PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Impact of preconception vaginal microbiota on women's risk of spontaneous preterm birth: Protocol for a prospective case-cohort study
AUTHORS	Lokken, Erica; Mandaliya, Kishorchandra; Srinivasan, Sujatha; Richardson, BA; Kinuthia, John; Lannon, Sophia; Jaoko, Walter; Alumera, Hudson; Kemoli, Arthur; Fay, Emily; John-Stewart, G; Fredricks, DN; McClelland, Scott

VERSION 1 – REVIEW

REVIEWER	David Desseauve Lausanne University Hospital (CHUV), Department Woman-Mother-Child
REVIEW RETURNED	08-Nov-2019

GENERAL COMMENTS	Review : bmjopen-2019-035186 Identification of risk factor of preterm birth is a major obstetrical concern and probably the most obstetrical challenge for the XXI em century. Unfortunately, prediction of preterm birth in unselected patient failed in the identification of convincing risk factor. The authors hypothesized than vaginal bacteria present around conception may lead to SPTB. Since PREMEVA publication(Subtil et al., 2018), we known that early detection of Bacterial vaginosis in the first trimesters and its treatment doesn't affect the incidence of preterm birth. Methodologically the study presented here is well design without problem. But the justification of this study have to be clarify To justify this study: Authors have to explain why bacterial vaginosis around conception could be different from early during pregnancy. What is the rational to support that somes kinds of bacterial vaginosis affects preterm birth. The rational of this study well designed is very lights and have to be update face to recent literature on this subject References: Subtil, D., Brabant, G., Tilloy, E., Devos, P., Canis, F., Fruchart, A., Bissinger, M.-C., Dugimont, J.-C., Nolf, C., Hacot, C., Gautier, S., Chantrel, J., Jousse, M., Desseauve, D., Plennevaux, J.L., Delaeter, C., Deghilage, S., Personne, A., Joyez, E., Guinard, E., Kipnis, E., Faure, K., Grandbastien, B., Ancel, P.-Y., Goffinet, F., Dessein, R., 2018. Early clindamycin for bacterial vaginosis in pregnancy (PREMEVA): a multicentre, double-blind, randomised

	controlled trial. Lancet Lond. Engl. 392, 2171–2179. https://doi.org/10.1016/S0140-6736(18)31617-9

REVIEWER	Bo Jacobsson Institution of Clinical Sciences Sahlgrenska Academy University of Gothenburg
REVIEW RETURNED	18-Nov-2019

GENERAL COMMENTS	Manuscript BMJOpen-2019-035186 General comments: Very interesting study in an interesting setting. Specific comments: Use preterm delivery instead of birth. Don't use both. Skip abbreviaton for spontaneous preterm delviery. Keep it for PCR and RNA etc but avoid as many abbreviations as possible. It will make it easier to read. Introduction: "No previous study has ...." avoid such statements without attaching a search stratigy that can prove that. Material and Methods section: Please try to condence the text and make it more easy readable. Discuss how the incentive could affect the outcome and if there can be any way to reduce that rissk. Please clarify this sentence: "Enrollment will continue through approximately June 2019, with final deliveries expected in 2021". This is a longitudinal study but no strategy is clear in the description how the loss to follow up and drop outs should be handled. Describe if you have done any other longitudinal studies in the same setting. I suggest that there will be samples taken between the membranes and also don't only use swabs. Take biopsies for 16S A lot of sensible information is handled in the study including sexual behavior. How is this information protected?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: David Desseauve
Institution and Country:
Centre Hospitalier Universitaire Vaudois, Lausanne
Switzerland

Comments: Identification of risk factor of preterm birth is a major obstetrical concern and probably the most obstetrical challenge for the XXI em century.
Unfortunately, prediction of preterm birth in unselected patient failed in the identification of convincing risk factor.
The authors hypothesized than vaginal bacteria present around conception may lead to SPTB. Since PREMEVA publication(Subtil et al., 2018), we known that early detection of Bacterial vaginosis in the first trimesters and its treatment doesn't affect the incidence of preterm birth.
Methodologically the study presented here is well design without problem.
But the justification of this study have to be clarify To justify this study:
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What is the rational to support that somes kinds of bacterial vaginosis affects preterm birth.

The rational of this study well designed is very lights and have to be update face to recent literature on this subject

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Subtil, D., Brabant, G., Tilloy, E., Devos, P., Canis, F., Fruchart, A., Bissinger, M.-C., Dugimont, J.-C., Nolf, C., Hacot, C., Gautier, S., Chantrel, J., Jousse, M., Desseauve, D., Plennevaux, J.L., Delaeter, C., Deghilage, S., Personne, A., Joyez, E., Guinard, E., Kipnis, E., Faure, K., Grandbastien, B., Ancel, P.-Y., Goffinet, F., Dessein, R., 2018. Early clindamycin for bacterial vaginosis in pregnancy (PREMEVA): a multicentre, double-blind, randomised controlled trial. Lancet Lond. Engl. 392, 2171–2179. https://doi.org/10.1016/S0140-6736(18)31617-9

We thank the reviewer for their interest in this study and for the PREMEVA citation. We have provided additional rationale for our study in the introduction (lines ~138-146) and here.

The lack of association between treatment of BV during pregnancy and risk of subsequent preterm birth, as observed in multiple prior studies (1–3), provides important background for the hypothesis that underpins the present study. Bacteria in the vagina at conception may ascend to and be present in the uterus. Formation of the cervical mucus plug after implantation likely reduces further transfer of vaginal bacteria to the uterus. Bacteria colonizing the uterus at conception may then become sealed between the decidua capsularis and decidua parietalis, and could cause chronic low-level inflammation, contributing to preterm birth. Antibiotic treatment during pregnancy, when bacteria are already present in the uterine cavity or sealed in the decidua, is likely too late to change this outcome. If we find that vaginal bacteria present at conception predict preterm birth, this would suggest that in contrast to BV treatment during pregnancy, treatment to eradicate key bacteria prior to conception could be effective at reducing prematurity.

Reviewer: 2
Reviewer Name: Bo Jacobsson Institution and Country:
Institution of Clinical Sciences
Sahlgrenska Academy
University of Gothenburg

General comments:

Very interesting study in an interesting setting. **Thank you.**

Specific comment 1: Use preterm delivery instead of birth. Don't use both. Skip abbreviation for spontaneous preterm delivery. Keep it for PCR and RNA etc but avoid as many abbreviations as possible. It will make it easier to read. **We thank the reviewer for this observation and have chosen to use just the term preterm birth. In this context, we have retained the commonly used abbreviation for spontaneous preterm birth (SPTB). If the editorial team would prefer that we**

spell out spontaneous preterm birth in lieu of SPTB, we are happy to do so. Lastly, we removed a number of less frequently used abbreviations throughout the manuscript, per the reviewer's recommendation.

Specific comment 2: Introduction: "No previous study has ...." avoid such statements without attaching a search strategy that can prove that. **We have rewritten this sentence rather than specifying the search strategy (Line 138).**

Specific comment 3: Material and Methods section: Please try to condence the text and make it more easy readable. **We made edits throughout the Methods and Analysis section (Lines 172-555)**.

Specific comment 4: Discuss how the incentive could affect the outcome and if there can be any way to reduce that risk. **In this observational study, we do not believe the small transportation reimbursements for study visits (300KSh, approximately $3.00 USD) will affect whether women deliver preterm or not. The Kenyatta National Hospital Ethics and Research Committee restricts reimbursements to a rate that does not lead to coercion. The 300KSh rate is consistent with other studies conducted in Kenya and is meant to cover only transportation costs. This rate of reimbursement would be expected to have no impact on women's overall financial status. In response to this comment, we have revised the manuscript to clarify that the visit reimbursement is for transportation only (Lines 399-400).**

Specific comment 5: Please clarify this sentence: "Enrollment will continue through approximately June 2019, with final deliveries expected in 2021". **Enrollment is ongoing. We will continue to enroll participants through approximately June 2019. The timing for closing enrollment will be based on the number of participants required to reach 80 spontaneous preterm births and 240 controls. We enroll women who are not yet pregnant, follow them for 6-9 months of conception attempt time, then follow women who become pregnant through gestation (9 months) and until 6-weeks postpartum. Therefore, deliveries are expected to continue for approximately 18 months after the last enrollment. We have clarified this in the text (Lines 182-184).**

Specific comment 6: This is a longitudinal study but no strategy is clear in the description how the loss to follow up and drop outs should be handled. Describe if you have done any other longitudinal studies in the same setting.

Both study sites have recruitment and retention staff who call participants in advance of each study visit and follow-up any who have missed an appointment. We have added this to the manuscript (Line 291). In addition, we have developed a number of key retention measures to prevent loss to follow-up during pregnancy and postpartum. These include a SMS program beginning during the second trimester of pregnancy and weekly phone calls beginning at 35 weeks. We have clarified this in the manuscript (Line 342).

Importantly, for this case-cohort study, the most important pieces of data are the vaginal microbiota swab at the visit prior to pregnancy, date of delivery, and whether there was spontaneous onset of labor. These delivery data are easily collected over the phone when our nurses call or by SMS. To date, we have collected delivery date and onset data from 99% of the women who have become pregnant and did not miscarry. Therefore, we do not believe we are at a high risk of bias due to loss to follow-up.

The co-investigators and research teams in Nairobi and Mombasa are highly experienced in managing prospective studies. Dr. McClelland (Study PI) and co-authors Dr. Kinuthia (co-investigator, Nairobi Site PI), Dr. Mandaliya (co-investigator, pathologist), Dr. Jaoko (co-investigator, Mombasa Site PI), and Dr. John-Stewart (co-investigator, expert in prevention of maternal to child transmission of HIV and studies in pregnant women) have conducted numerous longitudinal research studies in Kenyan women, including during pregnancy. Dr. McClelland has led the research site at the Ganjoni Clinic in Mombasa, Kenya for >20 years and the research site at the Couples Counseling Center in Nairobi, Kenya for 7 years.

Specific comment 7: I suggest that there will be samples taken between the membranes and also don't only use swabs. Take biopsies for 16S. **We agree that taking placental biopsies for molecular microbiota assessment would be an interesting addition to this study. However, given the costs of molecular testing we were unable to include this in the study.**

Specific comment 8: A lot of sensible information is handled in the study including sexual behavior. How is this information protected? **Data are collected and secured per Good Clinical Practice procedures for data security. More specifically, data are entered into a password protected REDCap database on a password protected and encrypted computer. We have added additional clarification around data security to the manuscript (Lines 576-579).**

References:

1. Subtil D, Brabant G, Tilloy E, Devos P, Canis F, Fruchart A, et al. Early clindamycin for bacterial vaginosis in pregnancy (PREMEVA): a multicentre, double-blind, randomised controlled trial. Lancet. 2018;392(10160):2171–9.
2. Haahr T, Ersboll AS, Karlsen MA, Svare J, Sneider K, Hee L, et al. Treatment of bacterial vaginosis in pregnancy in order to reduce the risk of spontaneous preterm delivery - a clinical recommendation. Acta Obstet Gynecol Scand. 2016;95(8):850–60.
3. Brocklehurst P, Gordon A, Heatley E, Milan S. Antibiotics for treating bacterial vaginosis in pregnancy. Cochrane Collab. 2013;(1):1–123.

VERSION 2 – REVIEW

REVIEWER	David Desseauve Lausanne University Hospital (CHUV), Department Woman-Mother-Child
REVIEW RETURNED	13-Dec-2019

GENERAL COMMENTS	Thank you for your precisions and sounds modifications