**Author’s response to reviews**

**Title:** Effect of maternal vitamin D3 supplementation on maternal health, birth outcomes, and infant growth among HIV-infected Tanzanian pregnant women: study protocol for a randomized controlled trial

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Reviewer #1: Sudfeld and colleagues reported on a RCT protocol that aimed at determining the effect of adding vitamin to antiretroviral therapy on maternal and infants health. The trial recruitment and follow-up of participants are ongoing; meaning any comment by reviewers would not modified the trial procedure. The manuscript is well written and I have only some suggestions for the improvement of the paper. Below are my comments.

Response: Thank you Reviewer #1 for your thorough review.

Major comments

1) Page 5. Background. For the paragraph on the effect of the vitamin D on pregnancy neonatal outcomes, authors SHOULD cite and comment a systematic review and meta-analysis that included 13 studies.
Response: We have added this citation and a summary of the findings: “A recent meta-analysis of RCTs found that vitamin D supplementation in pregnancy significantly increased birth weight and birth length but had no effect on preeclampsia, gestational diabetes, SGA, preterm birth, and cesarean section [27].”

2) Study population

For the Inclusion criteria d), it is reported from some places in Africa that women doesn't know their last menstrual period. Since the recruitment is ongoing, how do clinical investigators to determine the gestational age of the pregnancy?

Response: We agree that LMP is prone to errors in most settings. We have added the following sentence and citation:

“We acknowledge use of LMP for gestational age dating may lead to misclassification of SGA [39]. Nevertheless, errors in maternal report of LMP are expected to be non-differential with respect to the randomized regimen and therefore would lead to underestimation of the effect of vitamin D supplements on SGA.”


3) Screening and Randomization

Page 9. Lines 238-240. What was/will be done in the case of undetermined/inconclusive results after the two rapid HIV testing? Please, give details.

Response: We have clarified the standard HIV testing algorithm used in Tanzania.

“Pregnant women receive HIV testing as part of routine antenatal care in Tanzania and women with unknown HIV status receive HIV testing with two licensed rapid assays [37]. If the HIV rapid assays are inconclusive (first test positive and second negative), the first and second HIV
rapid assay are repeated following same algorithm. If the results are still inconclusive upon repeat testing, the pregnant woman is advised that she may be in acute HIV infection period and asked to return for a repeat HIV testing in 2-4 weeks.”

4) It would be interesting if investigators also stratified randomized by WHO HIV stage (clinical profile) or initial viral load (virological profile); since the rationale for using vitamin D is based on immunological response in the presence of low/high vitamin D concentration. Initial profile may be source of heterogeneity on the effect of vitamin D on outcomes. I suggest that this heterogeneity be investigated during statistical analysis.

Response: Agreed. We have included an analysis of several potential effect modifiers including WHO HIV disease stage. We are not able to look at effect modification by initial viral load since this test is not performed at ART initiation in Tanzania. Viral load monitoring is only offered post ART initiation at 6 or 12 month intervals. The following sentence is included in the statistical analysis section:

“We will examine effect modification of any treatment effect by pre-defined baseline variables: maternal age, maternal BMI, socioeconomic status, gestational age at randomization, CD4-T cell count, hemoglobin concentration, WHO HIV disease stage, ART regimen, duration of ART, duration of exclusive and any breastfeeding, and trial regimen adherence.”

Minor comments

5) Study population. Please correct the second "(d)" to "(e)"

Response: Corrected.

6) Authors can remove all "(n)" in the figure since this is the protocol.

Response: Journal suggests keeping (n)

Discretionary revisions
7) I suggest to authors to add some sentences on dissemination plan.

Response: We have added “The trial results will be communicated in academic journals and at the national and local levels in Tanzania through policy briefs and dissemination meetings.”

Reviewer #2: I have finished the reading of the manuscript entitled "Effect of maternal vitamin D3 supplementation on maternal health, birth outcomes, and infant growth among HIV-infected Tanzanian pregnant women: study protocol for a randomized controlled trial". The authors have adhered to the SPIRIT checklist for protocols, according to the journal guidelines. The randomized controlled trial is focused on the effect of maternal vitamin D3 supplementation on maternal and child health outcomes, and the study has been conducted among HIV-infected pregnant women in Tanzania. I think it can be considered for publication in TRIALS after addressing the following issues:

Thank you for the thoughtful review.

- Inclusion criteria are mentioned in the 'study population' paragraph, but is there any specific exclusion criterion in this study?

Response: We noted the following exclusion criteria: “Women were excluded if they (a) did not intend to stay in Dar es Salaam for two years after enrollment, (b) were enrolled in any other clinical trial, or (c) did not provide informed consent.”

- The formula used for estimating the sample size needed, should be included or referenced.

Response: We have added the following sentence and reference:

“Sample size and power calculations for ToV5 were based on a 2-sided test of proportions and a z-statistic using the asymptotic variances of the observed proportions which assumed 1:1 randomization to vitamin D3 and placebo arms and a nominal Type I error rate (alpha) of 0.05 [33].”

- Some specific information about the instruments employed to assess depression, anxiety, social support, and physical activity, should be provided, adding references if needed.

Response: We have added the following sentence and references to this section: “The Hopkins Symptom Checklist (HSCL-25) will be administered to assess symptoms of depression and anxiety [40]. We have previously validated the HSCL-25 among Tanzanian women living with HIV against diagnosis of major depressive disorder by a trained psychiatrist using the Structured
Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [41]. We will measure functional dimensions of social support, a scale based on the Duke University–University of North Carolina Functional Social Support Questionnaire [42].”

- The Discussion should mention how will be managed the potential problems found during the recruitment of participants or in the data collection.

Response: We prefer to report any problems or changes to protocol (if needed) in the final report. To date there have been no problems in recruitment or follow-up.

- Since the number of participants is not being provided in any of the cells comprising the Figure 1, maybe the 'n's could be deleted.

Response: Journal suggests keeping (n)

Minor comments:

- At the end of p.6, you have missed '(j)' when you are enumerating the effects of maternal vitamin D3 supplementation regimen.

Response: Thank you, corrected

- There is a typo in line 373: "We will use the use the log-rank test to test…".

Response: Corrected

- In the 'Trial status' sentence, the status of the recruitment should be updated.

Response: Updated, we are still recruiting.

Reviewer #3: The manuscript describes the protocol of a blinded randomized control study to understand the effect of maternal vitamin D supplementation on maternal and infant outcomes among HIV-infected Tanzanian pregnant women. The manuscript is very well written and covers all the aspects of a RCT. The results of the study will answer important questions about prenatal
vitamin D supplementation in women living with HIV. I have a few minor suggestions/comments for the authors.

Thank you Reviewer #3.

1. In page 10/line 256 of the manuscript it is mentioned that randomization will be stratified by study clinics. How many study clinics will be there? Are the study clinic different from HIV clinic? Will the enrollment take place at the HIV clinic and follow-up will happen at study clinic? Where will the delivery take place? If you are using multiple study clinics then how are the clinics selected. Will you train all the staff in the clinics to have consistent methods for data collection? Please provide more information so the readers can understand how the data collection is managed.

Response: We have added: “The trial is conducted at five PMTCT sites in Dar es Salaam including: Mnazi Mmoja Hospital, Buguruni Health Center, Mbagala Rangi Tatu Hospital, Sinza Hospital, and Tabata A Dispensary. These sites were selected since they are among the largest PMTCTs in Dar es Salaam in terms of patient numbers according to program records. All PMTCT sites are attached to ANC clinics. Research nurses and clinic staff were uniformly trained before the start of the study and have refresher meetings at least every 6 months or as issues arise.”

We also clarify on deliveries: “Study nurses/midwives are available throughout the day and night to attend labor and delivery of participants. Deliveries which occur outside of Dar es Salaam are reached by phone to obtain relevant information from the mother and/or clinic staff.”

2. Are the house visits done by field workers? How are they trained and how frequently? Is there any mechanism in place to check for the accuracy of data collection?

Response: We have added “Home visits are conducted by trained research assistants who receive updated training on home visits in the context of HIV every 6 months. Accuracy of home visit information is assessed through spot checks by clinic supervisors.”
3. Is there any provision in the statistical analysis to account for multiple study clinics and multiple testing?

Response: Thank you for catching this issue. We have added that stratification in Kaplan-Meir models will be used: “We will use the log-rank test stratified by study clinic to assess differences in the incidence of maternal HIV progression of death and use the binomial proportion test for SGA births, and child stunting at 12 months of age between the treatment arms.”

Stratified K-M curves will only produce 1 estimate and as a result no multiple testing is needed. For more information on stratification in K-M models: http://www.lexjansen.com/wuss/2004/hands_on_workshops/i_how_kaplan_meier_and_cox_p.pdf

4. Though the content of placebo is generally known, it is advisable to mention it clearly in the manuscript instead of just mentioning that it is identical to the drug.

Response: We have added more information on the composition of the placebo: “The placebo inactive ingredients included: maltitol, gelatin, magnesium stearate, and titanium dioxide.”

5. Does the study provide any compensation to the participants?

Response: Women are not compensated financially or with gifts for participation in the trial.

6. How frequently does the study plan to provide information about AE and SAE to the DSMB? Will it be provided within the first 24 hours? Is there any specific reason for the DSMB to meet only at 6 months and not earlier?

Response: We clarified the ToV5 DSMB reviews event rates every 6-months (not starting at 6 months). We have also added: “All serious adverse events (SAEs) that are at least possibly related to study regimen or activities are reported to all IRBs within 24 hours of Principal Investigator notification per regulatory guidelines.”