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)	Supplementation of vitamin B12 in pregnancy and postpartum on growth and neurodevelopment in early childhood: Study Protocol for a Randomized Placebo Controlled Trial
AUTHORS	Chandyo, Ram; Ulak, Manjeswori; Kvestad, Ingrid; Shrestha, Merina; Ranjitkar, Suman; Basnet, Sudha; Hysing, Mari; Shrestha, Laxman; Strand, Tor A.

VERSION 1 - REVIEW

REVIEWER	Christopher Sudfeld Harvard T.H. Chan School of Public Health, USA
REVIEW RETURNED	04-Mar-2017

GENERAL COMMENTS	This manuscript reports the protocol for a vitamin B12 trial among pregnant women in Nepal. The trial will yield important results whether an available and relatively lost cost supplement may improve child growth and development. The manuscript has excellent information on the neurodevelopment tools, quality control, and data management but in my opinion could be much clearer for most other trial aspects, in particular the randomization procedures, description of investigational product, and statistical analysis. I would suggest the authors make sure all of these are clear so the trial is not inadvertently downgraded in a Cochrane review or meta-analysis. In addition, the order of the methods is hard to follow. I would suggest following the order of the SPIRIT checklist. The manuscript would also be improved with another check on grammar and punctuation. Major 1. Abstract- It would be helpful to explicitly state the primary outcomes in the abstract. 2. Methods -The primary outcome is not clear since the Bayley has multiple subscales. Is the primary endpoint the composite score? 3. Methods- The order of the paragraphs does not flow. Please consider changing to the SPIRIT order. 4. Some definitions of exclusion criteria need explanations on how they are assessed. How and when is hemoglobin assessed? Is this different from the baseline hemoglobin assessment mentioned later? What conditions require B12 treatment in this setting per standard of care? 5. Randomization list code in Stata…I assume you will also use block 8?

	6. How is allocation concealment performed? Where are bottles labeled etc? 7. “All pregnant women will also be given iron, folic acid and calcium supplements according to national guide lines.” What are doses and when are they delivered? A citation would also be helpful. 8. There is a lot more information needed on the investigational product. Who makes the B12? How long is shelf-life? How many pills are in a bottle? How many bottles do women receive? 9. SAEs and safety. There are no clear safety endpoints listed. Are minor side effects like headache, itching, etc collected? What about other known B12 side effects? 10. Sample size- more information needed in the text. Figure 2 has good information on statistics but the text is much more unclear. What equations were used? 11. Outcomes: Need citation for the z-score growth reference. I assume WHO. 12. Statistical Analysis: This section needs a lot more explicit detail on the exact tests that will be used. How will differences between subgroups be assessed, what test will determine effect modification? What will be done if there is baseline imbalance for factors? I caution the authors on this section, the statistical analysis in the main paper must identically match the statistical analysis plan in the protocol or it will be considered ad hoc. Minor Abstract Line 9. ‘lack vitamin B12’ is unclear. Is this in reference to dietary intake or blood levels? Please clarify and suggest change to ‘suboptimal’ or ‘low’ levels in the blood or diet. Abstract Line 11. “The most important time for neurodevelopment starts in utero.” Not sure this is entirely true for all metrics. Abstract Line 17. The first sentence of methods is not a complete sentence. Methods line 41: Individually randomized?

REVIEWER	Helen D Bailey INSERM, Université Paris-Descartes, Université Sorbonne-Paris-Cité, CRESS-EPICEA Epidémiologie des cancers de l’enfant et de l’adolescent, Paris, France
REVIEW RETURNED	10-May-2017

GENERAL COMMENTS	Specific points Consent: Participation in the trial will involve a level of burden for the women and the follow-up protocol is reasonably complex. Please give more details about how the study will be explained to the women before obtaining consent and by whom. Please also give details about how consent will be obtained (from both literate and non-literate women. Please describe whether separate consent will be obtained for the collection of the biological specimens. Biological specimen collection: Obtaining blood samples from in 6

	month old infants requires a skilled phlebotomist. Please give details about who will collect these.

REVIEWER	Elizabeth Prado University of California Davis, USA
REVIEW RETURNED	10-May-2017

GENERAL COMMENTS	Review of the Manuscript "Supplementation of vitamin B12 in pregnancy and postpartum on growth and neurodevelopment in early childhood: Study Protocol for a Randomized Placebo Controlled Trial" The manuscript describes a protocol for an individually randomized placebo-controlled trial of 50 mcg/d vitamin B12 supplementation from early pregnancy through six months postpartum in Nepal. The primary outcomes are Bayley-III scores at six and 12 months of age and LAZ, WAZ, and WLZ at 12 months of age. The study is well-designed and clearly presented and has the potential to contribute to the establishment of a causal role of moderate B12 deficiency in impairment in growth and development, as well as provide evidence inform maternal health policy. A few points require clarification. COMMENTS: 1) Page 5 line 16 "common" is vague, please provide prevalence or range. 2) Page 9 line 4-7. I would also suggest measuring maternal cognition. As stated in the introduction, B12 deficiency has been associated with cognition in children and the elderly and may also affect maternal cognition during pregnancy and postpartum. 3) Page 9, line 9-12. Maternal and infant Hb and infant growth could also be mediators for developmental outcomes. 4) Page 10, line 42-46. How will the study supplements and the IFA/Ca supplements be distributed? By whom? At home or clinic? Weekly or monthly? 5) Page 12, line 13. Does weekly monitoring of compliance include both the study supplements and the standard IFA/Ca supplements? 6) Page 15, line 48-52. The ASQ-3 is a screening tool designed to detect children at risk for delay, therefore typically developing children score at ceiling (no variance in scores) which will not allow assessment of developmental trajectories. 7) Page 16, line 44-53. The eye-tracking measure is not mentioned in the study objectives and it is not clear at what time point this will be collected. 8) Page 17, line 4-10. Likewise TIMP is not mentioned in the study objectives. 9) Page 18, line 38. What will be done if there is poor agreement between field worker and supervisor? 10) Methods: Many studies have shown the importance of adaptation of tools when transferring from one context to another. Many of the tools in the current study would require adaptation, including the Bayley-III, ASQ, BISQ, ADBB, TIMP, HOME, CKCDI, and possibly the stimuli in the eye-tracking task. I don't see a plan for adaptation and piloting of these tools or evaluation of the psychometric properties of the adapted tools, such as internal reliability and test-retest reliability. Many of these also require evaluation of inter-rater agreement for quality control. Refs: Greenfield, P. M. (1997). You can't take it with you: Why ability assessments don't cross cultures. American Psychologist, 52, 1115-

	1124. van de Vijver, F., & Tanzer, N. K. (2004). Bias and equivalence in cross-cultural assessment: an overview. European Journal of Applied Psychology, 54, 119-135. Vierhaus, M., Lohaus, A., Kolling, T., Teubert, M., Keller, H., Fassbender, I., . . . Schwarzer, G. (2011). The development of 3- to 9-month-old infants in two cultural contexts: Bayley longitudinal results for Cameroonian and German infants. European Journal of Developmental Psychology, 8(3), 349-366. 11) Discussion: requires editing for grammar. 12) Page 23, line 15-30. This is an interesting and important question, but if it is a study objective it should be presented as such, rather than in the Discussion.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Christopher Sudfeld
Institution and Country: Harvard T.H. Chan School of Public Health, USA
Competing Interests: None declared.

This manuscript reports the protocol for a vitamin B12 trial among pregnant women in Nepal. The trial will yield important results whether an available and relatively lost cost supplement may improve child growth and development. The manuscript has excellent information on the neurodevelopment tools, quality control, and data management but in my opinion could be much clearer for most other trial aspects, in particular the randomization procedures, description of investigational product, and statistical analysis. I would suggest the authors make sure all of these are clear so the trial is not inadvertently downgraded in a Cochrane review or meta-analysis. In addition, the order of the methods is hard to follow. I would suggest following the order of the SPIRIT checklist. The manuscript would also be improved with another check on grammar and punctuation.

Major

1. Abstract - It would be helpful to explicitly state the primary outcomes in the abstract.
Response: We have added the primary outcomes in the methods and analysis paragraph of the abstract in the revised manuscript.

2. Methods -The primary outcome is not clear since the Bayley has multiple subscales. Is the primary endpoint the composite score?
Response: The primary outcomes of the trial are scores on the cognitive, language (receptive and expressive separately and composite score) and motor (fine and gross separately and composite score) subscales of the Bayley Scales of Infant and Toddler Development 3rd ed. (Bayley-III) measured at 6 and 12 months of age, and growth (length and weight) measured at 12 months of age. We have revised this throughout the manuscript.

3. Methods- The order of the paragraphs does not flow. Please consider changing to the SPIRIT order.
Response: We have changed method section as per SPIRIT guidelines.

4. Some definitions of exclusion criteria need explanations on how they are assessed. How and when is hemoglobin assessed? Is this different from the baseline hemoglobin assessment mentioned later? What conditions require B12 treatment in this setting per standard of care?

Response: We agree, and have clarified the exclusion criteria. Unfortunately, in Nepal there are no specific guidelines for B12 treatment, but it is common to prescribe vitamin B12 for treating pernicious anaemia or in strictly vegans.

5. Randomization list code in Stata…I assume you will also use block 8?
Response: Yes, and we hope that this is clearer in the updated manuscript (page 11).

6. How is allocation concealment performed? Where are bottles labeled etc?
Response: We will ensure allocation concealment throughout the study as none of the investigators will have access to the list that links the group identity with the id-number until completion of data collection, analysis and interpretation. Nepalese and Norwegian scientists otherwise not involved in a trial has labelled supplements.

7. “All pregnant women will also be given iron, folic acid and calcium supplements according to national guidelines.” What are doses and when are they delivered? A citation would also be helpful.
Response: The revised manuscript includes doses and timing of antenatal supplements under the subheading Co-intervention on page 10 with the relevant reference.

8. There is a lot more information needed on the investigational product. Who makes the B12? How long is shelf-life? How many pills are in a bottle? How many bottles do women receive?
Response: We have updated the manuscript under the subheading Intervention on page 9 to include details on the investigational product. The vitamin B12 supplements are produced by GC Rieber Compact Norway with shelf life of 5 years, and it is a soft solid wafer like form. Each bar of supplement is sufficient for 8 days and participants are requested to consume one piece each day.

9. SAEs and safety. There are no clear safety endpoints listed. Are minor side effects like headache, itching, etc. collected? What about other known B12 side effects?
Response: There are no known adverse effects of giving vitamin B12 orally, the maximum capacity for absorption is 2-4 µg per day and there is no reason to believe that there will be an overload (as discussed on page 11). During our weekly visits, we will ask for general adverse events such as GI symptoms and symptoms related to possible allergic reactions. This is described under “Follow-up” on page 13.

10. Sample size- more information needed in the text. Figure 2 has good information on statistics but the text is much more unclear. What equations were used?
Response: We have rewritten and expanded this section on page 21, and added appropriate references for the equation used for calculating the sample size.

11. Outcomes: Need citation for the z-score growth reference. I assume WHO.
Response: It is the WHO references that we are using, we have added appropriate references.

12. Statistical Analysis: This section needs a lot more explicit detail on the exact tests that will be used. How will differences between subgroups be assessed, what test will determine effect modification? What will be done if there is baseline imbalance for factors? I caution the authors on this section, the statistical analysis in the main paper must identically match the statistical analysis plan in the protocol or it will be considered ad hoc.
Response: The plan of analysis has now been expanded.

Minor
Abstract Line 9. ‘lack vitamin B12’ is unclear. Is this in reference to dietary intake or blood levels? Please clarify and suggest change to ‘suboptimal’ or ‘low’ levels in the blood or diet.
Response: Now changed to “at risk for poor vitamin B12 status”.

Abstract Line 11. “The most important time for neurodevelopment starts in utero.” Not sure this is entirely true for all metrics.
Response: We agree and have deleted this statement.

Abstract Line 17. The first sentence of methods is not a complete sentence.
Response: Corrected

Methods line 41: Individually randomized?
Response: Yes, hope this is clearer now.

Reviewer: 2
Reviewer Name: Helen D. Bailey
Institution and Country: INSERM, Université Paris-Descartes, Université Sorbonne-Paris-Cité, CRESS-EPICEA Epidémiologie des cancers de l’enfant et de l’adolescent, Paris, France
Competing Interests: None

Specific points
Consent: Participation in the trial will involve a level of burden for the women and the follow-up protocol is reasonably complex. Please give more details about how the study will be explained to the women before obtaining consent and by whom. Please also give details about how consent will be obtained (from both literate and non-literate women. Please describe whether separate consent will be obtained for the collection of the biological specimens.
Response: Thank you for the suggestion. We have revised our description of the process of consenting under the subheading Recruitment procedure, confirmation of pregnancy and assessment of gestational age on page 10.

Biological specimen collection: Obtaining blood samples from in 6-month-old infants requires a skilled phlebotomist. Please give details about who will collect these.
Response: We have added the following text on page 12: “In the infants we will collect blood samples at 6 months by a trained lab technician with direct supervision by a study paediatrician”.

Reviewer: 3
Reviewer Name: Elizabeth Prado
Institution and Country: University of California Davis, USA
Competing Interests: none declared

The manuscript describes a protocol for an individually randomized placebo-controlled trial of 50 mcg/d vitamin B12 supplementation from early pregnancy through six months postpartum in Nepal. The primary outcomes are Bayley-III scores at six and 12 months of age and LAZ, WAZ, and WLZ at 12 months of age. The study is well-designed and clearly presented and has the potential to contribute to the establishment of a causal role of moderate B12 deficiency in impairment in growth and development, as well as provide evidence inform maternal health policy. A few points require clarification.

COMMENTS:
1) Page 5 line 16 “common” is vague, please provide prevalence or range.
Response: Corrected

2) Page 9 line 4-7. I would also suggest measuring maternal cognition. As stated in the introduction, B12 deficiency has been associated with cognition in children and the elderly and may also affect maternal cognition during pregnancy and postpartum.
Response: We agree that it would have been interesting to include a measure of maternal cognition, which we will consider in future studies. The present study is ongoing however, and since enrolment already has started it will not be feasible to include maternal cognition at this point. It should also be noted that this is a randomized, placebo controlled trial, and thus we expect an even distribution of maternal cognitive abilities between the study groups?

3) Page 9, line 9-12. Maternal and infant Hb and infant growth could also be mediators for developmental outcomes.
Response: We agree and changed accordingly.

4) Page 10, line 42-46. How will the study supplements and the IFA/Ca supplements be distributed? By whom? At home or clinic? Weekly or monthly?
Response: Thanks for pointing this out, the last version of the manuscript describes this much clearer on page 9. At enrolment, participants will get IFA/CA supplements in the hospital and then at home in the subsequent weekly visits. Field research workers will provide these supplements.

5) Page 12, line 13. Does weekly monitoring of compliance include both the study supplements and the standard IFA/Ca supplements?
Response: Yes, the manuscript has been updated at page 9.

6) Page 15, line 48-52. The ASQ-3 is a screening tool designed to detect children at risk for delay, therefore typically developing children score at ceiling (no variance in scores) which will not allow assessment of developmental trajectories.
Response: We agree that the ceiling effect is a potential problem when using a screening questionnaire such as the ASQ. We have used the ASQ-3 at this study site previously, and to avoid a ceiling effect we have altered the method and administer the next level questionnaire when there is a maximum score in the age appropriate questionnaire which reduces the ceiling effect. It should also be noted that the ASQ is a secondary outcome and the results from this test will supplement that of the main outcome. The description of the ASQ-3 has been updated on page 15.

7) Page 16, line 44-53. The eye-tracking measure is not mentioned in the study objectives and it is not clear at what time point this will be collected.
Response: This tool is still used on an explorative basis and we have decided to remove this test from this paper. Thanks for noticing.

8) Page 17, line 4-10. Likewise, TIMP is not mentioned in the study objectives.
Response: the TIMP is included because the main concern by the Norwegian IRB was that we were violating the principle of clinical equipoise. I.e. despite the fact that screening for vitamin B12 status, or that vitamin B12 supplementation, alone or with other nutrients are not recommended by the World Health Organization or any South Asian health bodies, the IRB was concerned that we were withholding vitamin B12 treatment for the placebo group. This concern was based on comments from an expert reviewer whom the IRB asked for advice. We met this concern by suggesting to measure TIMP when the infant was 45 days in the first 100 children. If we find an unexpected beneficial effect in this test, the DSMB will consider stopping the trial because giving a placebo would be unethical. We have added a description of the TIMP on page 16 in the manuscript, as well as discussed how and why it will be used in a separate paragraph in the discussion section.

9) Page 18, line 38. What will be done if there is poor agreement between field worker and supervisor?

Response: We have added the following sentence to the method section under the subheading Quality control of field activities: “In case of poor agreement, extra training will be carried out until acceptable agreement is achieved.”

10) Methods: Many studies have shown the importance of adaptation of tools when transferring from one context to another. Many of the tools in the current study would require adaptation, including the Bayley-III, ASQ, BISQ, ADBB, TIMP, HOME, CKCDI, and possibly the stimuli in the eye-tracking task. I don’t see a plan for adaptation and piloting of these tools or evaluation of the psychometric properties of the adapted tools, such as internal reliability and test-retest reliability. Many of these also require evaluation of inter-rater agreement for quality control.
Refs:
Greenfield, P. M. (1997). You can't take it with you: Why ability assessments don't cross cultures. American Psychologist, 52, 1115-1124.
van de Vijver, F., & Tanzer, N. K. (2004). Bias and equivalence in cross-cultural assessment: an overview. European Journal of Applied Psychology, 54, 119-135.
Vierhaus, M., Lohaus, A., Kolling, T., Teubert, M., Keller, H., Fassbender, I., . . . Schwarzer, G. (2011). The development of 3- to 9-month-old infants in two cultural contexts: Bayley longitudinal results for Cameroonian and German infants. European Journal of Developmental Psychology, 8(3), 349-366.
Response: Thank you for the suggested readings. We agree that adaptation and piloting is critical when transferring tools from one context to another. All the included tests, except for the TIMP have been translated, culturally adapted when necessary and piloted in the field site for previous trials. This information has been added in the manuscript at page 14. We will however, carry out further training and standardisation ahead of the project start, as well as conduct ongoing quality controls.

11) Discussion: requires editing for grammar.
Response: The discussion section has been checked for grammar and edited.

12) Page 23, line 15-30. This is an interesting and important question, but if it is a study objective it should be presented as such, rather than in the Discussion.
Response: We agree, and have removed the discussion on B12 and folic acid in the discussion section.

Other modifications: We have simplified the text and removed some of the secondary outcomes such as measuring lead exposure, telomere length etc. This is because we do not yet have funding to undertake these analyses.

VERSION 2 – REVIEW

REVIEWER	Christopher Sudfeld Harvard T.H. Chan School of Public Health, United States of America
REVIEW RETURNED	11-Jul-2017

GENERAL COMMENTS	No comments. Very nice protocol.

REVIEWER	Helen Bailey Epidémiologie des Cancers de l'Enfant et de l'Adolescent UMRS-1153 Equipe 7 (EPICEA), INSERM, Université Paris Descartes
REVIEW RETURNED	17-Jul-2017

GENERAL COMMENTS	Thank you. The revisions have improve the submission. I still have some minor comments that need to be addressed. Page 22 of 83 (of PDF), page 14 of word document. Line 50: The authors state that 'a detailed information regarding the study will be provided. Do they mean 'information sheet? Will someone explain the study in detail to illiterate women (as well as getting their thumbprints) and who will be responsible for this? Page 33 of 83 (of PDF), page 15 of word document. Line 31: Please change 'staffs' to 'staff' Page 39 of 83 (of PDF), page 31 of word document. Line 44: The abbreviations Haz, Waz and Whz need to be defined the first time used.

REVIEWER	Elizabeth Prado University of California Davis
REVIEW RETURNED	19-Jul-2017

GENERAL COMMENTS	No further comments.