Author's response to reviews

Title: Subclinical iron deficiency is a strong predictor of bacterial vaginosis in early pregnancy

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Author's response to reviews: see over
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To The Editor
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Revised Manuscript Submission

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Manuscript title: Subclinical iron deficiency is a strong predictor of bacterial vaginosis in early pregnancy

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Dear Editor,

Please find attached a revised version of our manuscript entitled “Subclinical iron deficiency is a strong predictor of bacterial vaginosis in early pregnancy”.

We would like to express our gratitude to the Reviewers for their insightful comments and suggested revisions, which allowed us to improve the content of our manuscript.

We addressed all comments made by the Reviewers and herewith provide a point-by-point response to them (see below). We revised our manuscript accordingly and we believe that we have fully complied with nearly all suggested revisions, while a few suggested revisions that were impossible to properly account for are discussed as limitations to the study in the revised manuscript.

We hope that the Reviewers may approve the revised manuscript and that it is now suitable for publication in the Journal.

Sincerely yours,

(On behalf of all authors)

Hans Verstraelen

(Corresponding author)
We enclose below the complete Reviewer’s reports and inserted our point-by-point responses following each issue raised. Author’s responses are marked by [Author] followed by our response in blue font.

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Reviewer's report – Reviewer 1

Title: Subclinical iron deficiency is a strong predictor of bacterial vaginosis in early pregnancy

Version: 1

Date: 8 March 2005

Reviewer: Phillip Hay

Reviewer's report:

General

Subclinical iron deficiency is a strong predictor of bacterial vaginosis in early pregnancy

Bacterial vaginosis is an important risk factor for adverse pregnancy outcomes and acquisition of sexually transmitted infections including HIV. Those women who experience frequent recurrences of bacterial vaginosis after treatment also experience distress and frustration from their condition. It is likely that most women develop bacterial vaginosis at some stage although it may well remain asymptomatic. Our lack of understanding of the factors which predispose to relax of bacterial vaginosis is reflected in our inability to control it.

This paper reports a study investigating iron status in early pregnancy in relation to the diagnosis of intermediate flora and bacterial vaginosis. Natural history study suggests that BV becomes less common during a pregnancy and may well go through the intermediate stage before becoming normal(1). It therefore appears appropriate to classify intermediate and bacterial vaginosis as abnormal flora for the purposes of a study like this. This is a substudy of a larger study investigating risk factors for preterm birth. The authors refer to recent findings identifying associations between genetic differences such as mutations in the Toll 4-like receptor gene and phenotypic differences in the expression of anti inflammatory cytokines as being associated with susceptibility to bacterial vaginosis. Their hypothesis was that micronutrient status might also affect the incidence of BV. Iron in particular is known to affect bacterial colonisation. The principal finding was that conventional markers of iron deficiency such as haemoglobin and serum ferritin were not associated with abnormal vaginal flora, but more sensitive and specific indicators of iron deficiency including soluble transfer and receptor levels and the log [sTfR/ferritin] index were
significantly associated with abnormal flora. They concluded that subclinical iron deficiency probable related to inadequate preconceptional iron status was a risk factor for abnormal vaginal flora in early pregnancy.

The women were screened at a median of 9.2 weeks of gestation. In the discussion the authors identify many of the limitations of the study which include the small sample size with only 18 women with abnormal flora and 80 with normal flora. Clearly these findings need to be confirmed in larger prospective cohort studies. They controlled for most of the established confounders including age, gestational age, body mass index, smoking habit and parity, but did not collect data on sexual behaviour related characteristics and did not present data on ethnicity which may be an important risk factor for bacterial vaginosis and could also be related to iron status. They identified a cut-off sTfR levels but the sensitivity and positive predictive value were actually quite low for disturbed vaginal flora.

Many of the women had been taking iron supplementation during the pregnancy but this may not yet have had time to alter the iron stores. Again the study did not have the power to examine this in a meaningful way. The authors conclude that if iron status is important, it would be better to correct it in a preconceptional phase than during an established pregnancy.

This is an interesting study and gives us one more risk factor to consider in the etiology of bacterial vaginosis. It does need to be replicated and it would be interesting to examine a non pregnant population.

Natural history study suggests that BV becomes less common during a pregnancy and may well go through the intermediate stage before becoming normal. It therefore appears appropriate to classify intermediate and bacterial vaginosis as abnormal flora for the purposes of a study like this.

I do not feel qualified to comment on the statistics used, although they appear valid.

Reference List


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**Major Compulsory Revisions**

(that the author must respond to before a decision on publication can be reached) None

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Minor Essential Revisions

(such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

✓ p16 para 3 line 1-2: change ..dampen down.. to reduced

[Author] The wording has been changed according to the reviewer’s suggestion and rephrased in the revised manuscript (Discussion section, page 17, 2nd paragraph) as
“… intestinal iron absorption during the first trimester is also reduced …”

✓ p17 para 2 line 10: You cannot identify a risk factor in a cross sectional study. I suggest changing risk factor to the acquisition… to is associated with bacterial vaginosis...

[Author] We agree with the Reviewer that this phrasing is indeed speculative, considering we had no information on the timing of the exposure relative to the onset of the outcome of interest. The wording has been changed according to the reviewer’s suggestion and rephrased in the revised manuscript (Discussion section, page 18, 1st paragraph line 8-9) as
“… our observations on the association between iron deficiency and bacterial vaginosis concur with …”

✓ p20 conclusions line 1-3: Similarly you should refer to association rather than risk factor and also amend the conclusion in the abstract on page 3.

[Author] We adjusted the wording in the conclusion section of the text and in the conclusion of the abstract according to the reviewer’s suggestion, phrased in the revised manuscript as
“… subclinical iron deficiency … is strongly and independently associated with vaginosis-like microflora during early pregnancy”

Discretionary Revisions

(which the author can choose to ignore)

✓ It would be useful to comment on ethnicity as a potential confounding factor
[Author] Dr. Hay already made this remark in his general comment above and stated that we “did not present data on ethnicity which may be an important risk factor for bacterial vaginosis and could also be related to iron status”.

We agree with Dr. Hay that ethnicity is definitely a very important factor with regard to this association considering e.g. that black women tend to have a considerably higher incidence of bacterial vaginosis while they also tend to show vastly different values for most iron indicators, including sTfR values, as compared to white women. It would therefore be very interesting to elaborate on this association among black women.

We did not omit, however, this demographic information regarding our study population and actually stated in the Results section of the original manuscript that (page 9, Results section, 2nd paragraph, lines 6-7):

“Basic clinical characteristics of study participants, who were all of white Caucasian origin, are displayed in table 1”.

Therefore, ethnicity is not further commented on, as it was not a confounder in our study that exclusively comprised women of white Caucasian origin (this notion has been added in the revised manuscript to the Discussion section, page 19, lines 19-20, when addressing potential drawbacks of the study).

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes

Declaration of competing interests:

'I declare that I have no competing interests
Author's response to Reviewer 2

Reviewer's report – Reviewer 2

Title: Subclinical iron deficiency is a strong predictor of bacterial vaginosis in early pregnancy

Version: 1 Date: 7 April 2005

Reviewer: George Schmid

Reviewer's report:

General

This is a nicely-written and interesting article which associates subclinical iron deficiency with the presence of bacterial vaginosis-like flora (which I'll call BV). The article, using a case-control study design of patients from a cohort study of pregnant women, finds no association of BV with traditional, "macro" indicators of iron deficiency but does with more sensitive indicators, which presumably also measure more specifically tissue iron levels. The paper builds on recently conducted work looking at basic pathophysiologic reasons for having BV and provides an association well worth exploring, as the authors suggest, in subsequent studies better designed to study the effect they have described, and in populations other than pregnant women. That this study itself is derived from such a larger study suggests that the authors themselves have at least some of the data that they call for, inasmuch as the cohort was recruited March 3-November 6, 2003 (see Major Compulsory Revisions). If these data are available, I think the paper should be altered to include relevant data.

Comments (number/page/paragraph)

Major Compulsory Revisions

✔ 1.6/1 The cohort was recruited March 3-November 6, 2003. The authors collected data on the first antenatal care (ANC) visit. But, the paper is a case-control study nested within a cohort study. Thus, one wonders what the design of the cohort study was. Was it simply to enroll women at the first ANC visit and then follow the women for pregnancy outcome? Or, were other data collected during the study, e.g., subsequent determinations of BV or iron status that might impact
on or answer some of the questions, which this study raises. Other readers will wonder the same. Thus, at a minimum, I suggest the authors place more information about the cohort study (which is obviously completed by now, given the dates of enrollment of the pregnant women). If they have data that impact on some of the questions raised by this paper, e.g., do women receiving iron supplementation revert to a non-BV flora, or, do subsequent cross-sectional looks at the women later in pregnancy find the same associations, the authors should consider either including this information or indicate why not.

[Author] Since the manuscript (including the methods as well as the main results) is entirely based on a case-control study, we aimed at providing the reader with all relevant information and study details on this case-control study as such, rather than on a cohort study which is not the subject of the manuscript anyway. We believe that listing details, in the Methods section of a paper, on a study that is not the subject of the paper, nor of any relevance to its results, is likely to confuse the reader.

In this respect, the dates of enrolment should not be misconstrued, as they represent the exact time frame of the recruitment of the study participants that were included in the case-control analysis. This, we believe, has been unambiguously stated in the original manuscript (Methods section, 1st paragraph, pages 5-6) as

"we conducted a nested case-control study comprising 115 unselected pregnant women, which were consecutively enrolled on the occasion of their first antenatal visit, between March 3 and November 6, 2003 ...".

So these 115 women were enrolled during this period, not the entire cohort.

We do understand however, that the Reviewer makes a plea to reveal any additional data obtained from the cohort that might impinge on the (preliminary) results presented in the manuscript. However, at present, there are no such data and obviously, if we did have any further data that might be of interest to the reader with regard to the study as set out in the manuscript, we would have added these data to the paper.

However, to comply with the Reviewer’s requests, we did add some notes on the cohort study both in the Methods and in the Discussion section, which we hope may take away any possible doubts raised.

First of all, we rephrased the initial description of the study design in a way that more properly describes what the cohort study was actually about (Methods section, 1st paragraph, page 6 of the revised manuscript):

“As part of a prospective cohort study basically involving the study of vaginal microflora during early pregnancy in relation to pregnancy outcome, we conducted a nested case-control study ..."
Secondly, we also spelled out – as a limitation to our research - why the cohort study itself wasn’t of any further use to the findings obtained from the case-control study (Results section, <Limitations of the study> subsection, first paragraph, page 18 of the revised manuscript):

“Our results should be taken with caution considering our sample size was limited. Therefore our findings undeniably need to be confirmed in much larger, prospective cohort studies, preferably including non-pregnant women as well. Though the present case-control study was actually nested within a larger cohort study, failure to consistently follow-up patients enrolled at entry to prenatal care prevented any further conclusions that might be of interest to our results presented above, being drawn from it.”

To the attention of the reviewer we would like to explain why – indeed somewhat counterintuitively – the cohort study failed to do so.

The prospective cohort study was actually finished in August 2004, this is subjects were enrolled until August 2004, and the last subjects were or are to be delivered this month (April 2005). In this prospective cohort study, we basically aimed at a longitudinal assessment (serial assessment at three points in time during pregnancy) of potential markers of (idiopathic) spontaneous preterm birth, through repeated collection of vaginal swabs and maternal serum. However, the study soon deviated from its original goals as a result of the following:

1/ Mainly due to logistic constraints in terms of lack of study personnel and of financial resources, serial assessment was only accomplished among a small number of women, and therefore data available for analysis are largely confined to those obtained in the first trimester, rather than having true longitudinal data as intended. Furthermore, financial constraints also prevented us to perform a large deal of the planned analyses and therefore a number of the samples obtained were merely stored without being analyzed …

2/ Cross-sectional, rather than longitudinal study of the vaginal microflora during (early) pregnancy eventually evolved as the main focus of our study, following the discovery at the beginning of the study of a series of ‘novel’ bacterial vaginosis-associated organisms through culture-independent identification techniques (Verhelst et al. BMC Microbiol 2004, Verstraelen et al. Am J Obstet Gynecol 2004). The focus of the study further shifted towards a laborious study of the vaginal microflora as comparison of Gram stain, culture and various molecular techniques again lead to some new discoveries in this particular research area.

✓ 2.4/3 The authors have identified some risk factors for BV but apparently did not collect data on all in their study. As noted later in the multivariate analysis (11/3), some may be true
confounders when it comes to iron deficiency and its relationship to BV. But, some may not be currently recognized as confounders. For instance, vaginal douching could conceivably, to my thinking, affect vaginal tissue iron stores as well as cause BV (maybe the douching alters the vaginal tissue). A multivariate analysis can control only for the potential confounding variables entered into it. Could the authors address the issue of risk factors for BV other than those they have used in their analysis and comment on whether these might be "true" risk factors and tissue iron itself only a confounder.

[Author] We agree with the Reviewer that lack of control for vaginal douching - albeit an uncommon practice in our population - as a potential confounder should be addressed as a limitation to the study and this has been added to the limitations of the study in the discussion section (Results section, <Limitations of the study> subsection, first paragraph, page 19, lines 20-25 of the revised manuscript):

"We did not collect any data on sexual behaviour-related characteristics nor on vaginal douching, which have consistently been associated with BV, and therefore it cannot be precluded that differential sexual behaviour and differences in use of vaginal hygiene products between both groups may have confounded our results at least to some extent."

Though vaginal douching is an established risk factor for BV acquisition, we would like to emphasize that, according to the available evidence as referred to in the manuscript, the relationship between tissue iron shortage and vaginal overgrowth with anaerobes most likely, albeit speculative, relates to the effects of iron deficiency on the immune response, rather than to iron availability at the level of the vaginal mucosal surface. In contradiction with the mechanism as set out by the Reviewer, we have many arguments to assume that iron availability at the level of the vaginal mucosa may actually enhance the acquisition of vaginal pathogens and BV in particular, e.g. BV frequently occurs or relapses in association with menses (a setting with abundant local iron availability), high iron load is toxic to (the iron-abstaining) lactobacilli, *Gardnerella vaginalis* is capable of erythrocyte lysis through a specific haemolysin, and *Gardnerella vaginalis* also expresses a number of other mechanisms to acquire iron from human iron-binding proteins, to name a few.

Finally, we fully agree with the Reviewer that we took the contention that tissue iron might be merely a confounder insufficiently into consideration. Consequently, we explored a number of additional putative confounding interactions that were not or briefly discussed in the original manuscript. The Discussion section has been thoroughly revised and extended with regard to the limitations of the study according the Reviewer’s remarks (Results section, <Limitations of the study> subsection, second paragraph, pages 18-19 of the revised manuscript):

"As to confounding, we were able to control for most established confounders that may impinge on vaginal microflora status and on sTfR concentrations, including age, gestational length, BMI,
smoking habits, and parity. Among these body mass index and parity warrant particular scrutiny, considering these variables were significantly associated with vaginal microflora status in our series, while also being known determinants of maternal sTfR concentrations. Maternal sTfR concentrations were however not significantly correlated with BMI (p=0.4). The correlation between sTfR and parity was also not apparent from our data, this is when parity was handled as the raw, categorical variable (p=0.2), yet the correlation became highly significant when parity was handled as a binary variable (0.005). Therefore, it cannot be ignored that the observed association between maternal sTfR concentrations and vaginal microflora status concurs with the association between sTfR and parity. In particular, we found that multiparous women had on average a significantly higher sTfR (p=0.003) and that they were significantly more likely to have disturbed vaginal microflora (p=0.04) as compared to nulliparous women. Though these interactions were cancelled in the multivariable analysis by the correlation between sTfR and vaginal microflora status, it still needs to be considered that parity may act as a confounder to the former association. If anything, as it is only plausible that parity affects mean sTfR rather than sTfR concentrations determining parity, the most conceivable explanation would be that sTfR concentrations are the explanatory variable to the association between parity and vaginal microflora status.”

✓3.9/3 Here, and elsewhere, the authors have divided the flora into three categories, normal, BV-like, and BV, placing the latter two groups together for purposes of analysis. One of the means of evaluating causality is dose-response relationship. Thus, one might expect to see the most disturbed flora (grade III) to be associated with the lowest iron store scores. Yet, the authors have not presented nor mentioned such data. Would the authors comment, likely addressing it in their paper?

[Author] We agree with the Reviewer that documenting a dose-response relationship between maternal sTfR concentration and degree of vaginal microflora alteration is an elegant way to give further support to the association (and hypothesis) presented in the manuscript.

We did actually present data on this “dose-response” relationship, including the strength of this correlation, as there was indeed a linear trend between sTfR-concentrations (continuous) and degree of vaginal microflora alteration (three category scale).

As a matter of fact, we did mention this contention as the primary finding in the Results (sub) section <Soluble transferrin receptors in relation to vaginal microflora status> where it is stated (page 10, Results, third (sub) section, 1st paragraph):
“…we observed a trend by which maternal serum transferrin receptor (sTfR) concentrations during early pregnancy were negatively correlated with lactobacillary grading and hence positively correlated with the degree of vaginal microflora alteration (R=0.26, p=0.01)“.

We also took this finding into the Discussion section as the very first statement (page 13, Discussion, 1st paragraph) and hence as the primary finding:

“We found that maternal serum concentrations of soluble transferrin receptors during early pregnancy were positively correlated with decreased lactobacillary grading and hence with degree of vaginal microflora alteration.”

At the same time we have to acknowledge however, that there were too few cases with grade III flora to firmly establish a dose-response relationship, albeit this trend was statistically significant, and therefore we did not further elaborate on this observation.

4.11/3 Please reconcile the wording used, or groups examined in the analysis, when talking about the abnormal vaginal flora. The last sentence of this paragraph uses the term "vaginosis-like flora" when discussing the OR of 4.5. Is this only the Grade II flora (as the Results state, on page 9) or is it a combination of Grades II and III? If the latter, why were the grade III women excluded?

[Author] For the purpose of this study we pooled grade II and grade III microflora, which are designated as ‘BV-like’ microflora. This approach seems valid, considering grade II microflora typically show decreased numbers of lactobacilli and increased numbers of BV-associated microorganisms. Reviewer 1 has also approved this approach for the reasons he pointed out above. In addition, it has become increasingly apparent that there exists a continuous association between Nugent grading ≥ 4 and adverse pregnancy outcome (see e.g. Ugwumadu et al. Lancet 2003).

Nonetheless, we agree with the Reviewer that our wording in referring to grade II/III flora was indeed inconsistent and therefore potentially confusing in the original manuscript. We therefore added an unambiguous definition of the wording used, in the Methods section (second paragraph, page 6) and further consistently referred to this definition throughout the manuscript.

“Accordingly, Gram-stained vaginal smears were initially categorized as normal (grade I), intermediate (grade II), and bacterial vaginosis (grade III). To the purpose of the present study, we subsequently pooled the latter two categories into a single category unless otherwise specified, and therefore further denote two vaginal microflora status categories: normal or healthy microflora (corresponding to grade I microflora or a Nugent score 0-3) and disturbed or bacterial vaginosis-like microflora (corresponding to grade II and III or a Nugent score 4-10).”
I'm surprised by the finding that iron supplementation had no association with iron indices. The authors subsequently postulate that the women were likely recently begun on iron (page 19). But, perhaps not. Did the authors really not collect a start date for iron supplementation to support their guess? Would the authors please include some data about the lack of an association, e.g., at least the p values, as even if there was no statistically significant association there may be "trends"? The issue is quite important, as the logical approach to iron deficiency is to give iron—if this is ineffective, it would certainly be unusual and important to know.

How long does it take for supplemental iron to alter iron stores as measured in this study?

[Author] We registered the use of any pharmaceutical product (including commercial preparations of oligo-elements and vitamin supplements) from entry to prenatal care on, but not the use of these products before that time. Accordingly we did not obtain any data on duration of intake preceding the first antenatal visit.

We agree with the Reviewer, that in retrospect, this is a rather unfortunate lack in our data collection. However, even if we would had pursued detailed data on duration of supplemental iron intake, this probably wouldn't allow us to make any meaningful inferences from these data anyway, as Reviewer 1 stated.

We further agree with the Reviewer that we can't rule out some women having continued preconceptional iron intake into the first trimester, but based on our clinical experience this is usually not the case.

Finally, we agree with the Reviewer that 'lack of association' should be accompanied by the corresponding p-values, especially since there was indeed a trend for serum ferritin concentrations among women with supplemental iron intake during these early gestational ages, as the Reviewer suggested. Accordingly, we added the p-values and changed the phrasing of this paragraph (Results section, page 13, last paragraph):

"We found no significant association between iron supplementation (37/98 or 37.8%) and maternal sTfR concentrations (p=0.95) or with the combined sTfR-ferritin indexes following log transformation (p=0.20 to 0.97), while there was a marginally significant association between serum ferritin concentrations and supplemental iron intake (p=0.053)."
Minor Essential Revisions

✓ 6. Fig1 Here and in several places, it is not clear to me what happened to a Nugent score of 4. That is, healthy flora are categorized as grade 1-3, while abnormal as >4.

[Author] We thank the Reviewer for pinpointing this erroneous description of ‘abnormal’ microflora in the Figure and Table legends in the original manuscript. ‘Disturbed’ or BV-like microflora should have been defined as a Nugent score ≥ 4 (instead of >4) and this has been corrected in the revised manuscript.

Discretionary Revisions

✓ 7.8/3 Did the authors have a study hypothesis when they considered the collection of data on iron? That is, the authors employed sophisticated sampling for iron but there is no mention in the statistical analysis section, or elsewhere, about a hypothesis of the association of iron deficiency and BV (or other outcome) and sample size.

[Author] No power calculation was made prior to the data-collection and the sample size was merely determined by the available financial and human resources to conduct the study. Therefore we included a post hoc-power analysis in the original manuscript in the Results section (p12 – 13).

There are several lines of evidence emerging from the literature however – as discussed to some extent in the manuscript – that made us to decide to perform this large array of iron status-related assays in each and every patient enrolled during the first year of the study.

For instance, several investigators have documented a strong association between the evolution of ferritin concentrations over pregnancy and spontaneous preterm birth in large cohorts, including the Camden study (Scholl 1998). So, the association between ferritin (and to a lesser extent transferrin and lactoferrin) and preterm birth is intriguing, but hampered to fit into a pathophysiologic framework, as it is unclear from these studies whether ferritin reflects iron status or an acute phase response during gestation.

We therefore wanted to assess sTfR as a sound marker of iron status (even in pregnancy), which is independent of an acute phase response, inflammation or infection. By measuring sTfR in parallel with CRP, ferritin, and transferrin we further expected to disentangle the relative contributions of iron status and subclinical infection to the observed trends in ferritin values.

In addition, we hypothesized that the relationship between iron status and susceptibility to infection (and hence to spontaneous preterm birth) may well be U-shaped, this is, both iron
deficiency as well as iron overload may enhance infection, as has been shown for infections at other anatomical sites.

With regard to the latter, one of us (Joris Delanghe, second author) has a vast expertise in this field, this is, the study of iron status and its genetic determinants in relation to infectious disease, including HIV-1.

8.11/2 What is meant by "accuracy" of the sTfR assay?

[Author] The three so-called ‘measures of accuracy’ are reported to document the accuracy of identifying patients as having BV-like microflora based on an sTfR-assay with a cut-off of > 1.45 mg/L. Whereas ‘sensitivity’ refers to the accuracy with which patients actually having this condition are picked up by the assay and conversely, ‘specificity’ refers to the accuracy with which women not having the condition are classified as such, ‘accuracy’ refers to the overall accuracy, this is, the extent to which women are correctly allocated as having BV-like microflora or not by use of a sTfR > 1.45 mg/L cut-off, which was about 80% in our study.

9.-- There is some minor grammatical awkwardness that should be corrected before publication, e.g., 13/3 ("pregnant cohort" should be "cohort of pregnant women") or 16/3 ("precluded" is not quite the correct word).

[Author]

1/ “pregnant cohort” has been replaced with “cohort of pregnant women” in the revised manuscript (Discussion section, p14, 2nd paragraph)

2/ “It can therefore not be precluded” has been omitted and is rephrased in the revised manuscript (Discussion section, p17, second paragraph) as

"It is therefore plausible that restrictive iron absorption during early pregnancy at the time of critical processes such as placental development and organogenesis also concurs with other protective mechanisms, notably anti-oxidant and anti-infectious defence [28, 40]."

10.18/1 Would the authors like to suggest what the cohort studies should examine? For example, serial iron and flora determinations?

[Author] The proof of the pudding would be to demonstrate that prepregnancy/periconceptional tissue iron shortage is indeed predictive of bacterial vaginosis, chorioamnionitis, and spontaneous preterm birth and that these conditions are preventable by adjusting prepregnancy iron shortage.
This should not only involve serial assessment of iron and vaginal microflora status starting before conception, but also sufficiently large cohorts, and therefore this goal (especially recruiting large numbers of women planning to get pregnant) may not be amenable at all.

Evidence may also come from micronutrient supplementation to women in developing countries or to inner city, socially deprived women, but then again such intervention studies should be carefully designed to account for any source of confounding such as insidious infectious diseases, comorbidity patterns, sexual behaviour-related characteristics, etc.

As mentioned above, future studies may also address this issue in the study of the so-called ‘black-and-white gap’ in the prevalence of bacterial vaginosis and preterm birth.

Finally, we believe that micronutrient status (including iron, zinc, copper, etc) definitely warrants further scrutiny among those (non-pregnant) women who fail to respond to antibiotic treatment with metronidazole or clindamycin.