Reviewer's report

Title: Second trimester amniotic fluid cytokine concentrations, Ureaplasma spp. colonisation status and sexual activity as predictors of preterm birth in Chinese and Australian women

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Reviewer: Peta L Grigsby

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Summary:
This study examines different immunological markers, amniotic fluid Ureaplasma colonization, lifestyle factors, such as smoking and sexual intercourse during pregnancy and their association with preterm birth. The main analysis is of the potential association between these lifestyle factors, infection and the immune and inflammatory state of the pregnant woman leading to preterm birth. A novel aspect of this study is the comparison of these factors in two populations, Australian and Chinese women, with differing rates of preterm birth. The use of two very different racial, cultural and geographical populations to examine these factors poses a very interesting opportunity to pare out the pathological role of infection that leads to premature birth. Rates of Ureaplasma spp. colonization of amniotic fluid were unexpectedly very low in both populations. Some differences were identified in cytokine expression between the two populations, with IL-10 associated with preterm birth in the Chinese but not Australian population.

Sexual activity during pregnancy was not found to be associated with preterm birth.

Comments:
• The methods portion of the abstract lacks any mention of the lifestyle factors being measured.
• In the introduction, mention of the potential role of sexually transmitted infections, bacterial vaginosis and organisms, other than Ureaplasma, in the progression of infection-associated preterm birth would improve this section and the rationale of the study.
• Was screening for other organisms considered? Could the
• Previous studies or supporting evidence for the rationale of studying the role of sexual activity during pregnancy and its association with preterm birth is missing from the introduction. Please add this.
• Hypothesis should be written in the present tense (line 114).
• The examination of lifestyle factors in this study is omitted from the aims described in the introduction (lines 118-121).

• The final two sentences of the introduction may be better suited to inclusion in the discussion (lines 121-126). Consider deleting or moving.

• Methodological data for the sexual activity and lifestyle questionnaire is lacking. Previous use or validation of the questionnaire for its use in two different cultural and language settings is necessary when comparing behavioral data between varied populations. This is particularly an issue, given the much lower response rate to the questionnaire in the Chinese population, which may greatly skew the results reported. Self-reporting of smoking is also known to be highly inaccurate in many settings. Were any objective measures used to assess the validity of this data?

• Please clarify the statistical comparisons made between the populations for data other than the cytokine analysis (lines 240-242) eg. gestational age, smoking and sexual intercourse data.

• Racial/ethnic background of both populations is not described other than Australian and Chinese. Given the rationale behind the study and discussion of the involvement of racial factors in determining immune and inflammatory responses, the inclusion (or discussion) of ethnic background would enhance the paper.

• The discussion does not include mention of the poor response rate from the Chinese population and the implications for the reliability of this data.

• Description of the clinical management, method of dating pregnancies, choice to perform amniocentesis in the different populations that may confound the data collected should be clarified in the methods or commented on in the discussion. These factors, for example, could influence the incidence of reported threatened abortion prior to amniocentesis if different courses of management are prescribed in the different settings. Please clarify and discuss.

• Selection of the population by those high-risk pregnancies indicating the need for genetic amniocentesis also warrants comment. Please discuss if this could affect the outcomes measured in this study?

• Given that infection-associated preterm birth generally occurs earlier in gestation, could the absence of preterm birth at <28 weeks in the Chinese population explain the low rate of infection in the population studied? Please provide more discussion on the low incidence of Ureaplasma infection.

• Data relating to the frequency of sexual activity during pregnancy is duplicated from Table 1 in Figure 2. Please consider deleting the graph or the data from the table to avoid unnecessary repetition.

• Differences in parity and presence of previous preterm birth in the Australian population (with much lower incidence of nulliparity) are not mentioned in the results or discussion. Other factors that should be included in the demographic data or at least discussed are the presence of other sexually transmitted infections, short cervix and use of antibiotics during pregnancy, particularly given the incidence of PROM in the populations.
• Given that the identification of Ureaplasma infection was a primary aim of this study, why was the presence of Ureaplasma in vaginal/cervical swabs (at amniocentesis or delivery) or amniotic fluid at the time of birth not determined in this study?

• Some data on the presence of vaginal/cervical Ureaplasma infection is available in some Chinese populations. This should at least be mentioned alongside the similar Australian data (line 369).

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests