

The role of maternal choline, folate and one-carbon metabolism in mediating the impact of prenatal alcohol exposure on placental and fetal development

Sarah E Steane, James S M Cuffe, and Karen M Moritz

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The following individual(s) involved in review of this submission have agreed to reveal their identity: Maureen Keller-Wood (Referee #2)

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Senior Editor: Laura Bennet

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Dr Moritz,

Re: JP-SR-2022-283556 "The role of maternal nutrition and one-carbon metabolism in mediating the impacts of prenatal alcohol exposure on placental and fetal development" by Sarah E Steane, James S M Cuffe, and Karen M Moritz

Thank you for submitting your invited Review-Symposium to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

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I hope you will find the comments helpful and have no difficulty in revising your manuscript within 4 weeks.

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Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

This is a well written and interesting summary of the mechanisms mediating the impact of prenatal alcohol exposure on fetal development. It is a valuable synthesis of the area. The figures are really helpful in summarising the findings.

Line 80 - This sentence refers to guidelines in countries but only cites NHMRC. Can other guidelines also be cited?

Line 99 -to understand HOW to develop.....

Line 101 and elsewhere - In most cases, that is preferred over which. When 'which' is used, it is generally preceded by a comma with the exceptions of 'in which', 'by which', etc.

Line 102 - which is fine but should be preceded by a comma.

Line 117 - led not lead?

Line 120 and elsewhere - However should be preceded by a semicolon.

REFEREE COMMENTS

Referee #1:

This a well written review discussing the potential role of folate and choline in mediating the effects of alcohol on the fetus with an emphasis on placental development. I have a few minor comments that should be addressed for the paper is considered for publication in the Journal of Physiology.

1. The title of 'the role of maternal nutrition....' is a little misleading when the paper focuses almost entirely on folate and choline. It might be better to be explicit and say the role of folate, choline and one carbon metabolism in....
2. Some mention of the metabolic effects of alcohol that may alter other elements of nutrition essential for feto-placental development would also be useful eg what effect does alcohol have on food intake, glucose and amino acid levels, other hormones and factors known to alter placental function. Perhaps as a table.
3. The paper would benefit from some discussion of placental folate and choline transport and the cellular mechanisms involved.
4. Graphical abstract and Figure 1. Identify that the circles on the DNA molecule represent methylation.
5. Figures 1, 2 and 3 legends should include the references from which the data has been drawn.

6. Figure 2B Should be titled 'Alcohol exposure and folate deficiency' otherwise it could be read as alcohol deficiency
7. The conclusions recommend a minimum choline dose but makes no mention of the need for a greater dose of folate during pregnancy. Should this be included?

Referee #2:

This is an interesting and well-written review, which summarizes work in this area and points out gaps in knowledge requiring further research on mechanisms, but also points out where clear associations suggest greater public health awareness and policy to improve outcomes.

The review makes a nice case for the effects of alcohol mediated through, and exacerbated by, reduced nutrients and effects on methylation. It is clear that there are different mechanism depending on the time in pregnancy of exposure to alcohol. I think it would be helpful to include a figure that shows the effects of alcohol/ reduced choline and folate over the course of pregnancy, ie early effects on blastocyst, later effects on placentation and early brain development, and even later effects on placental vascularity, "activity" (transporters etc) and fetal brain development/maturation.

Specific comments:

Page 6-7: Is there any information on folate or choline uptake into the blastocyst or early embryo in vivo (ie prior to placentation?)

Line 185-186: Is there a reference for 'This may be particularly important during early pregnancy as the developing blastocyst is highly sensitive to changes in micronutrient availability.'

Lines 248-249: The mechanism for the link between reduced availability of methyl groups and poor placentation is not discussed. What is the hypothesized mechanism? Is this due to reduced proliferative capacity or is it a specific epigenetic effect secondary to hypomethylation? Does folate also affect igf2 levels?

Concluding paragraph: makes a case for raising awareness about choline and folate intake in the periconception period, but the review also makes a case for the importance of both of these throughout pregnancy, and has evidence for an interaction of alcohol with either folate or choline deficiency in all stages of pregnancy.

END OF COMMENTS

Confidential Review

04-Jul-2022

Response to Reviewing Editor and Referees

PLEASE NOTE THAT LINE NUMBERS PROVIDED IN EACH RESPONSE REFER TO THE REVISED MANUSCRIPT WITH CHANGES HIGHLIGHTED

Reviewing Editor:

Line 80 - This sentence refers to guidelines in countries but only cites NHMRC. Can other guidelines also be cited?

Citations for guidelines from government agencies in the UK (NICE guidelines) and the US (CDC guidelines) have been added (line 82).

Line 99 -to understand HOW to develop.....

Corrected (line 101)

Line 101 and elsewhere - In most cases, that is preferred over which. When 'which' is used, it is generally preceded by a comma with the exceptions of 'in which', 'by which', etc.

Corrected (highlighted)

Line 102 - which is fine but should be preceded by a comma.

Corrected (highlighted)

Line 117 - led not lead?

Corrected (line 119)

Line 120 and elsewhere - However should be preceded by a semicolon.

Corrected (highlighted)

Referee #1:

1. The title of 'the role of maternal nutrition....' is a little misleading when the paper focuses almost entirely on folate and choline. It might be better to be explicit and say the role of folate, choline and one carbon metabolism in....

This has been altered as suggested to 'The role of maternal choline, folate, and one-carbon metabolism in mediating the impacts of prenatal alcohol exposure on placental and fetal development' (Line 1).

2. Some mention of the metabolic effects of alcohol that may alter other elements of nutrition essential for feto-placental development would also be useful eg what effect does alcohol have on food intake, glucose and amino acid levels, other hormones and factors known to alter placental function. Perhaps as a table.

The impact of PAE on intake and absorption of nutrients more broadly, has been mentioned in the introduction with reference to relevant studies (lines 108 – 110). An additional reference has been added here (Line 110, Kloss *et al*, 2022), to direct readers to a detailed description of the impact of PAE on a range of nutrients, with implications for feto-placental development.

While we appreciate that PAE has other impacts beyond nutrition (oxidative stress, inflammation, maternal HPA axis etc), we feel that this is outside the scope of the current review. We wanted to focus on the mechanism through which PAE may alter DNAm and gene expression and highlight evidence supporting an opportunity for nutritional intervention for PAE.

3. The paper would benefit from some discussion of placental folate and choline transport and the cellular mechanisms involved.

Mechanisms of placental folate transport have been added at lines 125 - 133, and placental choline transport at lines 151 – 158.

4. Graphical abstract and Figure 1. Identify that the circles on the DNA molecule represent methylation.

This has been added in the figure legends (Line 46 and line 184)

5. Figures 1, 2 and 3 legends should include the references from which the data has been drawn.

References have been added to the figure legends (Lines 188 - 189, 354 – 366, 469 – 471)

6. Figure 2B Should be titled 'Alcohol exposure and folate deficiency' otherwise it could be read as alcohol deficiency

This has been altered as suggested, figure 2, panel B.

7. The conclusions recommend a minimum choline dose but makes no mention of the need for a greater dose of folate during pregnancy. Should this be included?

A higher dose of folic acid would not currently be recommended in an uncomplicated pregnancy as there are concerns that the high levels of folic acid in some prenatal supplements (e.g. Elevit contains 800ug) together with fortification of foods is already resulting in high levels of unmetabolized folic acid in some pregnant women, which may have detrimental effects on fetal neurodevelopment. Rather, it is suggested to emphasise the importance of achieving the **recommended** intake. Choline on the other hand is not usually added to prenatal vitamin supplements and is not fortified in foodstuffs. Very few pregnant women meet the adequate intake level of 450mg from dietary sources and this amount is thought to underestimate requirements during pregnancy. In addition, while alcohol consumption has been demonstrated to reduce absorption of folate, there is currently no evidence that choline absorption is directly impacted. Given that up to 80% of women consume alcohol before realising they are pregnant, choline supplementation would be a safer and more effective option for supplying additional methyl groups than potentially over supplementing with folate.

Referee #2:

The review makes a nice case for the effects of alcohol mediated through, and exacerbated by, reduced nutrients and effects on methylation. It is clear that there are different mechanisms **depending on the time in pregnancy of exposure to alcohol**. I think it would be helpful to include a figure that shows the effects of alcohol/ reduced choline and folate over the course of pregnancy, ie early effects on blastocyst, later effects on placentation and early brain development, and even later effects on placental vascularity, "activity" (transporters etc) and fetal brain development/maturation.

After careful consideration, the authors felt that the complexities of timing, dose and duration of folate/choline intake and PAE in the available studies could not be clearly and accurately represented in a figure. For example, many of the animal studies that examine outcomes following PAE and choline/folate deficiency or supplementation, use models of chronic PAE throughout gestation, or for the majority of gestation. There are currently insufficient studies examining the effects of PAE at specific stages of pregnancy on different backgrounds of dietary folate/choline, to be able to determine outcomes at particular stages of placenta and fetal brain development. In addition, fetal brain development at the end of pregnancy in rodents is approximately equivalent to the end of the second trimester in humans. We have not included rodent studies of the impact of alcohol/folate/choline on the brain in the neonatal period (third trimester equivalent) as this does not include effects on placental function as a contributor to brain development.

Specific comments:

Page 6-7: Is there any information on folate or choline uptake into the blastocyst or early embryo in vivo (ie prior to placentation?)

As far as we are aware, folate and choline content of *in vivo* developed blastocyst/embryo has only been examined following *in vitro* culture with addition of labelled molecules to the culture medium. This information has been added in lines 125-130 and lines 146 – 151.

Line 185-186: Is there a reference for 'This may be particularly important during early pregnancy as the developing blastocyst is highly sensitive to changes in micronutrient availability.'

References have been added (lines 206 – 207).

Lines 248-249: The mechanism for the link between reduced availability of methyl groups and poor placentation is not discussed. What is the hypothesized mechanism? Is this due to reduced proliferative capacity or is it a specific epigenetic effect secondary to hypomethylation? Does folate also affect igf2 levels?

Reduced trophoblast viability, proliferation and expression of genes in pathways regulating invasion, have been hypothesised to underlie poor placentation. Further clarification of this has been added in lines 266 – 268 and lines 272 – 274.

Whilst there is evidence that Igf2 plays a role in regulating placentation (*Kent et al, Akt1 and insulin-like growth factor 2 (Igf2) regulate placentation and fetal/postnatal development. Int J Dev Biol. 2012;56(4):255-61*), there is currently no evidence that folate impacts expression of Igf2 **in the placenta**.

Concluding paragraph: makes a case for raising awareness about choline and folate intake in the periconception period, but the review also makes a case for the importance of both of these throughout pregnancy, and has evidence for an interaction of alcohol with either folate or choline deficiency in all stages of pregnancy.

This has been clarified in lines 480 – 487, lines 490 – 491, and line 495.

Dear Professor Moritz,

Re: JP-SR-2023-283556R1 "The role of maternal choline, folate and one-carbon metabolism in mediating the impact of prenatal alcohol exposure on placental and fetal development" by Sarah E Steane
James S M Cuffe
Karen M Moritz

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EDITOR COMMENTS:

Reviewing Editor:

Thank you for revising the paper.

REFeree COMMENTS:

Referee #1:

I am happy with the alterations and explanations that the authors have made in response to my comments.

Referee #2:

The authors have addressed my comments.

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1st Confidential Review

16-Jan-2023
