

## Manuscript NO: 27731-manuscript revision

### REGULATION OF MITOCHONDRIAL FUNCTION AND ENDOPLASMIC RETICULUM STRESS BY NITRIC OXIDE IN PLURIPOTENT STEM CELLS

Caballano-Infantes Estefania et al.

#### RESPONSE TO REVIEWERS

##### Reviewer 1.

The manuscript entitled Regulation of mitochondrial function and endoplasmic reticulum stress by nitric oxide in pluripotent stem cells by Caballano-Infantes et al addresses very interesting issue of undifferentiated cells metabolism and NO involvement in pluripotency maintenance. The manuscript is interesting however there are some drawbacks that makes it difficult to read. Firstly it is a huge number of abbreviations that are used sometimes only once like TEM transmission electron microscopy or instead are used very often like embryonic stem cells (or PGC-1a) but each time providing both full and abbreviated (ESCs) forms. Some abbreviations are not explained (RER???)

*I would recommend to Authors to read the manuscript carefully and remove all unimportant abbreviations however to combine all meaningful abbreviations on one page.*

Response: We have carefully reviewed the abbreviations and added a section of the most important at the beginning of the manuscript

*It is also surprised me that in PSCs mitochondria are large (see section 1.4)*

Response: it was a mistake. In this version it has been corrected.

It said:

“the Pluripotent Stem Cells (PSCs) have a low mitochondrial population . Mitochondria are large, with low energy potential”

Now says:

“pluripotent stem cells (PSCs) have a low mitochondrial population with low energy potential”

##### Reviewer 2.

The authors review the role of NO on mitochondrial function and ERS in pluripotent stem cells. The authors also described the mechanism by which NO regulates embryonic development and signaling pathways. The topic is of considerable interest since NO has important role in controlling differentiation and survival on the embryonic stem cells, but the molecular mechanisms by which it modulates these functions unknown. Therefore, review on the relation between NO levels, ER stress and mitochondrial dysfunction that control stem cell fate and the molecular mechanisms by which it modulates these functions is a beneficial. Overall the review is complete, and contains the most important

information about the role of NO on mitochondrial function and ERS in stem cells. However, there are several concerns about the manuscript, and needs to be improved. Therefore, I cannot recommend acceptance of the manuscript in its present form.

I believe that the manuscript will be suitable for publication in the Journal after minor revision.

#### **Minor Comments:**

*1. The manuscript is long, and there are some typo errors that require attention.*

Response:

We have reviewed the manuscript and deleted paragraphs we consider dispensable.

In addition, American Journal Express has edited the manuscript. We attach certification.

*2. During the first occurrence of an acronym, should spell out a technical term first and then present the acronym abbreviation in parentheses and use the abbreviation only after that. For example, on page 3, the authors should spelled out human embryonic stem cells first and then abbreviated (hESc), and then on page 10 should use abbreviation only. Some more of these examples can be found throughout the manuscript. The authors should go over the entire manuscript for this correction.*

Response:

We have carefully reviewed the abbreviations and added a section of the most important at the beginning of the manuscript

*3. Some of the references were old. For example, reference number 4, 30, 53, ext. The authors need to add new, up-to-date references to the reference section.*

Response:

We agree with the reviewer. But we believe that these references to be the first to report those results or concepts are important to mention.

*4. Figure 1 legend is not complete and needs to explain more detail.*

Response:

We have reviewed the legend of Figure 1.

We have completed the drafting. We believe that now is better explained

#### **Reviewer 3.**

The manuscript by Caballano-Infantes et al. is an interesting review about the role of NO in pluripotent stem cells. More particularly, the authors have focused on the modulation of the mitochondrial function and endoplasmic reticulum stress in pluripotent stem cells by NO. The paper is well structured and generally well written.

*There are however several typos and grammatical errors that need to be corrected/edited.*

Response:

American Journal Express has edited the manuscript. We attach certification.

**Reviewer 4.**

This article intends to review the roles of nitric oxide in regulating the mitochondrial function and endoplasmic reticulum stress, especially in pluripotent stem cells. The topic, i.e., NO levels, ER stress and mitochondrial dysfunction could regulate the stem cell fate, is interesting, but the concerns are:

- 1) *The whole article is unbalanced, with a too long INTRODUCTION (almost 5 pages long).*
- 2) *The 2nd part of the article, i.e., NO AND MITOCHONDRIAL BIOGENESIS, is also too long, since, this part should not be the major focus of the article, even though it may be necessary.*

Response:

We have reviewed the manuscript and deleted paragraphs we consider dispensable

- 3) *Overall, even the major take home message is well received, the underlying rationale/logic should be tighter, the language needs to be polished, and the flow of the idea should be more natural.*

Response:

American Journal Express has edited the manuscript. We attach certification