Reviewer’s report

Title: Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin

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Reviewer: Michael Gravett

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Preterm birth is now the leading cause of childhood death under the age of 5, and is preceded by preterm premature rupture of the membranes (PPROM) in 30-40% of cases. Causes of PPROM, and in particular the relationships among the vaginal microbiome and PPROM, however, are poorly understood. In general, the vaginal microbiome becomes less diverse and Lactobacillus abundant during pregnancy, but has not been characterized in women destined to experience PPROM. The authors characterize in this manuscript the cervical-vaginal microbiome of the largest prospective, longitudinal cohort of pregnant women who experience PPROM to date, supplemented by a second group of cases enrolled following PPROM, compared to women who do not experience PPROM. The significant findings from this study are that: 1. Women with subsequent PPROM have vaginal dysbiosis characterized by Lactobacillus depletion, and increased diversity of other bacteria represented, and 2. That treatment with erythromycin following PPROM (as is the current standard of care in most high income countries) exacerbates Lactobacillus depletion and increases other potential vaginal pathogens.

This is an excellent well designed longitudinal study by respected, established investigators and is an important contribution to our understanding of PPROM. The methods are well described, and results clearly presented. An additional strength of the study is in correlating the vaginal microbiome with placental histopathology and neonatal outcome.

Specific suggestions include:

1. P3, L12. "PPROM occurs prior to 40% of all…” is inconsistent with the abstract that states "30%".

2. P10, L6-14. The sample size is small (only 15 patients among the 250 prospectively followed had PPROM), making generalization to other populations difficult. The small sample size, however, is understandable given the expense and constraints of the study.

3. P10, Results. The gestational ages at sampling among the groups is confusing and may CONFOUND the data set.

   a. For example, those enrolled following PPROM were sampled at 27 +0 weeks whereas those samples prior to PPROM were samples at 34 +4 weeks. This is a 7 week difference. Did differences in gestational age affect the microbiome?
b. Of particular concern, those who were prospectively enrolled (and I think ruptured at 34 weeks) were samples at a mean gestational age of 30 +1 weeks. This is a 4 week interval from sampling to PPROM. Could this have affected the microbiome, and do the authors have data that it did not?

4. Only 43 patients were sampled following 48 hours of erythromycin prophylaxis (from Figure 1, P29, L13). This is a small sample, and yet effects of erythromycin seems to be the main focus of the discussion.

a. While interesting, these findings will need to be confirmed by larger studies. The characterization of the microbiome preceding PPROM is really the strongest point, and should be the focus of discussion.

In summary, this is a meritorious manuscript, representing the culmination of an enormous effort and body of work. It significantly contributes to our knowledge, and, if confirmed, may change current practices of antibiotic prophylaxis flowing PPROM or suggest novel methods, e.g., probiotic therapy, to prevent PPROM.

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

Yes

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.

Yes

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.

I am able to assess the statistics

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