Reviewer's report

Title: Myometrial contractility influences oxytocin receptor (OXTR) expression in term trophoblast cells obtained from the maternal surface of the human placenta

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Reviewer: Eiji Shibata

Reviewer's report:

Overview
This manuscript address potential differences in OXT and OXTR expression levels and localization in the placenta. The authors use immunohistochemistry and ELISA methods to estimate protein levels and expression patterns. The authors report that OXTR levels are higher in both placenta samples and cultured trophoblast from placentas delivered by labor compared to C/S. The importance of this finding is associated with 1) the generation and propagation of an action potential signal from the placenta periphery to the uterine wall, and 2) placental separation during vaginal delivery.

Major Compulsory Revisions

General comments
#1 - The introduction contains excessive history and unrelated info about oxytocin and its receptor (OXT, OXTR). It would be more useful to include the (additional) facts regarding the function of OXT and its receptor during pregnancy. Specifically, argue as to why OXTR expression in trophoblast may be important and, based on observations and a current model, what may be gained from this data.

#2 - Methods is also very long with unimportant information for some sections. Figure 1, demonstrating sample collection points, is not necessary.

#3 - Writing: English should be checked to improve clarity and proper word choice (Introduction, methods, results). It reads as if an auto-translator was used without follow-up checking (for example- may we presume that “carbon dioxide snow” = dry ice?).

#4 - Results do not clearly describe what is being tested and type of data collected. It would help to state, for example, to compare OXTR protein levels in the placenta from vaginal and CS births, placental extracts were analyzed by a standard ELISA assay. And then go on with the description of important findings (starting at line 284). The specifics of the methods are in the methods section, but the why and outcome of doing the experiments are in the results section. Additionally, the results must be described much more fully. Figure 3, for example should include samples from groups I and II, the figure must be explained in the legend, and the observations made by IHC/IF must be explained in the text.
- Discussion is written with greater depth and clarity on the subject than the rest of the paper. Please be consistent in the level of attention given to manuscript production. The review process suffers when the manuscript is not at its best before submission. The discussion presents many important theories/models of vaginal delivery. It should be more focused on how the data, and how it supports, rejects, or otherwise relate to such.

Specific comments
#6 -(Line 284-297, Figure 2) Results presented in Figure 2 are confusing. Is it necessary to see each individual sample value? Is there some reason to compare samples #1, #2, etc., between normal and CS births? If not, the data values can be averaged and presented more concisely, as a vertical dot plot for each category, or even simple bar graph. Data values from Figure 2A and 2B can be presented on the same axis or table, and significance differences may be clearly labeled.

#7 -(Line 298-301, Figure 3) It is not clear what is being presented in the Results section referring to Figure 3. How is contractile activity of the uterus being examined or measured or presented here? What are the labels in the figures? What are the different colors? Please elaborate on these points in both the figure legend and the text.

#8 -(line 298-301, Figure 3) When presenting IF or IHC results, a verbal description or interpretation by the author is needed. Readers can decide to agree or not if they see what you see.

#9 -In this study, which population(s) of trophoblasts are being focused on and measured? The results describing both placenta section and primary cultures should address this, as well as the discussion.

#10 -Wasn`t CD31 used here in IHC/IF experiments to estimate the vascular endothelial cell content of samples? I don`t see any description of results for this.

#11 -(Line 304, Figure 4) In Figure 4, OXTR staining levels do appear to be higher in group I compared to group 2. But no change or “augmentation” is observed in levels here with a sample type. Only a single time point and condition is presented for each sample type, so no measure of augmentation is possible.

#12 -For Figure 4 data, what is the standard, total protein? How about a trophoblast marker to control for culture conditions and cell differentiation?

#13 -Figure 5 appears to be a (vestigial?) copy of Figure 2A.

#14 -The results, in summary, need to be presented in a more detailed, complete manner. The reader must be able to follow clearly both why and how samples were analyzed, as well as how data was collected, processed, and presented.

#15 -(Line 324-343) A large discussion of action potential is presented, but it is much less clear what the connection between myometrium contractions and placenta-localized action potentials. Is there evidence of signaling between the
two, for example in refs 43 and 44? Please elaborate on this more, and reduce the preceding discussion to focus on the role of OXT/OXTR.

#16 -(Line 381-393) It is not clear that this paragraph is directly related to OTX/OTXR observations made here.

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

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests