BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

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Reviewer 01236209

1. We are afraid that we do not quite understand what is incorrect about this statement. We would be more than happy to make the necessary corrections if you could elaborate on our error.

2. You have highlighted an excellent point, in reactivating dormant SLCs that are within the testes. We have included additional trials in which investigators explore the mechanisms through which SLCs proliferate and differentiate into steroidogenic cells. It is my hope that from these in vitro studies, future researchers will administer these growth factors in an in vivo model.

3. We have made the corrections to the grammatical errors that you have mentioned.

Reviewer 02446119

We greatly appreciate your feedback and believe that a table providing the various formulas that lead to Leydig cell differentiation is incredibly valuable. As such, we had included in our original submission a table that summarizes all of the *in vivo* and *in vitro* studies, including the growth factors utilized. As of today, there is no formula that can be standardized for the differentiation of Leydig cells. Researchers are experimenting with many different formulas in order to determine the ideal growth media. Because of this, we have sought to provide an overview of some of the critical components of these media that researchers believe play a critical role in differentiation.

We have discussed the location of the stem Leydig cells in the section “Stem Leydig Cells”. At this point, there are two leading hypotheses as to their location: the peritubular and vascular hypotheses. To explain such a discrepancy it is believed the location of these stem cells might result from differing conditions within the testicular tissue. It may be that SLCs reside in both peritubular and interstitial locales. A third plausible hypothesis that researchers have put forth is that the adult Leydig cells differentiate not from stem cells, but rather from myoid cells, vascular smooth muscle cells, or pericytes that have transdifferentiated.

We have included additional trials in which investigators explore the mechanisms through which SLCs proliferate and differentiate into steroidogenic cells. In the future, researchers will hopefully utilize this information in order to administer the necessary growth factors in an in vivo model to produce differentiated Leydig cells.

Reviewer 02459030

1. I have added more updated citations to further support the studies that are specifically evaluating the mechanisms that lead to Leydig cell dysfunction.

Duan T, Fan K, Chen S, Yao Q, Zeng R, Hong Z, et al. Role of peroxiredoxin 2 in H2O2 induced oxidative stress of primary Leydig cells. Mol Med Rep. 2016;13(6):4807-13.

Matzkin ME, Miquet JG, Fang Y, Hill CM, Turyn D, Calandra RS, et al. Alterations in oxidative, inflammatory and apoptotic events in short-lived and long-lived mice testes. Aging (Albany NY). 2016;8(1):95-110.

Sokanovic SJ, Janjic MM, Stojkov NJ, Baburski AZ, Bjelic MM, Andric SA, et al. Age related changes of cAMP and MAPK signaling in Leydig cells of Wistar rats. Exp Gerontol. 2014;58:19-29.

2. There is, unfortunately, not as much research exploring the reactivation process of SLCs. Most work has focused on the use of stem cells from other tissues. This may in part be due to the controversy of the exact location of these stem cells and how to isolate them. However, we have included additional trials in which investigators use select growth factors to explore the mechanisms through which SLCs proliferate and differentiate into steroidogenic cells.

3. As compared to BMSCs and ADSCs, there are fewer studies utilizing umbilical cord-derived stem cells. We have included two studies by Yazawa et al. and Wei et al, respectively.