intervention ($\beta_{ajusté}$=113.2 secondes; 95% IC =96.6-129.9). L'anémie maternelle a été identifiée comme un facteur modifiant. Chez les enfants nés d'une mère anémique, le rapport de cotes pour l'anémie est de 0.59 pour chaque minute où la coupure du cordon est retardée. Ces résultats supportent le retardement de la coupure du cordon comme mesure pouvant réduire l'anémie infantile.

Preface

The present thesis focuses on the implementation of a new hospital policy on delayed umbilical cord clamping. First, an introduction to delayed umbilical cord clamping and infant anemia is given (Chapter 1). In Chapter 2, a detailed overview of anemia is presented followed by an in-depth description of the timing of umbilical cord clamping. A summary of the epidemiological literature to date of the effects of delayed cord clamping is given as well as a summary of the most up-to-date recommendations (Chapter 2). Following is an outline of the primary and secondary objectives of the thesis (Chapter 3). Next, the study methodology including study location, population, design, and statistical analyses is described (Chapter 4). The results are presented in Chapter 5, summarized in two manuscripts. Finally, a discussion of the results and concluding remarks make up Chapters 6 and 7.

The following thesis has been prepared according the guidelines for a "Manuscript-Based Thesis". The results are outlined in two manuscripts:

> Brittany Blouin, Martin Casapia, Eder Aguilar, Hermánn Silva, Mary E. Penny, Hilary Creed Kanashiro, Serene A. Joseph, Mathieu Maheu-Giroux, Theresa W. Gyorkos. Delaying cord clamping: the effect of a two-component intervention to change practice in the Peruvian Amazon. Submitted to *Health Policy and Planning*
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> Brittany Blouin, Martin Casapia, Eder Aguilar, Hermánn Silva, Mary E. Penny, Hilary Creed Kanashiro, Serene A. Joseph, Mathieu Maheu-Giroux, Elham Rahme, Anita Gagnon, Theresa W. Gyorkos. The effect of maternal anemia on the association between timing of umbilical cord clamping and infant anemia: observations of hospital practice in the Peruvian Amazon. Submitted to *Pediatrics*

Details of authors' contributions are described on pages v-vi.

In addition, results from this thesis have been presented or submitted for presentation at scientific conferences. These include:

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Blouin B, Casapia M, Aguilar E, Silva H, Penny ME, Creed-Kanashiro HM, Joseph SA, Gyorkos TW. Implementation of a hospital-based training initiative in later cord clamping: observations of change in practice in the Peruvian Amazon. *Canadian Conference on International Health*. Oral Presentation. Ottawa, Canada; October 26, 2009 – presented

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Blouin B, Casapia M, Aguilar E, Silva H, Penny ME, Creed-Kanashiro HM, Joseph SA, Maheu-Giroux M, Gyorkos TW. Maternal anemia modifies the effect of the timing of umbilical cord clamping on infant anemia in the Peruvian Amazon. *L'Axe de recherché en santé mondiale du RRSPQ Colloque sur les bonnes pratiques de recherche en santé mondiale.* Montreal, Canada; June 1, 2010 - presented

Blouin B, Casapia M, Aguilar E, Silva H, Penny ME, Creed-Kanashiro HM, Joseph SA, Maheu-Giroux M, Rahme E, Gagnon A, Gyorkos TW. Maternal anemia modifies the effect of the timing of umbilical cord clamping on infant anemia in the Peruvian Amazon. *Global Congress of Maternal and Infant Health*. Barcelona, Spain; September 22-26, 2010 – abstract submitted

Blouin B, Casapia M, Aguilar E, Silva H, Penny ME, Creed-Kanashiro HM, Joseph SA, Maheu-Giroux M, Gyorkos TW. The effect of maternal anemia on the association between timing of umbilical cord clamping and infant anemia. *Canadian Conference on Global Health.* Ottawa, Ontario; October 31 – November 3, 2010 – abstract submitted

Contributions of Authors

The original research idea was developed by Dr. Mary Penny, Mrs. Hilary Creed-Kanashiro and Dr. Theresa Gyorkos. Specific study objectives and design were conceptualized by Ms. Brittany Blouin, Ms. Serene Joseph and Dr. Gyorkos. The study design and objectives were presented to Dr. Martin Casapia, Dr. Hermánn Silva and Dr. Eder Aguilar who offered valuable input.

Ms. Blouin wrote the study protocol which was subsequently reviewed and revised by Ms. Joseph and Dr. Gyorkos. The protocol was also reviewed by Dr. Penny, Mrs. Creed-Kanashiro, Dr. Casapia, Dr. Elham Rhame, and Dr. Anita Gagnon.

The intervention component of the study was organized by Ms. Joseph, Salome Chapiama and Dr. Penny (in collaboration with two Peruvian Ministry of Health officials). Following conversation with Dr. Casapia, Dr. Aguilar and Dr. Silva were also involved in the study intervention.

The four questionnaires were designed by Ms. Blouin with help from Dr. Gyorkos and Ms. Joseph. Ms. Joseph translated all questionnaires from English to Spanish. All questionnaires were pre-tested in Spanish by three local nurse-midwives (Obst. Evelyn Burga, Obst. Nohelia Camboa Paredes, and Obst. Ever Lazaro Panduro) under the direct supervision of Ms. Blouin. All interviews, collection of biological samples, collection of anthropometric measurements and outcome measurements were undertaken in Spanish by the three local nurse-midwives under the direct supervision of Ms. Blouin. Ms. Blouin oversaw all aspects of data collection, ensured quality control, and performed all data entry, cleaning and management. Ms. Blouin performed all statistical analyses and interpretation of data with help from Mr. Mathieu Maheu-Giroux under the supervision of Dr. Gyorkos.

Complete drafts of the two manuscripts entitled: “Delaying cord clamping: the effect of a two-component intervention to change practice in the Peruvian Amazon” and “The effect of maternal anemia on the association between timing of umbilical cord clamping and infant anemia: observations of hospital practice in the Peruvian Amazon” were written by Ms. Blouin and subsequently reviewed and revised by Dr. Gyorkos, Dr. Casapia, Dr. Aguilar, Dr. Silva, Dr. Penny, Mrs. Creed-Kanashiro, Dr. Rhame, Dr. Gagnon, Ms. Joseph, and Mr. Maheu-Giroux. One manuscript (Chapter 5, section 5.2) has been submitted for publication in *Health Policy and Planning*. The second manuscript (Chapter 5, section 5.4) has been sent to all co-authors for their comments prior to being submitted to *Pediatrics*.

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Table of Contents

List of Tables

Chapter 2

Chapter 5: Section 5.2.

Chapter 5: Section 5.4.

List of Figures

Chapter 4

Chapter 5: Section 5.2.

Chapter 5: Section 5.4.

List of Appendices

List of Abbreviations

adj: Adjusted

aOR: Adjusted odds ratio

CI: Confidence interval

CIHR: Canadian Institutes for Health Research

cm: Centimetre

DALYs: Disability adjusted life years

DCC: Delayed cord clamping

ECC: Early cord clamping

EU: European Union

g: Grams

g/L: Grams per litre

HAI: Hospital Apoyo Iquitos

Hb: Hemoglobin

Hct: Hematocrit

IIN: Instituto de Investigación Nutricional

IQ: Intelligence quotient

mg: Milligrams

mg/dL: Milligrams per decilitre

min.: Minutes

mL: Millilitres

mL/kg: Millilitres per kilogram

MINSA: Ministerio de Salud, Peru

NICU: Neonatal intensive-care unit

OPS: Organización Panamericana de la Salud

OR: Odds ratio

PAHO: Pan American Health Organization

PRCT: Packed red cell transfusion

RCT: Randomized controlled trial

sec.: Seconds

sd: Standard deviation

T_0: Timing start point

µg: Micrograms

UNICEF: United Nations Children's Emergency Fund

WHO: World Health Organization

1. Introduction

Iron deficiency anemia is a global problem. Although it remains to be a significant health burden in developed countries, iron deficiency anemia is particularly devastating in developing countries, where it is highly associated with poverty (UNICEF and WHO, 1999). Iron deficiency is the most prevalent nutrient deficiency worldwide and is the most common cause of anemia (WHO et al., 2001). The age groups most susceptible to iron deficiency anemia include pregnant women and young children, as during pregnancy and early childhood, iron requirements are increased (Benoist *et al.*, 2008). During the first year of life, iron is needed for growth and normal brain development (Lozoff and Georgieff, 2006; Lawless *et al.*, 1994). Iron deficiency anemia during infancy is associated with poorer cognitive, motor and social/emotional outcomes. The most devastating finding regarding iron deficiency anemia during infancy, is that its consequences are usually permanent and do not improve following iron supplementation (Lozoff and Georgieff, 2006). The life-long, irreversible effects of iron deficiency anemia during infancy highlight the notion that preventing iron-deficiency anemia is preferable to treating it, especially during infancy.

One of the major interventions known to prevent iron deficiency anemia during the first six months of life is delayed umbilical cord clamping at birth (Stoltzfus, 2008). Waiting approximately three minutes to clamp the umbilical cord following the birth of the baby allows placental transfusion (blood transfer from the placenta to the infant) to run to completion (McDonnell and Henderson-Smart, 1997). This can provide the newborn with an additional 30-50 mg of iron which will increase iron reserves and decrease the risk of iron deficiency anemia later in infancy (Weckert and Hancock, 2008; Levy and Blickstein, 2006). In experimental settings, delayed umbilical cord clamping has been shown to be particularly beneficial to the iron status of infants (Strauss *et al.*, 2008; Ultee *et al.*, 2008; Kugelman *et al.*, 2007; van Rheenen *et al.*, 2007; Chaparro *et al.*, 2006; Ceriani Cernadas *et al.*, 2006; Emhamed *et al.*, 2004; Gupta and Ramji, 2002;

Grajeda *et al.*, 1997; Rabe *et al.*, 2000) and in 2007, the Pan American Health Organization released new recommendations favouring delayed cord clamping over immediate cord clamping (Chaparro and Lutter, 2007). This intervention has not only been proven effective, but it is cost-free, making it a particularly appropriate and sustainable intervention for low-resource areas of the world. No research has been conducted which has attempted to change hospital policy from early to delayed umbilical cord clamping, nor on the effectiveness of delayed clamping in decreasing infant anemia in a real life setting. This is however, a very important step and one that needs to be addressed in order to put evidence-based research into practice.

2. Literature Review

2.1. ANEMIA

2.1.1. Anemia as a public health problem

Anemia is defined as a reduction in the normal number of circulating red blood cells and in the quantity of hemoglobin in the blood (Reveiz *et al.*, 2007). It primarily results from iron deficiency, but can also occur as a result of chronic infections, folate deficiency, vitamin B12 deficiency, bone marrow suppression, hemolytic diseases, blood loss (ie. from schistosomiasis, hookworm infection, hemorrhage and trauma), and underlying malignancies (UNICEF and WHO, 1999; WHO, 1992). In severe cases of iron deficiency, a lack of iron leads to an inadequate amount of red blood cells and this is termed iron deficiency anemia. Iron deficiency is the primary cause of anemia in 50% of cases worldwide (WHO *et al.*, 2001). In the most recent WHO report on global burden of disease, iron-deficiency anemia was ranked in the top ten leading global causes of years lost to disability (DALYs) in low and middle income countries. Moreover, worldwide, more individuals have iron-deficiency anemia than any other health problem (WHO, 2008a).

Anemia is a major health problem occurring in both developed and developing countries. Globally, it affects an estimated 1.62 billion people or 24.8% of the world's population (Benoist *et al.*, 2008). Anemia has been identified as a severe public health problem (prevalence ≥ 40%) in 69 countries of the world (Benoist *et al.*, 2008; UNICEF and WHO, 1999). The highest risk groups are preschool-aged children, pregnant women and non-pregnant women of child-bearing age (Benoist *et al.*, 2008). Few countries exist where anemia is not at least a mild public health problem in all three high risk groups. The highest proportions affected are in Africa where up to 68% of individuals have been found to be anemic (Benoist *et al.*, 2008). The greatest absolute numbers affected are reported from South-East Asia where an estimated 315 million individuals in high risk groups are anemic (Benoist *et al.*, 2008). In South America, anemia is confirmed as a severe public health problem in preschool-aged children in Peru, Brazil, Guyana and Bolivia; in

non-pregnant women of child-bearing age in Peru and Guyana; and in pregnant women in Peru and Guyana (Benoist *et al.*, 2008).

2.1.2. Determinants of anemia

The major determinants for iron deficiency anemia include low iron intake, poor absorption of iron from diets high in phytate or phenolic compounds, and period of life when iron requirements are especially high (infancy, young childhood, adolescence and pregnancy) (Benoist *et al.*, 2008; UNICEF and WHO, 1999). Increased iron requirements during periods of growth are often not met, which commonly leads to iron deficiency anemia. Other determinants of anemia include heavy blood loss (ie. from menstruation), repeated pregnancies, parasite infections, acute and chronic infections, other micronutrient deficiencies, pathological blood losses, processes that impair iron absorption and use, and genetic causes (Benoist *et al.*, 2008; UNICEF and WHO, 1999). Low socio-economic status is also associated with iron deficiency anemia. This is due to lack of food security, inadequate access to health care and poor sanitation and personal hygiene (UNICEF and WHO, 1999). Interestingly, some studies also suggest that males may have a higher predisposition for iron deficiency than females during the first year of life (Miller *et al.*, 2006; Thorsdottir *et al.*, 2003; Domellof *et al.*, 2002; Sherriff *et al.*, 1999; Wharf *et al.*, 1997). The mechanism for this is unknown but may be a result of differential testosterone and estrogen production that can effect erythropoietin production, differences in growth during this time or differences in birth iron stores.

2.1.3. Consequences of anemia

Anemia has major consequences both for human health and for social and economic development. Severe anemia is associated with an increased risk of death (Brabin *et al.*, 2001). Anemia affects the immune system which increases morbidity due to infectious diseases for all age groups (WHO *et al.*, 2001). Additionally, blood transfusions are often prescribed in cases of severe anemia which may lead to increased exposure to infections, especially in developing

settings (Lackritz, 1998). Severe anemia also decreases the body's ability to monitor and regulate body temperature when exposed to the cold (UNICEF and WHO, 1999). At all stages of life, iron deficiency can impair cognitive performance. Iron plays a vital role in brain function, and deficiencies have been found to alter neurotransmitters (WHO *et al.*, 2001). The cognitive effects of iron deficiency anemia in infancy and early childhood are often not reversed following iron supplementation. Physical work capacity is decreased during iron deficiency and this is due to decreased transport of oxygen to the muscles and alterations in how muscles use energy (WHO *et al.*, 2001). Work capacity, however, is restored following iron supplementation. Iron needs are higher during periods of growth and iron-deficiency impairs growth rates. The effect of iron supplementation in restoring proper growth depends on the frequency of diarrhea and other infections, age at iron depletion and diet (WHO *et al.*, 2001). The economic effects of anemia primarily result from loss of productivity, health care costs and lifetime costs related to the permanently impaired cognitive development of young children who develop iron deficiency anemia.

2.1.4. Indicators of anemia

Anemia typically commences when total storage iron is less than 200 mg (Handelman and Levin, 2008). Storage iron can be estimated from plasma ferritin such that 1μg of ferritin corresponds to approximately 8 mg of storage iron (Cook, 1982; Walters *et al.*, 1973). Inflammation, however, elevates plasma ferritin, making this indicator less reliable. Other indicators of iron-deficiency anemia include transferrin saturation, zinc-protoporphyrin-IX, soluble transferrin receptors and hemoglobin concentration (Handelman and Levin, 2008; Zimmermann, 2008). When iron stores drop below 200 mg, there is not enough iron for erythropoeisis and transferrin iron saturation and hemoglobin levels decrease. When iron stores are depleted, plasma iron and iron supply to the bone marrow decreases. Consequently, the bone marrow increases the expression of transferrin receptors, which act by taking iron from the bloodstream. Insufficient quantities of iron to convert protoporphyrin to heme leads to an increased fraction

of the non-iron containing heme precursor, Zn-protoporphyrin-IX in red blood cells (Handelman and Levin, 2008).

The most reliable indicator of iron deficiency anemia at the population level is hemoglobin concentration (Benoist *et al.*, 2008). As iron deficiency anemia progresses, mean corpuscular hemoglobin levels decrease and the percent of red blood cells with insufficient hemoglobin can increase from 2-3% (normal) to 20-30% (Handelman and Levin, 2008). The WHO standard for classifying iron deficiency anemia varies with age and sex. The definitions can be found in Table 1 (Benoist *et al.*, 2008).

Table 1: WHO age and sex specific thresholds for defining iron deficiency anemia

Age or sex group	*Hemoglobin threshold (g/L)*
Children (males and females) < 6 months	Not specified
Children (males and females) 6 months – 5 years	110
Children (males and females) 5 – 12 years	115
Children (males and females) 12 – 15 years	120
Non-pregnant women ≥ 15 years	120
Pregnant women (any age)	110
Men ≥ 15 years	130

2.2. MATERNAL ANEMIA

Pregnancy is one of the periods of life when iron requirements are increased. More than 500 mg of storage iron is required to avoid iron deficiency during pregnancy (Baker, 2000). Maternal iron is needed for transfer to the fetus making it more difficult to maintain iron needs with diet alone. Moreover, physiological changes that occur with pregnancy include expansion of plasma volume by 46-55% with expansion of red-cell volume by only 18-25%. This leads to

hemodilution (van den Broek, 2003). Hemodilution occurs during the first and second trimester and causes a decrease in hemoglobin and hematocrit concentration (Taylor *et al.*, 1982; Bothwell and Charlton, 1981). In the third trimester, both hemoglobin and hematocrit levels rise back to pre-pregnancy levels in women with adequate iron intake (Goodman, 1989; Svanberg *et al.*, 1975). Between 1993-2005, globally, approximately 42% of all pregnant women were anemic (Benoist *et al.*, 2008). Anemia during pregnancy is more common in developing countries, where up to 60% of pregnant women are affected by anemia (WHO, 1992).

Increased iron needs during pregnancy are supported by a significant increase in iron absorption. The majority of iron transfer occurs after 30 weeks gestation. Serum transferrin carries iron from the maternal circulation to receptors on the placental surface. Iron is released into the placenta, binds to ferritin and is carried to the fetal side of the placenta where it enters the fetal circulation (Allen, 2000). When maternal iron status is poor (ie. during maternal iron deficiency anemia) the number of placental receptors increases, such that transport of iron to the fetus increases. Excessive transport to the fetus is regulated by limiting production of ferritin in placental cells used to carry iron to the fetal circulation (Allen, 2000).

The major concern regarding anemia during pregnancy is that it increases the risk of maternal mortality and morbidity. Associations between severe maternal anemia and maternal mortality have been shown; however, the actual cause of death is debatable. For example, maternal hemorrhage is more likely to be fatal when the woman is anemic (van den Broek, 2003). The reported cause of death, however, is hemorrhage, not anemia. Maternal anemia has also been associated with lower infant birth weights (Singla *et al.*, 1997; Hemminki and Rimpela, 1991; Agarwal *et al.*, 1991), lower infant APGAR scores (Preziosi *et al.*, 1997; Rusia *et al.*, 1995) and preterm delivery (Allen, 2000). The increased risk of preterm delivery is only present when anemia is detected in the first or second trimester (Scholl *et al.*, 1992).

2.3. INFANT ANEMIA

Iron is crucial for growth and development and has particular importance from gestation through the first year of life. During gestation, the fetus has a high red blood cell mass, which requires iron. These red blood cells provide oxygen needed both for development as well as to overcome the relatively hypoxic (lacking oxygen) uterine environment (Chaparro, 2008). The iron needed to sustain the large red blood cell mass is actively transported across the placenta from maternal to fetal circulation (Beard, 2008). In the case of iron deficiency during pregnancy, protective mechanisms attempt to maximize iron transfer to the fetus; however, the extent of this protection is not well known (Chaparro, 2008). Iron transport to the fetus occurs primarily during the last trimester of pregnancy (Bradley *et al.*, 2004) adding to the importance for the fetus to reach term.

2.3.1. Hemoglobin levels during gestation and first year of life

At 9-10 weeks gestation, hemoglobin concentration in the fetus is approximately 90 g/L (Brown, 1988). This increases to 140 g/L by mid-gestation and reaches 170 g/L, on average by the last eight weeks of gestation (Brown, 1988). Hemoglobin concentration is higher at birth than at any other period of life and decreases from approximately 170 g/L at birth to a low of about 112 g/L in the first two months of life (Brown, 1988). This stage is referred to as the "physiological anemia of infancy" and is a result of the combined effect of the shorter lifespan of fetal erythrocytes, decreased erythrocyte production and a dilution effect from increased blood volume related to growth (Brown, 1988). From two months onward, hemoglobin concentration increases slightly and usually reaches 118 g/L, on average, by four to six months of age (Domellof *et al.*, 2001).

Accompanying changes in hemoglobin concentration during the first few months of infancy is a redistribution of total body iron. Newborns are estimated to have approximately 260 mg of total body iron of which 70% is in the form of hemoglobin, 24% is stored as ferritin and the remaining 6% is in myoglobin and

iron-containing enzymes (Dallman *et al.*, 1993). By four months of age, although there is little change in total body iron content, the distribution changes significantly. Iron stores are mobilized for growth and therefore ferritin stores decline to approximately 12%, iron in hemoglobin increases to approximately 76%, and iron in myoglobin and iron-containing enzymes increases to 12% (Dallman *et al.*, 1993). After four to six months of age, the breast-fed infant begins to receive exogenous sources of iron such that total body iron increases to approximately 420 mg; however, the distribution of the iron content remains essentially the same as at four months of age (Dewey and Chaparro, 2007).

2.3.2. Determinants of birth iron stores

Maternal iron status, infant birth weight, and gestational age are the physiological factors that likely contribute the most to total body iron at birth (Chaparro, 2008). The effect of maternal iron deficiency during pregnancy on infant iron stores at birth, however, is controversial. Cross-sectional studies have produced conflicting results. This is likely due to differing degrees of iron deficiency in the women studied and differing attributes of the pregnancy and delivery that can alter the indicators used to assess anemia (Chaparro, 2008). Several longitudinal studies, including one randomized controlled trial on iron supplementation during pregnancy, have found that maternal anemia during pregnancy is associated with infant anemia during the first year of life (de Pee *et al.*, 2002; Kilbride *et al.*, 1999; Preziosi *et al.*, 1997; Colomer *et al.*, 1990). In the two studies that measured iron status at birth, however, maternal anemia during pregnancy was not associated with cord blood iron levels (Kilbride *et al.*, 1999; Preziosi *et al.*, 1997). These two studies were limited by using cord blood as a proxy measure for body iron levels. Conversely, another study that measured total body iron at birth as the sum of circulating hemoglobin iron and body storage iron showed that infants of mothers with the lowest hemoglobin concentrations had the lowest total body iron at birth (Miller *et al.*, 2003).

The effect of birth weight on iron stores at birth is more well known. A linear relationship between body iron and birth weight exists such that iron stores at birth increase with increasing birth weight (Widdowson and Spray, 1951). Although gestational age is correlated with the amount of body iron stores at birth, the correlation between birth weight and gestational age may, to a large degree, account for this (Chaparro, 2008). During the last eight weeks of gestation there is an increase in the total amount of liver iron because of an increase in liver size, which is related to birth size (Chaparro, 2008).

2.3.3. Depletion of birth iron stores during first year of life

During the first six months of an infant's life, the optimal feeding practice is exclusive breastfeeding. Breastmilk, however, does not provide the infant with a significant amount of iron (Oski and Landaw, 1980). The amount of iron absorbed from breast milk is approximately equivalent to the amount of total iron lost (the majority lost by sloughing of epithelial cells of the gastrointestinal tract) (Oski and Landaw, 1980). Body iron stores at birth, therefore, are essential to ensure adequate iron levels for the first six months of life and are required to sustain growth and development during this time (Chaparro, 2008). Postnatal factors that contribute to early depletion of iron stores and development of anemia include feeding practices and growth rate (Chaparro, 2008). Although breast milk is not high in iron, exclusive breastfeeding has been shown to be the optimal feeding practice to prevent iron deficiency anemia, especially in areas where iron-fortified foods are uncommon (Oski and Landaw, 1980). Not only is iron from other complementary foods not well absorbed during the first six months, it can interfere with the absorption of iron in human milk (Oski and Landaw, 1980). Additionally, the introduction of complementary foods may introduce pathogens that can potentially increase iron losses through increased morbidity or increased diarrheal episodes (Oski and Landaw, 1980; Saarinen and Siimes, 1979). Fresh cow's milk, for example, is often introduced in the first six months of life in developing countries and can increase iron loss by causing gastro-intestinal bleeding (Fomon *et al.*, 1981). As an infant grows, and his/her blood volume

expands, more iron is needed in the form of hemoglobin. Infants with greater weight gain are at an increased risk of iron deficiency (Wharf *et al.*, 1997; Michaelsen *et al.*, 1995). Low birth weight infants are at a higher risk of iron deficiency during the first six months of infancy because they begin life with lower iron stores and require more iron to sustain their faster rate of post-natal growth (Chaparro, 2008). The high prevalence of anemia in six-to-nine month olds raises the concern that birth iron stores in some infants are inadequate to sustain growth and development through the first six months of life (Chaparro, 2008). It does appear, however, that under optimal conditions, adequate iron status among infants can be maintained through at least six months of age (Dewey and Chaparro, 2007). In areas in which optimal conditions cannot be achieved, however, iron supplementation may be necessary to achieve appropriate iron levels in infants during their first six months of life (Dewey and Chaparro, 2007).

2.3.4. Consequences of fetal/infant anemia

Iron deficiency anemia during gestation and infancy can have severe adverse effects on neural development and behavioural outcomes. Iron is a critical nutrient for normal brain development (Lozoff and Georgieff, 2006). The structures of the brain can become abnormal due to iron deficiency *in utero* or in early postnatal life as iron is essential for proper neurogenesis and differentiation of certain brain cells and brain regions (Rao *et al.*, 2007; Ward *et al.*, 2007; Rao *et al.*, 2003). In the fetus, red blood cells receive priority for iron over other tissues, including the brain (Lozoff and Georgieff, 2006). When iron supply does not meet iron demand, as in the case of iron deficiency, the fetal brain may be at risk of developmental problems (Lozoff and Georgieff, 2006). During the first year of life, the brain develops rapidly and when morphological, biochemical and bioenergetic alterations occur, they can influence future functioning (Rao and Georgieff, 2007; Lozoff *et al.*, 2006). Furthermore, based on animal models, it has been proposed that iron deficiency impairs myelination, dendritogenesis, synaptogenesis, and neurotransmission as these processes are highly dependent on

iron-containing enzymes and hemoproteins (Chaparro and Lutter, 2007; Lozoff and Georgieff, 2006).

Iron deficiency during infancy is associated with poorer cognitive, motor, and/or social/emotional outcomes later in life (Lozoff and Georgieff, 2006). In children, the effect of iron deficiency on IQ has been estimated to be 1.73 points lower for each 10 g/L decrease in hemoglobin (Ezzati *et al.*, 2004). Behavioural effects of iron deficiency in infants include being more fearful, shy, wary, hesitant, unhappy, tense, exhibiting less pleasure, and tending to be more attached to their mothers during play (Beard, 2008; Grantham-McGregor and Ani, 2001). The developmental and behavioural effects of iron deficiency are interconnected. The iron-dependent effects on neural development have obvious effects on behavioural outcomes, and likewise, certain behavioural outcomes will affect neural development (Lozoff and Georgieff, 2006). What is particularly alarming regarding iron deficiency during infancy is the finding that many negative consequences are not reversed following iron therapy (Lozoff and Georgieff, 2006). The ability of iron therapy to reverse adverse effects depends both on the timing of therapy and the severity of the iron deficiency (Lozoff and Georgieff, 2006). The best strategy to prevent and/or reverse adverse effects is to give iron therapy earlier in development or before iron deficiency becomes severe or chronic (Lozoff and Georgieff, 2006). This highlights the importance of prevention of fetal and infant iron deficiency.

2.3.5. Preventing iron deficiency anemia

The optimal strategy to prevent iron deficiency in infancy is to scale up interventions with a history of evidence-based efficacy and effectiveness. There is an increasing need to translate research into cost-effective and sustainable programs. Four such interventions exist that are known to be effective in preventing iron deficiency during the first six months of life. These include iron supplementation to pregnant women, delayed umbilical cord clamping at birth, immediate exclusive breast-feeding within the first hour of birth and continued

exclusive breast-feeding for six months (Stoltzfus, 2008). If iron deficiency anemia persists, iron supplementation may be necessary. Particularly in low resource settings, a gap exists between discovery of efficacious interventions and large scale implementation (Darmstadt *et al.*, 2005). Researchers and policymakers need to collaborate, particularly in these settings, to attempt to bridge this gap so that optimal health care can be provided to at-risk populations.

2.4. UMBILICAL CORD CLAMPING

2.4.1. Active vs. physiological management of the third stage of labour

Umbilical cord clamping occurs during the third stage of labour. Two fundamental management options have been developed to guide practice for this stage of labour. The first is referred to as active management (also called routine or prophylactic management) (Weckert and Hancock, 2008) and involves prophylactic administration of an oxytocic drug, early umbilical cord clamping and cutting (immediately following the birth of the baby), and controlled cord traction to remove the placenta following signs of separation (Enkin *et al.*, 2000). The second management option is referred to as physiological (or expectant) management. This option relies on normal birth occurring without intervention, leaving the umbilical cord to finish pulsating and awaiting expulsion of the placenta by natural uterine contraction without the use of prophylactic oxytocin or cord traction (Enkin *et al.*, 2000). Active management became part of clinical practice in the 1960s, following documentation of its effect in decreasing the risk of maternal postpartum hemorrhage by 50% compared to physiological management (Duley *et al.*, 2009; Enkin *et al.*, 2000). Recently, however, it has come to light that the effects of the individual components of active management of labour have not been adequately studied. The effect of active management on the decrease in risk of post-partum hemorrhage is almost entirely due to the use of prophylactic oxytocic drugs and not a result of the three interventions combined, as was originally thought. Following this realization, the impact of the timing of umbilical cord clamping has undergone intensive scientific investigation.

2.4.2. Placental transfusion

The umbilical cord contains arteries that carry blood from the infant to the placenta and veins which carry blood from the placenta to the infant (Dewey and Chaparro, 2007). Following birth, blood continues to flow through arteries (from the infant to the placenta) for approximately 20-25 seconds and is negligible by 45 seconds (Yao and Lind, 1974). In the umbilical vein, however, blood continues to flow from the placenta to the infant for up to three minutes after delivery (Dewey and Chaparro, 2007). Placental transfusion is defined as the additional blood volume transferred from the placenta to the infant at birth through the vein of the umbilical cord (Duley *et al.*, 2009).

Placental transfusion is affected by three main factors: uterine contractions following delivery, placement of infant following delivery, and timing of umbilical cord clamping (Duley *et al.*, 2009). The uterine contraction that occurs naturally between one to three minutes following the birth contraction is thought to be responsible for placental transfusion following birth (Yao *et al.*, 1968). Administration of a prophylactic uterotonic drug will cause this uterine contraction to occur earlier, which will in turn cause transfusion to be completed earlier (Duley *et al.*, 2009). If such a drug is administered intravenously, the rapid contraction leads to an increased rate of transfusion; however, the overall blood volume transfused is not affected (Yao *et al.*, 1968). Administration intramuscularly (which is preferable (National Collaborating Centre for Women's and Children's Health, 2007)) following birth, on the other hand, is not likely to influence the rate of transfusion because it takes approximately 2.5 minutes to take effect and cause a contraction when administered intramuscularly (Embrey, 1961). Even if it is administered immediately following birth, by the time the contraction occurs, placental transfusion is nearly complete.

The placement of the infant following delivery also has a significant effect on placental transfusion (Dewey and Chaparro, 2007). This effect is due to gravity (Chaparro and Lutter, 2007). If the infant is placed at the level of the placenta or

within 10 cm above or below the placenta, complete placental transfusion is expected to take approximately three minutes (Chaparro and Lutter, 2007). If the infant is placed 50 cm above the level of the placenta, gravity will prevent placental transfusion by stopping blood flow through the umbilical vein (Yao and Lind, 1974). If the infant is placed below the level of the placenta, the rate of transfusion will increase, and transfusion can be completed within one to two minutes of delivery (Linderkamp, 1982).

Finally, the timing of umbilical cord clamping will affect the extent of placental transfusion. Obviously, placental transfusion cannot occur if the umbilical cord is clamped or cut, blocking or severing the link between the infant and the placenta. Delaying umbilical cord clamping until placental transfusion can be completed will maximize the blood volume transfused to the infant.

2.4.3. Timing of umbilical cord clamping

The timing of umbilical cord clamping will affect the amount of blood volume transferred to the infant via placental transfusion. The average term infant weighs approximately 3500 g at birth, and has a blood volume of 80-85 mL/kg which equates to approximately 290 mL of blood volume (Prendiville *et al.*, 2000). During placental transfusion, an additional 15-40 mL of blood volume per kg birthweight is transferred from the placenta to the infant through the umbilical cord. Therefore, waiting to clamp the umbilical cord and allowing placental transfusion to complete can increase a term neonate's blood volume by approximately 30-50% (Levy and Blickstein, 2006; Dewey and Chaparro, 2007). Approximately 25% of the transfer occurs in the first 15 – 30 seconds after birth, 50-78% of the transfer by one minute and the remaining by three minutes (Yao *et al.*, 1969).

Providing the neonate with additional blood volume by delaying umbilical cord clamping has many subsequent effects on child and maternal health outcomes. The results from two recently published meta-analyses performed to compare

early and delayed umbilical cord clamping on a variety of outcomes are presented in Table 2 (McDonald and Middleton, 2008; Hutton and Hassan, 2007).

Table 2: Description of outcomes of meta-analyses comparing early and delayed umbilical cord clamping[1]

First Author, Year	*# of studies; % RCT*	*Location of included studies*	*Definition of DCC/ECC*	*Positive neonatal outcomes of DCC*	*Negative neonatal outcomes of DCC*	*Outcomes not associated with DCC*
McDonald 2008	15 studies; 53% RCT	Canada, Germany, United Kingdom, Sweden, United States, Argentina, Libya, Egypt, Guatemala, India, Mexico	DCC = > 1 minute; ECC = < 1 minute	-Higher Hb levels at birth and up to 24 hours -Lower prevalence of anemia at 6 hours and 24-48 hours of life -Higher ferritin levels at 3 and 6 months	-More infants required phototherapy for jaundice -Lower levels of cord Hb -Lower rate of breastfeeding at 1 month	-APGAR score -Admission to special care baby nursery or neonatal intensive care unit -Respiratory distress -Clinical jaundice -Polycythemia -Hb levels at 2-4 and 6 months -Anemia at 4 months -Rate of breastfeeding at discharge or at 2-4 and 6 months -Maternal postpartum hemorrhage -Maternal mean blood loss -Maternal postpartum Hb levels -Maternal need for blood transfusion -Need for manual removal of placenta -Length of third stage of labour -Therapeutic uterotonics
Hutton 2007	11 studies; 100% RCT	Argentina, Mexico, Libya, India, United Kingdom, Canada, United States, Zambia, Unspecified for 2 studies	DCC = ≥ 2 minutes; ECC = Immediately after birth (in most trials)	-Higher Hct at 6, 24-48 hours, 5 days and 2 months -Higher Hb levels at 7 hours -Increased ferritin levels at 2-3 and 6 months -Lower prevalence of low ferritin (<50μg/L) -Increased blood volume at 2-4 hours of life -Decreased risk of anemia at 24-48 hours and at 2-3 months -Higher blood viscosity in first 2-4 hours and at 5 days	-Increased risk of polycythemia at 7 hours and 24-48 hours of life	-Hct levels at 6 months -Mean Hb levels at 2-3 and 6 months -Mean serum bilirubin levels in first 24 and 72 hours -Plasma viscosity at 24 hours and 5 days after birth -Proportion of infants with elevated bilirubin levels that require use of phototherapy -Neonatal jaundice in first 24-48 hours of life and at 3-14 days after birth -Risk of tachypnea -Risk of respiratory grunting -Admission to neonatal intensive care unit

[1] Abbreviations used throughout table: DCC = Delayed cord clamping; ECC = Early cord clamping; Hb = Hemoglobin; Hct = Hematocrit

In summary, the major benefit of delayed umbilical cord clamping was reported to be its association with a more favourable iron status of the infant at birth, which appears to be sustained over the long term. The only negative consequence is a potential increased risk of jaundice requiring phototherapy associated with delayed cord clamping. This warrants further investigation.

It should be noted that meta-analyses on this topic are difficult to conduct for three major reasons. First, there is significant variability in the definitions of early and delayed umbilical cord clamping across studies. The definition of early umbilical cord clamping varies between immediately following delivery up to the first minute following delivery, and the definition of delayed umbilical cord clamping varies from greater than 30 seconds to up to 10 minutes. Second, the starting point for timing the duration between delivery and clamping varies widely among studies. Some studies use head crowning as the start point, others use delivery of shoulders, and some do not specify a start point. These factors make interpretation and comparison of results among studies difficult. Lastly, the large variability in specific outcomes ascertained creates difficulties in performing a meta-analysis. Although many studies have documented hematological effects, the same specific indicator (eg. hematocrit, hemoglobin, ferritin) may not always be reported.

2.4.4. Hematological effect of timing of umbilical cord clamping

The greatest benefit of delayed umbilical cord clamping is its association with an improved hematological status in the newborn when compared to early umbilical cord clamping. Allowing placental transfusion to complete can provide the infant with an additional 30-50 mg of iron which will increase iron reserves and decrease the risk for iron deficiency anemia later in infancy (Weckert and Hancock, 2008; Levy and Blickstein, 2006). Several studies have recently investigated the effect of delayed umbilical cord clamping on infant hematological outcomes. A summary of the randomized controlled trials (RCTs) conducted on this topic can be found in Table 3.

Table 3: Characteristics of randomized controlled trials comparing hematological outcomes between early and delayed umbilical cord clamping[1]

First Author, Year	*Study Location*	*Study Population*	*Sample Size*	*Definition of randomized intervention*	*Loss to Follow-up[2]*	*Hematological Outcome(s)*		*Blinding*	*Significant Measure of Effect(s)*	*Major Limitations*
						Type	*Time point*			
Strauss 2008	United States	Preterm infants (30-36 weeks)	105	ECC: 2 - 5 sec.; DCC: 60 sec.; T_0 not specified	0%	Serial Hct	< 24 hours of birth; between 48-72 hours after birth; day 7, 14, 21, 28	Laboratory staff blinded	Hct significantly different between groups at day 7 (p= 0.005); day 14 (p<0.0001); day 21 (p<0.0001); day 28 (p<0.0001)	Measure of Hct was a secondary outcome and study was not sufficiently powered
Jahazi 2008	Iran	Women with uncomplicated vaginal pregnancies between 38-42 weeks gestation	64	ECC: 30 sec.; DCC: 3 min.; T_0 = complete delivery of infant	0%	Cord blood Hct; neonatal Hct	Cord blood: after clamping; Neonatal blood: at 2 and 18 hours of life	Double-blind	No significant effect found	Did not control for maternal/ neonatal characteristics that differed between intervention groups
Ultee 2008	Not specified	Infants with gestational age between 34 - 37 weeks delivered vaginally to Caucasian women	34 (at 10 weeks); 37 (at 1 hour)	ECC: ≤ 30 sec.; DCC: >180 sec.; T_0 not specified	7.3%	Hb, ferritin	Hb: 1 hour after birth; Hb & Ferritin: 10 weeks	Outcome accessor blinded	Significantly higher Hb in DCC than ECC group at 1hr and 10 weeks (p<0.05)	10 week analysis underpowered
Kugelman 2007	Israel	Premature infants (gestational age between 24 - 34.9 weeks)	65	ECC: <10 sec.; DCC: 30-45 sec.; T_0 = delivery of infant	0%	Venous Hct	At admission; During first 24 hours in NICU	Not clearly stated	Vaginal deliveries: Hct higher in DCC group at admission (p = 0.03) and at 24 hours (p = 0.03) - adjusted for birth weight and gestational age	Under-powered; only 65 of eligible 151 participated - possible volunteer bias

van Rheenen 2007	Zambia	Women delivering full-term	91 (1 day post-partum); 84 (2 months); 78 (4 months); 72 (6 months)	ECC: ≤ 20 sec.; DCC: > cord stopped pulsating; T_0 > delivery	21%	Hb from finger-prick	2,4,6 months	Partially blinded	Decrease in Hb levels between blood sampled at 4 months and cord blood was smaller in DCC group: Difference = 1.1g/dL (95% CI: 0.2 - 2.1)	If infant Hb<7g/dL at follow-up, they were given iron supplements for 2 months
Chaparro 2006	Mexico	Women at full-term, not admitted at advanced stage labour, who didn't plan to deliver by c-section	358	ECC: 10 sec.; DCC: 2 min.; T_0 = delivery of the infant's shoulders	25%	Venous Hb, Hct and ferritin	6 months	Outcome accessors/ statistician blinded	At 6 months, mean ferritin, stored iron, and body iron significantly higher in DCC group than ECC group; Significant interaction effects between intervention group and maternal ferritin, infant birth weight, and infant feeding practices	Randomized protocol broken for 53 participants
Ceriani 2006	Argentina	Women with uneventful vaginal or cesarean section delivery with singleton pregnancy at term	272	ECC: ≤ 15 sec.; DCC: 1) 1 min., 2) 3 min.; T_0 not specified	1.4%	Venous Hct	6 hours after birth; 24-48 hours after birth	Outcome accessors blinded	At 6 hours: prevalence of anemia significantly higher in ECC (8.9%) vs. 1 min.(1%) and in ECC vs. 3 min.(0%); At 24-48 hours: Prevalence of anemia significantly higher in ECC (16.9%) vs. 1 min.(2.3%) and in ECC vs. 3 min.(3.3%)	No long term follow-up

Emhamed 2004	Libya	Women whose infants birth weight was ≥ 2500g, at term, singleton	102	ECC: ≤ 10 sec.; DCC: >cord stopped pulsating; T_0 = complete expulsion of the infant	2%	Cord Hb and Hct; Venous Hct	Cord blood: after clamping; Venous blood: 16-24 hours after birth (at discharge)	Not mentioned	At 24 hours: Hct levels significantly higher in DCC group (p = 0.0037), Hb levels significantly higher in DCC group (p=0.0005)	Univariate stats only with maternal hemoglobin levels and proportion anemic significantly different between intervention groups
Gupta 2002	India	Hospital term infants born vaginally to pregnant women with Hb <100 g/L at time of delivery	58	ECC: immediately; DCC: after placenta had descended into vagina; T_0 not specified	43%	Cord ferritin/Hb; Venous ferritin/Hb	Cord blood: at birth; Venous blood: at 3 months	Not mentioned	At 3 months, significantly higher Hb (p<0.001) and ferritin (p=0.02) in DCC group; At 3 months, risk of anemia significantly lower in DCC (OR = 7.7), and risk of low iron stores significantly lower in DCC (OR = 10.67)	Underpowered ; High loss to follow-up; Potential volunteer bias
Rabe 2000	Not specified	Single preterm infants of <33 weeks gestation	39	ECC: 20 sec.; DCC: 45 sec.; T_0 not specified	Not clearly stated	Require-ment of donor PRCT	During first 6 weeks of life	Unclear	Mean numbers of PRCT greater in ECC than DCC (2.4 vs. 1.2, p<0.05)	Small sample size; outcome is a proxy measure for anemia

Grajeda 1997	Guatemala	Women delivering vaginally in hospital, infant birth weight ≥ 2000g, gestational age ≥ 37 weeks, singleton birth	69	ECC: immediately; DCC: 1) cord stopped pulsating with infant at level of the placenta; 2) cord stopped pulsating with infant below level of placenta; T_0 = head crowning / appearance of shoulders	22%	Venous Hct; Venous Hb; Serum ferritin	24 hours in a subsample (last 41 subjects recruited) for Hct; At 2 months for all subjects for Hct, Hb, ferritin	Follow-up staff and laboratory personnel blinded	2 months: both DCC groups had higher Hct compared to ECC (p = 0.001 for both), significant difference in Hb levels between group 1 (ECC) and group 2 (infant placed at level of placenta with DCC)(p=0.03)	Possible volunteer bias: Those lost to follow-up had a significantly lower mean birthweight than infants who completed study; Quazi-randomized (by day of week)
Geethanath 1997	India	Hospital-born term infants born vaginally to women with uncomplicated pregnancies with Hb>10g/dL.	107	ECC: immediately; DCC: after placenta had descended into vagina; T_0 not specified	0%	Cord ferritin/ Hb; Serum ferritin, Hb	Cord blood: after birth; Venous blood: 3 months	Not mentioned	No significant effect found	Insufficient details to assess

[1] Acronyms used in table: Hb = hemoglobin; ECC = Early cord clamping; DCC = Delayed cord clamping; T_0 = Point at which timing began; sec. = seconds; min. = minutes; Hct = Hematocrit; Hb = Hemoglobin; PRCT = Packed red cell transfusion; NICU = Neonatal intensive-care unit

[2] Percentage that did not complete the study

Of the 12 RCTs presented, 10 documented at least one positive effect on the hematological status of the infant following delayed umbilical cord clamping compared to early umbilical cord clamping (Strauss *et al.*, 2008; Ultee *et al.*, 2008; Kugelman *et al.*, 2007; van Rheenen *et al.*, 2007; Ceriani Cernadas *et al.*, 2006; Chaparro *et al.*, 2006; Emhamed *et al.*, 2004; Gupta and Ramji, 2002; Rabe *et al.*, 2000; Grajeda *et al.*, 1997). The remaining two trials failed to show a statistically significant effect (Jahazi *et al.*, 2008; Geethanath *et al.*, 1997). The effect of delayed umbilical cord clamping on infant iron status had been documented both in babies born to term (van Rheenen *et al.*, 2007; Ceriani Cernadas *et al.*, 2006; Chaparro *et al.*, 2006; Emhamed *et al.*, 2004; Gupta and Ramji, 2002), as well as in preterm babies (Strauss *et al.*, 2008; Ultee *et al.*, 2008; Kugelman *et al.*, 2007; Rabe *et al.*, 2000; Grajeda *et al.*, 1997). Interestingly, possible modification of the documented effect has been proposed for maternal iron status, infant birth weight and infant feeding practices (Chaparro *et al.*, 2006). Chaparro *et al.* found that delayed umbilical cord clamping had a greater effect when infants were born to mothers with low ferritin levels compared to mothers with normal ferritin levels; in infants with birth weights between 2500 – 3000 g compared to infants with birth weights >3000 g; in infants still breastfeeding at six months compared to infants no longer breastfeeding at six months; and in infants receiving iron-fortified formula or milk at six months compared to those not receiving such products. Additionally, Gupta and Ramji's (2002) study which only included anemic women, found that the odds of developing infant anemia (Hb < 110g/L) was 7.7 times higher in infants born with early cord clamping compared to infants born with delayed cord clamping (OR = 7.7; 95% CI: 1.84 – 34.9). Because this odds ratio is large, they concluded that infants born to anemic mothers may benefit more from delayed umbilical cord clamping than those born to non-anemic mothers (Gupta and Ramji, 2002). Unfortunately, no non-anemic comparison group was included in the Gupta and Ramji (2002) study. Interaction effects influencing the timing of umbilical cord clamping require further investigation.

The most common limitations that occurred among these trials were small sample sizes leading to underpowered analyses, volunteer and loss to follow-up biases, and a lack of multivariate analyses to control for relevant confounders that were not equally distributed between the intervention groups (eg. maternal hematological status).

Only one of the trials had been performed in South America (Ceriani Cernadas *et al.*, 2006), and no studies on this topic have been performed in Peru. More local evidence is required to guide policymakers in Peru and South America.

2.5. RECOMMENDATIONS FOR PRACTICE

Following the recent body of literature documenting the hematological benefits of delayed umbilical cord clamping, both the World Health Organization (WHO) and the Pan American Health Organization (PAHO) have published new recommendations regarding the timing of cord clamping recommending delayed cord clamping (Chaparro and Lutter, 2007; Mathai *et al.*, 2007).

2.5.1. WHO recommendations

In a document entitled "WHO Recommendations for the Prevention of Postpartum Haemorrhage", published in 2007, a formal recommendation was made with regard to the timing of umbilical cord clamping: "Because of the benefits to the baby, the cord should not be clamped earlier than is necessary for applying cord traction in the active management of the third stage of labour. For the sake of clarity, it is estimated that this will normally take around 3 minutes. Early clamping may be required if the baby is asphyxiated and requires immediate resuscitation." (Mathai *et al.*, 2007) This recommendation was graded as "a weak recommendation with low quality evidence" (Mathai *et al.*, 2007) highlighting the need for more rigorous research.

2.5.2. PAHO recommendations

In 2007, PAHO also published a document entitled "Beyond Survival: Integrated delivery care practices for long-term maternal and infant nutrition, health and development" that formally supported the practice of delayed cord clamping: "The optimal time to clamp the umbilical cord for all infants regardless of gestational age or fetal weight is when the circulation in the cord has ceased, and the cord is flat and pulseless (approximately 3 minutes or more after birth). After cord pulsations have ceased (approximately 3 minutes after delivery), clamp and cut the cord following strict hygienic techniques… In the majority of cases, resuscitation can be performed simultaneously with delayed cord clamping." (Chaparro and Lutter, 2007) A detailed history of the timing of cord clamping and a summary of the evidence to support this recommendation was also provided in this PAHO document.

2.6. IMPLEMENTATION OF RECOMMENDATIONS

Following the publication of formal recommendations, the next step is the implementation of new guidelines in clinical practice. Bridging the "know-do" gap, which signifies putting evidence-based research into practice, is a recent area of scientific study. In order to successfully implement a new policy on delayed umbilical cord clamping in hospitals, it is first important to understand current practices with regard to the timing of cord clamping, the attitudes of health professionals in terms of accepting the new policy, and the effectiveness of the policy in a "real life setting" (ie. not in an experimental setting such as an RCT). With regard to delayed umbilical cord clamping, this is an intervention that now requires rigorous evaluation of implemented strategies in order to successfully put research into action.

2.6.1. Current practices of timing of umbilical cord clamping

One recent study attempted to describe current practices of the timing of umbilical cord clamping across Europe (Winter *et al.*, 2007). This study concluded that 66-90% of maternity units in Belgium, France, Italy, the Netherlands, Portugal, Spain, Switzerland, and the UK had policies of clamping and cutting the cord

immediately after birth (Winter *et al.*, 2007). Conversely, 65-74% of maternity units in Austria, Denmark, Finland, Hungary, and Norway had policies of waiting until the cord stopped pulsating (Winter *et al.*, 2007). When 303 certified American nurse-midwives were randomly sampled from all active members of the American College of Nurse-Midwives and asked to complete a questionnaire, 21% reported that they clamp the cord immediately, 5% clamp before one minute, 35.7% clamp between one to three minutes, 3.8% clamp after three minutes, and 29.3% clamp after cord pulsations have ceased (Mercer *et al.*, 2000). There did not appear to be any demographic determinants of timing of cord clamping and many respondents implied that practice was dictated by institutional policies, independent of their own beliefs (Mercer *et al.*, 2000). The high degree of variability in the timing of cord clamping and the even distribution between early and delayed cord clamping found in both of these studies is thought to reflect an inadequate scientific knowledge base among obstetric staff (Mercer *et al.*, 2000).

2.6.2. Attitudes of health professionals towards delayed cord clamping

A questionnaire-based study published in 2009 attempted to investigate the attitudes of obstetricians towards delayed cord clamping (Ononeze and Hutchon, 2009). They concluded that only 9.3% of obstetricians sampled from hospitals across the British Isles, other EU countries, USA, Canada, Australia, etc. always adhere to recommendations on delayed umbilical cord clamping (Ononeze and Hutchon, 2009). Reasons for non-adherence include difficulty implementing delayed cord clamping in clinical practice and being unaware of the scientific evidence (Ononeze and Hutchon, 2009). It is unclear whether this study included obstetricians from developing countries; therefore, it is uncertain whether these results can be applied in developing country settings.

2.6.3. Effectiveness of delayed cord clamping

There is little direct published evidence on the implementation of delayed umbilical cord clamping. The few studies that have been published show that there is a definite need for action to make policy makers more aware of the

current evidence and that it be disseminated more readily to hospital workers (Ononeze and Hutchon, 2009; Winter *et al.*, 2007; Mercer *et al.*, 2000). To date, there have been no studies that have documented the effectiveness of changing hospital policy regarding the timing of cord clamping. This type of research would not only guide policymakers with regard to the most efficient strategy for implementation and the feasibility of adopting a new hospital policy but may also provide information regarding its effectiveness in decreasing infant anemia.

3. Study Objectives

3.1. OVERALL OBJECTIVE

The overall objective is to reduce anemia in infants.

3.2. PRIMARY OBJECTIVE

The primary objective is to determine the effectiveness of a two-component intervention aimed at changing hospital policy from early to delayed umbilical cord clamping in decreasing infant anemia (at four months of age).

3.3. SECONDARY OBJECTIVES

There are five secondary objectives:

3.3.1. – To determine the effectiveness of a two-component intervention in changing hospital practice from early to delayed umbilical cord clamping.

3.3.2. – To determine the effectiveness of a two-component intervention aimed at changing hospital policy from early to delayed umbilical cord clamping in increasing infant hemoglobin levels (at four months of age).

3.3.3. – To determine the effect of time-to-clamp the umbilical cord on infant anemia (at four months of age).

3.3.4. – To determine the effect of time-to-clamp the umbilical cord on infant hemoglobin levels (at four months of age).

3.3.5. – To determine the effect of maternal anemia on the association between time-to-clamp and infant anemia (at four months of age).

4. Study Methodology

4.1. STUDY LOCATION

This study was conducted in Iquitos, Peru, capital of the Loreto region, which is the largest administrative region in the Peruvian Amazon. Iquitos can only be accessed by air or water, as there are no roads connecting it to the rest of Peru. In 2007, the population of Iquitos was estimated at 406,000 inhabitants (INEI, 2008). More than half of the population of Loreto lives in poverty and previous research has found that up to 70% of 6-month-old infants born in this area are anemic (unpublished data). In 2005, a multidisciplinary workshop was held to identify the top ten health priority problems in this area. Infant malnutrition, adolescent pregnancy, diarrhea, anemia and parasites were ranked as the top five health priority problems, respectively (Casapia *et al.*, 2007).

The host hospital for this study was Hospital Iquitos "César Garayar García" (commonly referred to as the Hospital Apoyo Iquitos) which is one of only two public hospitals in Iquitos with a Department of Obstetrics and Gynecology. The catchment area for this hospital includes Iquitos as well as surrounding communities, including Belen, Punchana and San Juan.

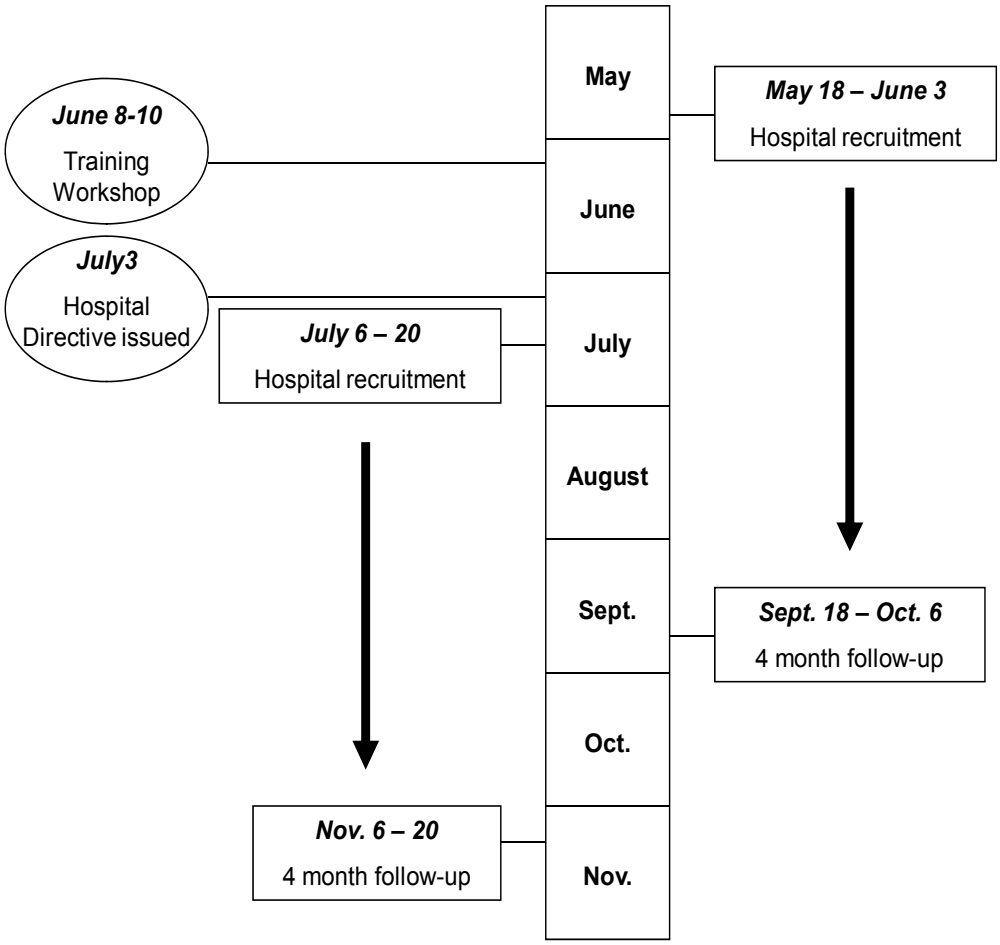
4.2. STUDY POPULATION

The study population was mother-infant pairs. All women who arrived at Hospital Apoyo Iquitos in labour between May 18 – June 3, 2009 and July 6 – July 20, 2009 were approached to participate in the study. Inclusion criteria included: not scheduled for a cesarean section, planned to be in the area when their baby turned 4 months old, and gave consent prior to entering the delivery room. Participants were excluded if they had an emergency cesarean section, their baby was stillborn, born with a tight nuchal cord that could not be unwrapped from the neck, born with any congenital abnormalities, or if they were transferred to another hospital before being discharged.

4.3. STUDY DESIGN

A pre/post study design was used to document the effectiveness of a two-component intervention aimed at changing hospital policy from early to delayed umbilical cord clamping in the Department of Obstetrics and Gynecology of Hospital Apoyo Iquitos (Figure 1).

Figure 1: Study timeline (May – November, 2009) showing the two recruitment and follow-up time periods and the dates corresponding to the two-component intervention.



4.3.1. Intervention

The two-component intervention consisted of: 1) A 3-day training workshop entitled "Estandarizacion de Habilidades Clinicas Basicas en Salud Materno-Neonatal [Standardization of basic clinical skills in maternal and neonatal health]" and 2) A written Hospital Directive sent to all nurse-midwives who worked in the hospital.

The training workshop took place from June 8 – June 10, 2009 with the purpose of training hospital personnel on delayed umbilical cord clamping. It was run by two Peruvian Ministry of Health (MINSA) trained health professionals from Lima (one Nurse-Midwife and one Obstetrician) and the Director of the Instituto de Investigación Nutricional, also from Lima. MINSA was charged with adapting the basic birthing standards to include and highlight delayed umbilical cord clamping (to reflect current PAHO recommendations). The first two days of the workshop consisted of "in-class" theoretical training on the techniques of normal birthing with emphasis placed on delayed cord clamping. The third day of the workshop was "hands-on" practice of techniques learned in the previous two days and took place in the labour/delivery room of Hospital Apoyo Iquitos. All nurse-midwives employed at the hospital were invited to attend this cost-free workshop.

The Hospital Directive was signed by the head of the Department of Obstetrics and Gynecology of the Hospital Apoyo Iquitos, Dr. Eder Aguilar, and was issued on July 3, 2009 to all nurse-midwives employed by the hospital. This document stated the new hospital policy with regard to the timing of umbilical cord clamping: "A partir de la fecha se recomienda que el clampaje del cordón umbilical en el Recién Nacido que no necesita atención inmediata en Neonatologia sera, entre los 2 y 3 minutos de nacido (cuando el cordon déjà de latir) de acuerdo a las recomendaciones de la OPS. [From this date forwards, I recommend that the clamping of the umbilical cord for newborns not needing immediate neonatal attention will be between 2 to 3 minutes after birth (when the cord stops pulsating), according to PAHO recommendations.]"

4.3.2. Recruitment

Recruitment of study participants took place in the labour room of Hospital Apoyo Iquitos between May 18 – June 3, 2009 (pre-intervention) and July 6 – July 20, 2009 (post-intervention). Three nurse-midwives (not employed by the hospital) were hired and trained to recruit the study participants and to collect the data for this study. They worked on a rotating shift basis of 8 hours each, such that at least one study nurse-midwife was present in the hospital at all times (24 hours/day). This approach ensured that all eligible women would be invited to participate in the study. Women were approached for participation when they entered the labour room. Generally, this occurred when they were approximately three centimetres dilated. The consent form was read to them, their questions were answered and, if they agreed to participate, their informed consent was obtained by signature or fingerprint. If a woman was less than 18 years of age, with her assent, an adult parent/guardian/spouse was asked to provide the written consent on her behalf (Appendix 1).

4.3.3. Hospital data collection: Procedures and independent variables

Following informed consent, a short questionnaire was administered to the participant by one of the three study nurse-midwives to obtain information on date of birth, marital status, residential address, education, occupation, attendance at antenatal care clinics, and medication and supplements taken during pregnancy (Appendix 2). Maternal hemoglobin levels were measured from finger-prick blood using a HemoCue® machine. Information on gestational age, date of last menstrual period, antenatal care, number of previous pregnancies, number of previous live births, abortions, stillbirths, pre-term deliveries and any medical conditions were collected from the participants' personal health record. Data on selected variables were verified using hospital registries and patient medical records. Additional information on current medication and any diagnoses and/or treatments during labour or delivery was obtained from participants' medical records. The woman was then given an appointment for a follow-up visit four months following the imminent birth of her baby.

When a study participant entered the delivery room (at approximately 9 centimetres dilated), the study nurse-midwife observed the delivery and using a digital stopwatch, recorded two time-points: 1) The time between the delivery of the first shoulder and the delivery of the entire body and 2) The time between the delivery of the first shoulder and clamping of the umbilical cord (Appendix 3). The stopwatch was started at the delivery of the first shoulder. When the entire baby was delivered, the time was recorded (to the nearest second) and when the umbilical cord was clamped, the stopwatch was stopped and time was recorded. The following information was also noted: date of delivery, time of entry into the delivery room, hospital personnel who delivered the baby, hospital personnel who clamped the umbilical cord, whether the umbilical cord had stopped pulsating before it was clamped, what was used to stop the blood flow in the umbilical cord, whether intramuscular oxytocin was administered, and whether the baby was born with any medical problems or complications. The following information was obtained from the hospital registry following the birth of the baby: weight, length and sex of the baby, APGAR score at 5 minutes, time of birth, date and time of discharge of the baby and any medical condition before or at discharge of the baby. The color of the amniotic fluid and if the baby was born with the umbilical cord wrapped around its neck were routinely reported in the comments section of the delivery room questionnaire.

The exposure variable for objective 3.2 (determining the effect of the intervention on infant anemia) was intervention status (whether the woman was recruited pre- or post- intervention). The exposure variable for objectives 3.3.3, 3.3.4 and 3.3.5 (determining the effect of time-to-clamp on infant anemia and infant hemoglobin levels and determining the role of maternal anemia on the association between time-to-clamp and infant anemia) was time-to-clamp.

4.3.4. Hospital data collection: Dependent variable

For objective 3.3.1 (determining the effect of the intervention on time-to-clamp), time-to-clamp, on a continuous scale, as measured in the labour room was the outcome.

4.3.5. Home data collection: Follow-up visit procedures and dependent variables

At approximately two to three weeks following delivery in the hospital, study nurse-midwives visited the participants and their babies in their homes. These visits took place between June 5-June 15, 2009 for the pre-intervention group and July 21-July 28, 2009 for the post-intervention group. The purpose of this visit was to locate and confirm addresses and to remind participants of the upcoming 4-month follow-up visit to minimize loss-to-follow-up and to ensure time efficiency during the visits. At this time, the study nurse-midwives reinforced one of the nutritional messages that are promoted in the communities: "Exclusive breastfeeding for infants up to 6 months of age" (Appendix 4).

Mother-infant pairs were followed-up for the final time within 1-2 days of each infant's 4-month birthday. These visits took place between September 18 – October 6, 2009 for the pre-intervention participants and November 6 – November 20, 2009 for the post-intervention participants (Figure 1). The same three nurse-midwives who undertook the recruitment and measurements/observations in the hospital performed these home-visits. At these visits, the study nurse-midwife administered a brief questionnaire to obtain information on: presence of any infant medical conditions, infant's health status in the previous two weeks (with regard to cough, difficulty breathing, diarrhea, fever and ear problems (as recommended by the Integrated Management of Childhood Illnesses guidelines of WHO (WHO, 2008b))), medication given to the infant since birth, whether the infant had been treated for jaundice, infant's feeding practices, and maternal iron supplementation after delivery (Appendix 5). The infant was then weighed using either a SECA digital scale (Model 354; Seca corp,

Baltimore, MD, USA) or a hanging clock scale (provided by UNICEF), and length was measured using a standard length board (provided by MINSA). Finally, maternal and infant hemoglobin levels were measured from finger-prick (maternal or infant) or from heel-prick (infant) blood, using a Hemocue® machine.

The outcome variable for objectives 3.2, 3.3.3 and 3.3.5 (determining the effect of the intervention and time-to-clamp on infant anemia and determining the role of maternal anemia on the association between time-to-clamp and infant anemia) was infant anemia at four months of age. Infant anemia was defined as having hemoglobin levels less than 110 mg/dL. Infant hemoglobin level, on a continuous scale, was the outcome variable for objectives 3.3.2 and 3.3.4 (determining the effect of the intervention and time-to-clamp on infant hemoglobin levels).

4.4. SAMPLE SIZE

The sample size estimation was based on an inference for proportions, comparing two independent samples. An expected difference of 20% in the prevalence of infant anemia following policy change was considered clinically relevant. Using a 2-sided chi-square test with a level of significance of 0.05, and 80% power, the sample size was calculated to be 93. Taking an estimated 20% attrition rate into account, the final sample size was then re-calculated to be 112 in each group (pre-intervention and post-intervention), for a total of 224 mother-infant pairs.

4.5. STATISTICAL ANALYSES

All statistical analyses were done in R version 2.6.1.

4.5.1. Descriptive statistics

Descriptive statistics (means, medians, quartiles, minimums and maximums) were calculated for all variables. For continuous variables, standard deviations were calculated. Descriptive statistics were calculated at baseline for all 224 participants, and then separately for those who completed the 4-month follow-up

and for those lost to follow-up, to determine comparability. Student t-tests (for continuous variables) and chi-square tests (for dichotomous variables) were used to compare demographic information between those lost to follow-up and those who completed the study, and to compare pre- and post-intervention groups.

Histograms and density plots were used to explore the distribution of the time-to-clamp variable and the infant hemoglobin variable, both for the entire population, and by intervention status.

Based on the literature, twelve variables were identified *a priori* as potentially being associated with the primary outcome (infant anemia). These included: number of years of education of the mother, whether the infant had been exclusively breastfed to four months of age, whether the mother was employed, maternal hemoglobin levels at delivery, maternal anemia at delivery, infant birth weight, infant gestational age, infant sex, infant weight gain, whether the infant had had a fever in the two weeks prior to hemoglobin determination, whether the infant had had diarrhea two weeks prior to hemoglobin determination, and whether the infant had had cough or difficulty breathing two weeks prior to hemoglobin determination. Correlation between the exposure and outcome variables and all mentioned covariates was explored by plotting each pair of variables and calculating their correlation coefficients.

4.5.2. Effect of intervention on cord clamping time

The effect of the intervention on time-to-clamp the umbilical cord was analyzed using simple and multivariate linear regression with time-to-clamp as the continuous dependent variable and intervention status as the primary independent variable. This was done with all participants for whom there were complete hospital data. Variables were included in the multivariate analysis if they were known to be associated with the exposure or outcome (on univariate analysis), and had sufficient variability.

4.5.3. Effect of intervention on infant anemia at 4 months of age

The effect of the intervention on infant anemia status at four months of age was analyzed using simple and multivariate logistic regression with infant anemia as the dichotomous dependent variable and intervention status as the primary independent variable. All participants who completed the 4-month follow-up visit were included in this analysis. Simple logistic regression was used to quantify the effect of the twelve identified potential confounders on infant anemia status. Variables were chosen to be included in the multivariate analysis if they were associated with the outcome (statistically significant association in the univariate logistic regression analysis or highly correlated to infant anemia) or were known determinants of the outcome in the literature, and had sufficient variability.

4.5.4. Effect of intervention on infant hemoglobin levels at 4 months of age

The effect of the intervention on infant hemoglobin levels at four months of age was analyzed using simple and multivariate linear regression with infant hemoglobin as the continuous dependent variable and intervention status as the primary independent variable for all participants who completed the follow-up visit. Simple linear regression was used to determine the effect of the twelve identified potential confounders on infant hemoglobin levels. Variables were chosen to be included in the multivariate analysis if they were associated with the outcome (statistically significant in the univariate linear regression or highly correlated to infant hemoglobin levels) or were known determinants of the outcome in the literature, and had sufficient variability.

4.5.5. Effect of timing of cord clamping on infant anemia at 4 months of age

The effect of time-to-clamp the umbilical cord on infant anemia status at four months of age was analyzed using simple and multivariate logistic regression with infant anemia as the dichotomous dependent variable and time-to-clamp as the primary independent variable. The cohort of participants who completed the follow-up visit was used for this analysis. Simple logistic regression was used to quantify the effect of the twelve identified potential confounders on infant anemia

status. Variables were chosen to be included in the multivariate analysis if they were associated with the outcome (statistically significant association in the univariate logistic regression or highly correlated to infant anemia), or were known determinants of the outcome in the literature, and had sufficient variability.

4.5.6. Effect of timing of cord clamping on infant hemoglobin levels at 4 months of age

The effect of time-to-clamp the umbilical cord on infant hemoglobin levels at four months of age was determined using simple and multivariate linear regression with infant hemoglobin as the continuous dependent variable and time-to-clamp as the primary independent variable for the cohort of participants who completed the follow-up visit. Variables were chosen to be included in the multivariate analysis if they were associated with the outcome (statistically significant in the univariate linear regression or highly correlated to infant hemoglobin levels), and had sufficient variability.

4.5.7. Investigation of effect modification

The variables maternal anemia, infant sex, whether the infant had been exclusively breastfed, and if the infant was born pre-term, had been identified in the literature as potential effect modifiers of the association between time-to-clamp and infant iron status (anemia and hemoglobin level). Therefore, their impact was investigated in two ways: 1) By stratifying the data by these variables; and 2) By creating an interaction term between time-to-clamp and the potential effect modifiers and including these interaction terms in the two models (effect of time-to-clamp on infant anemia and effect of time-to-clamp on infant hemoglobin levels). When an interaction term was included in the regression model, each component of the interaction term was also included. The distribution of the time-to-clamp variable was centered by subtracting the mean from each observation before being included in the interaction term to reduce collinearity.

4.5.8. Attributable risk

The adjusted attributable risk of time-to-clamp on infant anemia was calculated using the method proposed by Greenland (Greenland and Drescher, 1993). The multivariate regression model used to determine the effect of time-to-clamp on infant anemia was used to predict the number of cases of anemia that would occur if time-to-clamp was first set to one minute and then to three minutes for all participants. Confidence intervals were estimated by computing 1,000 bootstrap replicates using the 'boot' package of the R software.

4.6. ETHICS APPROVALS

All appropriate ethics reviews and approvals were obtained before beginning this study. Initial McGill University Health Centre ethics approval was obtained on January 22, 2009 and full approval obtained on March 17, 2009. Approval from Hospital Iquitos "César Garayar García" was obtained on April 6, 2009. Ethics approval for the recruitment of pre-intervention participants was obtained from the Instituto de Investigación Nutricional on March 30, 2009 and approval for the intervention and recruitment of post-intervention participants was received on May 27, 2009. Additionally, a letter of support for this project was obtained from the Peruvian Ministry of Health on June 26, 2009.

5. Study Results

5.1. PREFACE

The results of this thesis are presented in two manuscripts (Sections 5.2 and 5.4) and one chapter section (Section 5.3). The manuscripts include:

> Brittany Blouin, Martin Casapia, Eder Aguilar, Hermánn Silva, Mary E. Penny, Hilary Creed Kanashiro, Serene A. Joseph, Mathieu Maheu-Giroux, Theresa W. Gyorkos. Delaying cord clamping: the effect of a two-component intervention to change practice in the Peruvian Amazon. Submitted to *Health Policy and Planning*

> Brittany Blouin, Martin Casapia, Eder Aguilar, Hermánn Silva, Mary E. Penny, Hilary Creed Kanashiro, Serene A. Joseph, Mathieu Maheu-Giroux, Elham Rahme, Anita Gagnon, Theresa W. Gyorkos. The effect of maternal anemia on the association between timing of umbilical cord clamping and infant anemia: observations of hospital practice in the Peruvian Amazon. Submitted to *Pediatrics*

The first manuscript (Section 5.2) addresses objective 3.3.1, the effect of the intervention on changing hospital practice from early to delayed cord clamping. Following, in Section 5.3, objectives 3.2 and 3.3.2 are addressed. This includes the effect of the intervention on infant anemia status as well as the effect of the intervention on infant hemoglobin levels. Finally, the last manuscript, presented in Section 5.4, primarily deals with objective 3.3.5 and also addresses objectives 3.3.3 and 3.3.4. The focus of this manuscript is the association between time-to-clamp and infant anemia and the role of maternal anemia in this association.

Submitted to *Health Policy and Planning*

5.2. MANUSCRIPT 1: DELAYING CORD CLAMPING: THE EFFECT OF A TWO-COMPONENT INTERVENTION TO CHANGE PRACTICE IN THE PERUVIAN AMAZON

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Keywords: Obstetrics, Iron deficiency, Child health, Research to policy, Developing countries

Abbreviated Running Title: Changing practice on umbilical cord clamping

Key Messages: 1- Key involvement of MINSA and hospital administration; 2- Intervention included training and written Hospital Directive; 3- Pre/post study design demonstrates clear, timely results

Word Count: 3,017

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ABSTRACT

Background: Delayed umbilical cord clamping has recently been proven effective in decreasing the risk of infant anemia in the first six months of life. While recommendations from the Pan American Health Organization to wait three minutes before clamping the umbilical cord have been published, this intervention has not yet been implemented in hospital settings in Peru.

Objective: To investigate the effect of a two-component intervention on change in hospital practice with regard to the timing of umbilical cord clamping.

Methods: A pre/post study design was used to measure the effect of a two-component intervention on mean time-to-clamp the umbilical cord. The intervention consisted of 1) a 'Best Practice' three-day training workshop on birthing and 2) a Hospital Directive. A total of 224 women were recruited from the labour room of Hospital Iquitos (Iquitos, Peru): 112 women were recruited pre-intervention, from May 18-June 3, 2009 and 112 women were recruited post-intervention, from July 6-20, 2009. All deliveries were observed and the time between delivery of the first shoulder and clamping of the umbilical cord was measured with a digital stopwatch.

Results: The mean time between delivery and cord clamping before the intervention was 56.8 seconds (95% CI: 51.0, 62.7). This increased to 169.8 seconds (95% CI: 153.8, 185.8) following the intervention. The difference in mean time-to-clamp remained significant in multivariate analyses ($\beta_{adjusted}$ = 113.2 seconds, 95% CI: 96.6, 129.9).

Conclusion: A simple two-component training intervention was successful at changing hospital practice from early to delayed umbilical cord clamping.

INTRODUCTION

The timing of umbilical cord clamping has recently undergone scientific investigation. Although it was once thought that immediate clamping of the umbilical cord would be necessary to prevent maternal postpartum hemorrhage (Duley *et al.* 2009; Enkin *et al.* 2000), this has been proven false, and in fact, it is now widely accepted that delayed umbilical cord clamping is most beneficial to the infant without incurring any risk to the mother (Ultee *et al.* 2008; McDonald and Middleton 2008; Strauss *et al.* 2008; Hutton and Hassan 2007; Kugelman *et al.* 2007; van Rheenen *et al.* 2007; Chaparro *et al.* 2006; Ceriani Cernadas *et al.* 2006; Emhamed *et al.* 2004; Rabe *et al.* 2004; Gupta and Ramji 2002; Rabe *et al.* 2000; Grajeda *et al.* 1997). Allowing placental transfusion of blood to run to completion before clamping the umbilical cord can provide the infant with an additional 15-20 mL of blood volume per kilogram birthweight, increasing its total blood volume by an estimated 30% (McDonnell and Henderson-Smart 1997). This translates into an additional 30-50 mg of iron at birth (Weckert and Hancock 2008; Levy and Blickstein 2006). Maximizing placental transfusion of blood usually takes approximately three minutes (Yao *et al.* 1969). The two meta-analyses conducted on this topic have found that waiting more than one minute to clamp the umbilical cord leads to higher hemoglobin levels, decreased risk of anemia and higher ferritin levels in the first six months of life (McDonald and Middleton 2008; Hutton and Hassan 2007).

In 2007, the Pan American Health Organization (PAHO) released new recommendations for the timing of umbilical cord clamping (Chaparro and Lutter 2007). These state that: "The optimal time to clamp the umbilical cord for all infants regardless of gestational age or fetal weight is when the circulation in the cord has ceased, and the cord is flat and pulseless (approximately 3 minutes or more after birth). After cord pulsations have ceased ..., clamp and cut the cord following strict hygienic techniques…" (Chaparro and Lutter 2007).

Delaying umbilical cord clamping is a cost-free intervention that has been proven to be safe and effective in decreasing the risk of infant anemia. Poor communities struggle to combat anemia, as their ability to use other known effective interventions, such as iron supplementation, are limited by high costs and other difficulties. If successfully implemented, especially in poor areas of the world where the prevalence of infant anemia is high, delayed umbilical cord clamping could be an effective and sustainable means to improve child health and nutrition. Although this topic has been thoroughly researched, a gap often exists between research and practice. Bridging the "know-do" gap, which refers to putting evidence-based research into practice, is an increasingly important field of research. No study has yet been conducted that has examined changing hospital policy from early to delayed umbilical cord clamping. The objective of this study, therefore, was to investigate the effect of a simple two-component intervention consisting of a three day workshop and a Hospital Directive on changing hospital practice with regard to the timing of umbilical cord clamping.

MATERIALS AND METHODS

The host hospital for this study was the Hospital Iquitos "César Garayar García", one of the two public health facilities in Iquitos with an obstetrics and gynecology unit, located in the Peruvian Amazon. The catchment area for this hospital includes Iquitos and surrounding communities, with an estimated total population of 406,000 (INEI 2008). Approximately 5,000 births are recorded in this hospital annually.

A pre/post study design was used to document the effectiveness of a simple two-component intervention in changing hospital practice in the Department of Obstetrics and Gynecology of Hospital Iquitos. The goal of the intervention was to implement new national government guidelines for attendance at birth that included a change from early to delayed cord clamping. The study population was mother-infant pairs who had uncomplicated vaginal deliveries in the hospital. All women who arrived at the hospital in labour between May 18 – June 3, 2009

(pre-intervention) and between July 6 – July 20, 2009 (post-intervention) were approached to participate in the study (Figure 1). Inclusion criteria required that participants not be scheduled for a cesarean section, were and would be residing in Iquitos or neighbouring communities over the next four months, and that they gave written consent prior to entering the delivery room. Participants were excluded if they had an emergency cesarean section, their infant was stillborn, born with a tight nuchal cord that could not be unwrapped from their neck or with any congenital abnormalities, or if mother and/or infant were transferred to another hospital before being discharged.

The hospital level intervention consisted of: 1) A three-day training workshop entitled "Estandarización de Habilidades Clínicas Básicas en Salud Materno-Neonatal [Standardization of basic clinical skills in maternal and neonatal health]" and 2) A written Hospital Directive sent to all nurse-midwives in the hospital.

The training workshop took place from June 8 – June 10, 2009 with the purpose of training hospital personnel on delayed umbilical cord clamping. It was run by two Peruvian Ministry of Health (MINSA) trained health professionals from Lima (one nurse-midwife and one obstetrician) and the Director of the Instituto de Investigación Nutricional (MEP), also from Lima. The MINSA professionals were charged with training the hospital staff in the new Ministry guidelines that included delayed umbilical cord clamping. The first two days of the workshop consisted of in-class theoretical training on the practical techniques of the new birthing practice guidelines with emphasis placed on delayed cord clamping. The third day of the workshop took place in the labour/delivery room of Hospital Iquitos with "hands-on" practice of techniques learned in the previous two days. All nurse-midwives of the study hospital were invited to attend this official workshop.

The Hospital Directive was signed by the head of the Department of Obstetrics and Gynecology of Hospital Iquitos, Dr. Eder Aguilar (EA), and was issued on

July 3, 2009 to all nurse-midwives employed by the hospital. This document declared that the new hospital policy with regard to the timing of umbilical cord clamping would be consistent with that of PAHO: "A partir de la fecha se recomienda que el clampaje del cordón umbilical en el Recién Nacido que no necesita atención inmediata en Neonatologia sera, entre los 2 y 3 minutos de nacido (cuando el cordón deja de latir) de acuerdo a las recomendaciones de la OPS. [From this date forwards, I recommend that the clamping of the umbilical cord for newborns not needing immediate neonatal attention will be between 2 to 3 minutes after birth (when the cord stops pulsating), according to PAHO recommendations.]"

Pre and post-intervention data collection was identical. Three experienced and trained study nurse-midwives (not employed by the hospital) comprised the in-hospital research team. They recruited the study participants and collected the data in three 8-hour shifts, such that recruitment and data collection was on-going 24 hours/day. Upon obtaining informed consent, the study nurse-midwife administered a short questionnaire to each participant in the labour room to obtain demographic and antenatal information. Maternal hemoglobin levels were then obtained from finger-prick blood using a HemoCue® machine. Additional medical information was abstracted from the woman's personal health record. This information was subsequently verified from hospital registries and medical charts. When the woman entered the delivery room, the study nurse-midwife observed the delivery and, using a digital stopwatch, recorded the time between the delivery of the first shoulder and the clamping of the umbilical cord. Observations of the delivery were also noted. Infant characteristics at birth were obtained from hospital registries.

Statistical Analyses

The sample size was calculated to measure the effect of the change in practice on infant anemia at four months of age (results reported separately (Blouin *et al.* 2010)). It was based on an inference for proportions, comparing two independent

samples using a 2-sided chi-square test with a level of significance of 0.05, 80% power, and an expected meaningful clinical difference of 20% in infant anemia following change in practice. Taking an estimated 20% attrition rate into account, the total sample size was calculated to be 112 in each group, for a total of 224 mother-infant pairs.

Student t tests and chi-square tests were used to compare baseline characteristics between the pre- and post-intervention groups. Univariate and multivariate linear regression was used to assess the effect of the intervention on the change in the timing of cord clamping. All statistical analyses were done in R (version 2.6.1).

Ethical considerations

Ethics approvals were obtained from the Research Ethics Office of the McGill University Health Centre (Canada), the Hospital Iquitos (Peru) and the Instituto de Investigación Nutricional (Peru). Written informed consent was obtained from all participants.

RESULTS

During the study period, a total of 270 women were approached for inclusion in the study. Of these, 6 refused participation, 3 did not meet inclusion criteria, and 37 were excluded from the study due to exclusion criteria. Complete hospital data were obtained for a total of 224 mother-infant pairs (83% of those approached).

Pre- and post- intervention groups were comparable on all demographic maternal and infant characteristics with the exception of gestational age and presence of a nuchal cord at birth, which differed significantly between the two groups (Table 1). Pre-intervention, the mean time between delivery and cord clamping was 56.8 seconds (95% CI: 51.0, 62.7). This ranged from a minimum value of 8.9 seconds to a maximum value of 191.7 seconds. Following the intervention, the mean time-to-clamp increased to 169.8 seconds (95% CI: 153.8, 185.8) (Figure 2), with

a range of 13.4 seconds to 397.3 seconds. Before the intervention took place, the distribution of the time-to-clamp variable was tightly centered around the mean (Figure 3). This distribution shifted towards delayed cord clamping following the intervention, with greater spread around the higher mean. The proportion of cord clamping times greater than or equal to one minute increased from 39.3% pre-intervention to 85.7% post-intervention. Additionally, in the post-intervention group, only 27.7% of cord clamping times occurred at less than 2 minutes, whereas, in the pre-intervention group, 95.5% of cord clamping times occurred at less than two minutes.

Univariate linear regression showed that the mean cord clamping time increased by 113.0 seconds following the intervention (β = 113.0 seconds, 95% CI = 95.2, 129.9). The magnitude of this effect remained unchanged in the multivariate analysis, after adjusting for gestational age, type of health personnel clamping the cord, sex of health personnel clamping the cord, presence of infant medical complication at birth, presence of meconial amniotic fluid at delivery, presence of a nuchal cord, infant sex and maternal age ($\beta_{adjusted}$ = 113.2 seconds, 95% CI: 96.6, 129.9).

DISCUSSION

Although the benefits of delayed over early umbilical cord clamping have been thoroughly studied under experimental conditions, no study had previously investigated specific operational interventions to implement this policy.

Of the limited research done regarding implementation of delayed umbilical cord clamping, it has been shown that the evidence to date is not being appropriately disseminated to policy makers and is not reaching obstetricians and midwives working in hospital settings. A recent study that aimed to investigate whether obstetricians around the world were willing to adopt recommendations on delayed cord clamping showed that they were reluctant to do so and that this was mainly due to being unaware of the scientific evidence (Ononeze and Hutchon 2009).

Another study in which American nurse-midwives were asked to report their current practices regarding the timing of umbilical cord clamping found an almost equal distribution in practice between early, intermediate and late cord clamping and concluded that this was a result of confusion and an inadequate scientific knowledge base (Mercer *et al.* 2000). These studies highlight the need for communication between researchers, policy makers and hospital staff. In order to successfully change practice towards delayed umbilical cord clamping, health workers need to be made aware of the evidence.

Although the issue of changing hospital policy from early to delayed umbilical cord clamping has not previously been investigated, other hospital-based policy changes have undergone serious investigation. One historical example is the success of changing hospital breastfeeding policy with the 'Ten Steps to Successful Breastfeeding' program, which was developed to increase the duration of breastfeeding. The United Nations Children's Fund and the World Health Organization developed a list of 10 practices to guide change in hospital policy with regard to breastfeeding. The first two steps are: 1) Have a written breastfeeding policy that is routinely communicated to all health care staff; 2) Train all health care staff in skills necessary to implement this policy. Implementation of these steps has been shown to successfully change hospital policy and practice with regard to breastfeeding practices (Wright *et al.* 1996). These techniques can be used to guide other attempts at hospital-based policy change.

Our study measured the effectiveness of a two-component intervention designed to implement the new national policy on delaying cord clamping. The mean and tight distribution of the time-to-clamp variable pre-intervention represents adherence to the previous hospital policy that instructed cord clamping to take place at approximately one minute. Following the intervention, there was a shift towards delayed cord clamping. As to be expected in any policy change setting, the distribution of time-to-clamp following the intervention showed large spread.

There are many possible reasons for the 27.7% of cord clamping times demonstrating sub-optimal practice (less than two minutes) post-intervention, including: not having attended the workshop, not having read the hospital directive, not knowing how much time had elapsed and perceived need for immediate neonatal attention. We expect that, as in other policy-to-practice changes, the proportion of sub-optimal practice will diminish with time and practice, and that the distribution of cord clamping times will exhibit a higher peak at the recommended time as this becomes routine in the delivery room.

One challenge that arose during delivery was that it was unreasonable to expect nurse-midwives to accurately measure the time between delivery and cord clamping. Waiting for the umbilical cord pulsations to cease may be an acceptable alternative; however, in many cases, judging exactly when the cord has stopped pulsating can also be difficult. Accurately judging when three minutes has passed is a technique that requires practice for the nurse-midwives.

We believe that the observed success in implementing the new policy of delayed cord clamping in Hospital Iquitos was a result of three contributing factors: 1) Incorporation of delayed cord clamping into official Ministry of Health policy , 2) Motivating hospital staff by training them on the scientific reasoning behind delayed cord clamping; and 3) Collaboration at the hospital level.

Changing hospital policy was possible because delayed cord clamping had recently been incorporated into official Peruvian Ministry of Health (MINSA) policy. As a result, this study was supported by health care officials from the Department of Specialized Health Services at MINSA. In 2008, MINSA had begun drafting updated guidelines entitled “Atencion Integral de Salud Materna y Perinatal [Integrated Health for Maternal and Perinatal Attention]”. These drafted guidelines included an update on the recommendation with regard to the timing of umbilical cord clamping; specifically, to extend it to allow for complete placental transfusion, usually between 2-3 minutes following delivery. As MINSA was

eager to receive local epidemiological evidence on this topic, they readily agreed that one of the first training workshops on the new guidelines could be undertaken in Iquitos by two obstetric professionals with experience in Ministry of Health training workshops and that the Director of IIN (MEP) could be involved to provide information on the scientific background regarding delayed cord clamping. The workshop clearly demonstrated how delayed cord clamping was an essential component in the management of the third stage of labour. The incorporation of delayed umbilical cord clamping into this MINSA-led training workshop provided the assurance that this was official policy. This was essential in terms of gaining support from the hospital director and ultimately, for ensuring hospital uptake of the new policy.

Training the hospital staff in an engaging manner with the three-day workshop was a crucial step. Most nurse-midwives were not aware of the scientific evidence favouring delayed cord clamping. Having the epidemiological evidence and reasoning behind delayed cord clamping explained in a focussed manner throughout a training workshop provided motivation that helped ensure that what might have been just another change in standard routine guidelines resulted in a change in practice.

Finally, hospital officials also collaborated in this study and were key in terms of planning the data collection, organizing the training workshop, and issuing the Hospital Directive. Although all nurse-midwives of the hospital were invited to attend the workshop, not all were able to attend. In order for the message regarding the new policy to reach all appropriate staff, the Director of the Department of Obstetrics and Gynecology of Hospital Iquitos issued a Hospital Directive to every nurse-midwife in the hospital. This personnel directive explained the new policy and referred to the PAHO recommendations for further information.

CONCLUSION

In order to combat infant anemia, especially in low-resource settings, the most effective strategy is to scale up interventions that are known to be effective. Delayed umbilical cord clamping has not only been proven effective in reducing infant anemia, it is also cost-free and therefore an appropriate and sustainable intervention. Despite the body of scientific evidence supporting delayed umbilical cord clamping, a gap exists between research and practice. One method to bridge this gap is collaboration among researchers, government officials and hospital staff members. Using a comprehensive capacity-building approach, hospital policy and practice can successfully be changed from early umbilical cord clamping to delayed umbilical cord clamping.

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Table 1: Baseline demographic maternal/infant characteristics comparing pre/post intervention groups, Iquitos, Peru, May-July, 2009.

Variable	*Pre-Intervention (n = 112)*	*Post-Intervention (n = 112)*	*p-value*
Mother's age (years) [mean (sd)]	23.5 (6.5)	23.8 (5.5)	0.723
Mother's marital status: married /common law	90.2 %	86.6 %	0.532
Residence: Urban	54.5 %	49.1 %	
Residence: Peri-urban	37.5 %	42.0 %	0.725
Residence: Rural	8.0 %	8.9 %	
# Years of education [mean (sd)]	8.9 (2.8)	9.4 (2.5)	0.165
Mothers employed	23.2 %	12.5 %	0.055
# Antenatal care visits [mean (sd)]	6.8 (2.4)	6.7 (2.6)	0.641
Mother who took iron supplements during pregnancy	94.6 %	93.8 %	1.000
Maternal hemoglobin (g/dL) [mean (sd)]	12.02 (1.34)	11.77 (1.22)	0.158
Mothers anemic (Hb<11.0g/dL)	18.8 %	23.2 %	0.512
Gestational age (weeks) [mean (sd)]	38.8 (1.6)	38.2 (2.2)	0.021*
# of previous pregnancies [mean (sd)]	1.3 (1.5)	1.5 (1.4)	0.577
# of previous miscarriages [mean (sd)]	0.2 (0.5)	0.3 (0.5)	0.156
Personnel attending delivery (% nurse-midwife interns)	91.1 %	81.3 %	0.053
Personnel clamping cord (% nurse-midwife interns)	94.6 %	89.3 %	0.219
Sex of health personnel clamping cord (% males)	11.6 %	8.0 %	0.500

Presence of infant complication at birth[1]	7.1 %	8.0 %	1.00
Presence of meconial amniotic fluid at delivery	11.6 %	17.9 %	0.258
Presence of nuchal cord at delivery[2]	26.8 %	11.6 %	0.007*
Birth weight (g) [mean (sd)]	3152 (424.9)	3182 (389.5)	0.582
Birth length (cm) [mean (sd)]	50.05 (2.2)	50.2 (2.0)	0.521
Infant sex (% males)	54.5 %	50.0 %	0.593

* : p-value < 0.05

[1] = medical complications included neonatal depression (including infant not crying at birth) and infant born with fractured collarbone

[2] = nuchal cord was present but able to be unwrapped easily during delivery

Figure 1: Study timeline (May – July, 2009) showing the two recruitment periods and intervention components

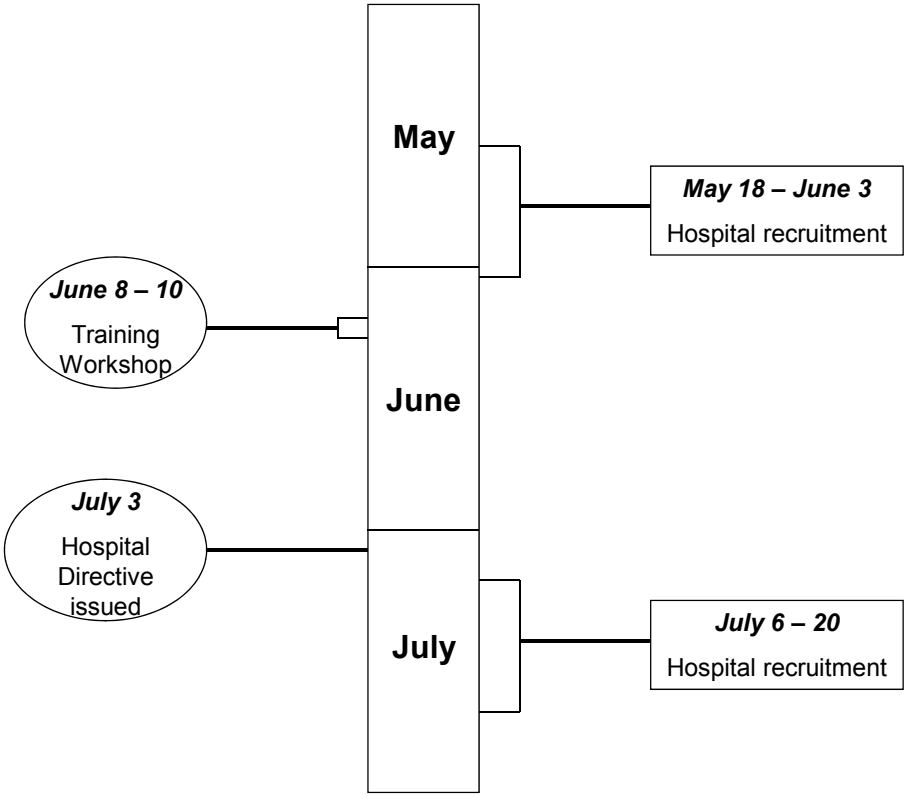


Figure 2: Barplots showing the increase in mean time between the delivery of the first shoulder and clamping of the umbilical cord (with 95% confidence intervals) in the pre-intervention group and the post-intervention group

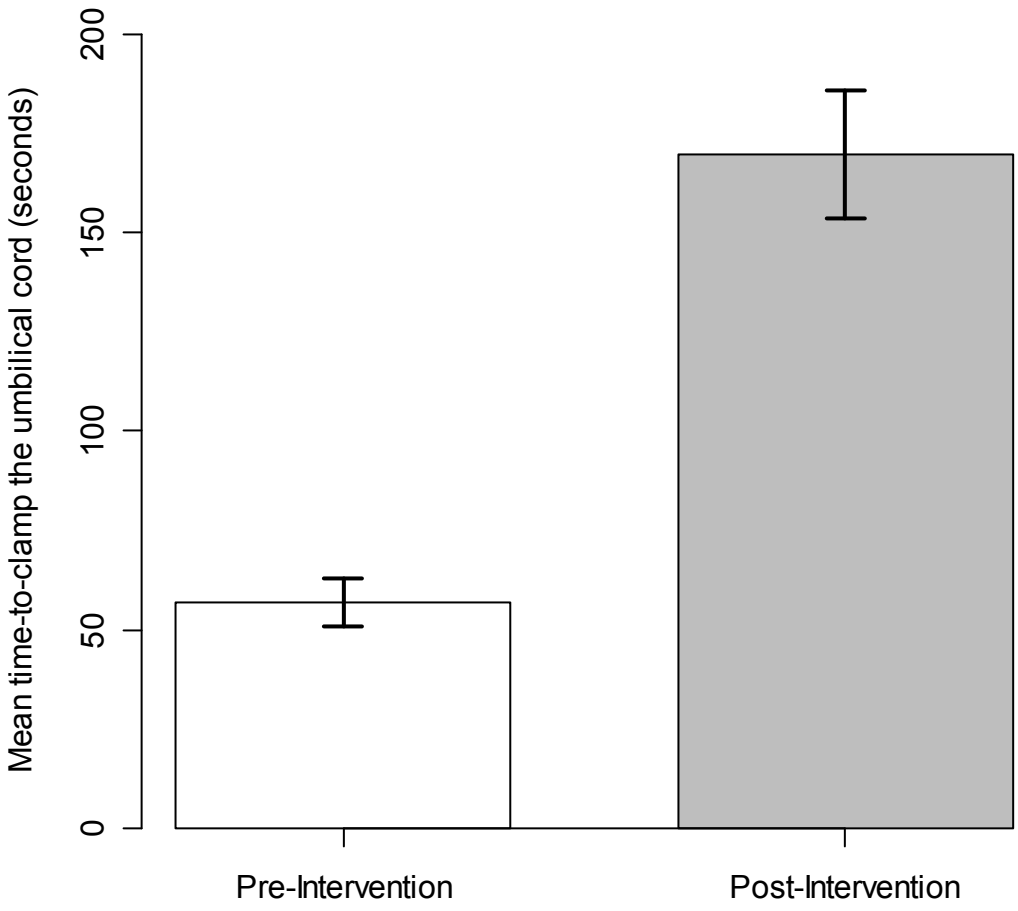
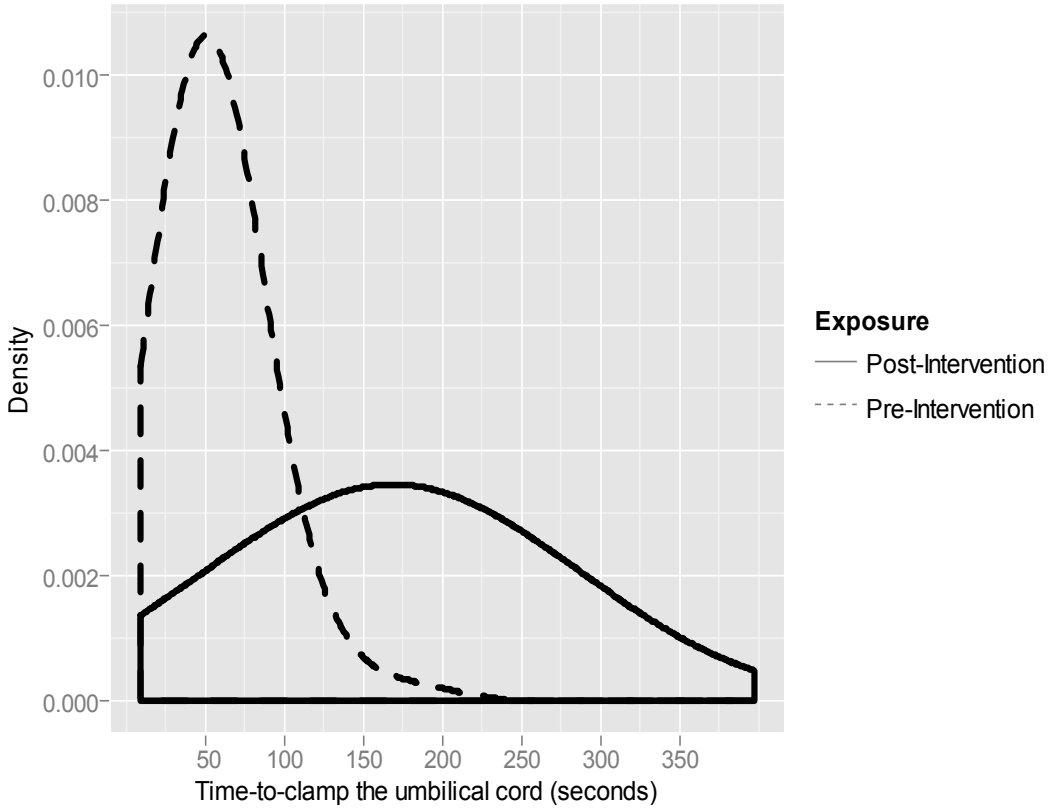


Figure 3: Density plots demonstrating the distribution of time-to-clamp (in seconds) in the pre-intervention and post-intervention groups



5.3. INTERVENTION AND INFANT ANEMIA: THE EFFECT OF A HOSPITAL TRAINING INTERVENTION PROMOTING DELAYED CORD CLAMPING ON INFANT HEMOGLOBIN LEVELS AND INFANT ANEMIA AT FOUR MONTHS OF AGE

Complete hospital data were collected for 224 mother-infant pairs (112 pre-intervention and 112 post-intervention). Of these, 207 completed the 4-month follow-up (7.6% loss-to-follow-up). Demographic, delivery and infant characteristics were comparable between those lost to follow-up (N=17) and those who completed the study (N=207) with the exception of urban/rural status (64.7% urban vs. 50.7% urban), mean number of antenatal care visits (5.5 vs. 6.9) and number of previous pregnancies (0.8 vs. 1.4).

For the 207 mother-infant pairs who completed the study, pre- and post-intervention groups were comparable on all demographic, delivery, and infant characteristics, with the exception of the presence of a nuchal cord at birth (25.5% pre vs. 12.4% post) and gestational age (38.9 weeks pre vs. 38.3 weeks post).

In the univariate linear regression analysis, mean infant hemoglobin levels at 4 months of age did not differ significantly between the pre- and post-intervention groups ($\beta = 0.06$; 95% CI: -2.84, 2.95). In the pre-intervention group, mean infant hemoglobin level was 104.07 mg/dL, and in the post-intervention group, this was 104.10 mg/dL. The effect of the intervention on infant hemoglobin levels remained non-significant when adjusted for relevant covariates: number of years of education of the mother, whether the infant had been exclusively breastfed since birth, maternal hemoglobin levels at delivery and infant weight gain from birth to 4 months of age ($\beta = 0.114$; 95% CI: -2.77, 3.00).

Similarly, infant anemia status was not significantly affected by the intervention. The percentage of anemic infants at 4 months of age in the pre-intervention group was 72.6%. In the post-intervention group, this was 73.3%. Logistic regression analysis revealed that the odds of developing infant anemia at 4 months of age

were not changed by the intervention (OR = 1.04; 95% CI: 0.56, 1.92). The odds ratio for infant anemia remained non-significant, both clinically and statistically in the multivariate analysis, after adjustment for the number of years of education of the mother, whether the infant had been exclusively breastfed since birth, maternal hemoglobin levels at delivery, and infant weight gain from birth to 4 months of age (aOR = 1.09; 95% CI: 0.57, 2.06). Gestational age and presence of a nuchal cord at delivery were investigated for inclusion in the multivariate model and were not included because they were not associated with the outcome.

Submitted to *Pediatrics*

5.4. MANUSCRIPT 2: THE EFFECT OF MATERNAL ANEMIA ON THE ASSOCIATION BETWEEN TIMING OF UMBILICAL CORD CLAMPING AND INFANT ANEMIA: OBSERVATIONS OF HOSPITAL PRACTICE IN THE PERUVIAN AMAZON

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Short Title: Maternal anemia and the timing of cord clamping

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Conflict of Interest: No conflict of interests

ABSTRACT

Objective: To determine the effect of maternal anemia at delivery on the association between timing of umbilical cord clamping and infant anemia at four months of age.

Patients and Methods: The patient population consisted of a cohort of women who entered the delivery room of Hospital Iquitos (Iquitos, Peru) during two recruitment periods in 2009. Maternal hemoglobin levels were measured before delivery and the time between the delivery of the first shoulder and clamping of the umbilical cord was recorded for each delivery. At four months post-delivery, mothers and infants were followed-up and infant hemoglobin levels were measured.

Results: Complete baseline and follow-up data were available for a total of 207 mother-infant pairs (92% of an initial 224 pairs). Multivariate analyses revealed that the association between the timing of cord clamping and infant anemia (at 4 months of age) was moderated by the mother's anemia status ($\beta_{interaction,\ adj.}$ = -0.65; 95% CI = -1.21, -0.09). Among infants born to anemic women, the adjusted odds of developing infant anemia were 0.59 (95% CI: 0.36, 0.96) times lower for every one minute increase in the timing of cord clamping.

Conclusion: Delaying cord clamping decreases the odds of developing anemia in infants born to anemic mothers. The effect of timing of cord clamping on infant anemia should always be reported separately by maternal anemia status. Our findings provide empirical evidence in support of delaying umbilical cord clamping.

INTRODUCTION

Anemia is a major health problem worldwide. It is estimated to affect 24.8% of the global population in both developed and developing countries.[1] The primary cause of anemia (in 50% of cases[2]) is iron deficiency which is among the most important contributing factors to the global burden of disease[3]. Iron deficiency during gestation and infancy can have devastating effects on both neural development and behavioural outcomes, and many of these negative consequences are not reversed following iron therapy.[4]

Delayed umbilical cord clamping has recently been identified as one of four effective interventions used to combat iron deficiency during the first six months of life.[5] An additional 15-20 mL of blood volume per kg birthweight can be delivered to the infant through the umbilical cord by allowing placental transfusion of blood to complete.[6] This can increase total blood volume by an estimated 30 %[6] which amounts to an additional 30-50 mg of iron at birth.[7,8] Maximizing placental transfusion of blood usually takes approximately three minutes.[9]

Several randomized controlled trials have documented improved iron status in infants when cord clamping was delayed compared to when cord clamping was more immediate.[10-20] Two recent meta-analyses of randomized controlled trials (RCTs) on this topic agree on the short term benefits of delayed umbilical cord clamping.[21,22] Infants born with delayed cord clamping had higher hemoglobin levels than infants born with early cord clamping, at birth and up to 24 hours later.[21,22] The long term benefits of delayed cord clamping on hemoglobin levels, however, are more controversial. Although some studies have reported long term positive effects of delayed cord clamping on infant hemoglobin levels beyond two months,[11,13,17] other studies (including meta-analyses) have not.[14,21-23] It has been proposed that maternal iron status, infant birth weight, and infant feeding practices may be potential effect modifiers of the relationship between the timing

of cord clamping and infant iron status.[14] Although several previous studies did adjust for maternal anemia, only one, an RCT, raised the issue of maternal anemia as a potential effect modifier.[14] This distinction is important to accurately characterize the true effect of timing of cord clamping on infant anemia.

The objective of this study was therefore to determine the role of maternal anemia as a potential effect modifier on the association between the timing of umbilical cord clamping and infant anemia. Hemoglobin data from a pre/post study design investigating a change in hospital policy from early to delayed cord clamping provided the data source for this study.[24]

PATIENTS AND METHODS

Participants

The study was conducted at Hospital Iquitos "César Garayar García", which is located in Iquitos, in the Peruvian Amazon. It serves both Iquitos and surrounding communities, with a total population of 406,000 (INEI, 2008)[25] and is one of only two health facilities in the region with an obstetrics and gynaecology unit.

The study population was a cohort of pregnant women admitted to the labour room of Hospital Iquitos during two recruitment periods (May 18 – June 3, 2009 and July 6 – July 20, 2009) (Figure 1). The inclusion criteria were: 1) that the participants were not scheduled for a cesarean section, 2) they were and would be residing in Iquitos or neighbouring communities for the next four months, and 3) that they give consent prior to entering the delivery room. Women were excluded if 1) they had an emergency cesarean section, 2) their baby was stillborn, 3) their baby was born with a tight nuchal cord that could not be unwrapped from the neck, 4) their baby was born with any congenital abnormalities, or 5) if they were transferred to another hospital before being discharged.

Data collection

Three experienced and trained nurse-midwives recruited the study participants and collected the data for this study daily in three 8-hour shifts. After obtaining informed consent, the study nurse-midwife administered a short questionnaire to the woman in the labour room to obtain demographic and antenatal information. The woman was given an appointment for a follow-up visit to occur four months following the birth of her baby. Maternal hemoglobin levels were obtained in the labour room from finger-prick blood using a HemoCue® machine. Additional medical information was taken from the woman's personal health record and verified from hospital registries and medical charts. When a woman entered the delivery room, the study nurse-midwife accompanied her to observe the delivery and to record the time between the delivery of the first shoulder and clamping of the umbilical cord, using a digital stopwatch. Infant characteristics at birth were obtained from hospital registries. All aspects of data collection were supervised on site by one author (BB).

At approximately two to three weeks following delivery in the hospital, a brief visit to the new mothers' homes was made by the study nurse-midwives to confirm addresses and to remind the woman of the upcoming 4-month follow-up visit. Women were also encouraged to breastfeed exclusively for six months.

Mother-infant pairs were followed-up within 1-2 days of each infant's 4-month birthday (Figure 1). The same three nurse-midwives who conducted the in-hospital assessments also made the home-visits, accompanied by the primary author (BB). At these visits, the study nurse-midwife administered a brief questionnaire to obtain information on: presence of any infant medical conditions, infant's health status in the previous two weeks, medication or vitamins given to infant since birth, whether infant had been treated for jaundice, infant's breastfeeding and other feeding practices, and maternal iron supplementation after delivery. The infant was then weighed using either a Seca digital scale (Model

354; Seca corp, Baltimore, MD, USA) or a hanging clock scale (provided by UNICEF), and length was measured using standard length boards. Maternal hemoglobin levels were ascertained from finger-prick blood using a Hemocue® machine. Similarly, infants' blood was obtained by either finger or heel prick and hemoglobin levels measured using a Hemocue® machine.

Statistical Analyses

The sample size calculated for the original policy intervention study was based on an inference for proportions, comparing two independent samples using a 2-sided chi-square test. An expected difference of 20% in infant anemia following policy change was considered clinically relevant. A sample size of 180 participants would have 80% power to detect a difference significant at the 5% level. This was increased to 224 to take into account a possible 20% loss to follow-up at 4 months.

Student t tests and chi-square tests were used to compare baseline characteristics between the final study population and those lost to follow-up. Univariate and multivariate logistic regression models were used to assess the effect of timing of cord clamping on infant anemia (a dichotomous variable). Infant anemia was defined as hemoglobin levels less than 110 mg/dL. Univariate and multivariate linear regression models were used to ascertain the effect of timing of cord clamping on infant hemoglobin levels, on a continuous scale. Based on the initial literature review, maternal anemia at delivery (hemoglobin<110 mg/dL) was identified as a potential effect modifier of the relationship between timing of cord clamping and infant anemia. The time-to-clamp variable was centered by subtracting the mean from all observations before being included in the interaction term. Multivariate models included all measured known confounders that were either significant in univariate analysis or considered clinically relevant from the literature review that were judged to have sufficient variation, small measurement error, and few missing observations (i.e., years of education of the

mother, whether or not the infant had been exclusively breastfed up to four months, and maternal anemia at delivery).

To calculate population attributable risk, the exposure variable, time-to-clamp, was dichotomized at relevant cut-off points. Adjusted population attributable risk was calculated using the method proposed by Greenland[26] and confidence intervals were estimated by computing 1,000 bootstrap replicates using the 'boot' package of the R software. All statistical analyses were done in R version 2.6.1.

Ethical considerations

Ethics approvals were obtained from the Research Ethics Office of the McGill University Health Centre (Canada), the Hospital Iquitos (Peru) and the Instituto de Investigación Nutricional (Peru). Written informed consent was obtained from all participating women.

RESULTS

During the study period, a total of 270 women were approached for inclusion in the study. Of these, 6 refused participation, 3 did not meet inclusion criteria, and 37 were excluded. Complete hospital data was obtained for a total of 224 mother-infant pairs. Of these, 17 were lost to follow-up at four months (8% loss-to-follow-up). The final sample size was therefore 207 mother-infant pairs. Baseline maternal, infant and delivery characteristics were comparable between women who remained in the study and those lost to follow-up, differing only with regard to the proportion of women living in urban areas (50.7% urban vs. 64.7% urban), mean number of antenatal care visits (6.9 vs. 5.5) and mean number of previous pregnancies (1.4 vs. 0.8). The mean time-to-clamp the umbilical cord was 114.6 seconds (Table 1). This ranged from a minimum value of 8.9 seconds to a maximum value of 397.3 seconds.

For all infants, univariate analyses revealed a small increase in infant hemoglobin levels for each one minute delay in cord clamping, however, this result did not reach statistical significance ($\beta = 0.06$ mg/dL; 95% CI = -0.95, 1.08). Similarly, for all infants, the odds of developing infant anemia were slightly lower for each one minute delay in cord clamping, but again these results did not achieve statistical significance (OR = 0.94; 95% CI: 0.76, 1.16) (Table 2).

Multivariate analyses revealed both a clinically and statistically significant interaction effect of maternal anemia on the association between time-to-clamp and infant anemia at 4 months of age. The interaction effect remained significant when adjusted for years of education of the mother and whether or not the infant had been exclusively breastfed up to 4 months ($\beta_{interaction,\ adj.} = -0.65$; 95% CI = -1.21, -0.09). Infant weight gain, birth weight and gestational age were examined for inclusion in the multivariate model and were not included because they were conclusively not associated with the outcome.

In babies born to non-anemic women, there was no significant effect of the timing of cord clamping on the odds of developing anemia at 4 months of age, adjusted for years of education of the mother and whether or not the infant had been exclusively breastfed up to 4 months, (aOR = 1.12; 95% CI = 0.87, 1.44). In babies born to anemic mothers, however, the adjusted odds of developing anemia were approximately 40% lower for each minute that clamping of the umbilical cord was delayed (aOR = 0.59, 95% CI = 0.36, 0.96). In babies born to anemic mothers, as the time between birth and cord clamping increased, the odds of developing anemia at four months of age decreased (Figure 2). Moreover, if all cord clamping times increased from one minute to three minutes, this would decrease the number of anemic infants at four months by 22.8% (in infants born to anemic women) (population attributable risk = -22.8%, 95% CI = -46.5%, -3.4%).

DISCUSSION

The safety and short term benefits of delayed umbilical cord clamping on infant iron status have been established;[10-22] however, the long term benefits on infant hemoglobin levels are less well understood. One possible reason for this could be due to a lack of understanding of the impact of maternal anemia on the relationship between timing of cord clamping and infant anemia. Gupta and Ramji[17] found that delayed cord clamping had beneficial effects on the odds of developing infant anemia at 3 months of age in infants born to anemic women (OR = 7.7; 95% CI = 1.84, 34.9). Furthermore, Chaparro *et al.*[14] proposed that delayed umbilical cord clamping has a greater effect on infant hemoglobin levels at six months in babies born to mothers with low ferritin levels (β = -6.5 mg/kg; 95% CI = -10.2, -2.8) than in babies born to mothers with normal ferritin levels (β = -0.8 mg/kg; 95% CI = -5.0, 3.4). This effect; however, had not been investigated further. Our study supports the finding by Chaparro *et al.*[14] and provides further insight into the long term effect of delayed umbilical cord clamping on infant anemia. We have found that the timing of umbilical cord clamping has a different effect in infants born to anemic mothers than in infants born to non-anemic mothers, such that the effect cannot be interpreted independent of maternal anemia status. In infants born to non-anemic mothers, we found no significant effect of delayed cord clamping on infant anemia at four months of age; however, in infants born to anemic mothers, for every one minute increase in cord clamping, we found that the odds of developing anemia at four months were significantly decreased.

This has many implications for the interpretation of results from previous studies. If maternal anemia is not treated as an effect modifier, the magnitude of the effect of delayed cord clamping on infant anemia will depend on the prevalence of maternal anemia in the study population. As the prevalence of maternal anemia in a study population increases, the effect of the timing of cord clamping on infant anemia at four months would appear greater at the population level. However, if

the prevalence of maternal anemia in a study population is low, the observed magnitude of the effect of the timing of cord clamping is more likely to be smaller. Results from previous research that have not taken into account maternal anemia as an effect modifier are likely affected by the prevalence of maternal anemia at birth in their study population. This could account for the fact that some studies report a long term statistically significant effect of the timing of cord clamping on infant iron status[13,17] while others fail to find a long term statistically significant effect at this longer term follow-up.[14,23]

The present study adds to the mounting support for delaying umbilical cord clamping. Although benefits appear to be greater for infants born to anemic mothers, no harm has been shown from delayed umbilical cord clamping in infants born to non-anemic women.

Considering that delaying umbilical cord clamping is a cost-free intervention and that routine screening for maternal anemia before birth is not feasible in most developing country settings, delayed umbilical cord clamping should be implemented for all infants. Future research is needed, however, to determine the true effect of timing of umbilical cord clamping in infants born to non-anemic mothers both in developing and developed countries. The only study that has investigated the effect of delayed cord clamping in babies born to non-anemic women found comparable infant hemoglobin and ferritin levels at three months of age between early and late cord clamping groups.[23]

CONCLUSION

The significant long term effect of the timing of cord clamping on infant anemia at four months of age in infants born to anemic women provides further support for this safe and cost-effective intervention. We have found that if cord clamping were increased from one to three minutes (the recommended time published by the Pan American Health Organization[27]), the number of cases of infant anemia

would decrease significantly. In areas around the world where anemia is a major health problem in infants, a simple and cost-effective practice such as delayed umbilical cord clamping is an ideal solution to improve child health and nutrition.

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Table 1: Baseline demographic maternal and infant characteristics of the 207 (of 224 (92%)) mothers for whom both baseline and follow-up (at 4 months) data were complete, Hospital Apoyo Iquitos, May – November, 2009

Maternal and infant characteristics		Study Population (n = 207)
Time between delivery of first shoulder and cord clamping (seconds) [mean (sd)]		114.6 (85.9)
Mother's age (years) [mean (sd)]		23.9 (5.9)
Mothers married / common law (%)		89.4 %
Residence:	Urban (%)	50.7 %
	Peri-urban (%)	40.6 %
	Rural (%)	8.7 %
# Years of education of mother [mean (sd)]		9.2 (2.7)
Mothers employed (%)		18.4 %
# Antenatal care visits [mean (sd)]		6.9 (2.5)
Mothers who took iron supplements during pregnancy (%)		93.7 %
Maternal hemoglobin (mg/dL) [mean (sd)]		118.9 (13.1)
Maternal anemia (Hb<110mg/dL)(%)		21.7 %
Gestational age (weeks) [mean (sd)]		38.6 (1.8)
# Previous pregnancies [mean (sd)]		1.4 (1.4)
# Previous miscarriages [mean (sd)]		0.2 (0.5)
Sex of infant (% males)		52.2 %
Birth weight (g) [mean (sd)]		3167 (411)
Birth length (cm) [mean (sd)]		50.1 (2.1)
APGAR Score at 5 minutes [mean (sd)]		9.8 (0.7)

Table 2: The association between the timing of umbilical cord clamping and other important covariates on infant anemia, univariate and multivariate results

Variables	Univariate *OR* (95%CI)*	Multivariate *aOR† (95%CI)*
Time-to-clamp (min)	0.94 (0.76, 1.16)	1.12 (0.87, 1.44)
Years of education	**0.82 (0.72, 0.93)**	**0.84 (0.73, 0.96)**
Exclusive breastfeeding	1.98 (0.97, 4.08)	1.58 (0.74, 3.38)
Maternal anemia	1.19 (0.56, 2.55)	1.27 (0.55, 2.95)
Interaction term: Maternal anemia * Time-to-clamp (min)	-	**0.52 (0.30, 0.92)**

Statistically significant level ($p<0.05$) are presented in bold
*OR: odds ratio
†aOR: adjusted odds ratio for years of education of the mother, whether or not the infant had been exclusively breastfed up to four months, maternal anemia at delivery, and the interaction term (maternal anemia * time-to-clamp.

Figure 1: Study timeline (May – November, 2009) showing the two recruitment and follow-up time periods from the original study[24] that make up the cohort of women analysed in this study

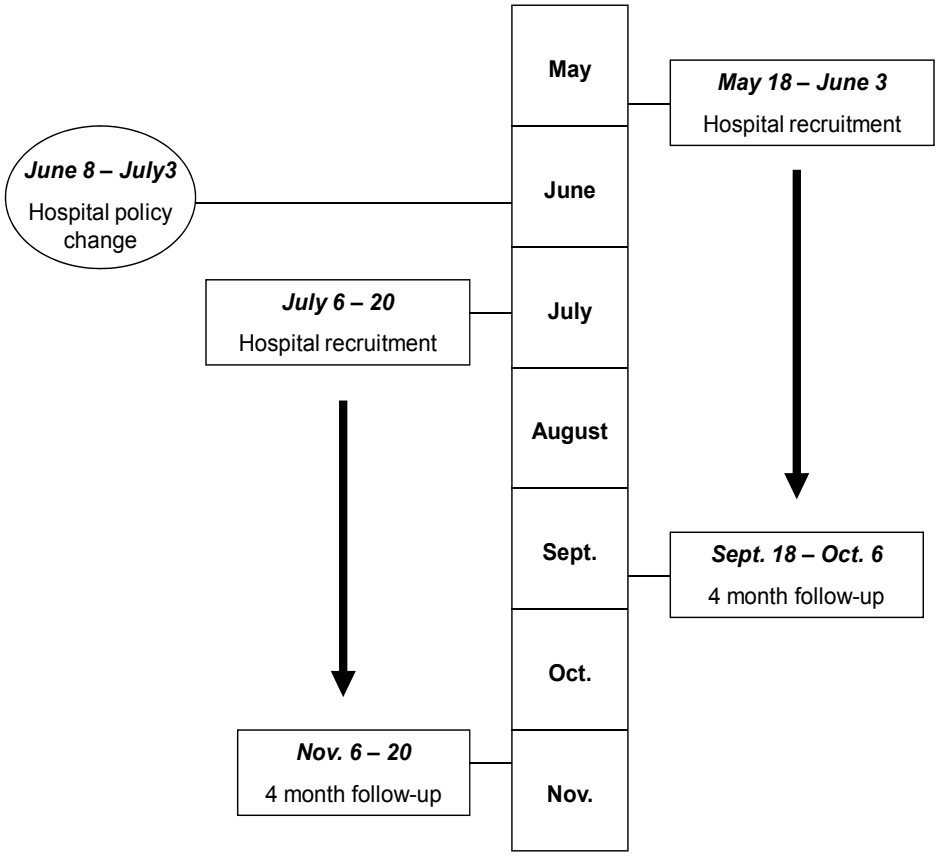
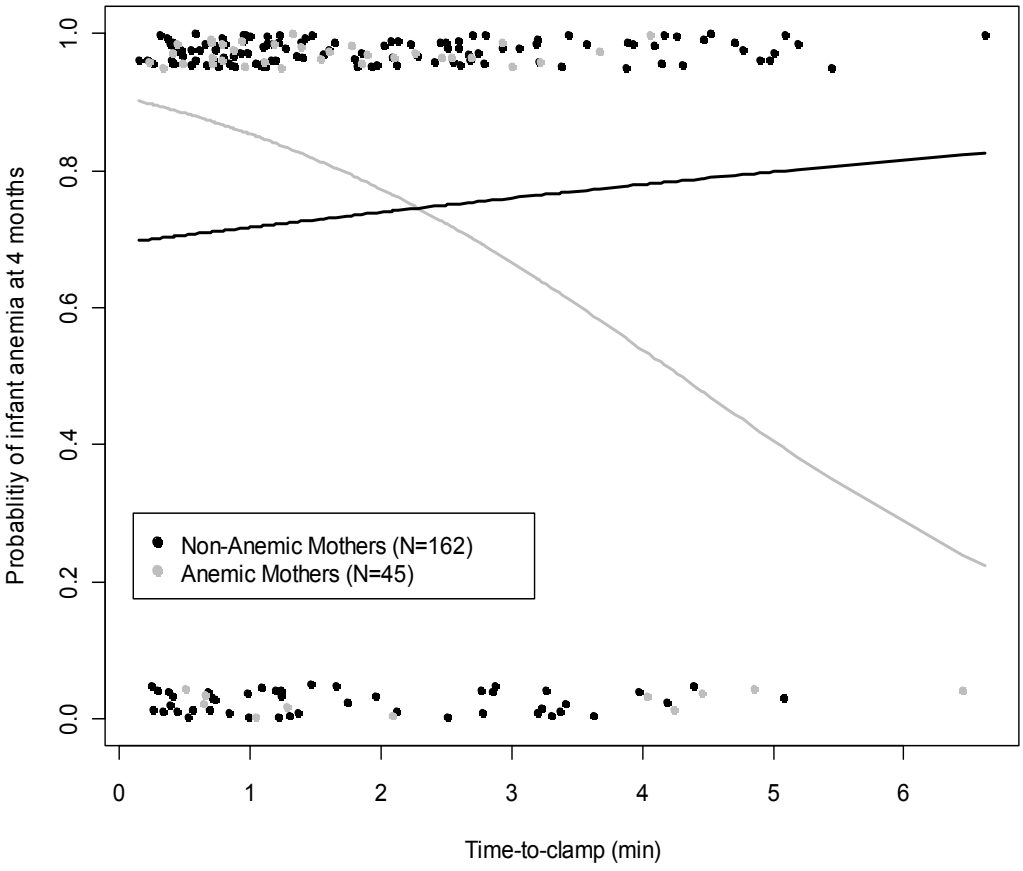


Figure 2: Maternal anemia as an effect modifier on the relationship between time-to-clamp and infant anemia at 4 months – (Fitted logistic regression curves for infant anemia at 4 months as a function of time-to-clamp by maternal anemia status, with all other covariates set at their mean)



6. Discussion

6.1. INTERPRETATION AND IMPLICATION OF RESULTS

The study results reported in the two manuscripts have important implications for clinical practice. First, the two-component intervention was successful in changing hospital practice from early to delayed umbilical cord clamping. Although the distribution of the time-to-clamp variable shows considerable spread, suggesting that more implementation time is needed to solidify the new practice in the hospital, there was a considerable and significant shift towards delayed cord clamping following the intervention. Interventions including both theoretical training and distribution of a written policy might be considered in future attempts to change hospital practice from early to delayed umbilical cord clamping.

Second, the intervention did not show a significant effect on infant anemia at four months of age and this is likely due to the large degree of overlap and variability in the time-to-clamp variable pre- and post- intervention (Section 5.2: Figure 3). The pre-intervention group represented observations of current hospital practice. Although in the majority of cases, cord clamping occurred at approximately one minute, there was a large degree of variability in practice. There were several instances where cord clamping took place much earlier and much later than one minute. Similarly, in the post-intervention group, even more variability was present in the time-to-clamp variable. As to be expected with any change in policy, a learning curve will most likely be associated with implementation efforts. Although the average time-to-clamp was close to the recommended time (2.8 minutes), the distribution exhibited very large spread both above and below the mean. This is most likely due to hospital workers being unaware of the new policy and not knowing exactly when three minutes had elapsed. For these reasons, the pre-intervention group, as a whole, could not be considered to consistently reflect “early umbilical cord clamping” and the post-intervention

group, as a whole, could not be considered to consistently reflect "delayed umbilical cord clamping".

In order to determine the effect of timing of cord clamping on infant anemia, the data were analysed using the actual "time-to-clamp" (on a continuous scale) as the exposure variable and infant anemia as the outcome. In this analysis, it was found that the effect of the timing of cord clamping was modified by maternal anemia status. In infants born to anemic mothers, the odds of developing infant anemia at four months of age is decreased by 0.59 for every one minute delay in cord clamping; however, in infants born to non-anemic mothers, there did not appear to be any effect of a one minute delay in cord clamping on the odds of developing infant anemia. Although the short term benefits of delayed umbilical cord clamping on infant iron status have been established in the literature, less agreement exists on how long these effects last. This lack of agreement could be due to the fact that previous studies have not taken into account maternal anemia as an effect modifier. In these circumstances, the magnitude of the effect would depend on the prevalence of maternal anemia in the study population.

6.2. STRENGTHS AND LIMITATIONS

A major strength of this study was the accurate measurement of the exposure and outcome variables making any information bias unlikely. The time-to-clamp variable was accurately measured with a digital stopwatch, and in all circumstances, the timing began at the delivery of the first shoulder of the infant and stopped as soon as the umbilical cord was clamped. Additionally, the accuracy of using a Hemocue® machine to measure hemoglobin levels has been established (Neufeld *et al.*, 2002). Misclassification, therefore, of the main outcome, infant anemia and the main covariate, maternal anemia at delivery, is unlikely.

Another strength of this study was the high participation rate. Having a study nurse-midwife present in the labour room 24 hours a day allowed us to approach

all women admitted to the labour room for participation in the study. Only six women refused participation (3% of eligible participants) and only 17 participants were lost-to-follow-up (7.6%). Follow-up with the participants in their homes two weeks after they were recruited into the study to locate their addresses and to confirm their four-month appointment was crucial to minimizing loss to follow-up.

One limitation is the fact that although loss-to-follow-up was very small, those lost differed significantly from the remaining study population with regard to urban/rural status, number of antenatal care visits and number of previous pregnancies. Although none of these variables were found to be associated with infant anemia, and none appeared to be relevant covariates based on the literature review and data analyses, losing these individuals meant that the final study population may not represent the entire target population.

A second limitation was that the presence of the research team in the labour and delivery room of the hospital may have affected the nurse-midwives' practice regarding the timing of umbilical cord clamping. Although the hospital nurse-midwives were not aware of the research objectives, it is impossible to know if the practices reported in Chapter 5: Section 5.2 would have been identical had the research team not been present.

6.3. AREAS FOR FUTURE RESEARCH

This is the first study to document any attempt at changing hospital policy and practice from early to delayed umbilical cord clamping. Collaboration between researchers and local and national decision makers is needed to further disseminate the scientific evidence favouring delayed cord clamping. This is a cost-effective intervention that could have significant health benefits, especially to infants born in low-resource settings, if research findings are disseminated appropriately. In addition, more epidemiological studies are needed on the

optimal methods for implementing this intervention both in hospital and other delivery settings (ie. local health centres).

Researchers who performed previous studies that failed to treat maternal anemia as an effect modifier should consider re-analysing their data. This study corroborates the result of Chaparro *et al.* (2006) in finding that maternal anemia modifies the effect of time-to-clamp on infant anemia. Therefore, previous published effect measures that combined results from anemic and non-anemic women may obscure an important timing-anemia relationship. Especially where combined results were found not to be statistically significant, this may lead to undervaluing the importance of delayed cord clamping. Effect measures should always be reported separately for infants born to anemic and non-anemic women in order to provide the most detailed evidence possible to inform clinical decision-making.

Finally, longer follow-up studies are needed to identify how long the effect of delayed cord clamping on infant iron status can last, especially in infants born to anemic women. The longest study to date followed infants up to six months of age and found that the effect persisted, especially in infants born to women with low ferritin levels (Chaparro *et al*, 2006). Determining exactly how long the effects can last would add to the mounting evidence favouring this intervention and may help promote policy change. A follow-up study beyond six months would prove to be a challenging task due to the fact that substances other than breast milk which are normally introduced into the diet of infants from six months of age, would have a considerable effect on infant iron status. Any such study that looks beyond six months would have to take diet into consideration, a variable that is difficult to measure.

7. Conclusion

Delayed umbilical cord clamping is a safe and effective means to decrease infant anemia at the population level. This research has shown that it is possible to change hospital practice from early to delayed cord clamping and that the effects of delayed cord clamping in decreasing infant anemia persist up to four months in infants born to anemic women.

Considering that this intervention is cost-free, and that it has been proven to decrease infant anemia, it could be of particular benefit in low-resource settings where other, more costly interventions are not feasible.

This research highlights the need for collaboration between health researchers and key decision makers, at the local and national level, in order to effect policy change. In this way, cost-effective and sustainable health interventions can be evaluated and implemented to improve health outcomes in a timely manner.

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APPENDIX 1: INFORMED CONSENT

INFORMED CONSENT FOR PARTICIPATION IN A SCIENTIFIC STUDY:

"The effect of timing of umbilical cord clamping on infant anemia: Implications for clinical practice in the Peruvian Amazon"

Investigators:

Dr. Martín Casapía, Asociación Civil Selva Amazónica, Iquitos. Telephone.: 236-277

Dr. Mary Penny and Nutricionist Hilary Creed-Kanashiro, Instituto de Investigación Nutricional (IIN), Av. La Molina 1885, La Molina, Lima. Telephone.: Lima 3496023

Dr. Theresa Gyorkos, Dr. Elham Rahme and Dr. Anita Gagnon, McGill University, Montreal, Canada.

Dr. Eder Aguilar and Dr. Hermánn Silva, Hospital Apoyo, Iquitos

Introduction

This is a collaborative study between the Instituto de Investigación Nutricional (Lima), the Asociación Civil Selva Amazónica (Iquitos), the Hospital Apoyo (Iquitos) and McGill University (Canada). These institutions have collaborated for several years working to improve the health and nutrition of the population of Iquitos. As this time, we are conducting a study to determine the effect of changing the moment that the umbilical cord is clamped and cut following delivery which will be implemented at the Ministry of Health and at Hospital de Apoyo Iquitos. This study has already received ethics approval from the McGill University Health Centre, the Instituto de Investigación Nutricional and Hospital Apoyo de Iquitos.

What is the study about?

The moment that the umbilical cord is clamped and cut after delivery can have an effect in preventing anemia in the baby. This study will determine if a difference exists in the prevalence of anemia between the babies whose umbilical cord is clamped and cut according to the current hospital practice compared to the new procedure of clamping and cutting the cord slightly later. We would like to invite you and your baby to participate in this study.

Who can participate in this study?

Women giving birth in Hospital Apoyo, Iquitos and their newborn babies can participate in this study. Participants are in the early stages of labour when they are recruited. A total of 224 mother and their babies will participate in this study.

What will I be asked to do if I participate in this study?

When you arrive at the hospital, before your delivery, when you are comfortable, we will ask you a few questions about yourself and about your pregnancy. We will take a sample of blood from your finger to determine if you are anemic. Later, in the delivery room, a

nurse-midwife will be present during your delivery and will note the time that the umbilical cord is clamped and cut following delivery.

When your baby turns 4 months old, we will visit you and your baby at your home and take a sample of blood from your finger and from your baby's heel or finger (whichever you prefer) to determine if either of you are anemic. We will also ask you a few questions about your baby's diet from birth up until 4 months. We will be in contact with you at your home in two to three weeks to confirm the appointment for your 4 month follow-up.

Additionally, we would like to consult your medical charts and that of your baby to obtain additional information about your delivery, your baby, and your baby's CREDE visits.

How much time will my and my baby's participation in the study take?
Your participation would start when you arrive at the hospital (before your delivery) and will end when your baby is 4 months old. Your baby would participate until he/she is 4 months old. The first interview (before your delivery) and the follow-up visit will both take between 15 and 20 minutes.

Are there any risks to me or my baby from participating in the study?
There are no foreseeable risks to you or your baby from participating in this study. However, it is possible that you and/or your baby feel slight discomfort when the blood sample is taken from your finger (or in your baby's case, the finger or heel).

Will I receive any benefit(s) from participating in the study?
If you and your baby participate in this study, we will give you the results of your and your baby's anemia test. If you or your baby are anemic, we will refer you to the hospital or a health centre so that you can receive iron supplements for treatment. If the health establishment does not have iron supplements, we will provide them with such. The results from this study will help us to determine the best time to clamp and cut the umbilical cord and to improve clinical practice with regard to delivery in Iquitos to benefit the baby.

Can I withdrawal myself or my baby from the study at any moment?
Your participation and that of your baby in this study is completely voluntary and you can decide not to participate or to withdrawal from the study at any moment without penalty to the attention provided to you and your baby from health services. You can decide to terminate the interview at any moment simply by informing the project personnel.

Confidentiality: Who will know my and my baby's identity?
Your identity and all of the personal information you provide us with during this study will be treated as confidential. We will not reveal your name or that of your baby's to anyone that is not a member of the research team, unless we are required to do so by Peruvian law. Your names will not appear in any publications or information.

Who can I call if I have questions about the study?
If you have any questions about the study, please communicate with Evelyn Burga, the research assistant for this study (Asociación Civil Selva Amazónica, Urbanización Jardín 27 – Iquitos, cell: 965995814). If you decide to participate in this study, you can contact Evelyn at any moment if you have any questions or concerns. We will provide you with an appointment card for your follow-up visit with Evelyn's contact information. You may also contact the investigators of this project: Dr. Martín Casapía of ACSA, Urbanización Jardín 27 - Iquitos, telephone 65-236-277 or Dr. Mary Penny or Nutr. Hilary Creed-Kanashiro of the IIN in Lima at 01-3496023, or Dr. Eduardo Verne, member of the IIN ethics committee, also at 01-3496023.

Voluntary Declaration of Informed Consent
I, after having received information on all aspects of the study described in this form and after having:

- ✓ Had the opportunity to ask questions about the project,
- ✓ Understood the procedures that will take place,
- ✓ Understood that the information will be treated as confidential without revealing my identity in any information or publication of the results of this study, and
- ✓ Received satisfactory answers to all of my questions and doubts,
- ✓ I freely and voluntarily accept participation of myself and my baby in this study
- ✓ I authorize the personnel of the project to review my and my baby's medical charts at Hospital Apoyo

:

_____ _____
Participant:(woman) Name Signature

_____
Participant:(baby) Name

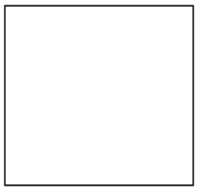
_____ _____
Father/Mother/Guardian: Name Signature
(for participants less than 18 years old)

_____ _____
Project Personnel: Name Signature

_____/_____/_____
Date (dd/mm/yy)

In the case that the mother cannot sign

The witness who signs this Consent Form declares that all aspects of this project have been explained in detail, including the purposes and procedures, the risks and benefits, confidentiality of information and all aspects described in this form to the participant mentioned previously, who has accepted by manner totally voluntarily to participate in this project. All of the participant's questions have been answered satisfactorily. To confirm, the participant has stamped her fingerprint in this form.

Fingerprint 

Date: ___ / ___ / ___
(dd / mm / yy)

Participant's Name _____

Witness Signature _____

Name of witness _____

ASSENT

In the case of a girl less than 18 years old, we ask that you provide your signed assent in the same document as the signature of consent provided by your mother, father, guardian or spouse (over 18 years of age).

_____
Signature of the minor (participant)

_____
Name of the minor (participant)

APPENDIX 2: QUESTIONNAIRE 1 – LABOUR ROOM

LABOUR ROOM QUESTIONNAIRE

ID: ☐ - ☐☐ - ☐☐
obst. day participant

Interviewer's Initials: _____

Name of Participant: _____ / _____
LAST NAME(S) First Name

1) Date of Birth: ____ /____ /_____ Age: ☐☐
dd mm yyyy

2) Marital Status:

Married or common law ☐
Single ☐

3) Address: _____

District: _____ Urban ☐ Peri-urban ☐ Rural ☐ Telephone #: _____

4) Education:

Primary: Incomplete ☐ → # Grades completed ____ Complete ☐
Secondary: Incomplete ☐ → # Grades completed ____ Complete ☐
Post-secondary: Incomplete ☐ → # Years completed ____ Complete ☐
None: ☐

5) Do you work? No ☐ ¿What do you do? _____
Yes ☐ ¿Occupation? _____

6) Have you attended pre-natal care visits? No ☐ Yes ☐ → Hospital ☐ Name: _____ *Number?* ☐
Health Center ☐ Name: _____ *Number?* ☐
Health Post ☐ Name: _____ *Number?* ☐
Private Clinic ☐ Name: _____ *Number?* ☐

7) Did you take any iron supplements (eg. iron sulfate) during your pregnancy? No ☐ Yes ☐

8) Did you take any other vitamins/medication during your pregnancy? No ☐ Yes ☐ _____

Maternal Hb Level: _____mg/dL Anemia? No ☐ Yes ☐ (Anemia < 110 mg/dL)

2 Week Visit:

morning ☐
afternoon ☐

4 Month Follow-up Appointment:

Date: _____ ____ /____ /_____ morning ☐ afternoon ☐
day of the week dd mm yyyy

Location: _____

1

	Carnet	Registry	Clinical History
9) Gestational age (weeks)	_____	_____	
10) Date of last menstrual period	____ / ____ / _____ dd mm aaaa		
11) Number of PNC visits	_____		
12) Location of PNC visits	_____		
13) Number of pregnancies	_____	_____	
14) Number of live births	_____	_____	
15) Previous abortions?	No ☐ Yes ☐ _____ _____	No ☐ Yes ☐ _____ _____	
16) Stillbirths?	No ☐ Yes ☐ _____ _____		No ☐ Yes ☐ _____ _____
17) Previous pre-term deliveries?	No ☐ Yes ☐ _____ _____	No ☐ Yes ☐ _____ _____	
18) Medical conditions?	No ☐ Yes ☐ _____ _____		No ☐ Yes ☐ _____ _____
19) Currently taking medication?			No ☐ Yes ☐ _____ _____ _____
20) Treatment during labour or delivery?			No ☐ Yes ☐ _____ _____ _____
21) Diagnoses during labour or delivery?			No ☐ Yes ☐ _____ _____ _____

Comments: _____

_____

_____

_____

2

APPENDIX 3: QUESTIONNAIRE 2 – DELIVERY ROOM

DELIVERY ROOM QUESTIONNAIRE

ID: ☐ - ☐☐ - ☐☐
obst day participant

Initials of person OBSERVING TIME: _____

Name of participant: _____/_____
LAST NAME(S) First Name(s)

1) Date of delivery: _____ /_____ /_____
dd mm yyyy

2) Time of entry into delivery room: _____ : _____
hour min

3) Health personnel **attending delivery**:

Nurse-Midwife ☐ *name:* _____
Nurse ☐ *name:* _____
Doctor ☐ *name:* _____
Nurse-Midwife Intern ☐ *name:* _____
Nurse Technician ☐ *name:* _____
Other ☐ *specify:* _____ *name:* _____

4) Health personnel **clamping the cord:**

Nurse-Midwife ☐ *name:* _____
Nurse ☐ *name:* _____
Doctor ☐ *name:* _____
Nurse-Midwife Intern ☐ *name:* _____
Nuse Technician ☐ *name:* _____
Other ☐ *specify:* _____ *name:* _____

RECORDING OF TIME

Delivery of Shoulders	Delivery of ENTIRE BODY	Clamping of UMBILICAL CORD
0:00	____:____ ____:____ ____ min sec 1/100 s	____:____ ____:____ ____ min sec 1/100 s

Was the cord still pulsating at the moment it was clamped? No ☐ Yes ☐ Unknown ☐

5) What instrument was used to stop the blood flow? _____

6) Oxitocin administered? No ☐ Yes ☐

7) Did the infant have any medical problems or complications? No ☐ Yes ☐ _____

_____

8) Observations: _____

_____

1

REGISTRY

9) Newborn characteristics:

- Birthweight: _____ g
- Length: _____ cm
- Sex: Male ☐ Female ☐
- Any medical conditions? No ☐ Yes ☐, *specify*: _____

_____

_____

- APGAR score at 5 minutes: _____

10) Time of birth: _____ : _____
hour min

11) Date and time of discharge: _____ /_____ /_____ _____ : _____
dd mm yyyy hour min

APPENDIX 4: QUESTIONNAIRE 3 – 2 WEEK FOLLOW-UP

2 WEEK FOLLOW-UP QUESTIONNAIRE

ID: ☐ - ☐☐ - ☐☐
obst day participant

Interviewer's Initials:_____

Date: _____ / _____ / _____
dd mm yyyy

Participant's Name:_____/_____
LAST NAME(S) First Name(s)

Address:_____

_____

1) Baby's Name: _____ / _____
LAST NAME(S) First Name(s)

2) How is your baby's health? _____

_____

_____

_____

3) 4 Month Follow-up Appointment: _____ / _____ / _____ morning ☐
dd mm yyyy afternoon ☐

4) *Counsil*: "IF YOU WANT A HEALTHY AND STRONG BABY, YOU MUST PROVIDE HIM/HER WITH YOUR BREASTMILK FOR THE FIRST 6 MONTHS"

5) Comments regarding address (location of house):_____

_____

_____

Confirm: Urban ☐ Peri-urban ☐ Rural ☐

6) Other comments: _____

_____

APPENDIX 5: QUESTIONNAIRE 4 – 4 MONTH FOLLOW-UP

4 MONTH FOLLOW-UP QUESTIONNAIRE

ID: ☐-☐☐-☐☐

Interviewer's Initials: _____

Date of Interview: _____ / _____ / _____
dd mm yyyy

Participant's Name: _____ / _____
LAST NAME(S) First Name(s)

Address: _____

_____

1) ¿Do you have your baby's carnet? No ☐ Yes ☐

If **yes** → *use the carnet to answer this question*

If **no** → *ask*:

Have you taken your baby to Growth and Development control visits (during the consultation, the nurse plays with your baby)?

At 1 month: No ☐ Yes ☐ At 4 months: No ☐ Yes ☐

At 2 months: No ☐ Yes ☐

Name of Health Establishment: _____

2) ***From Carnet:*** Appropriate/recommended immunizations up to 3 months old?

		Yes	No
Newborn	Tuberculosis (BCG)		
	Antihepatitis (HvB)		
2 months	Antipolio (1st)		
	DPT (1st)		
	HVB (1st)		
	HiB (1st)		
	Rotavirus (1st)		
3 months	Antipneumococcal (1st)		

If carnet not available → How many immunizations has your baby had since birth? _____

1

3) Does your baby suffer from any medical problem(s)? No ☐ Yes ☐ → Specify: _____

_____

4) Has your baby taken any vitamins, supplements or medications since birth? No ☐ Yes ☐

If yes → Specify *(if she doesn't remember, ask if she still has the bottle(s))*:

1- _____

2- _____

3- _____

→How old was your baby when he/she was given each for the first time?

1- ____:____ ____ 2- ____:____ ____ 3- ____:____ ____
months days months days months days

5) Has your baby received any treatment for Jaundice (skin or eyes take the colour yellow)?

No ☐ → *go to question 6*

Yes ☐ → Was your baby put under a light? No ☐ Yes ☐

If no → Specify treatment: _____

_____

→ How long after birth? ____ : ____ ____
months days

6) Have you *(mother's name)* nursed *(baby's name)*?

No ☐ → *go to question 7*

Yes ☐ → Do you still nurse your child? No ☐ →Until what age did you nurse (name)?

_____ months

Yes ☐ → *go to question 7*

7) ¿Have you given your baby any tea or infusion/water?

No ☐ → *go to question 8*

Yes ☐ → Since what age? (In months or day) ____ : ____ ____
months days

→ Do you still give the tea or infusion/water? No ☐ →Until what age did you give it?

(In months or days) ___ : ___ ___
months days

Yes ☐ → *go to question 8*

8) ¿Have you given your baby any other milk?

No ☐ → *go to question 9*

Yes ☐ → Since what age? (In months or days) ____ : ____ ____
months days

→ What milk did you give? *Evaporated* ☐ *Cow's milk powder* ☐ *Baby Formula* ☐

Other ☐ _____ *Unknown* ☐

→ Do you still give this milk? No ☐ → Until what age did you give it?

(In months or days) ____ : ____ ____
months days

Yes ☐ → *go to question 9*

9) What was the first thing that you gave your baby to drink or try that was neither milk nor tea? (That was a liquid or drink other than milk or tea)
What drink was it?

fruit juice	☐	*yogurt*	☐	*unboiled water*	☐
soft drink	☐	*refresco / lemonade*	☐	*banana porridge/ mingado rice/ quaker*	☐
boiled water	☐	*masato*	☐	*broth*	☐
other	☐	*unknown*	☐		

→ At what age did you give this for the first time? **(In months and/or days)** ____ : ____ ____
months days

nothing ☐ → *go to question 10*

10) Have you given your baby masato? No ☐ → *go to question 11*

Yes ☐ → At what age did you give it for the first time?

(In months and/or days) ____ : ____ ____
months days

11) What was the first food that you gave *(name)* to eat or try?

What food was it? _____

broth	☐	*soup*	☐	*puree*	☐	*cookies w/ drink*	☐
porridge	☐	*food boiled and mashed*	☐	*segundo*	☐	*other*	☐
unknown	☐						

→ At what age did you give this food for the first time? ____ : ____ ____
months days

nothing ☐ → *go to question 12*

ID: ☐ - ☐☐ - ☐☐

12) Have you *(the mother)* taken any **iron** supplements or vitamins (anything for anemia) since your baby was born? No ☐ Yes ☐

If yes → Specify *(if she doesn't remember, ask her if she has the bottle(s))*:

1- _____

2- _____

3- _____

→ How long after birth was it when you took each for the first time?

1- ____:____ ____ 2- ____:____ ____ 3- ____:____ ____
months days months days months days

13) ***In the last 2 weeks …***

a) In the last 2 weeks, has your baby had any cough or difficulty breathing?
No ☐ Yes ☐

If yes → For how long? _____ days

→ What action did you take? _____

b) In the last 2 weeks, has your baby had diarrhea (**3 or more** liquid depositions (take the form of a container))? No ☐ Yes ☐

If yes → For how long? _____ days

→ What action did you take? _____

→ Was there blood in the feces? No ☐ Yes ☐

c) In the last 2 weeks, has you baby had a fever? No ☐ Yes ☐

If yes → Did you measure it with a thermometer? No ☐ Yes ☐

→ For how long? _____ days

→ What action did you take? _____

d) In the last 2 weeks, has your baby had any problems with his/her ears?
No ☐ Yes ☐

If yes → Ear pain? No ☐ Yes ☐

→ Ear discharge? No ☐ Yes ☐

→ What action did you take? _____

→ Medical diagnosis? No ☐ Yes ☐ _____

e) In the last 2 weeks, has your baby received breastmilk? No ☐ Yes ☐

If yes → Received breastmilk exclusively? No ☐ Yes ☐

→ How many times in 24 hours?_____ times

→ During the night? No ☐ → What does he/she drink/eat during the night?

_____

Yes ☐

f) In the last 2 weeks, what food or drink have you given your baby to eat or drink that is not your breastmilk?

_____

_____

_____

14) Infant weight: _____ kg

→ SECA scale ☐ Clock scale ☐ From carnet ☐ From EESS ☐

15) Infant length: _____ cm

→ Length board ☐ From carnet ☐ From EESS ☐

Maternal Hb Level: _____mg/dL

Anemia? No ☐ Yes ☐ (Anemia < 120 mg/dL; WHO 1999)

Infant Hb Level: _____mg/dL

Heel prick ☐

Finger prick ☐

Comments: _____

_____

_____

ID: ☐ - ☐☐ - ☐☐