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Elective caesarean – is it enough to delay cord clamping 30 seconds to ensure sufficient iron stores at four months of age – a prospective observational study

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Elective caesarean, cord clamping and iron stores at 4 mo

Abstract

Objective To assess iron stores in infants born after elective caesarean section and a 30 seconds delay of umbilical cord clamping as compared to children born vaginally after early (≤10 seconds) or delayed (≥ 180 seconds) cord clamping.
Design Prospective observational study
Setting Swedish county hospital
Population 64 infants born after elective caesarean section, as a historical control;166 early clamped and 168 delayed clamped after vaginal birth.
Methods Blood and iron status were estimated by blood samples collected at birth, 48-96 h after birth, four and 12 months of age.
Primary and Secondary Outcome Measures Ferritin (primary), other indicators of iron status, and haemoglobin, at 4 months of age
Results At four months of age infants after elective caesarean had more positive iron status as compared to vaginally born infants subjected to early cord clamping, shown by higher adjusted mean difference of ferritin concentration (39 µg/L [95% CI 10 to 60]) and mean cell volume (1.8 fL [95% CI 0.6 to 3.0]); and lower levels of transferrin receptors (-0.39 mg/L [95% CI -0.69 to -0.08]). No differences were seen between infants born after elective caesarean and delayed clamped vaginally born infants at four months. No differences were found between groups at 12 months of age.
Conclusions Waiting to clamp the umbilical cord for 30 seconds after elective caesarean results in higher iron stores at four months of age compared to early cord clamping after vaginal birth, and seems to ensure iron status comparable with those achieved after 180 seconds delayed cord clamping after vaginal birth.

•Keywords: umbilical cord, cord clamping, pregnancy, vaginal birth, caesarean section, iron deficiency

Strengths and Limitations

- The present study report new data on iron status and haematological parameters in term infants after CS up to 12 months of life as compared to vaginal deliveries and in relation to time to umbilical cord clamping.
- The prospective design of the study helps to reduce possible sources of bias and confounding factors, but not being a randomised controlled trial, the interpretation of the study's results are limited by its possibilities of bias and confounding factors.
- Nutrition and growth rate is expected to influence iron status at a later age, and we could control for these data.
- Only 35-40% of eligible pregnancies were included, why readiness to participate may be a confounding factor.

Introduction

During the two last decades, evidence has accumulated regarding the benefits of waiting to clamp the umbilical cord for two to three minutes in term births.[1-3] Research has mainly included vaginal births, omitting the rising numbers of elective caesarean sections (CS) globally.

Newborns subjected to delayed cord clamping (DCC) have higher haemoglobin (Hb) concentrations at 24-48 hours of life, and improved iron stores at four to six months.[4] After delivery, the newborn may receive up to 30 ml/kg blood from its placental circulation within three to five minutes,[5 6] contributing 75 mg iron which is equivalent to the infant's requirements for three to four months.

Iron deficiency is associated with impaired development,[7] and a main reason for adopting DCC has been to reduce iron deficiency. Recently, we have shown that DCC is associated with improved fine motor skills at four years of age.[8]

Less is known about the placental transfusion after elective CS before start of labour. Pioneering physiological studies in the 1960s by Lind et al showed that there was less placental transfusion after CS than after early cord clamping.[9] More recently it was demonstrated that it is possible to harvest a higher volume of blood to stem cell banks after CS, also pointing to a reduced placenta to child transfusion after CS.[10]

Elective CS can be performed for several reasons, including maternal, fetal and preferential factors.[11] The obstetrician has to weigh possible benefits against possible disadvantages for mother and child. To ensure that this decision is evidence based, research must contribute to a wider understanding of long-term consequences of these actions.

Retrospective studies have shown associations between delivery by elective CS and later asthma and gastroenteritis as well as weak associations with diabetes mellitus type 1 and certain cancer forms.[12] Among the prospective studies found, short-term outcomes are

reported, such as an increased risk for neonatal intensive care and neonatal mortality after CS as compared to vaginal delivery during the first week of life.[13] CS were associated with anaemia at 12 and 58 months in two large longitudinal Chinese birth cohorts,[14] and a systematic review and meta-analysis found that caesarean section compared with vaginal delivery was associated with a reduced placental transfusion and poor iron-related hematologic indices in both cord and peripheral blood.[15]

We set out to prospectively study infants after elective CS, and follow them with the same protocol used for our cord clamping trial, using the vaginally born children as a historic control group.

Our hypothesis was that iron stores measured by ferritin at 4 months in children delivered by CS with cord clamping after 30 seconds would be lower than children born vaginally after DCC and thus similar to those born vaginally after ECC.

Method

Study design

This is a prospective observational study of children delivered by CS, using reference data from a study[3] of children randomized to DCC vs ECC after vaginal delivery.

Setting

During the period of June 6, 2010 and February 29, 2012, women planned for elective CS were approached by the midwife, informed of the study and asked for consent. The historical control group consisted of 382 term newborns included in a randomised, controlled trial between April 16, 2008 and May 22, 2009. The results from this trial have been reported in several papers.[3 8] The study was performed at the Hospital of Halland, Halmstad, Sweden.

Participants

Pregnant women were eligible if they met the following criteria: non-smoking; normal pregnancy (no pre-eclampsia, no diabetes, no prolonged rupture of membranes or signs of

infection) and term pregnancy (gestational age 37+0 to 41+6 weeks). The mother also had to understand Swedish well enough to participate in the study. Exclusion criteria were serious congenital malformations, syndromes, or other congenital diseases that could affect the outcome measures. For the elective CS group, additional eligibility criteria was planned CS. For the reference groups, eligibility also included being randomized to ECC or DCC in the performed randomized trial, having the intervention as allocated (per protocol), and being born vaginally.

After delivering the infant, the obstetrician placed the baby on the mother's thighs or beside her on the operation table and waited 30 seconds to clamp the umbilical cord, as advised by the present routine at the hospital. The timing at 30 seconds had been chosen by the board of obstetricians at the hospital before commencement of the current study. The timing of the clamping was noted. After clamping, blood samples for blood gas evaluation was taken routinely from the placental side of the umbilical vessels, and for the research project samples were taken for analysis of blood status; Hb and mean cell volume (MCV), and iron status; Transferrin saturation (TS), soluble transferrin receptor (sTfR) and ferritin.

Apgar scores, birth weight, length, and head circumference were recorded according to routine. At one and six hours after birth, the midwife assessed the infants well-being, and prospectively noted whether there were any respiratory difficulties (grunting, presence of nostril flaring, respiratory frequency above 60 breaths per minute and intercostal retractions) as well as if the baby had been breastfed.

At the time for routine blood sampling for metabolic screening at two days post partum, additional blood samples were gathered, i.e. blood and iron status, C-reactive protein (CRP) and bilirubin.

At three months of age, a letter was sent to ask the parents to return with their child at four months for sampling of blood status, iron status and CRP. Again, at eleven months of age, an

invitation to return at 12 months was sent. Blood samples for blood status, iron status and CRP were obtained.

Blood was collected in EDTA tubes (BD Vacutainer, Plymouth, UK) for blood status, and in serum separator tubes (BD Vacutainer) for iron status, bilirubin, and CRP.

Complete blood counts were analysed with an automated haematology analyser (Sysmex XE 2100, Sysmex, Kobe, Japan). Iron status indicators, bilirubin, and CRP were analysed with Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

At four months, mothers reported their infant's feeding habits in a three-day diary and infant´s length and weight was measured.

Definitions:

At two days

Anaemia: Hb <145 g/L[16], Polycythaemia: hematocrit >0.65[17].

At four months

Anaemia: Hb <105 g/L,[18] Iron deficiency: two indicators of iron status outside reference range (ferritin <20 µg/L,[18] MCV <73 fL,[19] TS <10%,[20] sTfR >7 mg/L[3]).

At 12 months

Anaemia: Hb <110 g/L. Iron deficiency: two indicators of iron status outside reference range (ferritin <12 µg/L, MCV <70 fL, and TS <10%, sTfR >5.6 mg).[21]

Outcomes

The primary outcome was infant serum ferritin at four months of age. Secondary outcomes included infant Hb and iron status (measured as serum ferritin, transferrin saturation, soluble transferrin receptors, reticulocyte Hb, mean cell volume) at four and 12 months of age.

In this paper we report neonatal morbidity (including anaemia, polycythaemia, and respiratory symptoms). Other secondary outcomes are beyond the scope of the current paper, and will be reported separately; Symptoms of infection during the first four months; IgG levels at birth

and at four months; psychomotor development at four and 12 months of age assessed by the Ages and Stages Questionnaire.

Confounders

To be able to compare the included children with the historical reference group and to ensure that inclusion criteria were met, data on the mother (reported illness, medication, parity, weight, height, smoking habits, blood group Rhesus factor status, and haemoglobin concentration at the time of admission to antenatal care) was obtained from medical records. Nutrition and growth may affect iron status at four months; to adjust for this we controlled feeding habits at four months of age. As birth weight can be affected by the size of placental transfusion, we decided to only use length as a proxy for growth from birth to four and 12 months.

Sample size

Our hypothesis was that the difference in Ferritin between DCC and CS at four months would be the same as shown between DCC and ECC in a previous study,[3] that is a difference in log10 Ferritin between 2.07 and 1.90 with a SD of 0.34. To show this difference, a sample size of 63 was needed.

Statistical analysis

For group comparisons of continuous variables, we used one-way analysis of variance (ANOVA) for variables with normal distribution and Bonferroni as post hoc test for pairwise comparisons. Categorical variables were compared between pairwise groups by using Fisher's exact test and across all three groups with Pearson Chi-square test. Ferritin concentration was log10 transformed for analysis. We used SPSS, version 22.0 (IBM, Armonk, NY, USA).

For adjusted analyses, analysis of covariance (ANCOVA) was used for test scores with Bonferroni post hoc test for pairwise comparisons. For adjustment variables, we decided to choose those background variables that differed between groups with a $p<0.1$. A $p<0.05$ was considered significant.

Results

During the inclusion period, 505 infants were born after CS, 98 (19 %) preterm and 34 (7 %) post term, Figure 1. Among the term newborns (n=373), CS were classified as acute (174, 47%), elective with a medical reason (145, 39%), and elective with no medical reason (54, 14%). From the 199 elective term CS, 26 could not be included due to maternal disease (diabetes, n=12), preeclampsia, n=6, intra uterine growth restriction (IUGR), n=6 and combination of preeclampsia and IUGR, n=2. Additionally, five women smoked at admission to antenatal care, leaving 168 possible for inclusion. One hundred and four declined participation, resulting in the inclusion of 64 deliveries with elective CS. Furthermore, 166 ECC and 168 DCC controls were available for analysis, Figure 1. We did compare data between the 64 included EC with available data from the 104 who declined inclusion and no significant differences in maternal age, gestational age, infants´ birth weight, length or head circumference was found, nor any differences in Apgar score or umbilical blood gases, pointing to our sample being representative for the whole cohort (results not shown).

At four months 59 (92.2%) infants in the elective CS group returned for blood sampling between October 6, 2010 and June 28, 2012. Corresponding blood samples had been obtained from 153 (92.2%) in the ECC group and from 156 (92.9%) in the DCC group between August 8, 2008 and October 1, 2009. At 12 months, 56 (87.5%) infants returned in the elective CS group between May 31, 2011 and Feb 20, 2013, while in the control group, 144 (86.7%) samples were available from the ECC and 149 (88.7%) from the DCC group (collected between April 8, 2009 and May 21, 2010) (Figure). The sex distribution was comparable between groups; 28 (44%) were males in the CS group, 83 (50%) in the ECC group and 73 (44%) in the DCC group, p=0.44.

For baseline characteristics, see Table 1. The gestational age in the elective CS group was lower than in the ECC group, -1.2 weeks (95% CI -1.5 to -0.8, P<0.001), and DCC group, -1.1 weeks (95% CI -1.5 to -0.8, P<0.001). The maternal age was also higher in the elective CS group than the in DCC group, 2.2 years (95% CI 0.5 to 3.9, p=0.005). As described in the method section, we chose to include mothers' age and gestational age as adjustment variables accordingly.

Apgar scores at one and five minutes were comparable between groups.

Haemoglobin was lower in umbilical cord blood in the elective CS group, when compared to the ECC group, adjusted mean difference (AMD) -13.5 (95% CI -20.3 to -6.6, P<0.001) g/L and the DCC group, AMD -8.5 g/L (95% CI -15.4 to -1.5, P=0.01). However, at 48-72 hours of age, the Hb level did not differ between groups. The Hb level after delivery increased more in the DCC (31.3 g/L [7.8], n 121) and CS (35.6 g/L [16.8], n=38) groups as compared to the ECC group (11.5 g/L [16.8], n=121), p<0.001, indicating a larger placental transfusion.

At four months, differences in ferritin, MCV, and transferrin receptors indicated better iron status in the CS group compared to the ECC group (Table 2). The proportions of infants having abnormal values for iron status parameters did not differ between the CS group, and the ECC and DCC groups, respectively (Table 3).

At 12 months, no differences between groups in iron status or blood status could be shown (Table 2 and 3).

Auxiliary analysis

Postnatally, children born after CS were more likely to not having been breastfed at one hour after delivery as compared to the ECC, relative risk (RR) 2.1 (95% CI 1.5 to 2.9) and DCC groups, RR 2.5 (95% CI 1.7 to 3.5). The CS group had a higher risk of respiratory distress at six hours after birth compared to ECC, RR 3.4 (95% CI 1.1 to 10.5) and DCC, RR 4.4 (95%

CI 1.4 to 14.9). Respiratory distress at one hour of age and breast-feeding frequency at six hours did not differ between groups.

At four months, exclusive breast-feeding was equally prevalent among the groups, CS 27 (47%), ECC 78 (52%) and DCC 84 (56%), p=0.45. Exclusive breast-feeding correlated positively correlated to the infants' serum ferritin level (r=0.144, p=0.007), but not to any other blood sample analysed for at four months. If 'exclusive breast-feeding' was included in the ANCOVA, results was not changed for any variable in any significant way, except for transferrin saturation, where the elective CS group attained a significant higher value than ECC: AMD 2.0% (95% CI 0.0 to 4.0), p=0.049.

Length and weight at four and 12 months of age were comparable across groups, also when adjusted for gestational age. Also weight and length gain increase from birth was comparable between groups at four and 12 months of age (data not shown). Adding 'length gain' into the adjusted model did not alter differences in any significant way.

Discussion

Main findings

The findings in this prospective observational study indicate that in infants born after elective CS with umbilical cord clamping after 30 seconds, iron stores at four months are comparable to iron stores in vaginally born infants subjected to DCC (≥180 s), and improved compared to vaginally born infants subjected to ECC (≤10 s).

Strengths and Limitations

The present study report new data on iron status and haematological parameters in term infants after CS, as compared to vaginal deliveries and in relation to time to umbilical cord clamping. Haematological and iron status after different timing of umbilical cord clamping have previously been reported in several studies.[4] Among available studies with four months or longer follow-up on iron stores, three excluded infants born after CS[2 3 22] while one

included CS but did not separate results from vaginal birth.[23] The prospective design of the study helps to reduce possible sources of bias and confounding factors, but not being a randomised controlled trial, the interpretation of the study's results are limited by its possibilities of bias and confounding factors. These include in particular unidentified differences in baseline characteristics between groups, including prenatal maternal as well as perinatal and postnatal infant influences. Except for iron stores at birth, nutrition and growth rate is expected to influence iron status at a later age, and we could control for these data that did not alter the main outcomes. In all three groups, only 35-40% of eligible pregnancies were included, why readiness to participate may be a confounding factor. Data from the included EC were not significantly different from with available data from those who declined inclusion, indicating similarity between included and 'declined inclusion' pregnancies.

Interpretation

Previous studies have implied less placental transfusion after CS. Consequently; our findings are not in line with the relatively scarce literature on this subject. One explanation to our finding that CS rather improves iron stores compared to ECC at four months of age could be that the obstetrician actually waited 30 second to clamp the cord, timing to umbilical cord clamping after CS has usually not been reported in other studies. Another potentially contributing factor to the improved iron stores is that infants born after elective CS have a lower blood pressure due to less circulating adenosine and catecholamines,[24 25] facilitating a faster blood transfusion from the placenta. Unfortunately, we did not record the time for the first breath/cry, but earlier reports indicate that most new-borns had commenced breathing before the cord was clamped in the CS group.[26]

Haemoglobin in the umbilical cord blood sample was significantly lower after elective CS compared to ECC and DCC, a finding in coherence with a recent systematic review and meta-

analysis.[15] This finding suggests that umbilical cord Hb is not a reliable marker of iron status in newborns.

Our study give support to waiting for 30 seconds before clamping after CS, as we could not demonstrate any negative effect on iron homeostasis compared to the vaginally born groups. Our findings might imply that whatever negative consequences on the child's health CS is associated with, waiting for 30 seconds to clamp the cord reduces those that could possibly be explained by a less placental blood transfusion.

Conclusion

Erickson-Owens et al have suggested umbilical cord milking as a possible procedure to facilitate the placental transfusion after CS in term infants.[27] Our results suggest that the less invasive method of a 30 s DCC might be sufficient to ensure the placental transfusion after elective CS. Large observational studies, most preferably prospective with vaginally born matched controls, are indicated and warranted.

In summary, in this study comparing infants born after elective CS with children born vaginally after DCC and ECC, we could demonstrate that infants born after elective CS had iron stores similar to DCC and better than ECC at four months of age.

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Disclosure of Interests

None.

Contribution to Authorship

OA, LHW and MD planned the study. OA was responsible for staff training, study management and data collection with support from LHW and MD. OA, LHW and MD

analysed the data. OA drafted the manuscript. All authors revised the manuscript and accepted the final version. Ola Andersson is the guarantor.

Details of Ethics Approval

The study was approved by the regional ethical review board at Lund University (2008/41, amendment 2009/344).

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Data sharing statement

None available

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Table/Figure Caption List

Table 1. Baseline characteristics and early outcomes after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Table 2. Laboratory status at four and 12 months of age after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Table 3. Proportion of infants with iron status indicators outside reference limits at four and 12 months after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Figure 1. Trial profile. Flow diagram.

Elective caesarean, cord clamping and iron stores at 4 mo

Table 1. Baseline characteristics and early outcomes after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

							Mean Difference (95% CI)[b]			
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[d]	CS vs DCC	*P*-value[d]
Maternal characteristics											
At admission to antenatal care											
Weight, kg	69.3 (14.8)	56	66.4 (12.0)	164	67.3 (12.2)	168	0.32				
Length, cm	166.8 (6.6)	56	167.9 (6.4)	147	167.5 (5.1)	141	0.44				
Body mass index, kg/m2	24.8 (4.3)	56	23.6 (3.8)	146	23.9 (3.6)	141	0.16				
Haemoglobin, g/L	126.4 (10.1)	57	128.0 (8.8)	161	128.0 (10.8)	168	0.55				
At day of giving birth											
Age, years	33.0 (5.6)	64	31.7 (4.2)	166	30.8 (4.9)	168	0.006	1.4 (-0.3 to 3.0)	0.15	2.2 (0.5 to 3.9)	0.005
Parity (including study child)	1.9 (1.0)	64	1.8 (0.9)	166	1.8 (0.7)	168	0.88				
Early infant characteristics							Adjusted[x] Mean Difference (95% CI)				
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[e]	CS vs DCC	*P*-value[e]
Gestational age, weeks	38.9 (0.6)	64	40.0 (1.1)	166	40.0 (1.1)	168	<0.001	-1.2 (-1.5 to -0.8)	<0.001	-1.1 (-1.5 to -0.8)	<0.001
Apgar score at 1 minute	9.0 (1.0)	64	8.8 (0.8)	166	9.0 (0.4)	168	0.008	0.2 (-0.1 to 0.4)	0.42	-0.1 (-0.3 to 0.2)	>0.99
Length, cm	50.4 82.0)	64	50.7 (1.9)	165	50.9 (1.9)	168	0.23	0.7 (0.0 to 1.3)	0.046	0.4 (-0.2 to 1.1)	0.31
Birth weight, gram	3537 (567)	64	3523 (483)	166	3632 (464)	168	0.10	224 (51 to 398)	0.006	104 (-70 to 277)	0.45
Head circumference, cm	35.8 (1.5)	64	34.7 (1.3)	166	35.0 (1.37)	168	<0.001	1.7 (1.2 to 2.1)	<0.001	1.4 (0.9 to 1.9)	<0.001
Umbilical cord haemoglobin, g/L	147.9 (19.0)	52	163.3 (14.9)	144	158.0 (17.6)	144	<0.001	-13.5 (-20.3 to -6.6)	<0.001	-8.4 (-15.4 to -1.5)	0.01
Ferritin, µg/L[f]	160 (8 to 853)	61	181 (12 to 1112)	163	183 (25 to 735)	164	0.38	-24 (-70 to 22)	0.30	-15 (-61 to 31)	0.51
pH in umbilical cord artery	7.29 (0.05)	57	7.27 (0.08)	159	7.26 (0.08)	144	0.04	0.02 (-0.01 to 0.05)	0.33	0.025 (-0.01 to 0.06)	0.16
Base deficit	2.0 (2.5)	56	4.4 (3.4)	158	4.8 (3.7)	143	<0.001	-2.0 (-3.4 to -0.6)	0.002	-2.4 (-3.8 to -1.0)	<0.001

CS=Elective Caesarean Section, ECC=Early Cord Clamping, DCC=Delayed cord clamping, CI=confidence interval. [a] Data are mean (SD) or mean difference (95% CI). [b]Adjusted for maternal age and gestational age. P values were calculated using [c]One-way ANOVA, [d]One-way ANOVA with Bonferroni post hoc comparison, [e]Analysis of covariancewith Bonferroni post hoc comparison. [f]Ferritin is presented as geometric mean (geometric standard deviation)[f]

Elective caesarean, cord clamping and iron stores at 4 mo

Table 2. Laboratory status at four and 12 months of age after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

								Adjusted[c] Mean Difference (95% CI)			
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[c]	CS vs DCC	*P*-value[c]
4 months											
Haemoglobin, g/L	113.4 (7.5)	57	113.0 (7.1)	153	112.8 (7.5)	147	0.88				
MCV, fL	79.3 (2.6)	57	77.9 (3.1)	153	79.1 (3.1)	147	<0.001	1.8 (0.6 to 3.0)	0.001	0.5 (-0.7 to 1.7)	0.96
Ferritin, µg/L[b]	103 (14 to 401)	55	80 (6 to 760)	153	117 (20 to 880)	149	<0.001	39 (10 to 60)	0.007	2 (-41 to 33)	>0.99
Transferrin saturation, %	17.1 (6.5)	56	15.8 (5.6)	153	18.2 (6.1)	148	0.002	2.1 (-0.3 to 4.5)	0.11	-0.3 (-2.7 to 2.1)	>0.99
Transferrin receptors, mg/L	3.70 (0.75)	55	4.00 (0.80)	153	3.72 (0.69)	149	0.002	-0.39 (-0.69 to -0.08)	0.007	-0.10 (-0.40 to 0.21)	>0.99
12 months											
Haemoglobin (g/L)	117.5 (8.0)	52	119.4 (8.2)	131	117.6 (7.8)	129	0.14				
MCV, fL	76.8 (3.6)	52	76.9 (3.3)	131	76.6 (3.3)	129	0.77				
Ferritin, µg/L[b]	35 (8 to 107)	48	34 (8 to 135)	136	35 (10 to 281)	129	0.84				
Transferrin saturation, %	16.2 (7.1)	49	15.4 (7.3)	135	15.3 (6.0)	130	0.72				
Transferrin receptors, mg/L	4.40 (0.82)	49	4.48 (0.99)	136	4.37 (0.87)	130	0.61				

ECC=Early Cord Clamping, DCC=Delayed cord clamping, CI=Confidence Interval, MCV=Mean Cell Volume. [a] Data are mean (SD) or mean difference (95% CI). [b] Ferritin is presented as geometric mean (geometric standard deviation). [c] Adjusted for maternal age and gestational age. *P* values were calculated using [d]One-way ANOVA, [e]Analysis of covariance with Bonferroni post hoc comparison

Elective caesarean, cord clamping and iron stores at 4 mo

Table 3. Proportion of infants with iron status indicators outside reference limits at 4 and 12 months after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

								Absolute risk reduction (95% CI), %	
	CS	n	ECC	n	DCC	n	*P*-value	CS vs ECC	CS vs DCC
4 months									
Anaemia (Hb < 105 g/L)	6 (10.5%)	57	20 (13.1%)	153	20 (13.6%)	147	0.84		
Anaemia and iron deficiency	0 (0%)	52	2 (1.3%)	153	0 (0%)	148	0.27		
Iron deficiency (2 out of 4)[b]	0 (0%)	52	8 (5.2%)	153	1 (0.7%)	144	0.02	5.2 (-2.9 to 5.2)	0.7 (-1.8 to 0.7)
MCV < 73 nm	0 (0%)	57	8 (5.2%)	153	3 (2.0%)	147	0.09	5.2 (-2.3 to 5.2)	2.0 (-3.0 to 2.0)
Ferritin < 20 umol/L	1 (1.8%)	55	11 (7.2%)	153	0 (0%)	149	0.002	5.4 (-3.8 to 7.7)	-1.8 (-1.8 to 0.5)
Transferrin saturation < 10%	6 (10.7%)	56	22 (14.4%)	153	8 (5.4%)	148	0.03	3.7 (-8.9 to 12.0)	-5.3 (-14.5 to 2.8)
Transferrin receptors < 7 mg/L	0 (0%)	55	0 (0%)	153	0 (0%)	149	NA		
12 months									
Hb < 110 g/L	9 (17.3%)	52	16 (12.2%)	131	22 (17.1%)	129	0.49		
Anaemia and iron deficiency	1 (2.1%)	46	1 (0.8%)	130	0 (0%)	128	0.30		
Iron deficiency (2 out of 4)[b]	2 (4.3%)	47	7 (5.3%)	132	3 (2.3%)	128	0.464		
MCV < 73 nm	0 (0%)	52	3 (2.3%)	131	3 (2.3%)	129	0.54		
Ferritin < 20 umol/L	2 (4.2%)	46	3 (2.2%)	136	2 (1.6%)	129	0.58		
Transferrin saturation < 10%	6 (12.2%)	49	25 (18.5%)	135	22 (16.9%)	130	0.60		
Transferrin receptors < 5.92 mg/L	4 (8.2%)	45	10 (7.4%)	136	9 (6.9%)	130	0.96		

[a] Data are numbers (%). MCV=Mean Cell Volume, NA=not applicable. [b] Defined as having 2 or more of iron status indicators (low Ferritin, low MCV, low Transferrin saturation and/or high Transferrin receptors) out of reference range.

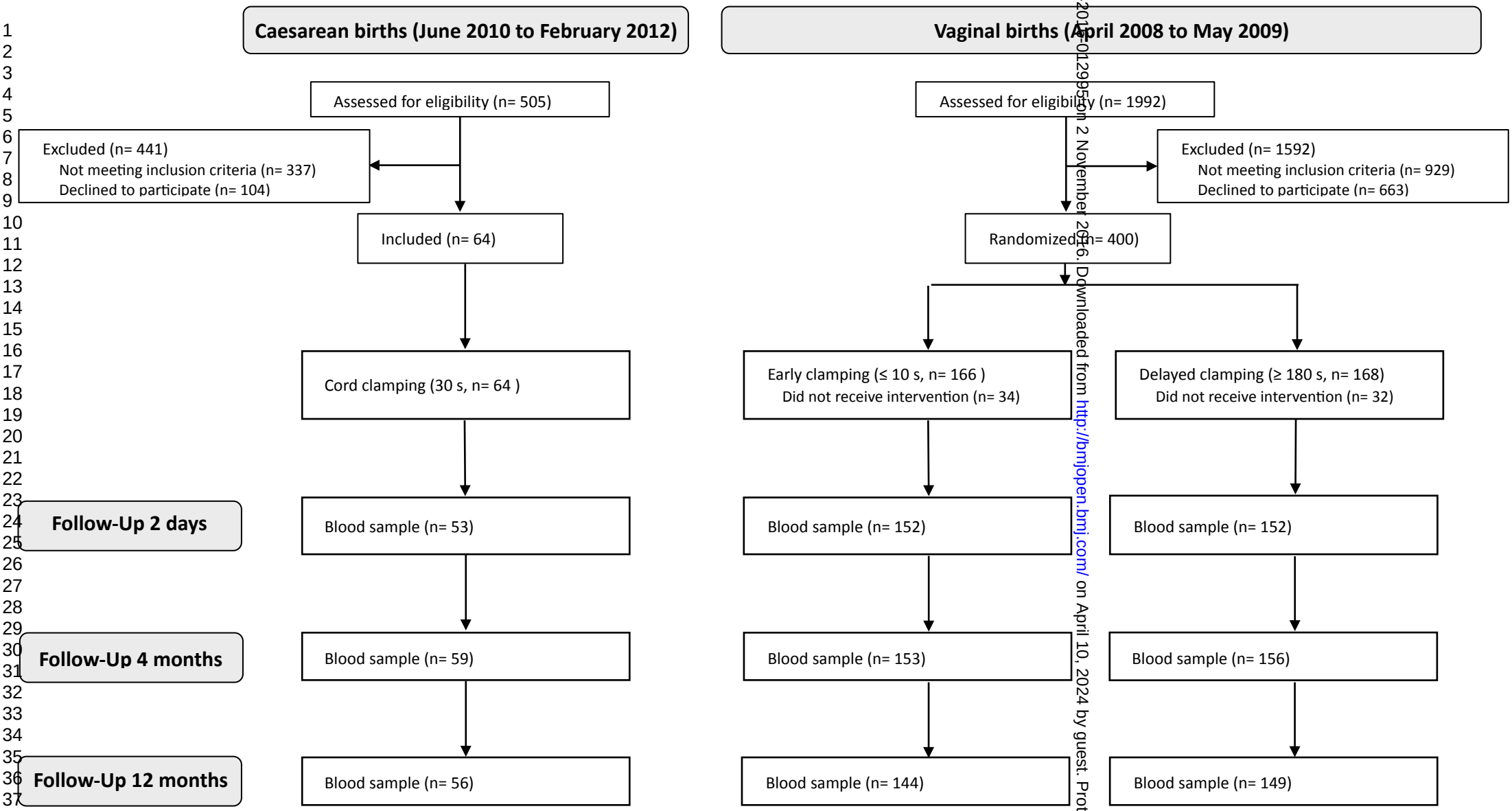

Figure. Trial profile. Flow diagram.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	5-7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	7

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure, page 8
		(b) Give reasons for non-participation at each stage	Figure, page 8
		(c) Consider use of a flow diagram	Figure
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7, table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, table 1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Results and tables
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2 & 3
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Results
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Elective caesarean – is it enough to delay cord clamping 30 seconds to ensure sufficient iron stores at four months of age? – a historical cohort control study

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Elective caesarean – is it enough to delay cord clamping 30 seconds to ensure sufficient iron stores at four months of age? – a historical cohort control study

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Elective caesarean, cord clamping and iron stores at 4 mo

Abstract

Objective To assess iron stores in infants born after elective caesarean section and a 30 seconds delay of umbilical cord clamping as compared to children born vaginally after early (≤10 seconds) or delayed (≥ 180 seconds) cord clamping.
Design Prospective observational study with historical control.
Setting Swedish county hospital
Population 64 infants born after elective caesarean section was compared with a historical control;166 early clamped and 168 delayed clamped after vaginal birth.
Methods Blood and iron status were estimated by blood samples collected at birth, 48-96 h after birth, four and 12 months of age.
Primary and Secondary Outcome Measures Ferritin at 4 months of age was the primary outcome, second outcome measures were other indicators of iron status, and haemoglobin, at four and 12 months of age, as well as respiratory distress at one and six hours after birth.
Results At four months of age infants after elective caesarean had more positive iron status as compared to vaginally born infants subjected to early cord clamping, shown by higher adjusted mean difference of ferritin concentration (39 µg/L [95% CI 10 to 60]) and mean cell volume (1.8 fL [95% CI 0.6 to 3.0]); and lower levels of transferrin receptors (-0.39 mg/L [95% CI -0.69 to -0.08]). No differences were seen between infants born after elective caesarean and delayed clamped vaginally born infants at four months. No differences were found between groups at 12 months of age.
Conclusions Waiting to clamp the umbilical cord for 30 seconds after elective caesarean results in higher iron stores at four months of age compared to early cord clamping after vaginal birth, and seems to ensure iron status comparable with those achieved after 180 seconds delayed cord clamping after vaginal birth.

•Keywords: umbilical cord, cord clamping, pregnancy, vaginal birth, caesarean section, iron deficiency

Strengths and Limitations

- The present study report data on iron status and haematological parameters in term infants after CS up to 12 months of life as compared to vaginal deliveries and in relation to time to umbilical cord clamping.
- By being a observational trial, the interpretation of the study's results is limited by its possibilities of bias and confounding factors.
- Nutrition and growth rate is expected to influence iron status at a later age, and we could control for these data.
- Only 35-40% of eligible pregnancies were included, why readiness to participate may be a confounding factor.
- A limitation for the conclusion is that cord clamping at 30 sec at elective CS has not been compared to the usual practice; immediate clamping at elective CS.

Introduction

During the two last decades, evidence has accumulated regarding the benefits of waiting to clamp the umbilical cord for two to three minutes in term births.[1-3] Research has mainly included vaginal births, omitting the rising numbers of elective caesarean sections (CS) globally.

Newborns subjected to delayed cord clamping (DCC) have higher haemoglobin (Hb) concentrations at 24-48 hours of life, and improved iron stores at four to six months.[4] After delivery, the newborn may receive up to 30 ml/kg blood from its placental circulation within three to five minutes,[5 6] contributing 75 mg iron which is equivalent to the infant's requirements for three to four months.

Iron deficiency is associated with impaired development,[7] and a main reason for adopting DCC has been to reduce iron deficiency. Recently, we have shown that DCC is associated with improved fine motor skills at four years of age.[8]

Less is known about the placental transfusion after elective CS before start of labour. Elective CS can be performed for several reasons, including maternal, fetal and preferential factors.[9] The obstetrician has to weigh possible benefits against possible disadvantages for mother and child. To ensure that this decision is evidence based, research must contribute to a wider understanding of long-term consequences of these actions.

Pioneering physiological studies in the 1960s by Lind et al showed that there was less placental transfusion after CS than after early cord clamping following vaginal delivery.[10] More recently it was demonstrated that it is possible to harvest a higher volume of blood to stem cell banks after CS, also pointing to a reduced placenta to child transfusion after CS.[11] CS was associated with anaemia at 12 and 58 months in two large longitudinal Chinese birth cohorts,[12] and a systematic review and meta-analysis found that CS compared with vaginal

delivery was associated with a reduced placental transfusion and poor iron-related hematologic indices in both cord and peripheral blood.[13]

We set out to prospectively study infants after elective CS, and follow them with the same protocol used for our cord clamping trial, using the vaginally born children as a historic control group. After delayed umbilical cord clamping at 180 seconds after vaginal births was introduced at the hospital, the board of obstetricians chose to perform cord clamping at 30 seconds on elective CS as an pragmatic attempt to allow for at least some placental blood transfusion.

Our hypothesis was that iron stores measured by ferritin at 4 months in children delivered by CS with cord clamping after 30 seconds would be lower than children born vaginally after DCC and thus similar to those born vaginally after ECC.

Method

Study design

This is a prospective observational study of children delivered by CS, using reference data from a study[3] of children randomized to DCC vs ECC after vaginal delivery.

Setting

During the period of June 6, 2010 and February 29, 2012, women planned for elective CS were approached by the midwife, informed of the study and asked for consent, which was then signed by both parents. The historical control group consisted of 382 term newborns included in a randomised, controlled trial between April 16, 2008 and May 22, 2009. The results from this trial have been reported in several papers.[3 8 14] The study was performed at the Hospital of Halland, Halmstad, Sweden.

Participants

Pregnant women were eligible if they met the following criteria: non-smoking; normal pregnancy (no pre-eclampsia, no diabetes, no prolonged rupture of membranes or signs of

infection) and term pregnancy (gestational age 37+0 to 41+6 weeks). The mother also had to understand Swedish well enough to participate in the study. Exclusion criteria were serious congenital malformations, syndromes, or other congenital diseases that could affect the outcome measures. For the elective CS group, an additional eligibility criterion was admission for a scheduled CS.

For the reference groups, eligibility also included being randomized to ECC or DCC in the performed randomized trial, having the intervention as allocated (per protocol), and being born vaginally.

After delivering the infant, the obstetrician placed the baby on the mother's thighs or beside her on the operation table and waited 30 seconds to clamp the umbilical cord, as advised by the present routine at the hospital. The timing at 30 seconds had been chosen by the board of obstetricians at the hospital before commencement of the current study. The timing of the clamping was noted. After clamping, blood samples for blood gas evaluation was taken routinely from the placental side of the umbilical vessels, and for the research project samples were taken for analysis of blood status; Hb and mean cell volume (MCV), and iron status; Transferrin saturation (TS), soluble transferrin receptor (sTfR) and ferritin. Although Ferritin is considered the most useful iron status marker, it is not sufficiently validated in children.[15] We chose to also include TS (lower in iron deficiency) and sTfR (higher in iron deficiency) as they, as well as Hb and MCV, may give additional information on the iron status of the infant. As inflammation is known to influence iron status markers,[16] blood samples with CRP $\geq$ 10 mg/L were excluded from analysis.

Apgar scores, birth weight, length, and head circumference were recorded according to routine. At one and six hours after birth, the midwife assessed the infants well-being, and prospectively noted in the protocol whether there were any respiratory difficulties (grunting,

presence of nostril flaring, respiratory frequency above 60 breaths per minute and intercostal retractions) as well as if the baby had been breastfed.

At the time for routine venous blood sampling for metabolic screening at two days post partum, additional blood samples were gathered, i.e. blood and iron status, and C-reactive protein (CRP).

At three months of age, a letter was sent to ask the parents to return with their child at four months for sampling of blood status, iron status and CRP. Again, at eleven months of age, an invitation to return at 12 months was sent. Venous blood samples for blood status, iron status and CRP were obtained.

Blood was collected in EDTA tubes (BD Vacutainer, Plymouth, UK) for blood status, and in serum separator tubes (BD Vacutainer) for iron status, and CRP.

Complete blood counts were analysed with an automated haematology analyser (Sysmex XE 2100, Sysmex, Kobe, Japan). Iron status indicators, and CRP were analysed with Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

At four months, mothers reported their infant's feeding habits in a three-day diary and infant´s length and weight was measured.

Definitions:

At two days

Anaemia: Hb <145 g/L[17], Polycythaemia: hematocrit >0.65[18].

At four months

Anaemia: Hb <105 g/L,[15] Iron deficiency: two indicators of iron status outside reference range (ferritin <20 µg/L,[15] MCV <73 fL,[19] TS <10%,[20] sTfR >7 mg/L[3]).

At 12 months

Anaemia: Hb <110 g/L. Iron deficiency: two indicators of iron status outside reference range (ferritin <12 µg/L, MCV <70 fL, and TS <10%, sTfR >5.6 mg).[21]

Outcomes

The primary outcome was infant serum ferritin at four months of age. Secondary outcomes included infant Hb and iron status (measured as serum ferritin, TS, sTfR, MCV) at four and 12 months of age, Apgar score at birth, and observations on breast feeding and respiratory symptoms at one and six hours after birth.

Confounders

To be able to compare the included children with the historical reference group and to ensure that inclusion criteria were met, data on the mother (reported illness, medication, parity, weight, height, smoking habits, blood group Rhesus factor status, and haemoglobin concentration at the time of admission to antenatal care) was obtained from medical records. Nutrition and growth may affect iron status at four months; to adjust for this we controlled feeding habits at four months of age. As birth weight can be affected by the size of placental transfusion, we decided to only use length as a proxy for growth from birth to four and 12 months.

Sample size

Our hypothesis was that the difference in Ferritin between DCC and CS at four months would be the same as shown between DCC and ECC in a previous study,[3] that is a difference in log10 Ferritin between 2.07 and 1.90 with a SD of 0.34. To show this difference, a sample size of 63 was needed.

Statistical analysis

For group comparisons of continuous variables, we used one-way analysis of variance (ANOVA) for variables with normal distribution and Bonferroni as post hoc test for pairwise comparisons. Categorical variables were compared between pairwise groups by using Fisher's exact test and across all three groups with Pearson Chi-square test. Ferritin concentration was log10 transformed for analysis. A $p<0.05$ was considered significant. We used SPSS, version 22.0 (IBM, Armonk, NY, USA).

For adjusted analyses, analysis of covariance (ANCOVA) was used for test scores with Bonferroni post hoc test for pairwise comparisons. For adjustment variables, background variables (Table 1) with a difference between groups with a $p<0.1$ were chosen, resulting in mothers' age and gestational age.

Results

During the inclusion period, 505 infants were born after CS, 98 (19 %) preterm and 34 (7 %) post term, Figure 1. Among the term newborns (n=373), CS were classified as acute (174, 47%), elective with a medical reason (145, 39%), and elective with no medical reason (54, 14%). From the 199 elective term CS, 26 could not be included due to maternal disease (diabetes, n=12), preeclampsia, n=6, intra uterine growth restriction (IUGR), n=6 and combination of preeclampsia and IUGR, n=2. Additionally, five women smoked at admission to antenatal care, leaving 168 possible for inclusion. One hundred and four declined participation, resulting in the inclusion of 64 deliveries with elective CS. We did not record the reason to decline by respect of parents integrity, but hesitance to return for repeated blood sampling was the most common objection. Furthermore, 166 ECC and 168 DCC controls were available for analysis, Figure 1. We did compare data between the 64 included EC with available data from the 104 who declined inclusion and no significant differences in maternal age, gestational age, infants´ birth weight, length or head circumference was found, nor any differences in Apgar score or umbilical blood gases, pointing to our sample being representative for the whole cohort (results not shown).

At four months 59 (92.2%) infants in the elective CS group returned for blood sampling between October 6, 2010 and June 28, 2012. Corresponding blood samples had been obtained from 153 (92.2%) in the ECC group and from 156 (92.9%) in the DCC group between August 8, 2008 and October 1, 2009. At 12 months, 56 (87.5%) infants returned in the elective CS group between May 31, 2011 and Feb 20, 2013, while in the control group, 144 (86.7%)

samples were available from the ECC and 149 (88.7%) from the DCC group (collected between April 8, 2009 and May 21, 2010) (Figure).

For baseline characteristics, see Table 1. The sex distribution was comparable between groups; 28 (44%) were males in the CS group, 83 (50%) in the ECC group and 73 (44%) in the DCC group, p=0.44. As expected, the gestational age was lower in the elective CS group than in the ECC group, -1.2 weeks (95% CI -1.5 to -0.8, P<0.001), and DCC group, -1.1 weeks (95% CI -1.5 to -0.8, P<0.001). The maternal age was also higher in the elective CS group than the in DCC group, 2.2 years (95% CI 0.5 to 3.9, p=0.005).

Apgar scores at one and five minutes were comparable between groups.

Haemoglobin was lower in umbilical cord blood in the elective CS group, when compared to the ECC group, adjusted mean difference (AMD) -13.5 (95% CI -20.3 to -6.6, P<0.001) g/L and the DCC group, AMD -8.5 g/L (95% CI -15.4 to -1.5, P=0.01). However, at 48-72 hours of age, the Hb level did not differ between groups. The Hb level after delivery increased more in the DCC (31.3 g/L [7.8], n 121) and CS (35.6 g/L [16.8], n=38) groups as compared to the ECC group (11.5 g/L [16.8], n=121), p<0.001, indicating a larger placental transfusion.

At four months, differences in ferritin, MCV, and transferrin receptors (but not in TS) indicated better iron status in the CS group compared to the ECC group (Table 2). The proportions of infants having abnormal values for iron status parameters did not differ between the CS group, and the ECC and DCC groups, respectively (Table 3).

At 12 months, no differences between groups in iron status or blood status could be shown (Table 2 and 3).

Auxiliary analysis

Postnatally, children born after CS were more likely to not having been breastfed at one hour after delivery as compared to the ECC, relative risk (RR) 2.1 (95% CI 1.5 to 2.9) and DCC groups, RR 2.5 (95% CI 1.7 to 3.5). The CS group had a higher risk of respiratory distress at

six hours after birth compared to ECC, RR 3.4 (95% CI 1.1 to 10.5) and DCC, RR 4.4 (95% CI 1.4 to 14.9). Respiratory distress at one hour of age and breast-feeding frequency at six hours did not differ between groups.

At four months, exclusive breast-feeding was equally prevalent among the groups, CS 27 (47%), ECC 78 (52%) and DCC 84 (56%), p=0.45. Exclusive breast-feeding correlated positively to the infants' serum ferritin level (r=0.144, p=0.007), but not to any other blood sample analysed at four months. If 'exclusive breast-feeding' was included in the ANCOVA, results was not changed for any variable in any significant way, except for transferrin saturation, where the elective CS group attained a significant higher value than ECC: AMD 2.0% (95% CI 0.0 to 4.0), p=0.049.

Length and weight at four and 12 months of age were comparable across groups, also when adjusted for gestational age. Also weight and length gain from birth was comparable between groups at four and 12 months of age (data not shown). Adding 'length gain' into the adjusted model did not alter differences in any significant way.

Discussion

Main findings

The findings in this prospective observational study indicate that in infants born after elective CS with umbilical cord clamping after 30 seconds, iron stores at four months are comparable to iron stores in vaginally born infants subjected to DCC (≥180 s), and improved compared to vaginally born infants subjected to ECC (≤10 s).

Strengths and Limitations

The main strength of the present study is to report data on iron status and haematological parameters in term infants after CS, as compared to vaginal deliveries and in relation to time to umbilical cord clamping. Haematological and iron status after different timing of umbilical cord clamping have previously been reported in several studies.[4] Among available studies

with four months or longer follow-up on iron stores, three excluded infants born after CS[2 3 22] while one included CS but did not separate results from vaginal birth.[23] From an ethical, and in many cases also medical point of view, it is impossible to randomise women to either elective CS or vaginal birth. As the trial is observational, and not randomised, the interpretation of the study's results is limited by the possibility of bias and confounding factors. These include in particular unidentified differences in baseline characteristics between groups, including prenatal maternal as well as perinatal and postnatal infant influences. Except for iron stores at birth, nutrition and growth rate is expected to influence iron status at a later age, and we could control for these data that did not alter the main outcomes. In all three groups, only 35-40% of eligible pregnancies were included, why readiness to participate may be a confounding factor. Data from the included EC were not significantly different from with available data from those who declined inclusion, indicating similarity between included and 'declined inclusion' pregnancies. A limitation for the conclusion is that cord clamping at 30 sec at elective CS has not been compared to the usual practice; immediate clamping at elective CS.

Interpretation

Previous studies have implied less placental transfusion after CS. Consequently; our findings are not in line with the relatively scarce literature on this subject. One explanation to our finding that CS rather improves iron stores compared to ECC at four months of age could be that the obstetrician actually waited 30 second to clamp the cord. Timing to umbilical cord clamping after CS has usually not been reported in other studies, but we presume it to have been performed immediately after delivery. Another potentially contributing factor to the improved iron stores is that infants born after elective CS have a lower blood pressure due to less circulating adenosine and catecholamines,[24 25] facilitating a faster blood transfusion from the placenta. Unfortunately, we did not record the time for the first breath/cry, but earlier

reports indicate that most new-borns had commenced breathing before the cord was clamped in the CS group.[26]

Haemoglobin in the umbilical cord blood sample was significantly lower after elective CS compared to ECC and DCC, a finding in coherence with a recent systematic review and meta-analysis.[13] This finding suggests that umbilical cord Hb may not a reliable marker of iron status in newborns, as the result may reflect not only iron status, but also mode of delivery. Our study give support to the pragmatic approach to wait for 30 seconds before clamping after CS, as we could not demonstrate any negative effect on iron homeostasis compared to the vaginally born groups. Our findings might imply that whatever negative consequences on the child's health CS is associated with, waiting for 30 seconds to clamp the cord reduces those that could possibly be explained by a diminished placental blood transfusion.

Conclusion

Erickson-Owens et al have suggested umbilical cord milking as a possible procedure to facilitate the placental transfusion after CS in term infants.[27] Our results suggest that the less invasive method of a 30 s DCC might be sufficient to ensure the placental transfusion after elective CS. Large observational studies, most preferably prospective with vaginally born matched controls, are indicated and warranted.

In summary, in this study comparing infants born after elective CS with children born vaginally after DCC and ECC, we could demonstrate that infants born after elective CS had iron stores similar to DCC and better than ECC at four months of age.

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Disclosure of Interests

None.

Contribution to Authorship

OA, LHW and MD planned the study. OA was responsible for staff training, study management and data collection with support from LHW and MD. OA, LHW and MD analysed the data. OA drafted the manuscript. All authors revised the manuscript and accepted the final version. Ola Andersson is the guarantor.

Details of Ethics Approval

The original study was approved by the regional ethical review board at Lund University (2008/41), and the new cohort including elective CS was approved by an amendment (2009/344).

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Data sharing statement

None available

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Table/Figure Caption List

Table 1. Baseline characteristics and early outcomes after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Table 2. Laboratory status at four and 12 months of age after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Table 3. Proportion of infants with iron status indicators outside reference limits at four and 12 months after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Figure 1. Trial profile. Flow diagram.

Elective caesarean, cord clamping and iron stores at 4 mo

Table 1. Maternal and birth characteristics and early outcomes after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

							Mean Difference (95% CI)[b]			
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[d]	CS vs DCC	*P*-value[d]
Maternal characteristics											
At admission to antenatal care											
Weight, kg	69.3 (14.8)	56	66.4 (12.0)	164	67.3 (12.2)	168	0.32				
Length, cm	166.8 (6.6)	56	167.9 (6.4)	147	167.5 (5.1)	141	0.44				
Body mass index, kg/m2	24.8 (4.3)	56	23.6 (3.8)	146	23.9 (3.6)	141	0.16				
Haemoglobin, g/L	126.4 (10.1)	57	128.0 (8.8)	161	128.0 (10.8)	168	0.55				
At day of giving birth											
Age, years	33.0 (5.6)	64	31.7 (4.2)	166	30.8 (4.9)	168	0.006	1.4 (-0.3 to 3.0)	0.15	2.2 (0.5 to 3.9)	0.005
Parity (including study child)	1.9 (1.0)	64	1.8 (0.9)	166	1.8 (0.7)	168	0.88				
Early infant characteristics							Adjusted[x] Mean Difference (95% CI)				
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[e]	CS vs DCC	*P*-value[e]
Gestational age, weeks	38.9 (0.6)	64	40.0 (1.1)	166	40.0 (1.1)	168	<0.001	-1.2 (-1.5 to -0.8)	<0.001	-1.1 (-1.5 to -0.8)	<0.001
Apgar score at 1 minute	9.0 (1.0)	64	8.8 (0.8)	166	9.0 (0.4)	168	0.008	0.2 (-0.1 to 0.4)	0.42	-0.1 (-0.3 to 0.2)	>0.99
Length, cm	50.4 82.0)	64	50.7 (1.9)	165	50.9 (1.9)	168	0.23	0.7 (0.0 to 1.3)	0.046	0.4 (-0.2 to 1.1)	0.31
Birth weight, gram	3537 (567)	64	3523 (483)	166	3632 (464)	168	0.10	224 (51 to 398)	0.006	104 (-70 to 277)	0.45
Head circumference, cm	35.8 (1.5)	64	34.7 (1.3)	166	35.0 (1.37)	168	<0.001	1.7 (1.2 to 2.1)	<0.001	1.4 (0.9 to 1.9)	<0.001
pH in umbilical cord artery	7.29 (0.05)	57	7.27 (0.08)	159	7.26 (0.08)	144	0.04	0.02 (-0.01 to 0.05)	0.33	0.025 (-0.01 to 0.06)	0.16
Base deficit	2.0 (2.5)	56	4.4 (3.4)	158	4.8 (3.7)	143	<0.001	-2.0 (-3.4 to -0.6)	0.002	-2.4 (-3.8 to -1.0)	<0.001

CS=Elective Caesarean Section, ECC=Early Cord Clamping, DCC=Delayed cord clamping, CI=confidence interval. [a] Data are mean (SD) or mean difference (95% CI). [b]Adjusted for maternal age and gestational age. P values were calculated using [c]One-way ANOVA, [d]One-way ANOVA with Bonferroni post hoc comparison, [e]Analysis of covariancewith Bonferroni post hoc comparison.

Elective caesarean, cord clamping and iron stores at 4 mo

Table 2. Laboratory status at different time points after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

								Adjusted[c] Mean Difference (95% CI)			
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[c]	CS vs DCC	*P*-value[c]
Umbilical cord											
Haemoglobin, g/L	147.9 (19.0)	52	163.3 (14.9)	144	158.0 (17.6)	144	<0.001	-13.5 (-20.3 to -6.6)	<0.001	-8.4 (-15.4 to -1.5)	0.01
Ferritin, µg/L[b]	160 (8 to 853)	61	181 (12 to 1112)	163	183 (25 to 735)	164	0.38				
48-72 hours after birth											
Haemoglobin, g/L	179.9 (20.5)	40	174.9 (18.6)	104	188.5 (16.4)	107	<0.001	7.5 (-0.7 to 15.8)	0.09	-6.6 (-14.9 to 1.7)	0.17
4 months											
Haemoglobin, g/L	113.4 (7.5)	57	113.0 (7.1)	153	112.8 (7.5)	147	0.88				
MCV, fL	79.3 (2.6)	57	77.9 (3.1)	153	79.1 (3.1)	147	<0.001	1.8 (0.6 to 3.0)	0.001	0.5 (-0.7 to 1.7)	0.96
Ferritin, µg/L[b]	103 (14 to 401)	55	80 (6 to 760)	153	117 (20 to 880)	149	<0.001	39 (10 to 60)	0.007	2 (-41 to 33)	>0.99
Transferrin saturation, %	17.1 (6.5)	56	15.8 (5.6)	153	18.2 (6.1)	148	0.002	2.1 (-0.3 to 4.5)	0.11	-0.3 (-2.7 to 2.1)	>0.99
Transferrin receptors, mg/L	3.70 (0.75)	55	4.00 (0.80)	153	3.72 (0.69)	149	0.002	-0.39 (-0.69 to -0.08)	0.007	-0.10 (-0.40 to 0.21)	>0.99
12 months											
Haemoglobin (g/L)	117.5 (8.0)	52	119.4 (8.2)	131	117.6 (7.8)	129	0.14				
MCV, fL	76.8 (3.6)	52	76.9 (3.3)	131	76.6 (3.3)	129	0.77				
Ferritin, µg/L[b]	35 (8 to 107)	48	34 (8 to 135)	136	35 (10 to 281)	129	0.84				
Transferrin saturation, %	16.2 (7.1)	49	15.4 (7.3)	135	15.3 (6.0)	130	0.72				
Transferrin receptors, mg/L	4.40 (0.82)	49	4.48 (0.99)	136	4.37 (0.87)	130	0.61				

ECC=Early Cord Clamping, DCC=Delayed cord clamping, CI=Confidence Interval, MCV=Mean Cell Volume. [a] Data are mean (SD) or mean difference (95% CI). [b] Ferritin is presented as geometric mean (geometric standard deviation). [c] Adjusted for maternal age and gestational age. *P* values were calculated using [d]One-way ANOVA, [e]Analysis of covariance with Bonferroni post hoc comparison

Elective caesarean, cord clamping and iron stores at 4 mo

Table 3. Infants with anaemia or abnormal iron indices outside reference ranges at 4 and 12 months after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

								Absolute risk reduction (95% CI), %	
	CS	n	ECC	n	DCC	n	*P*-value	CS vs ECC	CS vs DCC
4 months									
Anaemia (Hb < 105 g/L)	6 (10.5%)	57	20 (13.1%)	153	20 (13.6%)	147	0.84		
Anaemia and iron deficiency	0 (0%)	52	2 (1.3%)	153	0 (0%)	148	0.27		
Iron deficiency (2 out of 4)[b]	0 (0%)	52	8 (5.2%)	153	1 (0.7%)	144	0.02	5.2 (-2.9 to 5.2)	0.7 (-1.8 to 0.7)
MCV < 73 nm	0 (0%)	57	8 (5.2%)	153	3 (2.0%)	147	0.09	5.2 (-2.3 to 5.2)	2.0 (-3.0 to 2.0)
Ferritin < 20 umol/L	1 (1.8%)	55	11 (7.2%)	153	0 (0%)	149	0.002	5.4 (-3.8 to 7.7)	-1.8 (-1.8 to 0.5)
Transferrin saturation < 10%	6 (10.7%)	56	22 (14.4%)	153	8 (5.4%)	148	0.03	3.7 (-8.9 to 12.0)	-5.3 (-14.5 to 2.8)
Transferrin receptors < 7 mg/L	0 (0%)	55	0 (0%)	153	0 (0%)	149	NA		
12 months									
Anaemia (Hb < 110 g/L)	9 (17.3%)	52	16 (12.2%)	131	22 (17.1%)	129	0.49		
Anaemia and iron deficiency	1 (2.1%)	46	1 (0.8%)	130	0 (0%)	128	0.30		
Iron deficiency (2 out of 4)[b]	2 (4.3%)	47	7 (5.3%)	132	3 (2.3%)	128	0.464		
MCV < 73 nm	0 (0%)	52	3 (2.3%)	131	3 (2.3%)	129	0.54		
Ferritin < 20 umol/L	2 (4.2%)	46	3 (2.2%)	136	2 (1.6%)	129	0.58		
Transferrin saturation < 10%	6 (12.2%)	49	25 (18.5%)	135	22 (16.9%)	130	0.60		
Transferrin receptors < 5.92 mg/L	4 (8.2%)	45	10 (7.4%)	136	9 (6.9%)	130	0.96		

[a] Data are numbers (%). MCV=Mean Cell Volume, NA=not applicable. [b] Defined as having 2 or more of iron status indicators (low Ferritin, low MCV, low Transferrin saturation and/or high Transferrin receptors) out of reference range.

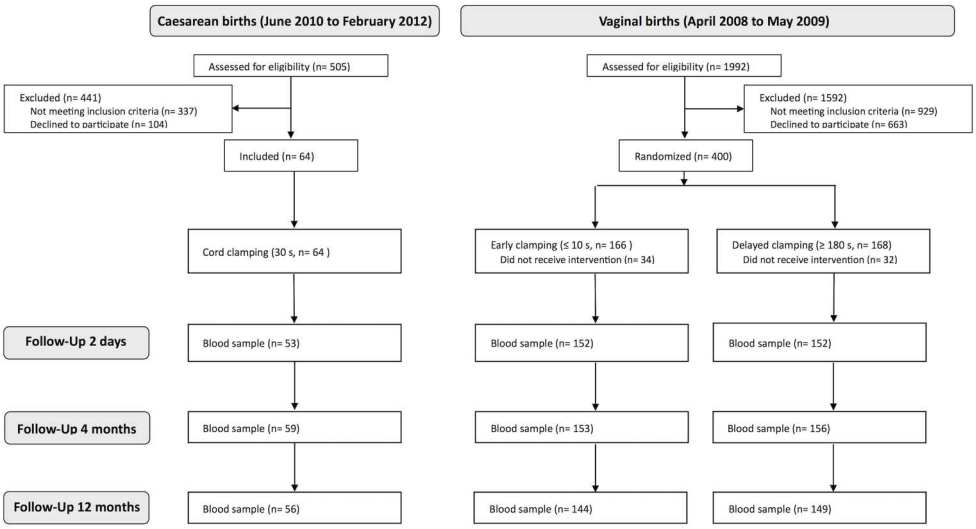

Figure. Trial profile. Flow diagram.

Trial profile. Flow diagram.
Figure
173x122mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	5-7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	7

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure, page 8
		(b) Give reasons for non-participation at each stage	Figure, page 8
		(c) Consider use of a flow diagram	Figure
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7, table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, table 1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Results and tables
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2 & 3
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Results
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Elective caesarean – does delay in cord clamping for 30 seconds ensure sufficient iron stores at four months of age? – a historical cohort control study

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Elective caesarean – does delay in cord clamping for 30 seconds ensure sufficient iron stores at four months of age? – a historical cohort control study

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Elective caesarean, cord clamping and iron stores at 4 mo

Abstract

Objective To compare iron stores in infants born after elective caesarean section (CS) and a 30 seconds delay of umbilical cord clamping with those born vaginally after early (≤ 10 seconds) or delayed (≥ 180 seconds) cord clamping.
Design Prospective observational study with historical control.
Setting Swedish county hospital
Population 64 infants born after elective CS were compared with a historical control of 166 early clamped and 168 delayed clamped after vaginal birth.
Methods Blood and iron status were measured in blood samples collected at birth, 48-96 h after birth, four and 12 months of age.
Primary and Secondary Outcome Measures Ferritin at 4 months of age was the primary outcome, second outcome measures were other indicators of iron status, and haemoglobin, at four and 12 months of age, as well as respiratory distress at one and six hours after birth.
Results At four months infants born by elective CS had better iron status than those born vaginally subjected to early cord clamping, shown by higher adjusted mean difference of ferritin concentration (39 µg/L [95% CI 10 to 60]) and mean cell volume (1.8 fL [95% CI 0.6 to 3.0]); and lower levels of transferrin receptors (-0.39 mg/L [95% CI -0.69 to -0.08]). No differences were seen between infants born after elective CS and delayed clamped vaginally born infants at four months. No differences were found between groups at 12 months of age.
Conclusions Waiting to clamp the umbilical cord for 30 seconds after elective CS results in higher iron stores at four months of age compared to early cord clamping after vaginal birth, and seems to ensure iron status comparable with those achieved after 180 seconds delayed cord clamping after vaginal birth.

•Keywords: umbilical cord, cord clamping, pregnancy, vaginal birth, caesarean section, iron deficiency

Strengths and Limitations

- This study compares iron status and haematological parameters up to 12 months in term infants after CS with those born vaginally in relation to time to umbilical cord clamping.
- As an observational study with historical controls, results must be interpreted with caution because of potential bias from confounding.
- Nutrition and growth rate is expected to influence iron status at a later age, and we could control for these data.
- Only 35-40% of eligible pregnancies were included, why readiness to participate may be a confounding factor.
- A limitation for the conclusion is that cord clamping at 30 sec at elective CS has not been compared to the usual practice of immediate cord clamping at elective CS.

Introduction

During the two last decades, evidence has accumulated regarding the benefits of waiting to clamp the umbilical cord for two to three minutes in term births.[1-3] Research has mainly included vaginal births, omitting the global increase in elective caesarean section (CS) births. Newborns subjected to delayed cord clamping (DCC) have higher haemoglobin (Hb) concentrations at 24-48 hours of life, and improved iron stores at four to six months.[4] After delivery, the newborn may receive up to 30 ml/kg blood from its placental circulation within three to five minutes,[5 6] contributing 75 mg iron which is equivalent to the infant's requirements for three to four months.

Iron deficiency is associated with impaired development,[7] and a main reason for adopting DCC has been to reduce iron deficiency. Recently, we have shown that DCC is associated with improved fine motor skills at four years of age.[8]

Less is known about the placental transfusion after pre-labour elective CS. Elective CS can be performed for several reasons, including maternal, fetal and preferential factors.[9] The obstetrician has to weigh possible benefits against possible disadvantages for mother and child. To ensure that this decision is evidence based, research must contribute to a wider understanding of long-term consequences of these actions.

Pioneering physiological studies in the 1960s by Lind et al showed that there was less placental transfusion after CS than after early cord clamping following vaginal delivery.[10] More recently it was demonstrated that it is possible to harvest a higher volume of blood to stem cell banks after CS, also pointing to a reduced placenta to child transfusion after CS.[11] CS was associated with anaemia at 12 and 58 months in two large longitudinal Chinese birth cohorts,[12] and a systematic review and meta-analysis found that CS compared with vaginal delivery was associated with a reduced placental transfusion and poor iron-related hematologic indices in both cord and peripheral blood.[13]

We set out to prospectively study infants after elective CS, and follow them with the same protocol used for our cord clamping trial, using the vaginally born children as a historic control group. After delayed umbilical cord clamping at 180 seconds after vaginal births was introduced at the hospital, the board of obstetricians chose to perform cord clamping at 30 seconds after the delivery of the child in elective CS as an pragmatic attempt to allow for at least some placental blood transfusion.

Our hypothesis was that iron stores measured by ferritin at 4 months in children delivered by CS with cord clamping after 30 seconds would be lower than children born vaginally after DCC and thus similar to those born vaginally after ECC.

Method

Study design

This is a prospective observational study of children delivered by CS, using reference data from a study[3] of children randomized to DCC vs ECC after vaginal delivery.

Setting

During the period of June 6, 2010 and February 29, 2012, women planned for elective CS were approached by the midwife, informed of the study and asked for consent, which was then signed by both parents. The historical control group consisted of 382 term newborns included in a randomised, controlled trial between April 16, 2008 and May 22, 2009. The results from this trial have been reported in several papers.[3 8 14] The study was performed at the Hospital of Halland, Halmstad, Sweden.

Participants

Pregnant women were eligible if they met the following criteria: non-smoking; normal pregnancy (no pre-eclampsia, no diabetes, no prolonged rupture of membranes or signs of infection) and term pregnancy (gestational age 37+0 to 41+6 weeks). The mother also had to understand Swedish well enough to participate in the study. Exclusion criteria were serious

congenital malformations, syndromes, or other congenital diseases that could affect the outcome measures. For the elective CS group, an additional eligibility criterion was admission for a scheduled CS.

For the reference groups, eligibility also included being randomized to ECC or DCC in the performed randomized trial, having the intervention as allocated (per protocol), and being born vaginally.

After delivering the infant, the obstetrician placed the baby on the mother's thighs or beside her on the operation table and waited 30 seconds to clamp the umbilical cord, as advised by the present routine at the hospital. The timing at 30 seconds had been chosen by the board of obstetricians at the hospital before commencement of the current study. The timing of the clamping was noted. After clamping, blood samples for blood gas evaluation was taken routinely from the placental side of the umbilical vessels, and for the research project samples were taken for analysis of blood status; Hb and mean cell volume (MCV), and iron status; Transferrin saturation (TS), soluble transferrin receptor (sTfR) and ferritin. Although Ferritin is considered the most useful iron status marker, it is not sufficiently validated in children.[15] We chose to also include TS (lower in iron deficiency) and sTfR (higher in iron deficiency) as they, as well as Hb and MCV, provided additional information on the iron status of the infant. As inflammation is known to influence iron status markers,[16] blood samples with CRP $\geq$ 10 mg/L were excluded from analysis.

Apgar scores, birth weight, length, and head circumference were recorded according to routine. At one and six hours after birth, the midwife assessed the infants well-being, and prospectively noted in the protocol whether there were any respiratory difficulties (grunting, presence of nostril flaring, respiratory frequency above 60 breaths per minute and intercostal retractions) as well as if the baby had been breastfed.

At the time for routine venous blood sampling for metabolic screening at two days post partum, additional blood samples were gathered, i.e. blood and iron status, and C-reactive protein (CRP).

At three months of age, a letter was sent to ask the parents to return with their child at four months for sampling of blood status, iron status and CRP. Again, at eleven months of age, an invitation to return at 12 months was sent. Venous blood samples for blood status, iron status and CRP were obtained.

Blood was collected in EDTA tubes (BD Vacutainer, Plymouth, UK) for blood status, and in serum separator tubes (BD Vacutainer) for iron status, and CRP.

Complete blood counts were analysed with an automated haematology analyser (Sysmex XE 2100, Sysmex, Kobe, Japan). Iron status indicators, and CRP were analysed with Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

At four months, mothers reported their infant's feeding habits in a three-day diary and infant´s length and weight was measured.

Definitions:

At two days

Anaemia: Hb <145 g/L[17], Polycythaemia: hematocrit >0.65[18].

At four months

Anaemia: Hb <105 g/L,[15] Iron deficiency: two indicators of iron status outside reference range (ferritin <20 µg/L,[15] MCV <73 fL,[19] TS <10%,[20] sTfR >7 mg/L[3]).

At 12 months

Anaemia: Hb <110 g/L. Iron deficiency: two indicators of iron status outside reference range (ferritin <12 µg/L, MCV <70 fL, and TS <10%, sTfR >5.6 mg).[21]

Outcomes

The primary outcome was infant serum ferritin at four months of age. Secondary outcomes included infant Hb and iron status (measured as serum ferritin, TS, sTfR, MCV) at four and 12 months of age, Apgar score at birth, and observations on breast feeding and respiratory symptoms at one and six hours after birth.

Confounders

To be able to compare the included children with the historical reference group and to ensure that inclusion criteria were met, data on the mother (reported illness, medication, parity, weight, height, smoking habits, blood group Rhesus factor status, and haemoglobin concentration at the time of admission to antenatal care) was obtained from medical records. Nutrition and growth may affect iron status at four months; to adjust for this we controlled feeding habits at four months of age. As birth weight can be affected by the size of placental transfusion, we decided to only use length as a proxy for growth from birth to four and 12 months.

Sample size

Our hypothesis was that the difference in ferritin at four month old childrenborn by elective CS and DCC would be the same as shown between DCC and ECC in a previous study,[3] that is a difference in log10 Ferritin between 2.07 and 1.90 with a SD of 0.34. To show this difference, a sample size of 63 was needed.

Statistical analysis

For group comparisons of continuous variables, we used one-way analysis of variance (ANOVA) for variables with normal distribution and Bonferroni as post hoc test for pairwise comparisons. Categorical variables were compared between pairwise groups by using Fisher's exact test and across all three groups with Pearson Chi-square test. Ferritin concentration was log10 transformed for analysis. A $p<0.05$ was considered significant. We used SPSS, version 22.0 (IBM, Armonk, NY, USA).

For adjusted analyses, analysis of covariance (ANCOVA) was used for test scores with Bonferroni post hoc test for pairwise comparisons. For adjustment variables, background variables (Table 1) with a difference between groups with a $p<0.1$ were chosen, resulting in mothers' age and gestational age.

Results

During the inclusion period, 505 infants were born after CS, 98 (19 %) preterm and 34 (7 %) post term, Figure 1. Among the term newborns (n=373), CS were classified as acute (174, 47%), elective with a medical reason (145, 39%), and elective with no medical reason (54, 14%). From the 199 elective term CS, 26 could not be included due to maternal disease (diabetes, n=12), preeclampsia, n=6, intra uterine growth restriction (IUGR), n=6 and combination of preeclampsia and IUGR, n=2. Additionally, five women smoked at admission to antenatal care, leaving 168 possible for inclusion. One hundred and four declined participation, resulting in the inclusion of 64 deliveries with elective CS. We did not record the reason to decline out of respect for parents privacy, but reluctance to return for repeated blood sampling was the most common objection. Furthermore, 166 ECC and 168 DCC controls were available for analysis, Figure 1. We did compare data between the 64 included EC with available data from the 104 who declined inclusion and no significant differences in maternal age, gestational age, infants´ birth weight, length or head circumference was found, nor any differences in Apgar score or umbilical blood gases, pointing to our sample being representative for the whole cohort (results not shown).

At four months 59 (92.2%) infants in the elective CS group returned for blood sampling between October 6, 2010 and June 28, 2012. Corresponding blood samples had been obtained from 153 (92.2%) in the ECC group and from 156 (92.9%) in the DCC group between August 8, 2008 and October 1, 2009. At 12 months, 56 (87.5%) infants returned in the elective CS group between May 31, 2011 and Feb 20, 2013, while in the control group, 144 (86.7%)

samples were available from the ECC and 149 (88.7%) from the DCC group (collected between April 8, 2009 and May 21, 2010) (Figure).

For baseline characteristics, see Table 1. The sex distribution was comparable between groups; 28 (44%) were males in the CS group, 83 (50%) in the ECC group and 73 (44%) in the DCC group, p=0.44. As expected, the gestational age was lower in the elective CS group than in the ECC group, -1.2 weeks (95% CI -1.5 to -0.8, P<0.001), and DCC group, -1.1 weeks (95% CI -1.5 to -0.8, P<0.001). The maternal age was also higher in the elective CS group than the in DCC group, 2.2 years (95% CI 0.5 to 3.9, p=0.005).

Apgar scores at one and five minutes were comparable between groups.

Haemoglobin was lower in umbilical cord blood in the elective CS group, when compared to the ECC group, adjusted mean difference (AMD) -13.5 (95% CI -20.3 to -6.6, P<0.001) g/L and the DCC group, AMD -8.5 g/L (95% CI -15.4 to -1.5, P=0.01). However, at 48-72 hours of age, the Hb level did not differ between groups. The Hb level after delivery increased more in the DCC (31.3 g/L [7.8], n 121) and CS (35.6 g/L [16.8], n=38) groups as compared to the ECC group (11.5 g/L [16.8], n=121), p<0.001, indicating a larger placental transfusion.

At four months, differences in ferritin, MCV, and transferrin receptors (but not in TS) indicated better iron status in the CS group compared to the ECC group (Table 2). The proportions of infants having abnormal values for iron status parameters did not differ between the CS group, and the ECC and DCC groups, respectively (Table 3).

At 12 months, no differences between groups in iron status or blood status could be shown (Table 2 and 3).

Auxiliary analysis

Postnatally, children born after CS were more likely to not having been breastfed at one hour after delivery as compared to the ECC, relative risk (RR) 2.1 (95% CI 1.5 to 2.9) and DCC groups, RR 2.5 (95% CI 1.7 to 3.5). The CS group had a higher risk of respiratory distress at

six hours after birth compared to ECC, RR 3.4 (95% CI 1.1 to 10.5) and DCC, RR 4.4 (95% CI 1.4 to 14.9). Respiratory distress at one hour of age and breast-feeding frequency at six hours did not differ between groups.

At four months, exclusive breast-feeding was equally prevalent among the groups, CS 27 (47%), ECC 78 (52%) and DCC 84 (56%), p=0.45. Exclusive breast-feeding correlated positively to the infants' serum ferritin level (r=0.144, p=0.007), but not to any other blood sample analysed at four months. If 'exclusive breast-feeding' was included in the ANCOVA, results was not changed for any variable in any significant way, except for transferrin saturation, where the elective CS group attained a significant higher value than ECC: AMD 2.0% (95% CI 0.0 to 4.0), p=0.049.

Length and weight at four and 12 months of age were comparable across groups, also when adjusted for gestational age. Also weight and length gain from birth was comparable between groups at four and 12 months of age (data not shown). Adding 'length gain' into the adjusted model did not alter differences in any significant way.

Discussion

Main findings

The findings in this prospective observational study indicate that in infants born after elective CS with umbilical cord clamping after 30 seconds, iron stores at four months are comparable to iron stores in vaginally born infants subjected to DCC (≥180 s), and improved compared to vaginally born infants subjected to ECC (≤10 s).

Strengths and Limitations

The main strength of the present study is to report data on iron status and haematological parameters in term infants after CS, as compared to vaginal deliveries and in relation to time to umbilical cord clamping. Haematological and iron status after different timing of umbilical cord clamping have previously been reported in several studies.[4] Among available studies

with four months or longer follow-up on iron stores, three excluded infants born after CS[2 3 22] while one included CS but did not separate results from vaginal birth.[23] From an ethical, and in many cases also medical point of view, it is impossible to randomise women to either elective CS or vaginal birth. As the trial is observational, and not randomised, the interpretation of the study's results is limited by the possibility of bias and confounding factors. These include in particular unidentified differences in baseline characteristics between groups, including prenatal maternal as well as perinatal and postnatal infant influences. Except for iron stores at birth, nutrition and growth rate is expected to influence iron status at a later age, and we could control for these data that did not alter the main outcomes. In all three groups, only 35-40% of eligible pregnancies were included. Data from the included EC cohort were not significantly different from those who declined consent, indicating similarity between included and 'declined inclusion' pregnancies. A limitation for the conclusion is that cord clamping at 30 sec at elective CS has not been compared to the usual practice; immediate clamping at elective CS.

Interpretation

Previous studies have implied less placental transfusion after CS. Consequently; our findings are not in line with the relatively scarce literature on this subject. One explanation to our finding that CS rather improves iron stores compared to ECC at four months of age could be that the obstetrician actually waited 30 second to clamp the cord. Timing to umbilical cord clamping after CS has usually not been reported in other studies, but we presume it to have been performed immediately after delivery. Another potentially contributing factor to the improved iron stores is that infants born after elective CS have a lower blood pressure due to less circulating adenosine and catecholamines,[24 25] facilitating a faster blood transfusion from the placenta. Unfortunately, we did not record the time for the first breath/cry, but earlier

reports indicate that most new-borns had commenced breathing before the cord was clamped in the CS group.[26]

Haemoglobin in the umbilical cord blood sample was significantly lower after elective CS compared to ECC and DCC, a finding in coherence with a recent systematic review and meta-analysis.[13] This finding suggests that umbilical cord Hb may not a reliable marker of iron status in newborns, as the result may reflect not only iron status, but also mode of delivery. Our study give support to the pragmatic approach to wait for 30 seconds before clamping after CS, as we could not demonstrate any negative effect on iron homeostasis compared to the vaginally born groups. Our findings might imply that whatever negative consequences on the child's health CS is associated with, waiting for 30 seconds to clamp the cord reduces those that could possibly be explained by a diminished placental blood transfusion.

Conclusion

Erickson-Owens et al have suggested umbilical cord milking as a possible procedure to facilitate the placental transfusion after CS in term infants.[27] Our results suggest that the less invasive method of a 30 s DCC might be sufficient to ensure the placental transfusion after elective CS. Large observational studies, most preferably prospective with vaginally born matched controls, are indicated and warranted.

In summary, our study demonstrated that infants born after elective CS with cord clamping at 30 seconds had iron stores similar to those born vaginally with DCC and better than those born vaginally with ECC at four months of age.

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Disclosure of Interests

None.

Contribution to Authorship

OA, LHW and MD planned the study. OA was responsible for staff training, study management and data collection with support from LHW and MD. OA, LHW and MD analysed the data. OA drafted the manuscript. All authors revised the manuscript and accepted the final version. Ola Andersson is the guarantor.

Details of Ethics Approval

The original study was approved by the regional ethical review board at Lund University (2008/41), and the new cohort including elective CS was approved by an amendment (2009/344).

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Data sharing statement

None available

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Table/Figure Caption List

Table 1. Baseline characteristics and early outcomes after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Table 2. Laboratory status at four and 12 months of age after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Table 3. Proportion of infants with iron status indicators outside reference limits at four and 12 months after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping

Figure 1. Trial profile. Flow diagram.

Elective caesarean, cord clamping and iron stores at 4 mo

Table 1. Maternal and birth characteristics and early outcomes after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

							Mean Difference (95% CI)[b]			
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[d]	CS vs DCC	*P*-value[d]
Maternal characteristics											
At admission to antenatal care											
Weight, kg	69.3 (14.8)	56	66.4 (12.0)	164	67.3 (12.2)	168	0.32				
Length, cm	166.8 (6.6)	56	167.9 (6.4)	147	167.5 (5.1)	141	0.44				
Body mass index, kg/m2	24.8 (4.3)	56	23.6 (3.8)	146	23.9 (3.6)	141	0.16				
Haemoglobin, g/L	126.4 (10.1)	57	128.0 (8.8)	161	128.0 (10.8)	168	0.55				
At day of giving birth											
Age, years	33.0 (5.6)	64	31.7 (4.2)	166	30.8 (4.9)	168	0.006	1.4 (-0.3 to 3.0)	0.15	2.2 (0.5 to 3.9)	0.005
Parity (including study child)	1.9 (1.0)	64	1.8 (0.9)	166	1.8 (0.7)	168	0.88				
Early infant characteristics							Adjusted[x] Mean Difference (95% CI)				
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[e]	CS vs DCC	*P*-value[e]
Gestational age, weeks	38.9 (0.6)	64	40.0 (1.1)	166	40.0 (1.1)	168	<0.001	-1.2 (-1.5 to -0.8)	<0.001	-1.1 (-1.5 to -0.8)	<0.001
Apgar score at 1 minute	9.0 (1.0)	64	8.8 (0.8)	166	9.0 (0.4)	168	0.008	0.2 (-0.1 to 0.4)	0.42	-0.1 (-0.3 to 0.2)	>0.99
Length, cm	50.4 82.0)	64	50.7 (1.9)	165	50.9 (1.9)	168	0.23	0.7 (0.0 to 1.3)	0.046	0.4 (-0.2 to 1.1)	0.31
Birth weight, gram	3537 (567)	64	3523 (483)	166	3632 (464)	168	0.10	224 (51 to 398)	0.006	104 (-70 to 277)	0.45
Head circumference, cm	35.8 (1.5)	64	34.7 (1.3)	166	35.0 (1.37)	168	<0.001	1.7 (1.2 to 2.1)	<0.001	1.4 (0.9 to 1.9)	<0.001
pH in umbilical cord artery	7.29 (0.05)	57	7.27 (0.08)	159	7.26 (0.08)	144	0.04	0.02 (-0.01 to 0.05)	0.33	0.025 (-0.01 to 0.06)	0.16
Base deficit	2.0 (2.5)	56	4.4 (3.4)	158	4.8 (3.7)	143	<0.001	-2.0 (-3.4 to -0.6)	0.002	-2.4 (-3.8 to -1.0)	<0.001

CS=Elective Caesarean Section, ECC=Early Cord Clamping, DCC=Delayed cord clamping, CI=confidence interval. [a] Data are mean (SD) or mean difference (95% CI). [b]Adjusted for maternal age and gestational age. P values were calculated using [c]One-way ANOVA, [d]One-way ANOVA with Bonferroni post hoc comparison, [e]Analysis of covariancewith Bonferroni post hoc comparison.

Elective caesarean, cord clamping and iron stores at 4 mo

Table 2. Laboratory status at different time points after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

								Adjusted[c] Mean Difference (95% CI)			
	CS	n	ECC	n	DCC	n	*P*-value[c]	CS vs ECC	*P*-value[c]	CS vs DCC	*P*-value[c]
Umbilical cord											
Haemoglobin, g/L	147.9 (19.0)	52	163.3 (14.9)	144	158.0 (17.6)	144	<0.001	-13.5 (-20.3 to -6.6)	<0.001	-8.4 (-15.4 to -1.5)	0.01
Ferritin, µg/L[b]	160 (8 to 853)	61	181 (12 to 1112)	163	183 (25 to 735)	164	0.38				
48-72 hours after birth											
Haemoglobin, g/L	179.9 (20.5)	40	174.9 (18.6)	104	188.5 (16.4)	107	<0.001	7.5 (-0.7 to 15.8)	0.09	-6.6 (-14.9 to 1.7)	0.17
4 months											
Haemoglobin, g/L	113.4 (7.5)	57	113.0 (7.1)	153	112.8 (7.5)	147	0.88				
MCV, fL	79.3 (2.6)	57	77.9 (3.1)	153	79.1 (3.1)	147	<0.001	1.8 (0.6 to 3.0)	0.001	0.5 (-0.7 to 1.7)	0.96
Ferritin, µg/L[b]	103 (14 to 401)	55	80 (6 to 760)	153	117 (20 to 880)	149	<0.001	39 (10 to 60)	0.007	2 (-41 to 33)	>0.99
Transferrin saturation, %	17.1 (6.5)	56	15.8 (5.6)	153	18.2 (6.1)	148	0.002	2.1 (-0.3 to 4.5)	0.11	-0.3 (-2.7 to 2.1)	>0.99
Transferrin receptors, mg/L	3.70 (0.75)	55	4.00 (0.80)	153	3.72 (0.69)	149	0.002	-0.39 (-0.69 to -0.08)	0.007	-0.10 (-0.40 to 0.21)	>0.99
12 months											
Haemoglobin (g/L)	117.5 (8.0)	52	119.4 (8.2)	131	117.6 (7.8)	129	0.14				
MCV, fL	76.8 (3.6)	52	76.9 (3.3)	131	76.6 (3.3)	129	0.77				
Ferritin, µg/L[b]	35 (8 to 107)	48	34 (8 to 135)	136	35 (10 to 281)	129	0.84				
Transferrin saturation, %	16.2 (7.1)	49	15.4 (7.3)	135	15.3 (6.0)	130	0.72				
Transferrin receptors, mg/L	4.40 (0.82)	49	4.48 (0.99)	136	4.37 (0.87)	130	0.61				

ECC=Early Cord Clamping, DCC=Delayed cord clamping, CI=Confidence Interval, MCV=Mean Cell Volume. [a] Data are mean (SD) or mean difference (95% CI). [b] Ferritin is presented as geometric mean (geometric standard deviation). [c] Adjusted for maternal age and gestational age. *P* values were calculated using [d]One-way ANOVA, [e]Analysis of covariance with Bonferroni post hoc comparison

Elective caesarean, cord clamping and iron stores at 4 mo

Table 3. Infants with anaemia or abnormal iron indices outside reference ranges at 4 and 12 months after elective caesarean section or vaginal birth after early (≤10 s) or delayed (≥180 s) umbilical cord clamping [a]

								Absolute risk reduction (95% CI), %	
	CS	n	ECC	n	DCC	n	*P*-value	CS vs ECC	CS vs DCC
4 months									
Anaemia (Hb < 105 g/L)	6 (10.5%)	57	20 (13.1%)	153	20 (13.6%)	147	0.84		
Anaemia and iron deficiency	0 (0%)	52	2 (1.3%)	153	0 (0%)	148	0.27		
Iron deficiency (2 out of 4)[b]	0 (0%)	52	8 (5.2%)	153	1 (0.7%)	144	0.02	5.2 (-2.9 to 5.2)	0.7 (-1.8 to 0.7)
MCV < 73 nm	0 (0%)	57	8 (5.2%)	153	3 (2.0%)	147	0.09	5.2 (-2.3 to 5.2)	2.0 (-3.0 to 2.0)
Ferritin < 20 umol/L	1 (1.8%)	55	11 (7.2%)	153	0 (0%)	149	0.002	5.4 (-3.8 to 7.7)	-1.8 (-1.8 to 0.5)
Transferrin saturation < 10%	6 (10.7%)	56	22 (14.4%)	153	8 (5.4%)	148	0.03	3.7 (-8.9 to 12.0)	-5.3 (-14.5 to 2.8)
Transferrin receptors < 7 mg/L	0 (0%)	55	0 (0%)	153	0 (0%)	149	NA		
12 months									
Anaemia (Hb < 110 g/L)	9 (17.3%)	52	16 (12.2%)	131	22 (17.1%)	129	0.49		
Anaemia and iron deficiency	1 (2.1%)	46	1 (0.8%)	130	0 (0%)	128	0.30		
Iron deficiency (2 out of 4)[b]	2 (4.3%)	47	7 (5.3%)	132	3 (2.3%)	128	0.464		
MCV < 73 nm	0 (0%)	52	3 (2.3%)	131	3 (2.3%)	129	0.54		
Ferritin < 20 umol/L	2 (4.2%)	46	3 (2.2%)	136	2 (1.6%)	129	0.58		
Transferrin saturation < 10%	6 (12.2%)	49	25 (18.5%)	135	22 (16.9%)	130	0.60		
Transferrin receptors < 5.92 mg/L	4 (8.2%)	45	10 (7.4%)	136	9 (6.9%)	130	0.96		

[a] Data are numbers (%). MCV=Mean Cell Volume, NA=not applicable. [b] Defined as having 2 or more of iron status indicators (low Ferritin, low MCV, low Transferrin saturation and/or high Transferrin receptors) out of reference range.

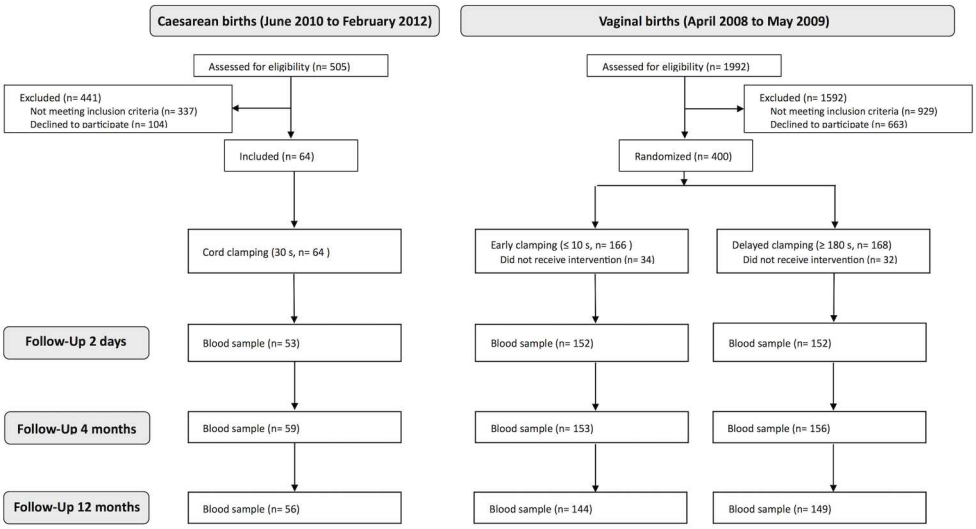

Figure. Trial profile. Flow diagram.

Trial profile. Flow diagram.
Figure
173x122mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	5-7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	7

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure, page 8
		(b) Give reasons for non-participation at each stage	Figure, page 8
		(c) Consider use of a flow diagram	Figure
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7, table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, table 1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Results and tables
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2 & 3
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Results
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.