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PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 70949

Title: In vitro induced pluripotency from urine-derived cells in porcine

Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05446072
Position: Peer Reviewer
Academic degree: MD
Professional title: Doctor
Reviewer's Country/Territory: Pakistan
Author's Country/Territory: Brazil

Manuscript submission date: 2021-08-23

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-08-23 12:35

Reviewer performed review: 2021-08-31 10:36

Review time: 7 Days and 22 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors have used porcine urine for iPSC generation and need to mention its significance in regenerative medicine in detail. Also was there any difference in comparison to murine and human models already established. The choice of vectors need to be discussed in detail and how the reprogramming efficiency can e improved. ICC image need to be presented better. Was RNA integrity analysis carried out before downstream qPCR experiments were performed. Please highlight any other novel aspect of the study apart from source of urine and how it presents a better model

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Peer-review model: Single blind

Reviewer's code: 06079635
Position: Peer Reviewer
Academic degree: BSc, MSc, PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: Pakistan

Author's Country/Territory: Brazil

Manuscript submission date: 2021-08-23

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-08-24 09:51

Reviewer performed review: 2021-09-01 11:51

Review time: 8 Days and 2 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The enclosed manuscript by the Bressan et al., reports the conversion of porcine urine-derived cells in to pluripotent cells. The present study is the first reposrted attempt to generate pluripotent cells from the cells isolated from urine. Such human equivalens to urine derived cells have previously been reported. This is an interesting study having potential applications for verterinary disease modelling and genetic improvements particulary in porcine model. Though similar reports of pluripotent cells generation from non-invasive origins does exist in humans, the importance of such studies in animals can not be discounted. The present study has applied previosuly established protocols of pluripotent cells generation but using the urine-derived cells of porcine. Few points that will aid in clarity of the manuscript are: a) The importance of such work in porcine models needs to be adequately described in the introduction section. b) In-text citations should be uniform throughout the manuscript, e.g. 1st sentence of paragrapgh 2 of Introduction section contains author-date in-text citation and rest of the introductin has numbered references c) Few abbreviations are used first time without explanation, e.g. NH in second last paragraph of Introduction section. d) Methodolgy section did not provide clarification as to why only female animals of reproductive age were used for urine collection. e) How endogenous and exogenous expression of reprogramming factors were distingusihed in PCR analysis? This needs to be elaborated. f) FP, IP and LP are the abbreviations not explained in Results and Disucssion section. g) There should be elaboration of the types of cells present in porcine urine and if thier heterogenity could influence the reprogramming efficiency. h) Supplementary Table 5 is hard to understand as is presented, that needs to be elaborated further.

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Peer-review model: Single blind

Reviewer's code: 05203277
Position: Editorial Board
Academic degree: PhD

Professional title: Senior Scientist

Reviewer's Country/Territory: India

Author's Country/Territory: Brazil

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Reviewer performed review: 2021-09-03 07:22

Review time: 10 Days and 2 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript entitled 'In vitro induced pluripotency from urine-derived cells in porcine' is a good attempt to derived cells from urine and then transduced those using reprogramming factors to generate iPS cells. The authors showed the derivation and characterization of generated iPS cells using immunocytochemistry and gene expression approaches followed by in vitro differentiation. There are many reports are available on successful generation of porcine iPS cells generation from different type of cells but definitely Urine derived cells may be first report of current manuscript. Recently many workers demonstrated to show the successful derivation of porcine iPS cells for example- using N2B27 base medium supplemented with FBS, LIF, activin A, vitamin C, knockout serum replacement and small molecule inhibitors such as GSK3 and Wnt. Similarly, Xu et al., 2019 demonstrated extended growth of porcine iPS cells using 2i condition media and these generated iPS cells were injected in early porcine embryo which subsequently developed into blastocyst where contributed competently to both ICM and trophectoderm cells. Authors have not tried these approaches to improve their efficiency. The gold standard of iPS cells id differentiation ability in vitro and in vivo. In current study, in vitro differentiated cells not showed the expression of ecdoderm. Further, in vivo study has not conducted. Based upon these reports generated cells has not qualifying the iPS cells and suggested to use iPS cell-like cells.

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Peer-review model: Single blind

Reviewer's code: 02937551
Position: Editorial Board
Academic degree: PhD

Professional title: Professor, Research Fellow

Reviewer's Country/Territory: China

Author's Country/Territory: Brazil

Manuscript submission date: 2021-08-23

Reviewer chosen by: AI Technique

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Reviewer performed review: 2021-09-05 10:15

Review time: 11 Days and 20 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Urine-derived iPSCs or urine-derived stem cells have been recently reported in humans, nonhuman primates,rabbit and canine models. This paper reports the isolation and reprogramming of cells derived through the noninvasive collection of urine in a porcine model. It is important for agricultural traits, genetic improvement and in vitro and in vivo modeling of several diseases. The comments: 1. How to distinguish the expression of endogenous factors Oct4, Sox2, Nanog, and exogenic OSKM? How to calculate relative expression (calculation formula)? 2. Figure 3 is too small to see clearly. 3. Is D6 embryos in Figure 4 marked with a dot just a sample? 4. The A-C superscript letters represent differences in Figure 5 is not clear. Figure 5 is wrongly marked as Figure 6. 5. Supplementary Table 1. What does "s" stand for in primers sOct4? The abbreviation “pb” is incorrect. It should be “bp” (base pairs). 6. Supplementary Table 2. hOSKM data is incorrect, please verify.