PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Elective caesarean – does delay in cord clamping for 30 seconds ensure sufficient iron stores at four months of age? – a historical cohort control study
AUTHORS	Andersson, Ola; Hellström-Westas, Lena; Domellof, Magnus

VERSION 1 - REVIEW

REVIEWER	Prof David Tudehope Professorial Research Fellow Mater Research Institute-UQ Matewr Health Services Raymond Terrace Sourth Brisbane 4101 Queensland Australia
REVIEW RETURNED	11-Jul-2016

GENERAL COMMENTS	BMJ OPEN review 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. The study compares levels of iron and iron stores between 64 infants born by elective CS with cords clamped at 30 sec [born 2010-2012] with infants born vaginally [2008-2009] with cords clamped<11 sec [152 infants]and >179 sec [156 infants].Hct and/or iron studies were performed on cord blood and at ages 48-72hrs and 4and 12 months . The introduction determines the need for further research on optimal timing for cord clamping in babies born by elective CS but does not provide the background evidence for clamping at 30 seconds as in this study .The obstetrician who has to weigh up possible benefits against possible disadvantages for mother and baby for selecting this intermediate time for clamping after elective CS.No justification is provided for clamping at 30 sec rather than at birth and no mention of any difficulties or potential benefits from this approach.. The title of a prospective study is misleading as the elective CS was followed prospectively but was compared with an historical cohort. Preferred terminology would be historical cohort control study Methods: Details of cord clamping and blood sampling techniques are notadequately described. Blood lood sampled at 48-72 hrs was heel prick but samples at4 and 12 months are not described. There are substantial differences in Hct,Hb and MCV between cord blood [UA

	or UV] heel prick and venous blood samples. Similarly definitions of anaemia and polycythaemia at 2 days ,4 months and 12 months need knowledge of where blood was sampled from .Another sentence on interpretation of iron stores by levels of TS, sTfR and ferritin would assist readership. Methods needs a clear statement as to which iron and blood studies were performed on cord blood, heel stick blood at 48-72hours and /venous blood at 4 and 12 months . Why were CRP and bilirubin levels included in study when results are not provided ? Similarly for IgG and reticulocytes . Estimate of Sample size required is adequate. Methods relating to auxiliary outcomes such as breast feeding at at 1 birth and at 6 hours after birth and respiratory distress at 1 and 6 hours are not described Outcomes: Primary outcome is clear but secondary outcomes studied in this paper are not clear to this reviewer Respiratory symptoms in first 4 months. I would recommend excluding additional studies such as bilirubin,IgG, psychometric testing using Ages and Stages questionairre as results are nmot reported in this study . Statistical Analysis: Readership and comprehension would be assisted by listing which background variable were analysed for ANCOVA and which were included in final model used . Strengths and limitations section needs re drafting as it is not clearly expressed .Point 1 is not relevant .Point 2 is not correct as comparative study use an historical cohort. ie historical cohort control study . Point 3 introduces a limitation to interpretation of existing data .Point 4 is relevant but provides no insight into why consent rate was only 35-40% Results : Acute CS is usually expressed as emergency CS and medical reason as maternal/obstetric/fetal indication.Of the 64 who consented for elective CS study what proportion had a specific indication versus no medical indication ? Was any analysis conducted on why women declined to be in study ? Rates of decline were almost identical between elective CS study[104/168[61.9%]] and historical cohorts 663/1063[62.4%] . Better values for transferrin saturation as well at 4 months but no differences between ECS and DCC. Resite sex distribution after " for baseline characteristics see table 1 " As expected the gestational age in elective CS was lower than in the ECC group . Delete we chose to include mother's age and GA from this section as already in methods. A better heading for Table 1 is .Maternal and birth characteristics . Table 2 For ease of understanding and readership I recommend removing Umbilical cord Hb and ? umbilical cord ferritin and placing all blood and iron studies on cord blood, 48-72 hrs heel stick blood and ?venous blood at 4 and 8 months in table 2 Addition of absolute values for Hb at 48-72 hrs is recommended to table 2 to better assess haemoconcentration and size of placental transfusion . Table 3 .Infants with anaemia or abnormal iron indices outside reference ranges - 4 months [iron deficiency ,ferritin transferrin saturation

	- 12 months not significant differences but high rates of low Hb - Discussion Make suggestions for editing of strengths and limations . Why is cord Hb not a reliable marker of iron status in newborns? Limitation; There is currently overlap between discussion and strengths and limitations section on page 2 which requires editing.Alimitation for conclusion that cord clamping at elective CS at 30 sec is recommended as it is equivalent to DCC for vaginal births is that it has not been compared to the usual practice immediate clamping at elective CS . Additional comments related to checklist : Abstract is unclear in several places. Population : 64 Study infants were born after elective CS and compared with historical controls born vaginally , 166 early cord clamping and 168 delayed clamping Secondary outcomes are not clearly separated from primary Study design – concerns already expressed Research ethics - process for obtaining parental consent is not described .Figure identifies high decline rates. There is no mention of ethics approval for the new cohort study on infants born by elective CS . Standard of English – overall paper needs some editing to improve English syntax

REVIEWER	Heike Rabe Brighton & Sussex Medical School, UK
REVIEW RETURNED	12-Jul-2016

GENERAL COMMENTS	The manuscript describes a prospective cohort study of 30 sec delayed cord clamping (DCC) time in term babies born by elective caesarean section (CS). As historic comparator groups babies enrolled into a RCT of immediate versus DCC (180 sec) delivered by vaginal delivery in the same institution were used. The study question of iron status at 4 months in all groups is valid and contemporary. The DCC group of the cohort study is highly selective (see exclusion criteria) and this needs to be kept in mind when interpreting the results. The authors found similar iron storage at 4 months in the 30 sec DCC CS group compared to 180 sec DCC vaginal group. I would recommend to think about the physiology a bit more and discuss this some what surprising finding. Elective CS babies might not have got the natural changes of receiving more placental blood prior to birth which normally occurs tow adds term. Thus even waiting for 30 sec did provide them with quite large amount of blood in a short time period? This is a main finding in the paper and I wonder whether the introduction of the paper needs to be re-written to reflect the logical thread of thinking throughout the paper? Minor typos: page 10 line 12: delete "correlated"

	page 10, line 14: delete "for" from "analyzed for at four months"

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Prof David Tudehope
Institution and Country: Professorial Research Fellow, Mater Research Institute-UQ, Matewr Health Services, Queensland, Australia
Competing Interests: None declared

The introduction determines the need for further research on optimal timing for cord clamping in babies born by elective CS but does not provide the background evidence for clamping at 30 seconds as in this study .The obstetrician who has to weigh up possible benefits against possible disadvantages for mother and baby for selecting this intermediate time for clamping after elective CS. No justification is provided for clamping at 30 sec rather than at birth and no mention of any difficulties or potential benefits from this approach.
++ Answer: We do understand the reviewer's objection on this matter. An explanation has been added to the introduction regarding the pragmatic decision by the board of obstetricians (page 4, line 132-135).

The title of a prospective study is misleading as the elective CS was followed prospectively but was compared with an historical cohort. Preferred terminology would be historical cohort control study
++ Answer: We have revised the title according to the reviewer's suggestion (page 1, line 2-3)

Methods:
Details of cord clamping and blood sampling techniques are not adequately described. Blood lood sampled at 48-72 hrs was heel prick but samples at4 and 12 months are not described. There are substantial differences in Hct,Hb and MCV between cord blood [UA or UV] heel prick and venous blood samples. Similarly definitions of anaemia and polycythaemia at 2 days ,4 months and 12 months need knowledge of where blood was sampled from .
++ Answer: As existing routine, also blood sampled at 48-72 hrs were sampled by venous blood samples, as also samples collected at 4 and 12 months. We did add this information. (Page 6, line 181 and 186)

Another sentence on interpretation of iron stores by levels of TS, sTfR and ferritin would assist readership.
++ Answer: We have added information on the interpretation of iron stores (page 5, line 169-174).

Methods needs a clear statement as to which iron and blood studies were performed on cord blood, heel stick blood at 48-72hours and /venous blood at 4 and 12 months .
++ Answer: The same method were used for analysis during the whole trial. (page 6, line 188-192)

Why were CRP and bilirubin levels included in study when results are not provided ?
++ Answer: Following the reviewer's suggestion, we omitted mentioning bilirubin. An explaination regarding why CRP was analysed has been added (page 5, line 173-174)

Similarly for IgG and reticulocytes .
++ Answer: Following the reviewer's suggestion, we omitted mentioning IgG and reticulocytes

Estimate of Sample size required is adequate.
Methods relating to auxiliary outcomes such as breast feeding at at 1 birth and at 6 hours after birth

and respiratory distress at 1 and 6 hours are not described
++ Answer: The method is described at page 5-6, line 175-180. It now also says that the midwife noted her observations in the protocol.

Outcomes:
Primary outcome is clear but secondary outcomes studied in this paper are not clear to this reviewer Respiratory symptoms in first 4 months.
I would recommend excluding additional studies such as bilirubin,IgG, psychometric testing using Ages and Stages questionairre as results are nmot reported in this study .
++ Answer: We have revised the outcome section according to the reviewer's suggestions.

Statistical Analysis: Readership and comprehension would be assisted by listing which background variable were analysed for ANCOVA and which were included in final model used .
++ Answer: We have elaborated this (page 8, line 251-252)

Strengths and limitations section needs re drafting as it is not clearly expressed .Point 1 is not relevant .
++ Answer: We do not see what reviewer 1 mean. Data comparing on iron status and haematological parameters in term infants after CS up to 12 months of life as compared to vaginal deliveries is to our knowledge not earlier reported from prospectively followed groups. The strength of this study is to present such data.

Point 2 is not correct as comparative study use an historical cohort. ie historical cohort control study .
++ Answer: We have rephrased point 2 (page 2, line 61)

Point 3 introduces a limitation to interpretation of existing data .

Point 4 is relevant but provides no insight into why consent rate was only 35-40%
Results : Acute CS is usually expressed as emergency CS and medical reason as maternal/obstetric/fetal indication.Of the 64 who consented for elective CS study what proportion had a specific indication versus no medical indication ? Was any analysis conducted on why women declined to be in study ? Rates of decline were almost identical between elective CS study[104/168[61.9%]] and historical cohorts 663/1063[62.4%] .
++ Answer: We have made a comment on this matter in the results section (page 8, line 261-263).

Better values for transferrin saturation as well at 4 months but no differences between ECS and DCC.
++ Answer: As the difference in transferrin saturation between CS and ECC was not significant, we chose not to write this in the results section. We have rephrased the sentence slightly (page 9, line 291)

Resite sex distribution after " for baseline characteristics see table 1 " As expected the gestational age in elective CS was lower than in the ECC group .
++ Answer: We have made changes according to the reviewer's suggestion (page 9, line 278-280)

Delete we chose to include mother's age and GA from this section as already in methods.
++ Answer: We have deleted the sentence (page 9, line 283-284).

A better heading for Table 1 is .Maternal and birth characteristics .
++ Answer: We have made changes according to the reviewer's suggestion

Table 2 For ease of understanding and readership I recommend removing Umbilical cord Hb and ?

umbilical cord ferritin and placing all blood and iron studies on cord blood, 48-72 hrs heel stick blood and ?venous blood at 4 and 8 months in table 2 Addition of absolute values for Hb at 48-72 hrs is recommended to table 2 to better assess haemoconcentration and size of placental transfusion .
++ Answer: We have made changes according to the reviewer's suggestion

Table 3 .Infants with anaemia or abnormal iron indices outside reference ranges - 4 months [iron deficiency ,ferritin transferrin saturation
++ Answer: We have changed the title according to the reviewer's suggestion

- 12 months not significant differences but high rates of low Hb
++ Answer: Different definitions for anaemia has been suggested at 12 months. We have chosen the most used (Hb < 110 g/L)

- Discussion
Make suggestions for editing of strengths and limations .
Why is cord Hb not a reliable marker of iron status in newborns?
++ Answer: We have added an explaination to our statement (page 11, line 382).

Limitation; There is currently overlap between discussion and strengths and limitations section on page 2 which requires editing. A limitation for conclusion that cord clamping at elective CS at 30 sec is recommended as it is equivalent to DCC for vaginal births is that it has not been compared to the usual practice immediate clamping at elective CS .
++ Answer: We had understood that the strengths and limitations section on page 2 is supposed to be a short version of the same paragraph in the discussion?

Additional comments related to checklist :

Abstract is unclear in several places.
Population : 64 Study infants were born after elective CS and compared with historical controls born vaginally , 166 early cord clamping and 168 delayed clamping
++ Answer: We have changed this sentence.

Secondary outcomes are not clearly separated from primary
++ Answer: We have changed this sentence.

Study design – concerns already expressed
++ Answer: We have changed this sentence in the abstract.

Research ethics - process for obtaining parental consent is not described .Figure identifies high decline rates.
++ Answer: Please see page 4, line 143-144

There is no mention of ethics approval for the new cohort study on infants born by elective CS .
++ Answer: We have made this clearer, page 13, line 412-414.

Standard of English – overall paper needs some editing to improve English syntax
++ Answer: We have made changes as seems appropriate.

Reviewer: 2
Reviewer Name: Heike Rabe
Institution and Country: Brighton & Sussex Medical School, UK

Competing Interests: None

The manuscript describes a prospective cohort study of 30 sec delayed cord clamping (DCC) time in term babies born by elective caesarean section (CS). As historic comparator groups babies enrolled into a RCT of immediate versus DCC (180 sec) delivered by vaginal delivery in the same institution were used.
The study question of iron status at 4 months in all groups is valid and contemporary.
The DCC group of the cohort study is highly selective (see exclusion criteria) and this needs to be kept in mind when interpreting the results.

The authors found similar iron storage at 4 months in the 30 sec DCC CS group compared to 180 sec DCC vaginal group. I would recommend to think about the physiology a bit more and discuss this some what surprising finding. Elective CS babies might not have got the natural changes of receiving more placental blood prior to birth which normally occurs tow adds term. Thus even waiting for 30 sec did provide them with quite large amount of blood in a short time period?
++ Answer: We agree that it is a surprising finding that warrants more studies. We chose to avoid to much speculation regarding the explanation, but have suggested some at page 11, line 363-378. All elective CS in our study was born at term, although earlier than in the groups born vaginally.

This is a main finding in the paper and I wonder whether the introduction of the paper needs to be re-written to reflect the logical thread of thinking throughout the paper?
++ Answer: We have changed the paragraphs in the introduction and hope that this makes it easier for the reader to realise the surprising finding

Minor typos:
page 10 line 12: delete "correlated"
page 10, line 14: delete "for" from "analyzed for at four months"
++ Answer: We have made changes as suggested.

VERSION 2 – REVIEW

REVIEWER	Prof David Tudehope Professorial Researcher ,Mater Research Institute-UQ, South Brisbane , Queensland,Australia
REVIEW RETURNED	13-Sep-2016

GENERAL COMMENTS	The authors have meticulously responded to all the numerous suggestions I made in my original review of manuscript to my satisfaction. These amendments have improved scientific writing and readership of manuscript However, I still have many minor suggestions for improvements in amended manuscript mainly of an editorial nature or related to English syntax. Your editorial process still needs to carefully review English expression ,syntax and plurality . Title of paper : change -is it enough to - does delay in cord clamping for 30seconds ..... Abstract : Use abbreviation elective CS throughout entire paper Objective: To compare .....umbilical cord clamping with those born Population: '.....were compared with an historical control cohort of ..... Methods: ..... measured in

	Results: At four months infants born by elective CS had better iron status than those born vaginally Strengths and Limitations: Line 57 . This study compares iron status and haematological parameters up to 12 months in term infants born by elective CS with those born vaginally in .... Line 61 . As an observational study with historical controls results must be interpreted with caution because of potential bias from confounding Line 68: .....the usual practice of immediate cord clamping Introduction; Line 76 ..... omitting the global increase in elective CS births Line 86: ....after pre-labour elective CS Line 107: ...WHAT WAS RELATIOSHIP TO TIME OF CORD CLAMPING? Participants : Line 150: as well as Hb and MCV ,provided additional information on iron status .... Line 158 : I am very surprised that ROUTINE VENOUS BLOOD is sampled for metabolic screening .I have never heard of this practice ,it is always heel stick blood .I would urge you to check that this is current practice at your hospital in Sweden . Line 173 : Once again I suggest this is capillary blood . SAMPLE SIZE: .....in ferritin at 4 month old children born by electiveCS Results : Line 227. .....decline out of respect for parents privacy ,but reluctance .... Strengths and Limitations: LINE 305: ....only 35-40% of eligible pregnancies were included. Data from the included ECC cohort were not significantly different from those who declined consent. Conclusions: Line339: 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 ..... I do not need to complete another re-review if authors and your editorial team appropriately respond to my suggestions .

VERSION 2 – AUTHOR RESPONSE

For this revision we have followed all of the reviewer's (Prof David Tudehope) suggestions except for the following:

Line 158 : I am very surprised that ROUTINE VENOUS BLOOD is sampled for metabolic screening. I have never heard of this practice, it is always heel stick blood. I would urge you to check that this is current practice at your hospital in Sweden .
Line 173 : Once again I suggest this is capillary blood.

Professor Tudehope seems very surprised that venepuncture is practiced in Sweden, instead of heel stick. The procedure is well established at Swedish hospitals, and has proven benefits.

The method was reported in two Swedish papers in 1998 and 1999 as less painful, and this has been further shown also by the Cochrane reviews. Please see references below.

1. Shah VS, Taddio A, Bennett S, Speidel BD. Neonatal pain response to heel stick vs venepuncture for routine blood sampling. Arch Dis Child - Fetal Neonatal Ed. 1997;77(2):F143-F144. doi:10.1136/fn.77.2.F143.
2. Larsson BA, Tannfeldt G, Lagercrantz H, Olsson GL. Venipuncture is more effective and less painful than heel lancing for blood tests in neonates. Pediatrics. 1998;101(5):882-6. doi:10.1542/peds.101.5.882.
3. Eriksson M, Gradin M, Schollin J. Oral glucose and venepuncture reduce blood sampling pain in newborns. Early Hum Dev. 1999;55(3):211-218. doi:10.1016/S0378-3782(99)00018-3.
4. Shah VS, Ohlsson A. Venepuncture versus heel lance for blood sampling in term neonates. Cochrane database Syst Rev. 2011;(10):CD001452. doi:10.1002/14651858.CD001452.pub4.