Effect of timing of umbilical cord clamping on iron status in Mexican infants: a randomised controlled trial

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Summary

Background **Delayed clamping of the umbilical cord increases the infant's iron endowment at birth and haemoglobin concentration at 2 months of age. We aimed to assess whether a 2-minute delay in the clamping of the umbilical cord of normal-weight, full-term infants improved iron and haematological status up to 6 months of age.**

Methods **476 mother-infant pairs were recruited at a large obstetrics hospital in Mexico City, Mexico, randomly assigned to delayed clamping (2 min after delivery of the infant's shoulders) or early clamping (around 10 s after delivery), and followed up until 6 months postpartum. Primary outcomes were infant haematological status and iron status at 6 months of age, and analysis was by intention-to-treat. This study is registered with ClinicalTrials.gov, number NCT00298051.**

Findings **358 (75%) mother-infant pairs completed the trial. At 6 months of age, infants who had delayed clamping had significantly higher mean corpuscular volume (81·0 fL *vs* 79·5 fL 95% CI −2·5 to −0·6, p=0.001), ferritin (50·7 μg/L *vs* 34·4 μg/L 95% CI −30·7 to −1·9, p=0·0002), and total body iron. The effect of delayed clamping was significantly greater for infants born to mothers with low ferritin at delivery, breastfed infants not receiving iron-fortified milk or formula, and infants born with birthweight between 2500 g and 3000 g. A cord clamping delay of 2 minutes increased 6-month iron stores by about 27–47 mg.**

Interpretation **Delay in cord clamping of 2 minutes could help prevent iron deficiency from developing before 6 months of age, when iron-fortified complementary foods could be introduced.**

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See Comment page 1956

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Introduction

In developing countries, up to 50% of children become anaemic by 12 months of age.[1] Risk factors for iron deficiency include low birthweight, maternal iron deficiency during pregnancy, and male sex.[1,2] Iron deficiency anaemia during infancy and childhood is of particular concern because of potentially detrimental effects on development, some of which might be irreversible even after iron treatment.[3] Prevention of iron deficiency and anaemia during infancy is therefore a priority. However, the types of interventions that can be implemented during this time are limited.

Delayed clamping of the umbilical cord might prevent or slow the onset of iron deficiency by increasing the infant's iron endowment at birth. Compared with early clamping, a delay of around 2–3 min provides an additional 40 mL of blood per kg of bodyweight.[4–6] For a 3·2 kg infant with a haemoglobin concentration of 170 g/L at birth, this additional blood amounts to an additional 75 mg of iron added to iron stores, sufficient to meet the needs of a 6–11 month-old infant for more than 3 months.[7]

The results of previous intervention studies on delayed clamping are mixed, and few followed up infants beyond 3 months of age.[8–14] Our main objectives, therefore, were to assess whether delayed clamping improves haematological and iron status at 6 months of age in a large sample of full-term, normal-birthweight infants, and whether the effect of delayed clamping is greater in subgroups with increased risk of developing iron deficiency.

Methods

Study design and participants

The study site was the Hospital de Gineco Obstetricia 4 "Luis Castelazo Ayala", a large obstetrics hospital in Mexico City, Mexico, belonging to the Mexican Institute of Social Security. From our observations of 76 vaginal deliveries, the average umbilical cord clamping time at the hospital was 17·7 (SD 7·2) s.

The study was approved by institutional review boards at the University of California, Davis, the National Institute of Public Health of Mexico, the Mexican Institute of Social Security, and by the hospital research committee. Primary outcomes were infant haematological and iron status at 6 months of age. Secondary outcomes included estimated maternal blood loss at delivery, newborn haematocrit (packed-cell volume), and reported clinical jaundice between birth and 14 days of age. Women were eligible to participate if they were not in advanced labour when admitted and did not have any of the following basic characteristics: planned delivery by caesarean section; pregnancy of 36 weeks or less, or 42 weeks or more, as established by the date of last menstrual period or estimated delivery date; or multiple gestation. Women were also excluded if they had a diagnosis of any of the following medical conditions during the current pregnancy: pre-eclampsia or eclampsia (also in any previous pregnancies), haemorrhage needing clinic or hospital admission, placental abnormalities, anomalies or Down's syndrome of the fetus, or had a diagnosis at any time of diabetes (all types), hypertension, cardiopathies, or chronic renal

disease. Behavioural criteria for exclusion were if the women were not planning to breastfeed for at least 6 months, if they smoked at all during pregnancy, were unwilling to return for follow-up visits at the same hospital, or were participating in another research study at the hospital. Written informed consent was obtained from mothers who were eligible and willing to participate.

We based the target sample size on examining the main effect of cord clamping on haemoglobin at 6 months of age, and the two-way interactions between treatment and sex of infant, initial infant iron status, and initial maternal iron status. We estimated that the increase in haemoglobin at 6 months attributable to delayed cord clamping would be 3·8 g/L (half the difference at 2 months of age between early and late clamped infants in Guatemala).[9] On the assumption of an SD of 7·2 g/L,[15] a level of significance of 5% for the main effect and 10% for interactions, 80% power, and allowing for 20% attrition, the target sample size was 448.

We randomly assigned participants to early or delayed clamping in blocks of four using the random digit generator in Microsoft Excel. Numbered index cards labelled with the assigned treatment were sealed in corresponding numbered opaque envelopes and ordered sequentially. Before delivery, the next envelope was opened by recruitment staff, who informed the obstetrician and the paediatrician attending the delivery of the assigned treatment. Study staff did not inform the mother of her assignment; however, because of the nature of the intervention, the mothers may have been aware of which intervention they were randomised to. If a mother already assigned to a treatment group later became ineligible (eg, method of delivery changed to caesarean), her assigned treatment card was not re-used.

At all births, a staff member was present to measure the time from the delivery of the infant's shoulders to the cord clamping. Obstetricians were asked to maintain the infant at the level of the mother's uterus until the cord was clamped. In the early-clamping group, the staff member informed the obstetrician when 10 s had passed and that the cord should be clamped. In the delayed-clamping group, the staff member informed the obstetrician of the passing time until 2 min (the intended clamping time). If the physician felt that the infant or the mother needed attention that could not be given while the infant remained attached to the umbilical cord, the cord was clamped before 2 min; however, original group assignment was not changed. Exclusion criteria applied after birth were low birthweight (<2500 g) and major congenital malformations.

Venous blood samples were taken from the mother before delivery and from the umbilical vein in the placenta immediately after its delivery. Obstetricians estimated the amount of maternal blood loss during the delivery and immediately afterwards as normal, high, or severe. Infant weight and length were measured shortly after birth. Recruitment and follow-up staff were trained and standardised (a formal process of repeated measurements to ensure comparability between individuals) in standard anthropometric techniques.

At 4–8 h after birth, a capillary blood sample was taken from the infant's heel. Before discharge from the hospital, study staff recorded medical chart information on complications or medical procedures for the infant and mother, and completed a socioeconomic and demographic interview with the mother.

At 3 and 14 days postpartum, a lactation consultant collected data on breastfeeding and maternal and newborn morbidity, and provided lactation guidance by telephone. At 2, 4, and 6 months after birth, infant morbidity (frequency and duration of symptoms, number of clinic and hospital visits), and dietary data (food-frequency, dietary recall, use of nutritional supplements) were obtained at hospital visits or by phone interview. When visits were at the hospital, we measured infant weight and length. Maternal height and weight were measured at 2 months postpartum. At 6 months, a venous blood sample was taken from the infant. Staff members responsible for collection of follow-up data did not participate in recruitment procedures, and were masked to treatment group.

As soon as possible after collection, whole blood was analysed with a haemoglobinometer (HemoCue, Lake Forest, CA, USA) and Coulter Ac T 8 automated analyser (Beckman Coulter, Fullerton, CA, USA) for haemoglobin and haematological profile, respectively. The calibration of both analysers was checked daily with appropriate controls. All venous blood samples were centrifuged (15 min, 1090×g, room temperature) and plasma and red blood cells were separated and stored at –20°C until transferred on dry ice to the University of California, Davis, for analysis of plasma ferritin (by radioimmunoassay; DPC, Los Angeles, CA, USA), transferrin receptor (by enzyme-linked immunoassay; Ramco, Stafford, TX, USA), and C-reactive protein (by radial immunodiffusion; Binding Site, Birmingham, UK). The individual responsible for the plasma analyses did not participate in any other aspect of the study and was masked to treatment group.

***Panel*: Body iron calculation**

HgbI=bodyweight (kg)×blood volume (L/kg)×Hgb concentration (g/L)×3·47 $\frac{\text{mg Fe}}{\text{g Hgb}}$

with blood volume estimated to be 0·075 L/kg bodyweight.[16] Non-HgbI was estimated as 20% of HgbI.[17] Stored iron was calculated from the following equation derived for infants:[18]

Stored body iron=($\log_{10}$plasma ferritin–1·345)/0·0439×bodyweight (kg)

The log ratio of transferrin receptor to ferritin, an index of depleted iron stores[19] was calculated for mothers and infants. Maternal total body iron (TBI) was calculated from the equation:[20]

TBI (mg/kg)=–[log(transferrin receptor/ferritin ratio)–2·8229]/0·1207

Hgb=haemoglobin.

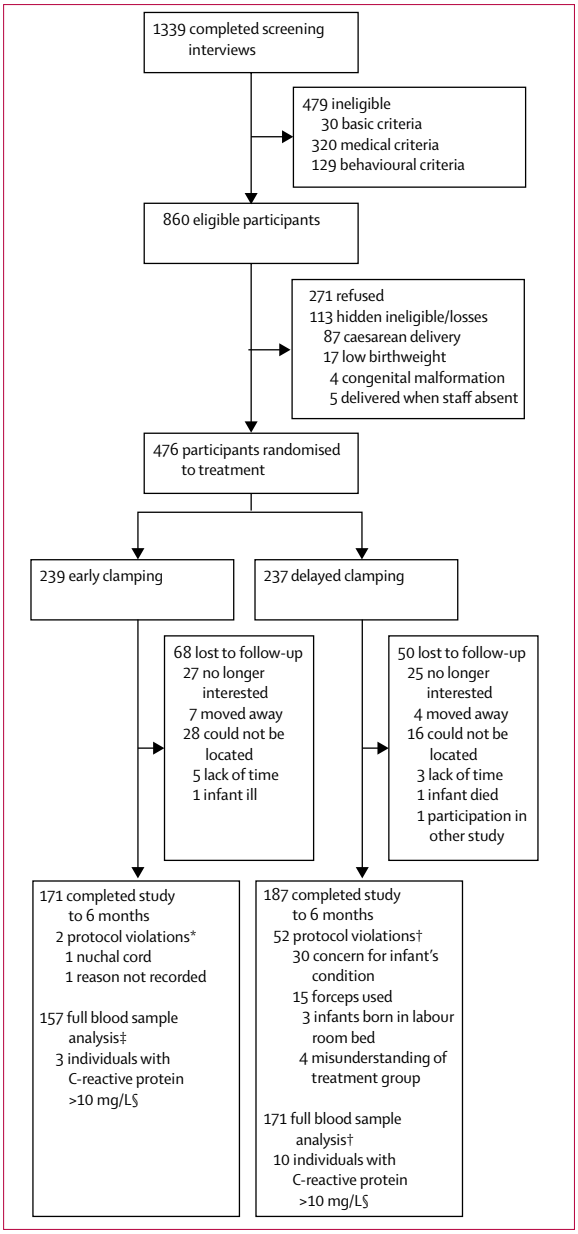

Figure 1: **Trial profile**

*Cord clamping more than 30 s after delivery of infant's shoulders. Individual clamping times were 38 s and 40 s for these two infants. †Cord clamping at less than 100 s after delivery of infant's shoulders. Mean time of cord clamping for this subgroup was 27·5 (19·7) seconds, range 7–97 seconds. ‡Included haematological profile, indicators of iron status (ferritin and transferrin receptor), and C-reactive protein. §Ferritin data for individuals with C-reactive protein >10 mg/L were excluded from analysis.

	Early clamping (n=171)	Delayed clamping (n=187)
Maternal socioeconomic and demographic characteristics		
Maternal age (years)	25·9 (5·6)	25·8 (5·8)
Maternal weight (kg)*	62·2 (10·7)	60·3 (9·0)
Maternal height (cm)	153·9 (5·2)	154·8 (5·7)
Maternal body-mass index (kg/m^2)	26·2 (4·1)	25·2 (3·5)
Number of children born alive (including study child)	1·8 (0·8)	1·8 (0·9)
Maternal education (years completed)	11·6 (3·1)	11·5 (3·3)
Marital status (% with partner)	91%	88%
Employed	51%	41%
Received prenatal care	98%	98%
Took prenatal iron supplements	91%	92%
Household belongings index†	8·6 (2·1)	9·0 (2·1)
Predelivery maternal blood sample		
Haemoglobin (g/L)	128 (15)	131 (13; n=185)
Anaemic‡	27%	18%
Haematocrit (fraction of 1)	0·400 (0·042; n=155)	0·406 (0·037; n=169)
Mean corpuscular volume (fL)	90·1 (7·1; n=155)	92·1 (5·9; n=170)
C-reactive protein >10 mg/L§	21%	25%
Ferritin (μg/L)¶‖	9·8 (6·0–18·1; n=132)	12·7 (7·4–22·5; n=135)
Iron deficiency**	62%	49%
Iron deficiency anaemia††	29%	21%
Transferrin receptor (μg/mL)‖	4·5 (3·5–5·7; n=166)	4·4 (3·4–5·5; n=179)
Ratio of transferrin receptor/ferritin‖	490·9(208·1–926·5;n=131)	348·8 (143·7–742·5; n=135)
Total body iron (mg/kg)	1·5 (3·9; n=131)	2·3 (3·6; n=135)
Infant characteristics		
Sex (% male)	52%	50%
Gestational age (weeks)‡‡	39·0 (1·1)	38·8 (1·1)
1 min Apgar score ≥7	95%	97%
Birthweight (g)	3196 (362)	3182 (369)
Birthweight <3000 g	29%	32%
Birth length (cm)	48·8 (1·6)	48·7 (1·6)

Data are number (SD) unless stated otherwise. Sample size in parentheses for comparisons with missing data. The following maternal characteristics differed between groups: body-mass index, employment, haemoglobin, mean corpuscular volume, and ferritin. *Maternal height and weight obtained at 2-month follow-up visit. †Additive index based on presence in home of: indoor bathroom, radio, microwave, television, blender, video cassette recorder, digital versatile disk player, computer, refrigerator, washing machine, telephone, motorcycle, and car. ‡Defined as haemoglobin <122 g/L. §Assessed for all maternal plasma samples analysed. ¶Excluding ferritin concentration data for all maternal samples with C-reactive protein concentrations >10 mg/L (n=79). ‖Median, the 25th percentile (quartile 1), and the 75th percentile (quartile 3) of untransformed data presented, data were $\log_{10}$ transformed for analyses. **Defined as ferritin <12 μg/L. ††Defined as ferritin <12 μg/L and haemoglobin <122 g/L. ‡‡Maternal report of estimated delivery date, verified with medical chart.

Table 1: **Baseline characteristics of participants who completed the study**

Infant body iron at 6 months of age was estimated as the sum of the iron contained in haemoglobin (HgbI), in storage, and in myoglobin and enzymes (non-HgbI). HgbI, stored iron, and maternal total body iron (TBI) were calculated as shown in the panel.

Maternal iron deficiency was defined as ferritin less than 12 μg/L,[21] and anaemia as haemoglobin less than 122 g/L, on the basis of the term pregnancy cutoff of 110 g/L,[22] adjusted for the altitude of Mexico City (2240 m).[23] High haematocrit in the newborn infant was defined as more than 0·70. Although the generally accepted definition of polycythaemia in venous blood is haematocrit more than 0·65, haematocrit from peripheral capillary blood is typically 15% higher (range 5–25%).[24] The more conservative haematocrit cutoff of more than 0·65 was used to refer newborn infants to hospital paediatricians for a clinical examination and a venous blood sample (obtained and analysed by hospital staff). We defined anaemia at 6 months as haemoglobin less than 117 g/L, on the basis of the recommended cutoff of 105 g/L at 6 months of age[25] adjusted for altitude.[23] Iron deficiency was defined as

	Early clamping (n=171)	Delayed clamping (n=187)	Difference or relative risk (95% CI)*	p
Delivery				
Time of cord clamping (seconds)	16·5 (6·4)	93·8 (44·2)	−79·1 (−85·7 to −72·5)	<0·0001
Maternal bleeding				
Normal (%)	94%	94%	1·1†(0·5 to 2·6)	0·83
High (%)	5%	5%		
Severe (%)	1%	1%		
Placental blood sample				
Ferritin (µg/L)‡	131·2	141·9	−10·7 (−28·8 to 7·4)	0·50
Quartile 1, quartile 3	84·6, 180·7 [159]	100·0, 199·2 [169]		
Newborn capillary sample				
Haemoglobin (g/L)	193 (23) [171]	199 (24) [183]	−7 (−12 to −2)	0·007
Haematocrit (1)	0·595 (0·072 [155])	0·620 (0·075 [166])	−0·025 (−0·041 to −0·008)	0·003
Haematocrit >0·70 (%)	8%	13%	0·6§ (0·3 to 1·2)	0·15
Neonatal jaundice				
Clinical jaundice (%)¶	22/160 (14%)	30/172 (17%)	0·79§ (0·48 to 1·31)	0·36

Data are number (SD) unless stated otherwise. Sample size in brackets for comparisons with missing data. *Treatment effect for continuous variables expressed as difference in means, and for binary outcomes calculated as the relative risk. †Relative risk calculated for severe and high blood loss categories combined versus normal blood loss. ‡Excluding one placental blood sample that had a corresponding C-reactive protein concentration of more than 10 mg/L. Median (IQR) of untransformed data presented, data were $\log_{10}$ transformed for analyses. §Relative risk (early clamping/delayed clamping). ¶Diagnosis in hospital or maternal report at 3 or 14 days of age.

Table 2: **Delivery, placental, and newborn characteristics**

ferritin less than 9 µg/L.[25] For women and infants, iron deficiency anaemia was defined as both ferritin and haemoglobin below the respective cutoffs. Ferritin values were excluded if the C-reactive protein value was more than 10 mg/L (indicative of an acute phase response, which can raise ferritin concentrations despite iron deficiency).[22]

Statistical analysis

All statistical analyses were done with SAS version 8.02. The individual responsible for primary data analysis was masked to treatment group during the first phase of analyses. Student *t* tests and χ^2 tests were used to compare baseline characteristics between treatment groups, and between participants who completed the study and those who were lost to follow-up. Outcome variable distribution was assessed for normality, and transformations were done when necessary.

Data were analysed as intention-to-treat. The haematological and iron indices at 6 months were analysed as continuous variables with analysis of covariance (ANCOVA) and as categorical variables with logistic regression. Baseline or follow-up characteristics that were not randomly distributed between treatment groups, and were also correlated with infant iron outcomes (maternal ferritin and employment), were controlled for in these analyses. The prespecified subgroups chosen for examination of interaction effects were based on maternal iron deficiency at delivery, iron status of infants at birth, and sex. Additional subgroups were based on a birthweight of between 2500 and 3000 g versus more than 3000 g, infant breastfeeding status at 6 months, and use of iron-fortified formula or milk at 6 months. The level of significance used was $p \leq 0 \cdot 05$ for main effects and $p < 0 \cdot 10$ for interaction terms.

	2 months		4 months		6 months	
	Early clamping (n=142)	Delayed clamping (n=160)	Early clamping (n=147)	Delayed clamping (n=166)	Early clamping (n=171)	Delayed clamping (n=187)
Any breastfeeding (%)	127 (89%)	137 (86%)	116 (79%)	124 (75%)	108 (63%)	125 (67%)
Breastfeeding, not receiving formula or iron-fortified milk (%)	59 (42%)	68 (43%)	60 (41%)	68 (41%)	54 (32%)	60 (32%)
Receiving any infant formula (%)	82 (58%)	91 (57%)	81 (55%)	91 (55%)	100 (58%)	111 (59%)
Receiving iron-fortified milk (%)	4 (3%)	1 (1%)	7 (5%)	9 (5%)	22 (13%)	26 (14%)
Receiving iron-fortified solid foods (%)*	8 (6%)†	2 (1%)	35 (23%)	29 (17%)	108 (63%)	134 (71%)
Receiving meat/fish/poultry/eggs (%)‡	0	0	13 (9%)§	6 (4%)	93 (54%)	90 (48%)
Receiving vitamin/mineral supplements (%)¶	27 (19%)	28 (18%)	23 (16%)	38 (23%)	34 (20%)	42 (22%)
Receiving supplements containing iron (%)	6 (4%)	10 (6%)	5 (3%)‖	15 (9%)	13 (8%)	20 (11%)

*Irrespective of breastfeeding or formula feeding status. Included industrialised, commercially available infant cereals, fruits, vegetables, and mixed vegetables, meat, and grain. †p=0·03. ‡Irrespective of breastfeeding or formula feeding status. Included beef, pork, poultry, fish, eggs, and any products made from these foods. §p=0·05. ¶Includes all types of supplements. ‖p=0·04.

Table 3: **Follow-up dietary data**

Role of funding source
The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 20, 2003, and July 31, 2004, 1339 women were screened for eligibility and 476 mother-infant pairs were assigned to treatment (more than the target sample size, to compensate for a higher than anticipated rate of attrition during follow-up; figure 1). At 6 months, 171 and 187 mother-infant pairs remained in the early-clamping group and delayed-clamping group, respectively. Baseline characteristics for women and infants who completed the study did not differ significantly from those of individuals lost to follow-up.

For those who completed the study, there were significant differences between treatment groups in maternal employment, body-mass index, haemoglobin, mean corpuscular volume, and ferritin, but not in any other maternal or infant characteristics (table 1). The results presented here are for the 358 mothers and infants who completed the study. At the time of delivery, 60% of mothers had iron deficiency and 25% had iron deficiency anaemia (table 1). Estimated maternal bleeding at delivery was not significantly different between groups (table 2). Mean cord-clamping time was under 20 s in the early-clamping group versus over 90 s in the delayed-clamping group (table 2). The cord was clamped after 30 s for two early-clamping infants and at less than 100 s for 52 delayed-clamping infants (figure 1). These 52 delayed-clamping infants did not differ in baseline maternal, socioeconomic, or infant characteristics from other infants in the delayed-clamping group.

Initial iron status (as measured by placental blood ferritin) did not differ between groups (table 2). Newborn capillary haemoglobin and haematocrit (measured at around 7 h of age) were significantly higher in the delayed-clamping group; however, the percentage of infants with capillary haematocrit more than 0·70 was not. In the subsample of infants for whom a venous haematocrit was also obtained (n=28), none in the early-clamping group and two in the delayed-clamping group had values greater than 0·65. The venous haematocrit of these two delayed-clamping infants was just above the cutoff (0·66 and 0·68) and no clinical symptoms or complications were reported while in hospital; neither infant was referred for additional follow-up in the days after birth. Clinical jaundice (as diagnosed in the hospital or by maternal report at 3 and 14 days of age) was not different between groups. In the days after discharge, each group had one hospitalisation for jaundice, and three early-clamping infants and four delayed-clamping infants attended a medical clinic for jaundice.

Early-clamping infants were more likely to consume infant foods fortified with iron at 2 months and products from the meat, fish, poultry, and eggs group at 4 months, whereas delayed-clamping infants were more likely to receive iron supplements at 4 months of age (table 3). There were no significant differences in growth between

	Unadjusted analyses				Analyses adjusted for maternal ferritin and employment*			
	Early clamping	Delayed clamping	Difference or odds ratio† (95% CI)	p	Early clamping	Delayed clamping	Difference or odds ratio† (95% CI)	p
Haemoglobin (g/L)	127 (9; n=171)	127 (11; n=185)	−0·1 (−2·2 to 1·9)	0·88	127 (9; n=132)	126 (11; n=133)	0·6 (−1·8 to 3·1)	0·61
Anaemic (%)‡	15%	13%	1·1§ (0·6 to 2.1)	0·65	13%	13%	1·0§ (0·5 to 2·0)	0·95
Haematocrit (l)	0·388 (0·031; n=146)	0·389 (0·033; n=159)	−0·001 (−0·009 to 0·006)	0·69	0·391 (0·028; n=113)	0·389 (0·032; n=116)	0·002 (−0·006 to 0·010)	0·60
Mean corpuscular volume (fL)	79·5 (3·7; n=148)	80·6 (3·2; n=160)	−1·1 (−1·8 to −0·3)	0·007	79·5 (3·8; n=115)	81·0 (3·2; n=117)	−1·5 (−2·5 to −0·6)	0·001
Mean corpuscular haemoglobin (pg)	25·9 (1·6; n=146)	26·2 (1·3; n=159)	−0·3 (−0·6 to 0·03)	0·07	25·8 (1·5; n=113)	26·3 (1·3; n=116)	−0·5 (−0·9 to −0·1)	0·007
Mean corpuscular haemoglobin concentration (g/L)	326 (12; n=146)	325 (9; n=159)	1 (−2 to 3)	0·56	325 (9; n=113)	325 (9; n=116)	−0·2 (−3 to 2)	0·87
Ferritin (µg/L) ¶ ||	34·9 (32·2; n=154)	46·7 (37·7; n=161)	−11·8 (−19·5 to −4·1)	0·001	34·4 (31·2; n=118)	50·7 (39·8; n=117)	−16·3 (−25·4 to −7·1)	0·0002
Iron deficiency (%)**	8%	2%	3·65§ (1·2 to 11·3)	0·02	7%	1%	5·05§ (1·0 to 24·1)	0·05
Iron deficiency anaemia (%)††	4%	0	..	0·01‡‡	.. §§	..	..	..
Transferrin receptor (µg/mL)	4·8 (1·4; n=157)	4·6 (1·3; n=171)	0·2 (−0·1 to 0·5)	0·30	4·6 (1·4; n=120)	4·6 (1·4; n=122)	0·03 (−0·3 to 0·4)	0·87
Ratio transferrin receptor/ferritin¶	129·3 (145·3; n=154)	93·8 (89·7; n=161)	35·5 (8·7 to 62·3)	0·002	127·9 (145·8; n=118)	86·5 (81·8; n=117)	41·4 (11·2 to 71·6)	0·001
Stored iron (mg)	32·0 (58·6; n=154)	51·9 (51·8; n=161)	−19·9 (−32·1 to −7·6)	0·002	30·7 (57·1; n=118)	57·6 (50·5; n=117)	−26·9 (−40·9 to −12·9)	0·0003
Body iron (mg)	318·7 (68·8; n=154)	339·5 (61·3; n=161)	−20·9 (−35·3 to −6·4)	0·005	316·3 (67·2; n=118)	343·4 (60·6; n=117)	−27·1 (−43·9 to −10·4)	0·002
Body iron (mg/kg bodyweight)	44·0 (8·7; n=154)	47·2 (7·7; n=161)	−3·2 (−5·0 to −1·4)	0·007	43·9 (8·7; n=118)	47·9 (7·8; n=117)	−4·0 (−6·1 to −1·8)	0·0003

Data are mean (SD) unless stated otherwise. Sample size in parentheses for comparisons with missing data. Analyses were also done adjusting for maternal haemoglobin instead of ferritin (because of the reduction in sample size when adjusting for maternal ferritin, due to exclusion of cases with high C-reactive protein) along with maternal employment. Results were similar except for the interaction between birthweight and treatment, which was not significant. *Data are adjusted means and SD of unadjusted means. †For continuous outcomes, treatment effect expressed as the difference between group means. For binary outcomes, treatment effect expressed as the odds ratio. ‡Defined as haemoglobin <117 g/L. §Odds ratio (early clamping/delayed clamping). ¶Ferritin and the ratio transferrin receptor/ferritin were $\log_{10}$ transformed for analysis. ||Excluding ferritin concentration data for all infant samples with C-reactive protein concentrations >10 mg/L (n=13). **Defined as ferritin concentration <9 µg/L. ††Defined as both haemoglobin <117 g/L and ferritin concentration <9 µg/L. ‡‡ Fisher's exact test. §§Logistic regression model could not be fit because of zero-cell.

Table 4: **6-month infant haematological and iron status by treatment group**

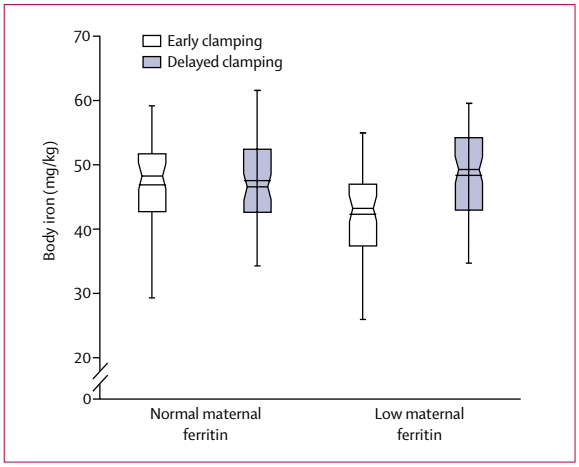

Figure 2: **Box-and-whisker plot of two-way interaction effect of treatment group and maternal ferritin on infant body iron (mg/kg) at 6 months of age**
Boxes represent the inter-quartile range (25th to 75th percentile), and whiskers indicate the 5th and 95th percentiles for the unadjusted data. The notch in each box represents CI about the median, represented by horizontal line at the middle of the notch. Additional horizontal line represents the mean of each subgroup. Treatment difference (early clamping *vs* delayed clamping, adjusted for maternal ferritin and employment) in body iron in infants born to mothers with normal ferritin concentrations was –0·8 mg/kg (95% CI –5·0 to 3·4 mg/kg). Treatment difference (adjusted) in body iron in infants born to mothers with low ferritin concentrations was –6·5 mg/kg (–10·2 to –2·8 mg/kg). Low maternal ferritin <12 µg/L; normal maternal ferritin ≥12 µg/L. p=0·008 for interaction term.

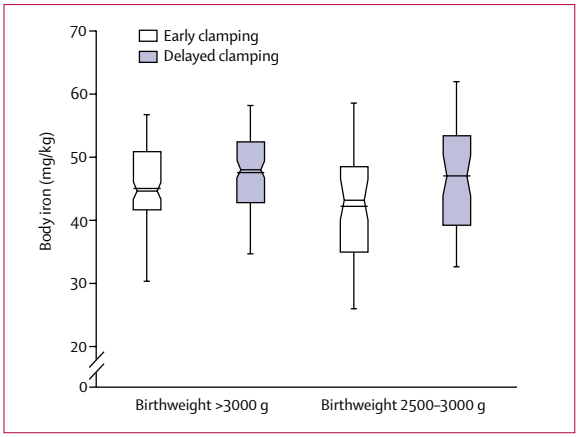

Figure 3: **Two-way interaction effect of treatment group and infant birthweight on infant body iron (mg/kg) at 6 months of age**
Treatment difference (early clamping *vs* delayed clamping, adjusted for maternal ferritin and employment) in body iron in infants with birthweight more than 3000 g was –2·5 mg/kg (95% CI –5·9 to 0·9 mg/kg). Treatment difference (adjusted) in body iron in infants with birthweight between 2500 g and 3000 g was –7·1 mg/kg (–11·9 to –2·4 mg/kg). p=0·04 for interaction term.

groups from birth to 6 months (weight-gain: early clamping 4054 [698] g *vs* delayed clamping 4080 [759] g, p=0·7; length gain: early clamping 17·1 [1·8] cm *vs* delayed clamping 16·9 [1·7] cm, p=0·3) or morbidity (data not shown).

At 6 months, delayed-clamping infants had significantly higher mean corpuscular volume, ferritin, body iron and stored iron, and a lower ratio of transferrin receptor to ferritin, than early-clamping infants (table 4). Significantly more infants in the early-clamping group had iron deficiency and iron deficiency anaemia than those with delayed clamping, although the percentage of infants with low haemoglobin was not different. Results did not differ when analyses were done either with or without control for baseline differences in maternal ferritin and employment. The results also did not change when additional analyses were done by actual clamping time rather than by intention-to-treat.

There were significant interaction effects between treatment group and maternal ferritin, infant birthweight, and infant feeding practices. Delayed clamping had a greater effect in infants born to mothers with low ferritin than those born to mothers with normal ferritin with respect to infant body iron (mg/kg, figure 2), stored iron (normal ferritin and early clamping *vs* normal ferritin and delayed clamping=–6·81 mg [95% CI –34·2 to 20·6]; low ferritin and early clamping *vs* low ferritin and delayed clamping=–42·7 mg [–66·9 to –18·6, p=0·01]), ferritin (p=0·01), and transferrin receptor/ferritin ratio (p=0·005). Delayed clamping increased infant ferritin (p=0·05) and body iron (mg/kg; figure 3) significantly more in infants with birthweight between 2500 and 3000 g than in those with birthweight more than 3000 g. Delayed clamping increased body iron more in infants still breastfed at 6 months than in those no longer breastfed (p=0·09 for interaction term; data not shown). Similarly, delayed clamping had a greater effect in infants not receiving any iron-fortified formula or milk at 6 months than in those receiving such products with respect to ferritin (p=0·06), body iron (figure 4), stored iron (iron-fortified formula or milk and early clamping *vs* iron-fortified formula or milk and delayed clamping=–16·9 mg [95% CI –38·6 to 4·9]; no formula or milk and early clamping *vs* no formula or milk and delayed clamping=–46·8 mg [95% CI –77·3 to –16·3]) and

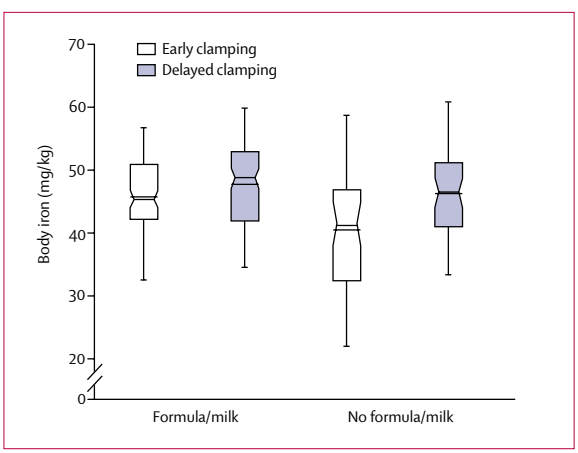

Figure 4: **Two-way interaction effect of treatment group and infant feeding practices on body iron (mg/kg) at 6 months of age**
Treatment difference (early clamping *vs* delayed clamping, adjusted for maternal ferritin and employment) in body iron in infants given iron-fortified formulas or milks was –2·6 mg/kg (95% CI –5·9 to 0·7 mg/kg). The treatment difference (adjusted) in body iron in infants not given any iron-fortified formulas or milks was –6·6 mg/kg (–11·3 to –2·0 mg/kg). p=0·07 for interaction term.

transferrin receptor to ferritin ratio (p≤0·06 for all interaction terms). There were no significant interaction effects between treatment group and placental ferritin concentration or sex of the infant, even though both of these variables were significantly correlated with iron status at 6 months (data not shown).

Discussion

Our results show that a 2-minute delay in clamping of the umbilical cord at birth significantly increases infant iron status at 6 months of age as measured by ferritin, mean corpuscular volume, transferrin receptor to ferritin ratio, estimated total body iron, and storage iron. They accord with the previous findings of shorter-term studies of delayed clamping that showed improved haematological and iron status at 2–3 months.[9,10] Our study shows that this effect lasts beyond 3 months, and is enhanced in infants born to iron-deficient mothers, in infants born weighing between 2500 and 3000 g, and in infants not receiving infant formulas or iron-fortified milk.

Our original hypothesis was that delayed cord clamping would increase infant haemoglobin at 6 months of age, as we had seen previously in younger infants.[9] However, although mean corpuscular volume and mean corpuscular haemoglobin were significantly different between groups, haemoglobin was not. The absence of difference in haemoglobin is probably because of the high iron status of this sample of infants. Haemoglobin and other erythrocyte values are late-stage markers of iron deficiency, and an effect on haemoglobin due to delayed clamping would not be expected until iron stores are depleted. Most infants in the study were receiving iron-fortified formulas or milk, many began consuming infant foods fortified with iron before 6 months of age, and a few infants were also receiving iron supplements. Previous research has shown that before 6 months of age, the mechanisms present in adults for regulating iron absorption might not yet be mature, and thus both haemoglobin and ferritin increase with increased iron intake, even in the presence of adequate iron stores.[15] The high intake of dietary iron in our population therefore could have masked haematological differences between groups.

Another consideration is that, of the anaemic infants, only 16% had low ferritin, indicating a potential role for other factors in the development of anaemia. Although iron deficiency at this age is usually the major cause of anaemia, other micronutrient deficiencies could contribute. In a similar population in Mexico City, the overall prevalence of anaemia was 28% (defined as haemoglobin <105 g/L with values adjusted for altitude) at 6–11 months of age but only a third of anaemia was attributable to iron deficiency, and around 5% of infants had folic acid deficiency.[26] Another possibility is that the haemoglobin adjustment for altitude could overestimate the prevalence of anaemia. In Bolivian preschool children living at an average altitude of about 2000 m above sea level, Cook and colleagues[27] noted that 45% were anaemic based on low haemoglobin, but only 12% were anaemic based on low total body iron.

Of the six previous intervention studies on the effects of early clamping versus delayed clamping on infant haematological and iron status, four were included in a recent systematic review.[8] In Guatemalan infants, clamping when cord pulsations stopped increased haemoglobin at 2 months of age.[9] In India, one study showed a beneficial effect of delayed clamping on haemoglobin and ferritin in 3-month-old infants born to anaemic mothers,[10] whereas another showed a non-significant trend for higher ferritin in the delayed-clamping group.[11] In the systematic review, the authors calculated a 15% reduction in absolute risk for anaemia at 2 or 3 months of age from the results of the former two studies.[8] Three studies, two from 1960 in South Africa[12] and China[13] and one from 1941 in the USA,[14] did not show significant differences in haemoglobin between early-clamped and delayed-clamped infants at 3, 6, and 9 months of age; however, no other indicators of iron status were measured. The heterogeneity of outcomes and the time at which they were assessed in previous studies,[9–14] as well as variability in the actual cord clamping interventions, makes a meta-analysis of all the existing trials difficult.

Despite a history of research beginning early in the 20th century, debate still exists as to the best time to clamp the cord, and recommendations and obstetric practices are varied. Concern about neonatal polycythaemia and jaundice are commonly mentioned deterrents to delayed clamping. We recorded no significant increase in the risk of these two conditions with delayed clamping, in agreement with other studies of term, normal-weight infants in which either of these outcomes has been measured.[6,9,28,29] However, we excluded mothers with any condition associated with risk of higher haematocrit in the newborn, which could limit the generalisability of our findings, and we did not measure serum bilirubin directly.

In 2002, early cord clamping was included as part of "active management of the third stage of labour" to prevent postpartum haemorrhage,[30] although more recent references do not include early cord clamping as a component of this protocol.[31] As far as we are aware there is no evidence that early clamping, independently from other active management techniques (administration of an oxytocic drug with delivery of the infant and controlled cord traction during delivery of the placenta), is associated with less maternal haemorrhage, or that delayed clamping would be contraindicated when other active management techniques are used. In our study, there was no significant difference between groups in the physician's assessment of maternal bleeding, although our sample size might have been too small to adequately detect modest differences in this outcome. A limitation of our study is that we were not able to quantitatively measure maternal blood loss.

Much of the variability in cord clamping recommendations and practices probably stems from a scarcity of research on long-term outcomes and inconsistency in definitions of delayed cord clamping. In more informal or

low-resource birth settings, a clock or stopwatch might not be readily available or visible, and a physiological definition, such as clamping at the end of cord pulsations or at placental separation, could be preferable. Our experience in a large obstetrics hospital with many births per day indicates that a clamping delay of around 2 min is both feasible and effective. This interval coincides roughly with the end of cord pulsations (between 1 and 3 min),[8] and allows for most of the blood transfer from the placenta to the infant (most of which takes place in the first minute).[32]

In most developing countries, the prevalence of maternal iron deficiency is high, as is the incidence of birthweight less than 3000 g; both conditions are associated with poorer infant iron status. Particularly for these infants, delayed clamping is an invaluable opportunity to increase an infant's iron endowment at birth, thereby ensuring adequate iron status until other interventions can be more easily implemented.

Contributors

C M Chaparro was responsible for study development, staff training, and development of study tools, study management, data analysis, and manuscript writing. L M Neufeld participated in study development and management, and manuscript writing. R Eguia-Líz Cedillo assisted with project management logistics and oversaw and coordinated participation of hospital pediatricians. G Tena Alavez assisted with project management logistics and oversaw and coordinated participation of hospital residents and obstetricians. K G Dewey was responsible for study development, review of study tools and data collection procedures, and oversight of data analysis and manuscript writing.

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Conflict of interest statement

We declare that we have no conflict of interest.

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studies on the prevalence of mental disorders in children were completed in Baghdad, Mosul, and Dohuk. This was part of a group of 15 research studies. In the first study we found that, of 600 primary school children from 16 schools in Baghdad (mean age 10·3 years), 283 (47%) reported exposure to a major traumatic event during the previous 2 years. 84 (14%) had post-traumatic stress disorder (PTSD; 21 of 225 boys [9%], 63 of 375 girls [17%]).

In the second study in Mosul, 1090 adolescents from eight secondary schools were screened for mental disorders. 323 (30%) had symptoms of PTSD (127 of 481 boys [26%], 196 of 609 girls [32%]). There was a higher rate of PTSD in the older adolescents. 301 (92%) of the ill adolescents had not received any treatment.

The third study, in Dohuk, involved 120 working street children and 120 school children. The prevalence of mental disorders was higher in the working children than the school children (42 [36%] *vs* 16 [13%]).[2]

There is an urgent need to address the needs of all children by means of preventive programmes such as life skills education and care programmes within the school setting.

Monaf Al Jadiry, T M Yasamy, Hussain Rustam, and R Srinivasa Murthy were technical advisers to the research studies. We declare that we have no conflict of interest.

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Timing of umbilical cord clamping

Camila Chaparro and colleagues (June 17, p 1997)[1] did a meticulous randomised trial on the timing of umbilical cord clamping and infants' iron status. However, we wondered why they departed from CONSORT guidelines on analysis by intention-to-treat.

The trial profile shows two groups of excluded mothers: one group being excluded before eligibility was assessed and one after, but both before randomisation. Because allocation was revealed only after delivery, it is not clear to us how the 87 caesarean deliveries or five women who delivered when staff were absent were exempt from randomisation. As stated in the paper, a planned caesarean clearly rendered women ineligible, and indeed those with planned caesareans were excluded before randomisation.

However, there seems to be a second group of women who were considered eligible at screening but who subsequently received an emergency caesarean. Since no caesarean births seem to be included as outcomes of the mothers who were reportedly randomised, these women have been excluded from the intention-to-treat analysis. The methods section explains only that "if a mother assigned to a treatment group later became ineligible (eg, method of delivery changed to caesarean), her assigned treatment card was not re-used." Of the remaining 113 "hidden ineligible/losses" we presume that the 17 low birthweight babies and four congenital malformations were based on prenatal diagnoses, but it is still not clear why the 271 who refused were not deducted from the 1339 screened, rather than the 860 eligible.

Intention-to-treat analysis could make an important difference to the findings.

We declare that we have no conflict of interest.

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Authors' reply

We appreciate the opportunity to respond to the comments by Ruth Bonita-Beaglehole and Laragh Gollogly regarding intention-to-treat analysis in our study. When planning the study, we carefully considered this part of the design, given that the CONSORT guidelines do not explicitly state what to do about participants whose eligibility cannot always be assessed before randomisation. We would like to clarify the situation here, since length limitations prohibited a full discussion of these issues in our original article.

The nature of the intervention was such that randomisation had to be done in anticipation of the delivery, and not in the delivery room after the infant's birth. Delayed cord clamping was not the standard practice in the study hospital before our study. Consequently, the obstetricians and paediatricians involved in the study asked to be notified of treatment assignment before the delivery of each participant to avoid confusion in the delivery room and to permit extra towels to be warmed and available to dry and hold each infant receiving delayed cord clamping. However, this created two complications: (1) some mothers assigned to a treatment group subsequently became ineligible, generally because they delivered by an unplanned caesarean section, and (2) some infants assigned to a treatment group became ineligible after delivery because they had a low birthweight or congenital malformation (both of which could only be definitively