**Reviewer’s report**

**Title:** Evaluation of apoptosis and angiogenesis in ectopic and eutopic stromal cells of patients with endometriosis compared to non-endometriotic controls

**Version:** 1  **Date:** 01 Oct 2019

**Reviewer:** Sandra Cecconi

**Reviewer's report:**

In this study, the authors investigated the differences in gene expression of apoptotic and angiogenic markers (Bcl-2, Bcl-xL, Bax, caspase 3, VEGF-A) among three types of stromal cells isolated from biopsies of endometriotic patients (ectopic: EESCs and eutopic: EuESCs) and non-endometriotic patients (CESCs). They found higher expression of Bcl-2, Bcl-xL in EESCs in comparison with EuESCs and CESCs, lower expression of caspase 3 in EuESCs in comparison with EESCs and CESCs, higher expression of VEGF-A in both endometriotic samples compared with control. Bax gene was similar among the three groups. The authors concluded that absence of apoptosis and the increased angiogenesis potential in EESCs could contribute to explain the pathogenesis of endometriosis.

Although of great interest, the following major revisions are required before publication.

**MAJOR REVISIONS:**

1. On the basis of literature data, the authors suggested the involvement of apoptotic proteins as important factors in the etiology and pathophysiology of endometriosis (Refs 6-8, 10, 11). As mentioned in these papers and considering the fact that endometriosis is notably an inflammatory disease, a pivotal role is played by Fas/FasL and TNF activation, both activators of the extrinsic apoptotic pathway. The partial activation of this pathway has been reported in a recent paper, highly pertinent to this Ms, that is not cited in the text (PMID: 31416694). Please add and discuss differences.

2. A large number of biomarkers are involved in the angiogenic mechanisms of endometriotic ectopic/eutopic lesions (PMID: 27541444), as VEGFR2, HIF1A, HGF, and PDGFB. It is important to add the analysis of some of these biomarkers to the panel of angiogenic factors studied to support conclusions. It is not correct to speculate about increased angiogenic potential by investigating only VEGFA.

3. The analysis of gene expression is not per se indicative of protein expression levels. Immunolocalization of proteins and/or WB analysis should better support results.

**Materials and methods:**

4. Paragraph "Sample collection":

   * Lines 118: "Finally, cells from 17 eutopic and 11 ectopic endometrial tissues of endometriotic patients and 15 eutopic endometrial tissue from non-endometriotic patients were used in this study". These numbers are in contrast with the total number of patients claimed in the beginning of "Patients" section. The authors enrolled for the study 25 endometriotic women and 20 non-endometriotic women. Here, they stated that they use 28 tissues of endometriotic patients. Did the AA take more than one sample for some patients? Please, explain.

5. In this section the paragraph "Statistical analysis" is missing. The authors reported statistical significance without explaining anywhere which test has been used. Additionally, it is not clear how experimental data were expressed. What is the "expression ratio"? Usually, value of each gene is
compared with the related housekeeping (gene expression/beta-actin). 

Results:
6. Line 146-48. Since the authors reported immunofluorescence staining as method for the evaluation of the purity of all ESCs groups, this reviewer suggests adding representative images for each ESCs group.

7. Figures: in all the legends the authors claimed that:
* "Data are expressed as mean and error". Please clarify.
* "Each bar represents levels of ... gene expression in two different endometrial cells". Do you mean two cell groups? If so, please correct accordingly.

Discussion:
8. Lines 195-202: The authors reported the results of Bax as not significant without discussing this result. Please, explain which could be the reason(s) of such Bax gene expression.
9. Lines 219-37: As stated before, since the authors evaluated only VEGF-A gene expression as marker of angiogenesis, this result is not sufficient to support the effective increase in angiogenesis. Please, add experiments with at least one angiogenic marker other than VEGFA (See point 2). Alternatively, the strength of this paragraph and conclusions needs to be modified.

MINOR REVISIONS:
10. Line 55: Correct "Despite being a quite common among women" with "Despite being quite common among women", or "Despite being a quite common pathology among women".
11. Line 162: Correct "as" with "is".
12. 437-38: Put "value" as subscripts.

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.

No

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 recommend additional statistical review

Quality of written English
Please indicate the quality of language in the manuscript:
Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

'I declare that I have no competing interests'.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal