Reviewer's report

Title: Identification of Streptococcus agalactiae by fluorescent in situ hybridization compared to culturing and the determination of prevalence of Streptococcus agalactiae colonization among pregnant women in Bushehr, Iran

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Reviewer: Michele Hacker

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Identification of Streptococcus agalactiae by fluorescent in situ hybridization compared to culturing and the determination of prevalence of Streptococcus agalactiae colonization among pregnant women in Bushehr, Iran

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The authors have substantially improved the manuscript. The only remaining issues relate to the 47 women added to increase sample size and the reporting of prevalence by FISH rather than culture—both are addressed in my comments below. Pending the authors addressing these issues, which are relatively easy to fix, I recommend publication of the manuscript.

I strongly suggest the authors consider removing all of the data in Tables 2 and 3 and the corresponding analyses comparing women who were and were not colonized with GBS. I understand the authors’ interest in presenting these results given that they have the data. However, this analysis was not an objective of the study, seems out of place and does not add to the paper in a meaningful way. While these findings take up nearly half of the Results section and two full pages of tables, it is telling that only a paragraph of the Discussion is dedicated to the all of these findings—they simply do not seem relevant to the paper as it is laid out. The paper would read better if the objectives, methods, results and discussion were all in sync. That can be accomplished by reporting only on the 285 samples tested by both culture and FISH and restricting the reported results to sensitivity, specificity and prevalence.

MAJOR COMPULSORY REVISIONS

Methods

3. Adequately addressed

a. More information has been added. However, the paper now states that samples were collected from “all eligible pregnant women” and then goes on to specify “depending on the availability of the providers”. Thus, stating samples were collected from “all eligible pregnant women” does not seem accurate. This also cannot be accurate unless all women approached also consented to participate. Perhaps what the authors mean to state is that eligible women were approached seven days per week during day and night shifts depending on the
availability of providers to screen and consent women and to collect their samples.

4. The authors’ revisions are an improvement over the previous version, but what is stated needs further revision. Now that the authors have clarified why they included the additional 47 women, my strong recommendation is for them to delete those additional 47 from the paper. They do not add anything. Although adding them increases the sample size, they do not change any conclusions of the paper. The primary objectives of the paper were to compare FISH to culture and establish GBS prevalence—both objectives are accomplished without the additional 47 women. The only significant finding is a one-year difference in maternal age. If this difference is not significant when excluding the 47, nothing is lost. Furthermore, prevalence results should be presented based on culture. It also would be acceptable to report prevalence for culture and for FISH, but reporting FISH alone is not appropriate given it is the method being tested in the paper. The prevalence is nearly the same with the two methods; so, again no information is lost by only using culture. The abstract would need to be revised to reflect reporting of prevalence via culture, see line 41-43.

This paper would be cleaner if the 47 FISH results were excluded and results in Tables 2 and 3 were reported by culture results rather than FISH. I am aware that the culture and FISH yielded the same results; however, data should be reported using the gold standard rather than the technique being tested. That said, I will not recommend against publishing the paper if the authors (and the editor) choose to leave in the 47 FISH results.

I can see the authors took the sentence in line 197 from my review, but I the way it was incorporated into the text does not make sense. Thus, I suggest revising the sentence that starts on line 194 to read as follows “To increase our sample size, we added 47 pregnant women to our study; thus, a total of 332 pregnant women were included.”

Line 196 should read “In other words, only…”.

Delete the sentence on line 197 beginning with “We did not need the additional 47 FISH results…”.

b. Previous review comments: The calculations regarding sensitivity in paragraph 2 of Results sound like post hoc calculations. How could the authors have known they would have 27 colonized participants until after the culture was performed unless they based the calculation on FISH results? If that is the case, please specify that. In addition, the authors state in the beginning of paragraph 2 of Results that the sensitivity and specificity of FISH were both 100%, which would have used culture as the gold standard. They then go on to explain why they only cultured 285 samples using sensitivity and specificity of culture as the justification. However, the aim of the paper was not to evaluate sensitivity and specificity of culture; culture was the gold standard used to assess FISH. Thus, this explanation does not make sense as stated. If it is the case that only 285 samples were cultured, rather than 332, due to time and resource constraints or something along those lines then that can simply be stated. A power calculation
is not needed—just some explanation for why 47 samples were only tested with FISH. The first few sentences in paragraph 3 of Results attempt to do this, but the explanation does not really make sense. It seems they did not need the additional 47 FISH results to establish GBS prevalence, but these 47 samples simply increased their sample size, which is acceptable.

5. Adequately addressed.

Results

6. Adequately addressed.

MINOR ESSENTIAL REVISIONS

Methods

1. Statistical analysis, line 181. The Mann-Whitney U test would be used due to a non-normal distribution of age in at least one of the two groups being compared. The unequal samples size in and of itself does not require a non-parametric method. Even with unequal sample sizes, if age were normally distributed in each of the groups, a t test would be appropriate.

DISCRETIONARY REVISIONS

Line 294: Delete “i.e., the trend was significant”.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

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