Reviewer’s report

Title: Transcriptomic analysis of fetal membranes reveals pathways involved in preterm birth

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Reviewer: Nardhy Gómez-López

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This study was focused on the analysis of RNA expression in the fetal membranes from women with severe preterm and term deliveries using RNA sequencing. After an evaluation of the overall gene expression the authors found a total of 270 genes that were differentially expressed, 252 were up-regulated and 18 were downregulated in the severe preterm cases when compared with term births.

After looking for enrichment for genetic association with KEGG pathways and Gene Ontology, an enrichment in the genes corresponding to biological processes associated with the immune response was found. When quantitative real-time PCR was performed to assess expression of IL1B, LCN2, MARCO, CASP5 and TNFSF15, these genes were significantly upregulated in severe preterm cases compared to controls.

Finally, the authors concluded that the identification of these differentially expressed genes could help to develop a gene profile for preterm birth. The authors also mentioned that the markers that they suggest were generated in a South American population where PTB has a high incidence and these markers should be compared to other populations.

Below there is a list of comments that should be addressed by the authors:

1. The authors should mention whether the fetal membrane samples included the decidua parietalis. I think they did. If so, the fetal membrane samples included amnion and choriodecidua. Previous studies have shown that the choriodecidua displays an inflammatory signature during the process of labor at term (PMIDs: 16890549, 25283845, 29362510, etc.). Did the authors identify common genes between the physiologic and pathologic processes of labor (term labor vs. preterm labor)? The differential expressed genes between term no labor and labor groups have been already published and is available. Therefore, this comparison can be done by the authors. This will improve the manuscript and somehow will dampen the fact that the authors could not include gestational age-matched controls for the preterm labor group, which is not possible: there are not women who delivered preterm without pathological conditions.

2. The fetal membranes have different zones: periplacental zone, middle zone, and rupture zone. These zones have been well-studied and showed differences in their transcriptome (for example: PMIDs: 14634589, 16638590, 20452490, 21763637, etc.). The authors
should mention which zone of the fetal membranes were used. If this was not recorded, this should be stated as a limitation of the study.

3. Half of the samples in the preterm group presented PPROM, and 25% of the term samples presented PROM. Is not the process of PPROM and PROM at term different? This should be discussed. In addition, the authors should perform analysis to see whether there are differences between these two groups, even if this is done with 2 samples per group. The differential expressed genes can later be validated using a larger sample size.

4. Since the authors reported a group of 252 upregulated genes by RNA sequencing analysis and the genes chosen for validation are associated with immune response, it would be important to enrich the discussion with literature supporting the role of the immune response in pregnancy. For example, there are several studies supporting a role for IL1beta and inflammatory caspases (Caspase 4 and 5) (via the inflammasome) in the physiologic and pathologic processes of labor (PMIDs: 26952361, 28122480, 28233423, 27806943, 24238269)

5. Regarding the upregulated genes in the fetal membranes from the preterm labor group: the authors should perform a literature search for associations between these genes (or their orthologs) and preterm birth. See point #4.

6. The figure legend from Additional file 2: Table 2 needs further explanation of the content.

7. Overall, the quality of the figures needs to be improved. It looks like the Figure 4 was copied and pasted from a website.

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.

No

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

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