**Reviewer’s report**

**Title:** Cervical microbiota in women with cervical intra-epithelial neoplasia, prior to and after local excisional treatment, a Norwegian cohort study.

**Version:** 0 **Date:** 17 Sep 2018

**Reviewer:** Yi-Chang Su

**Reviewer's report:**

This prospective observational study aimed to compare the cervical microbiota in women with CIN before and after LEEP and to assess whether cone depth and/or HPV persistence affects bacteria composition post-treatment. This research topic is interesting, and Zhang et al. had reported the same topic with the opposite result this March. (Zhang H, Lu J, Lu Y, Cai Q, Liu H, Xu C. Cervical microbiome is altered in cervical intraepithelial neoplasia after loop electrosurgical excision procedure in china. Sci Rep. 2018 Mar 21;8(1):4923. doi: 10.1038/s41598-018-23389-0)

https://www.nature.com/articles/s41598-018-23389-0

Though the sample size of this study is larger than Zhang's, there are several obvious flaws in the study design, which makes the results not solid and convincing. There are also some inadequate assumptions and inferences in the manuscript. The authors are suggested to revise and add more details about the participators, and analyze the effects of the related factors that may influence the cervical microbiota.

1. Since the cervical microbiota is the most important variables observed in this study, and the microbiota variety is closed related to the menopausal status, use of hormone replacement therapy, the time of menstruation, menstrual hygiene practices, use of vaginal douching, sexual behaviors, use of hormonal or barrier contraceptive products, numbers of sexual partners, history of recent sexual activity and antibiotics/probiotics (oral or topical) for other diseases; the authors should record and analyze the effects of these related factors of each participants instead stating these may impact the results as the study limitations.

2. The authors should mention the time of the sample collection. If the participants had menstruation or sexual activity in the previous days which may influence the cervical microbiota.

3. The participants in LEEP group were younger and more of them were single, whether their sexual behavior patterns and contraceptive use have changed after the diagnosis of CIN and receiving LEEP were not followed and recorded. Without the above information, the results are not convincing.
4. The discussion about the risk of preterm delivery (PTD) is not reasonable. PTD may result from other factors and pregnant women were excluded in the follow-up analysis. Meta-analyses published a few years ago in the Lancet and the BMJ showed an increased risk of adverse obstetric outcomes after treatment for CIN. The cause remains unclear, and potential mechanisms include anatomical changes, cicatrisation of the cervix, immunological factors, and alterations of the cervicovaginal flora. As the author said in the article: "The results were not significant possibly due to small sample size."(P.15, line12) In addition, the study couldn't control the interference factors, so the authors couldn't determine the reason why the study result was different from the previous research results, and couldn't explained the relation between LEEP therapy, microbial colonization and preterm delivery.

Other minor issues:

1. The Table 1 should be moved to the "Results."

2. In Table 4: the authors should explain in more detail about why authors group the women with the bacterial findings "Pos-pos, Neg-neg, Neg->pos" into one category.

Are the methods appropriate and well described?  
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?  
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?  
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?  
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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