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Dear Editors,

We thank the reviewers for their generous comments on the manuscript and have edited the manuscript to address their concerns.

We believe that the manuscript is now suitable for publication in PeerJ.

Prof. Guoning Huang

On behalf of all authors.

Reviewer 1

Basic reporting

1) Major Comment: It would be helpful to have some more specific functions of upregulated genes in the discussion section.

Thank you for pointing this out. We have added a discussion (line 279-281) of the function of upregulated gene HNRPUL1, and its role in embryonic development in mice.

Minor Comments:

a. Line 65: delete 'of'

b. Line 169: check grammar

c. Line 176: check grammar

d. Line 180: check grammar

e. Line 311: change exposure to exposed

f. Line 322: change cultured to culture

g. Line 355: Change leaded to lead

h. Line 362: Missing citation/ reference

i. Line 384: Check grammar

j. Line 385: Check grammar

k. Figure 4: Label Y axis

Thanks for the carefully review. We have corrected these errors according to reviewer's suggestions in line 65(a), line 169 (b), line 176(c), line 180(d), line 249(e), line 259(f), line 284(g), line 291(h), line 309 (i), line 309(j), Figure 4.

Experimental design

In the previous version 2 of the major concerns were 1) English, and 2) lack of qPCR validation of the upregulated genes. Both have been addressed.

Validity of the findings

This study is impactful since it gives a snapshot of differentially expressed genes in the

embryos cultured in the SI and TLI system.

Comments for the author

All the previous concerns have been addressed in the rebuttal.

Thank you for your suggestions. The suggestions have important guiding significance for my thesis writing and scientific research work.

Reviewer 2

Basic reporting

(1) There are still numerous grammatical issues throughout the manuscript that need addressing. For example, see:

(a) Line 125: "more information of on the process"

(b) Line 183: "which in turn lead"

(c) Line 276: "ICSI was performed with 5 h of oocytes retrieval"

(d) Lines 331-332: "without family heredity case history and smoke history."

(e) Lines 333-453: "Every RNA-Seq pools have 5 zygotes or embryos from 5 different patients, which can discount possible embryo heterogeneity from patient factor."

(f) Line 454: "were vitrification frozen"

(g) Line 458: "immediately every five embryos each tubes."

(h) Lines 459-460: "present study exhibited normal morphology, however didn't reach the transfer degree in this cycle."

(i) Line 652: "human embryos transcriptomes"

(j) Line 828: "especially the biological processes terms"

(k) Line 918: "changed when exposure"

(l) Line 1021: "possessed several different transcriptome."

(m) Line 1170: "which contain"

(n) Line 1176: "leaded to the blockage"

(o) Lines 1183-1184: "were several evidence showed"

(p) Line 1226: "needs to further research"

Thanks for the carefully review. We have corrected these errors. The details as below:

(a) Line 53: “more information of the process”

(b) Line 75: detected “which in turn lead to gene expression disorders”

(c) Line 95: “ICSI was performed within 5 h after oocyte~~s~~ retrieval”

(d) Lines 123-124: “without the history of ~~family~~ inherited diseases and smoking”

(e) Lines 126-127: “Each RNA-Seq pool has 5 zygotes or embryos from 5 different patients, which can discount possible embryo heterogeneity from patient factor” Should be “patient factors”

(f) Line 129: “were vitrification freezing storage” Still unclear. Do you mean: were in storage after vitrification?

(g) Line 132-133: “immediately each five embryos per tube” should read: “immediately, five embryos each per tube”

(h) Lines 133-134: “present study exhibited normal morphology without reaching the transfer degree in this cycle.” Clarify this.

(i) Line 173: “human embryo transcriptome”

(j) Line 214: “especially the biological process terms”

(k) Line 248: “changed when exposured” Line 250 is still wrong. Should be “when exposed”

(l) Line 265: “possessed several different transcriptomes.” One does not possess a transcriptome

(m) Line 274: “which contains”

(n) Line 283: “lead to the blockage”

Line 285 “and decreasing of SLC genes” should read “and decreased expression of SLC genes”

(o) Lines 290-291: “were several evidences showing” Still not grammatically correct

(p) Line 308: “needs further research”

Experimental design

(1) The authors do not specify if a DNase step was included or exon-spanning primers

used to remove contaminating genomic DNA (gDNA) and avoid amplification of gDNA, respectively, for quantitative RT-PCR (RT-qPCR).

Thanks for the carefully review. Amplification was performed using Single Cell-to-CT qRT-PCR Kit (Invitrogen, 4458237). According to the Single Cell-to-CT™ Kit workflow (Single Cell-to-CT™ Kit:https://www.thermofisher.com/order/catalog/product/4458237?SID=srch-srp-4458237#/4458237?SID=srch-srp-4458237), the first step is cell lysis with 10ul of Single Cell Lysis/Dnase I solution. Thus, the gDNA was removed in the first step.

(2) A minimum of 2 genes, preferably more, should be used for the normalization of RT-qPCR data in order to avoid individual gene variation between embryo groups. This is particularly important since the authors use GAPDH to normalize all the RT-qPCR data, but GAPDH has been shown to be more highly expressed in aneuploid human embryos (Vera-Rodriguez et al. Nat Comm 2015) and aneuploidy is common in cleavage-stage human embryos.

To avoid individual gene variation between embryo groups, each RT-qPCR pool has 3 embryos from different patients. The same expression trend of DEGs between RT-qPCR and RNA-Seq demonstrated the accuracy of DEGs.

The trend of DEGs does not address the concern that additional genes should have been used to normalize the data.

(3) Additional experimental details are needed in the Materials and Methods section even if described previously. For ICSI, the manipulator system used. For RNA-seq, the Illumina kit used (75, 150, 300 bp?). I assume paired-end?

Thank you for pointing this out. We have added the details to the Materials and Methods section according to reviewer's suggestions. For ICSI, CellTram® 4r from Eppendorf was used (line 96). For RNA-seq, 150-bp paired-end sequencing was performed (line 142).

Validity of the findings

(5) The word "clean" is not typically used to describe sequencing data after processing. A more appropriate term is "trimmed" or "processed."

Corrected.

(6) On Lines 1218-1220, the authors state: "In addition, it is well known that early embryos have the ability of correct the alterations in gene expression during the subsequent development." Do you have a reference for this statement b/c as far as I know this has not been established.

We changed the sentence to "it is well known that early embryos have the ability of self-correction during the subsequent development" (line 301-302). After fertilization, embryos are capable of self-correction in terms of several levels when there are errors during the development. For example, aneuploid embryos can become euploid after self-correction, especially for the tripronuclear human embryos; Oxidative stress during the in vitro culture can cause the DNA damage, as they can lead to the *alterations* of gene expression, and the self-correction named DNA damage repair pathway will be activated. In accordance with this, several DNA damage repair genes including *DOT1L*, *MMS19*, *POLL*, *SMARCB1*, *POLE*, *CEP164*, *TRRAP* and *RBM14* were found to be high regulated in SI group. Therefore, whether the high regulated genes will return the normal level remain unknown after the repair completed.

Comments for the author

In the revised version of their manuscript, Li and colleagues addressed some of my questions and concerns. However, there are still certain issues that need to be remedied.

We would like to thank you very much for your valuable comments and good suggestions that greatly helped to improve our manuscript.