

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) | Wait a minute? An observational cohort study comparing iron stores in healthy Swedish infants at four months of age after 10, 60 and 180 seconds' umbilical cord clamping |
| AUTHORS | Askelöf, Ulrica; Andersson, Ola; Domellöf, Magnus; Fasth, Anders; Hallberg, Boubou; Hellström-Westas, Lena; Pettersson, Karin; Westgren, Magnus; Wiklund, Ingela E; Götherström, Cecilia |

VERSION 1 – REVIEW

| | |
|------------------------|--|
| REVIEWER | Anup Katheria Sharp Mary Birch Hospital for Women and Infants |
| REVIEW RETURNED | 15-Apr-2017 |

| | |
|-------------------------|--|
| GENERAL COMMENTS | <p>Askelof et al attempt to answer an important question in the current era of placental transfusion and cord blood banking; that is, does clamping the cord earlier effect neonatal iron stores. The short answer is s short 60 second delay may not have significant effects. This is important data that needs to be made available to the public.</p> <p>The only areas I would elaborate on that pertain to variances in practices. For example in the US the infants are immediately placed on the mothers abdomen. 30 seconds of the infant being placed lower likely "speeds up" the transfusion and may explain why the authors still saw an increase compared to immediate (10 second) clamping. This needs to be stress for other institutions.</p> <p>The other limitation of this study is the lack of followup. One of the authors is a well known expert in the field of DCC and has produced 4 year followup in infants with DCC. This outcome is invariably more important then demonstrating differences in ferritin and cord Hb. That would make this study stronger.</p> |
|-------------------------|--|

| | |
|------------------------|--|
| REVIEWER | Judith Mercer University of Rhode Island, Kingston, Rhode Island, USA |
| REVIEW RETURNED | 03-May-2017 |

| | |
|-------------------------|--|
| GENERAL COMMENTS | <p>This study is well written with a clear explanation of all methodology. However, data are lacking to draw the conclusions that the paper presents.</p> <p>In the introduction to the paper, the authors clearly state that "a small cell dose in a transplanted unit is directly correlated with a delay in recovery of the immune system and lower incidence of sustained donor engraftment.</p> |
|-------------------------|--|

Large cell numbers are essential and closely correlated with the collected volume of UCB.” Having said that, it is essential to this paper to report the volume of blood obtain and the TNC count after a one–minute delay even if that information is not available for the historic comparative sample. I am sure that this information is readily available as cord blood banks historically collect data on the amount and cell count of each sample (Mousavi, 2016). The reader needs to know how many of the units obtained after 60 seconds were adequate in volume and number of TNC/stem cells.

In Figure 2, the information that boys were more likely to be anemic after the 60 sec delay is also critical information and is not discussed adequately. In Andersson’s paper on the 4-year evaluation of the children in the historic sample, he found that children had better social-emotional adjustment and fine motor skills – especially boys who also had faster process speed if they were in the 180 second group. These findings raise more concerns about the findings of more iron deficiency in boys with the 60 second delay and are not discussed adequately in this paper. Thus there is a risk for harm which must be considered especially if the units were not adequate for storage.

Without knowing the condition of the units collected, one cannot assess even the small risk of iron deficiency anemia in the boys (60 seconds) in this study. Thus, the conclusion in this paper are not appropriate.

It is not clear how the Swedish National Cord Blood Registry works and if there is a conflict of interest for the first author. Are they for profit, for instance? Do they do private banking or it is all public with no charges for parents? This information is needed to clear a conflict of interest for this paper.

I would recommend that the authors obtain the data on the quality of the units collected and include that information in this paper to attempt to balance out the risk of increased iron deficiency in the boys at four months of age.

Mousavi SH, Abroun S, Zarrabi M, Ahmadipanah M. The effect of maternal and infant factors on cord blood yield. *Pediatr Blood Cancer*. 2016.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Anup Katheria, Sharp Mary Birch Hospital for Women and Infants

Comment: Askelof et al attempt to answer an important question in the current era of placental transfusion and cord blood banking; that is, does clamping the cord earlier effect neonatal iron stores. The short answer is s short 60 second delay may not have significant effects. This is important data that needs to be made available to the public.

The only areas I would elaborate on that pertain to variances in practices. For example in the US the infants are immediately placed on the mothers' abdomen. 30 seconds of the infant being placed lower likely "speeds up" the transfusion and may explain why the authors still saw an increase compared to immediate (10 second) clamping. This needs to be stress for other institutions.

Answer from the authors: This is a very good point and of importance in order to estimate the generalizability of this study. We have now made this clearer in the method section on page 5 and we have also discussed potential consequences in the discussion on page 9.

Comment: The other limitation of this study is the lack of follow-up. One of the authors is a well-known expert in the field of DCC and has produced 4 year follow-up in infants with DCC. This outcome is invariably more important then demonstrating differences in ferritin and cord Hb. That would make this study stronger.

Answer from the authors: This is an important point and we agree that this study would be even stronger had we performed long term follow-up of the children. However, this particular study was not designed for long term follow-up of the children but in the conclusion on page 11, we have empathized that clinical studies are needed in order to determine long term consequences for children who donate UCB or who for some other reason are being subjected to limited cord clamping time.

Reviewer 2

Judith Mercer, University of Rhode Island, Kingston, Rhode Island, USA

This study is well written with a clear explanation of all methodology. However, data are lacking to draw the conclusions that the paper presents.

Comment: In the introduction to the paper, the authors clearly state that "a small cell dose in a transplanted unit is directly correlated with a delay in recovery of the immune system and lower incidence of sustained donor engraftment. Large cell numbers are essential and closely correlated with the collected volume of UCB." Having said that, it is essential to this paper to report the volume of blood obtain and the TNC count after a one-minute delay even if that information is not available for the historic comparative sample. I am sure that this information is readily available as cord blood banks historically collect data on the amount and cell count of each sample (Mousavi, 2016). The reader needs to know how many of the units obtained after 60 seconds were adequate in volume and number of TNC/stem cells.

Answer from the authors: This is an adequate comment that needs to be addressed. However, the aim of this study was to investigate the donor outcome after umbilical cord blood donation and its associated clamping time, and not the result of the collection.

There is a published article by Frändberg et al. describing the collection outcome from the Swedish National Umbilical Cord Blood Bank after different cord clamping times (Frändberg S, 2016). For this reason we are not discussing this further in the current study but we have made a clarification of the possibility that donated umbilical cord blood may not be used if the amount of collected UCB is too small and that parents considering donation should be clearly informed about this and take it into consideration in their decision to donate or not (page 10).

Comment: In Figure 2, the information that boys were more likely to be anemic after the 60 sec delay is also critical information and is not discussed adequately. In Andersson's paper on the 4-year evaluation of the children in the historic sample, he found that children had better social-emotional adjustment and fine motor skills – especially boys who also had faster process speed if they were in the 180 second group. These findings raise more concerns about the findings of more iron deficiency in boys with the 60 second delay and are not discussed adequately in this paper. Thus there is a risk for harm which must be considered especially if the units were not adequate for storage.

Without knowing the condition of the units collected, one cannot assess even the small risk of iron deficiency anemia in the boys (60 seconds) in this study. Thus, the conclusion in this paper are not appropriate.

Answer from the authors: This is another relevant issue, namely the importance of sex when it comes to early iron deficiency and long term effects. The current study was not designed to investigate differences according to sex (the groups were too small when dividing them according to sex):

N successfully analysed results Iron deficiency

10s-group: 75 girls, 78 boys

60s-group: 67 girls, 73 boys

180s-group: 82 girls, 65 boys

However, during data analyses the difference in iron stores at four months of age between boys and girls was identified. Since our findings support previous studies we chose to demonstrate these results in figure 2. Based on the data and on these results, we cannot draw strict conclusions on how sex and clamping time affects iron deficiency. We have now clarified this in the discussion on page 9.

Comment: It is not clear how the Swedish National Cord Blood Registry works and if there is a conflict of interest for the first author. Are they for profit, for instance? Do they do private banking or it is all public with no charges for parents? This information is needed to clear a conflict of interest for this paper.

Answer from the authors: The Swedish National Umbilical Cord Blood Bank is a public bank with altruistic donations of umbilical cord blood. It is financed by the Swedish government and has no profit interest. We have clarified this in the Method section on page 4 in the manuscript.

I would recommend that the authors obtain the data on the quality of the units collected and include that information in this paper to attempt to balance out the risk of increased iron deficiency in the boys at four months of age.

Mousavi SH, Abroun S, Zarrabi M, Ahmadipناه M. The effect of maternal and infant factors on cord blood yield. *Pediatr Blood Cancer*. 2016.

References

Frandsberg S, W. B., Konar J, Rydberg L, Fasth A, Holgersson J. (2016). High quality cord blood banking is feasible with delayed clamping practices. The eight-year experience and current status of the national Swedish Cord Blood Bank. Cell Tissue Bank. doi: 10.1007/s10561-016-9565-6

VERSION 2 – REVIEW

| | |
|------------------------|---|
| REVIEWER | Judith S Mercer University of Rhode Island; Women & Infants Hospital; Alpert School of Medicine, Brown University, Providence RI USA |
| REVIEW RETURNED | 11-Jun-2017 |

| | |
|-------------------------|---|
| GENERAL COMMENTS | <p>This study is well written with a clear explanation of all methodology. However, data are lacking to draw the conclusions that the paper presents. The authors did not address the points this reviewer made previously.</p> <p>In the introduction to the paper, the authors clearly state that “a small cell dose in a transplanted unit is directly correlated with a delay in recovery of the immune system and lower incidence of sustained donor engraftment. Large cell numbers are essential and closely correlated with the collected volume of UCB.” Having said that, it is essential to this paper to report the volume of blood obtained and the TNC count after a one-minute delay even if that information is not available for the historic comparative sample. I am sure that this information is readily available as cord blood banks historically collect data on the amount and cell count of each sample (Mousavi, 2016). The reader needs to know how many of the units obtained after 60 seconds were adequate in volume and number of TNC/stem cells.</p> <p>In Figure 2, the information that boys were more likely to be anemic after the 60 sec delay is also critical information and is not discussed adequately. In Andersson’s paper on the 4-year evaluation of the children in the historic sample, he found that children had better social-emotional adjustment and fine motor skills – especially boys who also had faster process speed if they were in the 180 second group. These findings raise more concerns about the findings of more iron deficiency in boys with the 60 second delay and are not discussed adequately in this paper. Thus there is a risk for harm which must be considered especially if the units were not adequate for storage.</p> <p>Without knowing the amount of blood (mL) and the TNC counts of the units collected, one cannot assess even the small risk of iron deficiency anemia in the boys (60 seconds) in this study. Thus, the conclusion in this paper are not appropriate.</p> <p>It is not clear how the Swedish National Cord Blood Registry works and if there is a conflict of interest for the first author. Are they for profit, for instance? Do they do private banking or it is all public with no charges for parents? This information is needed to clear a conflict of interest for this paper.</p> |
|-------------------------|---|

| | |
|--|---|
| | <p>I would recommend that the authors obtain the data on the quality of the units collected and include that information in this paper to attempt to balance out the risk of increased iron deficiency in the boys at four months of age. Otherwise, this paper should not be published as it is misleading.</p> <p>Mousavi SH, Abroun S, Zarrabi M, Ahmadipanah M. The effect of maternal and infant factors on cord blood yield. <i>Pediatr Blood Cancer</i>. 2016.</p> |
|--|---|

VERSION 2 – AUTHOR RESPONSE

Finally, in response to Dr Mercer's comment asking to report the volume of blood obtain and the TNC count after a one–minute delay, you may want to elaborate on this point referencing the published article by Frändberg et al. describing the collection outcome from the Swedish National Umbilical Cord Blood Bank after different cord clamping times (Frändberg S, 2016).

Answer from the authors. The data from a study by the Swedish National Umbilical Cord Blood Bank that shows that 37% of units collected after 60 second umbilical cord clamping met banking criteria compared to 47% after immediate umbilical cord clamping (Frändberg S. et al. 2016). This has been added to the discussion on page 11.

I would not worry about clarifying further the role of the funders since you already declared not having any conflicts of interest.

Reviewer(s)' Comments to Author

Reviewer 2

Judith Mercer, University of Rhode Island, Kingston, Rhode Island, USA

Comment: This study is well written with a clear explanation of all methodology. However, data are lacking to draw the conclusions that the paper presents. The authors did not address the points this reviewer made previously.

Answer from the authors: We have modified the conclusion (see answer above to editorial comment). We did indeed comment on all points raised by the editor and the reviewers in the first round, but it is difficult to answer the same questions again without any new feedback.

Comment: In the introduction to the paper, the authors clearly state that “a small cell dose in a transplanted unit is directly correlated with a delay in recovery of the immune system and lower incidence of sustained donor engraftment. Large cell numbers are essential and closely correlated with the collected volume of UCB.” Having said that, it is essential to this paper to report the volume of blood obtain and the TNC count after a one–minute delay even if that information is not available for the historic comparative sample. I am sure that this information is readily available as cord blood banks historically collect data on the amount and cell count of each sample (Mousavi, 2016). The reader needs to know how many of the units obtained after 60 seconds were adequate in volume and number of TNC/stem cells.

Answer from the authors: The objective of the current study was to investigate the donor outcome after umbilical cord blood donation and an associated umbilical cord clamping time of 60 seconds. The objective was not to study the impact of 60 second umbilical cord clamping time on result of the collection, which is described on the bottom of page 3 in the manuscript. The intention was to clearly separate studying effects of 60 second clamping time on the health outcome of the donor from studying 60 second clamping time on the quality of umbilical cord blood collection, i.e. to minimize mixing these different undertakings. For this reason we did not analyse collection outcome after 60 second umbilical cord clamping in this study.

Additionally, we do not have the requested data (volume, TNC, CD34 cells) available for all children that are a part of the current study since not all UCB collected were sent for banking in the Swedish National Umbilical Cord Blood Bank (mostly due to logistical reasons). This will of course introduce a bias in the suggested analyses. Lastly, the level of clinical banking of the UCB is directly correlated to the current TNC limit, which is continuously increasing. It has indeed changed during the course of the current study. So to state how many UCB units that are adequate in volume and number of TNC/stem cells will depend on that specific bank's current limits. However, we have included data from a study by the Swedish National Umbilical Cord Blood Bank on page 11 (Frändberg S. et al. 2016). Also see the answer to editorial comment above.

Comment: In Figure 2, the information that boys were more likely to be anemic after the 60 sec delay is also critical information and is not discussed adequately. In Andersson's paper on the 4-year evaluation of the children in the historic sample, he found that children had better social-emotional adjustment and fine motor skills – especially boys who also had faster process speed if they were in the 180 second group. These findings raise more concerns about the findings of more iron deficiency in boys with the 60 second delay and are not discussed adequately in this paper. Thus there is a risk for harm which must be considered especially if the units were not adequate for storage.

Without knowing the amount of blood (mL) and the TNC counts of the units collected, one cannot assess even the small risk of iron deficiency anemia in the boys (60 seconds) in this study. Thus, the conclusion in this paper are not appropriate.

Answer from the authors: The current study was not designed to investigate differences according to sex, but since this finding supports previous studies we chose to present these results in Figure 2. However, based on this data, we cannot draw stringent conclusions on how sex and clamping time affects iron deficiency and we are reluctant in performing further analyses such as comparing mean TNC and blood volume between groups on this very limited data. The number of children are distributed like this:

| Number of children diagnosed with iron deficiency | Number of children not diagnosed with iron deficiency |
|---|---|
| Boys 5 | 78 |
| Girls 2 | 69 |

The limitations in relation to the results are discussed on page 9 in the manuscript.

Comment: It is not clear how the Swedish National Cord Blood Registry works and if there is a conflict of interest for the first author. Are they for profit, for instance? Do they do private banking or it is all public with no charges for parents? This information is needed to clear a conflict of interest for this paper.

Answer from the authors: In the Method section on page 4 in the manuscript, we have made it clear already in the previous version of the manuscript that the Swedish National Umbilical Cord Blood Bank is a public bank with altruistic donations of umbilical cord blood and that it is financed by the Swedish government without a profit interest. All authors that have a connection to the Swedish National Umbilical Cord Blood Bank have a clear author affiliation to the bank. All authors have declared no conflicts of interest relevant to this article.

Comment: I would recommend that the authors obtain the data on the quality of the units collected and include that information in this paper to attempt to balance out the risk of increased iron deficiency in the boys at four months of age. Otherwise, this paper should not be published as it is misleading.

Answer from the authors: See answers to the comments above.

References

Frändberg S, Waldner B, Konar J, Rydberg L, Fasth A, Holgersson J.
 High quality cord blood banking is feasible with delayed clamping practices. The eight-year experience and current status of the national Swedish Cord Blood Bank.
 Cell Tissue Bank. 2016 Sep;17

VERSION 3 – REVIEW

| | |
|------------------------|---|
| REVIEWER | Judith S Mercer University of Rhode Island, Providence, Rhode Island None except that I am funded by the US NIH for a study on delayed cord clamping and brain development. |
| REVIEW RETURNED | 13-Aug-2017 |

| | |
|-------------------------|---|
| GENERAL COMMENTS | <p>This study is well written and clearly presents the work that was done. The glaring weakness of this study is that the authors do not report on the volume, cell count, and adequacy of the collections for umbilical cord banking.</p> <p>This is glaring because the importance of the number of hematopoietic stem cells is mentioned in the third sentence in the introduction. Negative effects of a small cell dose on the recipient of stem cell transplant is mentioned in line four. In line five, the need for large cell number recovery is stated.</p> <p>With so much emphasis on cell counts in the introduction, the authors at least need to 1) address this lack of reporting of volume and cell counts in their specimens as a major limitation – acutally a weakness – of this paper and 2) state why they elected not to report this information even though it is readily available for every banked umbilical cord blood sample.</p> <p>While they mention that 37% of units collected for the National Swedish UCB Bank after 60 seconds met the banking criteria, they do not state if the protocol followed for the study infants was the same. Were the infants lower for 30 of the 60 seconds as in Dr. Andersson’s study? There is much more research to be done and fully reported.</p> |
|-------------------------|---|

| | |
|--|--|
| | <p>And these results need to be shared with parents. If there is a 63% chance that the sample will be wasted or just used for research purposes, would parents still want to donate? Especially if the infants are boys who had a higher prevalence of anemia? These are ethical questions that still need to be raised and answered. One really cannot say that "60 sec UC...enables collection of UCB for transplant" since this paper only gives us 50% of the picture. The other 50% is "how many of these 60 sec units were adequate for transplant?"</p> |
|--|--|

VERSION 3 – AUTHOR RESPONSE

In the two previous revision rounds we have clarified the aim and objectives of the study, revised the manuscript and answered the comments from the reviewers.

Our objective was not to answer the question how many umbilical cord blood units that were banked, only if clamping at 60 seconds is safe for the child. In this context, we found no significant difference in iron status at 4 months of age between infants that had their cord clamped at 60 seconds compared to those clamped at ≥ 3 minutes. We agree that it is of interest to investigate also the cell dose, volume and adequacy of the UCB collection. However, due to ethical considerations we are not allowed to merge these data bases so the results are not readily available. As suggested by the reviewer, we have reduced the overall correlation of this study to umbilical cord blood collection and transplantation, and have accordingly deleted information about this practice in the abstract, introduction and discussion (page 2-3 and 11).

Also, our aim was not to discuss the ethics of umbilical cord blood collection or to discuss the information given to parents asked if they are willing to donate or not. Those issues are of course important, but they are important regardless of time of clamping the umbilical cord and not specific to our study. However, we have included information that parents considering donating UCB should receive information about the adequacy in UCB banking (page 11).

As suggested by the editor, we have changed from "s" to "seconds" in the title. Now the title reads as follows: Wait a minute? An observational cohort study comparing iron stores in healthy Swedish infants at four months of age after 10, 60 and 180 seconds' umbilical cord clamping.

We believe that the manuscript has been improved by the suggestions from the reviewers and hope that it is now acceptable for publication in BMJ Open. We thank the reviewers and editors for your time.

VERSION 4 – REVIEW

| | |
|------------------------|---|
| REVIEWER | Judith Mercer University of Rhode Island Kingston, RI |
| REVIEW RETURNED | 27-Oct-2017 |

| | |
|-------------------------|--|
| GENERAL COMMENTS | <p>Very nice job of addressing this reviewer concerns. I think that the fact that the babies were lowered for 30 of the 60 seconds needs to be mentioned in the abstract (line 10 and 29) and on page 4, line 35 and 44. It is possible that this practice influences the amount of blood that an infant receives and this needs to be studied before we assume that a 60 second delay, with an infant placed immediately skin-to-skin, results in the same transfer as the infant being lowered for 30 of the 60 seconds. But that is what people will take away from this study.</p> <p>Also, under limitations, after you mention "no record of ethnic or SE background... I think you should add "or no measure of adequacy of samples for donation." That study still needs to be done as well.</p> |
|-------------------------|--|

VERSION 4 – AUTHOR RESPONSE

As suggested by the reviewer, we have included information that the babies were lowered for 30 of the 60 seconds in the method part of the abstract: "The new-born baby was held below the uterine level for the first 30s before placing the infant on the mother's abdomen for additional 30s.", and in the conclusion part of the abstract "In this study of healthy term infants, 60s UC clamping with 30s lowering of the baby below the uterine level, resulted in higher serum ferritin concentrations at four months compared to 10s UC clamping.". In Materials and Methods "The new-born baby was held below the uterine level for the first 30s before placing the infant on the mother's abdomen for additional 30s." was added (page 4).

We have also added "...or no measure of adequacy of samples for donation, ..." under "Limitations of the study" in the discussion on page 10.

We believe that the manuscript has been improved by the revisions, and we thank the reviewers and editors for your time.