

March 10, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16743-edited revised author final).

Title: Spermatogonial Stem Cells: current biotechnological advances in reproduction and regenerative medicine

Author: Pedro M. Aponte

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 16743

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated

2. Revision has been made according to the suggestions of the reviewers:

Reviewer #02446204. The author is very grateful to reviewer 02446204 for his interesting suggestions and comments.

Comment: "SSC suffers from certain limitations due to a matter of imprinting. Authors should add some comments regarding this issue." **Answer:** Yes, indeed there are epigenetic defects in SSC derived pluripotent stem cells. New information was incorporated in lines 86, 259-266; accordingly, some new references have also been included.

Comment: "The SSC-based technique to produce gene manipulated animals will bring beneficial outcomes not only in the field of farm animal industries but also in the field of regenerative medicine because Professor Hitomitsu Nakauchi at Stanford School of Medicine has started his trials to produce human iPS-derived organs based on his theory of "organ niche" (Kobayashi T et al., Cell 42, 787-799, 2010) using genetically engineered pigs, for example, pancreas-ablated pigs produced by inserting hes1 transgene into the pdx1 locus. I would like to recommend the author to mention this topic". **Answer:** This is very interesting and relevant information to the topic covered. I tried to give a context to the possible insertion of SSCs on these technologies in lines 271-280. The suggested reference and others have been included.

Minor concerns:

- **Comment:** In line 8, page 3, the sentence "Since SSC are adult cells they do not bring about...." should be corrected as "Since SSC are adult cells, they do not bring about...." **Answer:** This has been corrected (now in line 85)
- **Comment:** In line 35, page 5, the sentence "However protocols need to be...." should be corrected as "However, protocols need to be....". **Answer:** This has been corrected (now in line 399)
- **Comment:** In line 19, page 6, the sentence "ES cell based transgenesis technologies are well develop" should be corrected as "ES cell-based transgenesis technologies are well developed" **Answer:** This has been corrected (now in line 305)
- **Comment:** In line 21, page 7, the sentence "This would be useful n extensive rearing animal industries, ..." should be corrected as "This would be useful in extensive rearing animal industries, ..." **Answer:** This sentence was rephrased to meet a comment from another reviewer. The

new sentence is now on line 402

- **Comment:** In line 30, page 8, the words “a feeder serum free culture” should be corrected as “a feeder-free and serum-free culture” or “a feeder/serum-free culture”. **Answer:** This has been corrected (now in line 167)

- **Comment:** In line 31, page 8, the phrase “which continue to improve” should be corrected as “which continues to improve” **Answer:** This sentence was rephrased to meet a comment from another reviewer. The new information is now in line 168

Reviewer # 02446163. The author is very grateful to reviewer 02446163 for his interesting suggestions and comments.

Comment: “First, we suggest to put SSC isolation and managing, as well as In vitro SSC differentiation just after "Introduction". **Answer:** This suggestion is very logical so I proceeded to reorganize the manuscript accordingly (please see the new manuscript version)

Comment: “Please, include topic about "pitfalls" of SSC isolation. Is this process very easy? Does the method of isolation will limit the future use of SSCs in biotechnology? **Answer:** I included a text block with new information (lines 122-160) in which I tried to cover the reviewer questions

Comment: “Please put together in correct order: 1. Isolation; 2 Markers of SSC, 3. in vitro differentiation and 4. pluripotency-teratomas production. Species specific difference in SSCs. 5. Gene technologies and SSC. 6. Germ cells transplantation. 7. Fertility restoration. 8. Final remarks.”

Answer: This suggestion was followed, so the manuscript was reorganized based on this scheme. Point 4 was grouped within the topic “SSC pluripotency”

Comment: “Introduction” should be improved, make it shorter, please. In introduction should be included critical statement about SSC and iPS cells derived from SSCs. Such as teratomas production and epigenetic marks.” **Answer:** I tried to do my best to improve the introduction. The introduction length was cut down by approximately 20%. Some very general facts are now covered in this section. More in-deep information about epigenetic marks and teratoma formation in the context of pluripotency of SSC derived stem cells is now included in the specific topics of the manuscript (please, see lines 86-88; 237-242; 259-266; 271-280).

Comment: “SSC stem cell niche in vivo, please include as well as, ref. Yoshida et al., 2007. Science 371, no5845, pp. 1722-1726.” **Answer:** this important aspect was included in the introduction (lines 96-105). Also, the suggested reference and others have been included.

Reviewer # 00504632. The author is very grateful to reviewer 00504632 for his interesting suggestions and comments. The manuscript has been carefully checked for proper use of English language. Furthermore, I have tried to eliminate all redundant and/or meaningless sentences. Several specific sentences pointed out and given as examples by the reviewer were eliminated or rewritten. In general, the text has been critically read and screened for this type of expressions. Where appropriate, the text has been condensed to communicate the desired ideas with less wording. I truly hope this time the manuscript is in a more reader-friendly format while at the same time conveying the message of new and potential uses of SSCs in biotechnology. Line numbers have been added to the manuscript.

Specific comments

Comment: ‘When awareness that SSC were capable to derive pluripotent stem cells...’ Should most likely be something like: ‘When it was discovered that pluripotent stem cell lines could be derived from SSCs..’ **Answer:** this idea was rewritten (please see lines 83-85)

Comment: In the Introduction ‘gene delivering faculties’ should be something like: ‘The facility/possibility of gene targeting...’ **Answer:** this idea was rewritten (please see lines 89-90)

Comment: In the Introduction: 'several techniques are totally developed' should be something like: 'several efficient techniques have been developed' **Answer:** this sentence has been eliminated

Comment: In the Introduction: 'is currently in the eyes of the scientific community' should be something like 'should be investigated' or 'is currently being investigated'. **Answer:** this idea was rewritten (please see lines 113-116)

Comment: 'Transgenesis involving SSCs would then be the next step, but culture conditions had to be first explored which actually became a breakthrough in SSC technologies' **Answer:** this idea was eliminated from the manuscript.

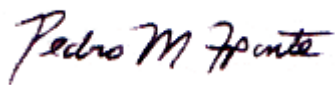
Comment: 'The advent of mGS cell research has brought important efforts that allowed advances in the field' **Answer:** this idea was eliminated from the manuscript.

Comment: 'Recently, new experiments have been done to try to settle the controversy' are meaningless and redundant. **Answer:** this sentence was eliminated from the manuscript

3. References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Stem Cells*.

Sincerely yours,



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