Author's response to reviews

Title: The vaginal microflora in relation to gingivitis

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Author’s response to reviews: see over
Responses to reviewers

We appreciate the comments made and have attempted to follow advice given to improve the manuscript.

We have inserted our responses on a point by point basis after each question.

Reviewer's report
Title: The vaginal microflora in relation to gingivitis
Version: 1 Date: 20 June 2008
Reviewer: Greg Spear

Reviewer's report:
This manuscript appears to have been extensively revised since the last submission in response to the reviews. An analysis was made that appear to indicate that gingivitis is more frequent in women with BV than in women that don’t have BV. Not surprisingly, many of the BV organisms such as P. bivia were found at higher levels in the BV+ samples in this analysis apparently 84 (BV+) plus 49 (BV-) women were analyzed. It is also stated that higher levels of several bacteria were found in gingivitis versus no gingivitis, but now 184 samples were analyzed since these probably included the samples from women that were Nugent intermediate. In the figure, comparisons between four groups were graphed. It is stated in that significant differences in a number of bacteria were found between BV+/G+ and BV-/G- groups, which is not surprising.

Major Compulsory Revisions;
1. Does this (results of figure 1) mean that there were no differences between BV+/G+ and BV+/G- or between BV-/G+ and BV-/G-? If so, this should be stated explicitly in the results. This would seem to argue against an association between gingivitis and genital bacteria.

Response:

We have added to the analysis assessments of in bacterial counts differences between BV/G+ and BV-/G-, and between BV+/G+ vs. BV+/G- subjects. The findings are introduced at the end of the result section, in the abstract, and in the discussion. We believe the new findings add to the concept but also that additional studies are needed with larger groups of subjects and perhaps using other definitions of BV and gingivitis.
2. Since the data is available, an analysis should be added to the figure and results showing values of intermediate/G+ compared with intermediate/G-.

Response

See above. We believe that the text is sufficient.

3. In the last review, it was asked if duplicate samples were ever run and if they agreed. While the samples for this study may all be used up, it is important to determine how reproducible the assay is and this can be done with other samples. To obtain enough sample, two or more samples could be pooled and then run in the assay. The variation should then be reported.

Response

Unfortunately we did not have enough of sample material to perform duplicate measures. This is an interesting question for us and we have in other publications considered this (Katsoulis et al. 2005) se ref 34). We also have an manuscript in press to discuss this issue. Nevertheless the dental literature on DNA-DNA checkerboard and duplicated measures does not exist. Your question is well taken.

4. In the abstract, it is stated that there were 83 BV- women and 58 BV+ women. On page 5 it states that there were 84 BV- and 49 BV-. Which is correct?

Response:

These questions have been addressed and reviewer 2 has also identified these typos. We have double checked that the present numbers are correct.

5. Minor Essential Revision; Figure 1 axis is not labeled.

Response

Added
Major compulsory revisions

Abstract
1. The results reported in the abstract are different from those reported in the text. In particular, in the abstract the Authors report that “a Nugent score of 0-3 was found in 83 women and a score >7 in 58 women”; in the Results section they report that “a Nugent score of 0-3 was found in 84 woman and a score of >7 was present in 49 women”.

Response

We have controlled the abstract, results, tables and the current numbers are now correct. All data have been cross-checked.

2. At the end of the abstract (pag. 2) the Authors write “The bacterial cell counts in vaginal samples were significantly higher in subject with bacterial vaginosis for 54 out of the 74 species” but in the Results section the species reported are 49 (listed at page 5 and 6).
3. The conclusion sentence of the abstract should be rephrased because it’s not clear.

Results
There is an improvement in the results exposition in comparison to the first version of this paper. Still, I have some observations:
1. Pag 5. “Among the 134 women…” which women? In the abstract and M&M section the total number of women is 180, whereas in table 2 the total number seems to be 184 (114 women with gingivitis, 70 women without gingivitis). Clearly there is a contradiction or a typing error somewhere.

Response

This has been corrected and crosschecked.

2. Pag 6. “The odds ratio for a diagnosis of BV was 5.3 for P. bivia and 4.4 for P. disiens.” It is known that the concentration of Prevotella increases in BV patients. Are these species isolated in gingivitis?

Response

P.disiens and bivia have been discussed by Holdeman et al around 1985. There are also some later papers with few subjects. The dental literature has focused on other bacteria and Prevotella nigrescens, and P.intermedia, P.melaninogenica. There are absolutely be no reasons why P.bivia, and P.disiens have been left out. Time, money, spezial interests ..maybe. we believe it is about time to include these species.

3. Pag 6. I think that the last part of the Results section (“Differences in bacterial levels by differentiation between subjects with any of four diagnostics...”)
combination”), in which the Authors look for correlations between BV and gingivitis, could be better explained. In particular, the Authors describe the significant differences between BV+/G+ and BV-/G- subjects, but these differences could be just related to the BV. In my opinion, it would be more noteworthy to report the significant differences between BV+/G+ and BV+/G- subjects.

Response.
The data have been revisited and new information inserted see comments to reviewer 1.

4. As the Authors recruited women “based on having a history of early preterm delivery or term delivery” (M&M section pag. 11) it would be interesting to include this parameter in the statistical analysis. Why the Authors didn’t do it?

Response
This number has been inserted (17) or about 10% and unfortunately too few for an analysis. We are working on this by expanding on the number of subjects.

5. It is not clear to me how the Authors calculated P values reported in tab. 3

Response:
We dichotomized the data by absence/presence > 1x10^4 and gingivitis or not (20%) Text added to table

Discussion
In my opinion, the discussion should be considerably shortened and more focused. In particular:

1. Pag 8. “This should be studied also in women without a recent history of pregnancy.” Why? Are the Authors interested in studying the association between gingivitis and an increate risk of preterm birth?

Response
The text has been revised

2. Pag 8-9. “Other recent studies have estimated the prevalence of gingivitis in adolescent subject at about the 50% level.” As commented above, since the Authors considered women with history of pregnancy, this sentence is irrelevant.

Response
We agree and have changed (deleted) both reference and text. The reference list has been adjusted

3. Pag 9. There is a whole paragraph (lines 5-14) about the gingivitis, the periodontitis and risk of preterm delivery. I think that it should be rewritten and shortened in order to better focus on gingivitis and preterm birth. In this paragraph the Authors could comment the results about the correlation of gingivitis, BV and preterm birth obtained by analyzing the 180 subjects enrolled
in the study.

Response
The text has been shortened and made more focused

4. Pag 10. “The analysis of bacterial levels based on the four different combinations of vaginal and periodontal diagnostic criteria demonstrated that the highest levels of a large number of different bacteria in the vaginal samples occurred among women who were positive for both BV and gingivitis.” This sentence doesn’t correspond to the results shown (pag.6-7 and Fig. 1). In the Result section the Authors report the statistically significant differences for a large number of bacteria found between groups BV+/G+ and BV-/G- (pag 7). Results about the comparison with the other two groups (BV+/G- and BV-/G+) were reported only for P. bivia and P. disiens (Fig. 1).

Response:
The issue has been addressed in response reviewer 1. New information has been added

5. Pag 10. “The analysis of bacterial levels based on the four different combinations of vaginal and periodontal diagnostic criteria demonstrated that the highest levels of a large number of bacteria in the vaginal samples occurred among women who were positive for both BV and gingivitis.” This is a repetition of a statement already reported in the Discussion section (see the above comment 4).

Response
Deleted

Materials and Methods
1. Page 11. “…were recruited based on having a history of early preterm delivery or term delivery.” How many of the 180 women recruited had history of preterm delivery? This info could be useful for the statistical analysis.

Response:
This number has been inserted (17) or about 10% and unfortunately too few for an analysis. We are working on this by expanding on the number of subjects

Minor Essential Revisions
1. Delete “?” (gingival ? disease) in the Background section (pag.4).

Response:
Deleted

2. Pag. 6. Delete the parenthesis in the title of the paragraph at the end of the page.
3. Pag 10. “The data also suggest that in the absence of gingivitis bacteria such as B. ureolyticus, P. bivia, P. disiens, M. curtisii, M. mulieris and V. cambriense may not present in vaginal samples.” I think “be” is lacking (typing error).