Reviewer’s report

Title: Association between cigarette smoking and the vaginal microbiota: A Pilot study

Version: 1 Date: 7 April 2014

Reviewer: Jimmy Twin

Reviewer’s report:

This manuscript introduces itself to shed more light on two important issues regarding the vaginal bacterial ecosystem: i) how closely is smoking related to community structure and ii) how valid is the theory that bacteriophage induction could disrupt a protective bacterial environment, potentially leading to conditions such as Bacterial Vaginosis. While this paper gives the first pilot data examining the changes smoking cessation has upon the vaginal bacterial community, the data is perhaps too limited and biased to address Phase A, and only gives a single case that shows a net positive effect from smoking cessation in Phase B, and indeed brings to question whether smoking is a confounding factor in previous studies.

Major points:

1. Although the authors did state that the smokers in this pilot study were more sexually active and reported a greater frequency of douching than non-smokers and took this into account, the small sample sizes are worrisome for the conclusions made, in particular when oral contraception usage was biased towards the non-smoking group. It is difficult to tell whether smoking was responsible for the higher low-Lactobacillus community structures, or whether oral contraception was promoting a L. crispatus- dominated environment (and perhaps this paper should be based on this). Therefore for Phase A, albeit interesting, cannot elude to smoking being responsible for a change in community structure, and may simply be a confounding factor.

2. Phase B is potentially quite informative as a methods-based paper and perhaps should be made the main focus of this paper, however will be limited based on descriptive data detailing one possible case of a response due to smoking cessation.

3. I am finding it difficult to see how the discussion leads with the data showing the effect smoking has on the vaginal bacterial environment when those followed in Phase B comprise of each of the described community types. Given the potential longitudinal variability of the vaginal bacterial community, would these cases be possible in those who did not smoke?

4. Do the bacterial genera Peptostreptococcus and Veillonella remain associated with smoking taking into account all factors including contraception usage?

Minor points:
5. Although phage induction is a plausible theory in the development of conditions such as Bacterial Vaginosis in certain cases, this paper does not directly address this, and perhaps should only be commented on in the discussion, in relation to the limited work that has been carried out in this area, and the need for further research. Indeed the perhaps “narrow” focus on bacteria should be changed to acknowledge other microbial elements such as bacteriophage that are most likely very much involved in the vaginal microbial environment in both health and disease states.

6. When describing Nugent scoring, it is perhaps time to start including other bacterial types more readily identified in high Nugent scoring samples with morphologies similar to Mobiluncus spp..

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests