Dear Reviewer 1:

Thank you for your comments concerning our manuscript entitled “Melatonin inhibits apoptosis and oxidative stress of mouse Leydig cells via a SIRT1-dependent mechanism”. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction and supplement which we hope meet with approval. Revised portion are marked with blue (revised according to reviewers) in the paper. The main corrections in the paper and the response to you comments are as following:

The authors reported that melatonin inhibited apoptosis and oxidative stress of mouse Leydig cell through a SIRT1-dependent mechanism. The research topic is interesting. Some major concerns should be addressed before further consideration.

1) The manuscript was poorly presented. The English must be polished by professional language editor.

Response: Thank you for your suggestion. We have completed the English revision of the manuscript by professional editors at “Editage”, a division of Cactus Communications (Job code: DHGIV_7). Certificate of English editing is among the files we uploaded.
2) The proliferative effect of melatonin on Leydig cells could not be determined by only MTT assay. Other assays must be performed.

**Response:** This comment is valuable and very helpful for improving our paper. We added a proliferation experiment—EdU (5-Ethynyl-2’-deoxyuridine) assay to demonstrated that melatonin promoted proliferation of mouse Leydig cells by detecting the ratio of EdU-positive cells. We also found that EX527 reversed the proliferative effect of melatonin in the EdU assay.

3) Why only 10 ng/ml melatonin showed activity, but not higher concentrations?

**Response:** For this problem, we considered that there are two reasons. First, we did not use drug stimulation (e.g. H$_2$O$_2$) to induce apoptosis and oxidative stress in this study, so lower concentrations of melatonin showed better results. Second, melatonin is often used in an effective concentration range, and higher concentrations of melatonin are not effective, which has been reported in many studies. We added discussion of this problem in the article as followed.

Treatment of mouse granulosa cells with 0.1, 1, 10, and 100 μM melatonin revealed that the optimal concentration to inhibit palmitic acid-induced apoptosis was 10 μM rather than 100 μM (Chen Z, 2019). In pig granulosa cells, melatonin with low concentration (0.1 μM) instead of high concentration (10 μM) could stimulate the synthesis of estradiol and make differentially expressed genes which associated with regulation of cell proliferation, cell cycle, and anti-apoptosis significantly enriched (Liu Y, 2019).

4) SIRT1 is a deacetylase. Could the authors speculate the direct targets of SIRT1 in mediating the effects of melatonin?

**Response:** SIRT1 as an NAD-dependent III histone deacetylase can interact with a variety of target proteins such as FOXO, tumor suppressor p53, and nuclear transcription factor NF-κB. At present, the direct targets of SIRT1 in mediating the effects of melatonin has not been reported. We speculated that SIRT1 could interact with Nrf2 to regulate oxidative stress in this study. However, we did not study the direct targets of SIRT1 in mediating the effects of melatonin, which is a limitation of this article.
Thank you for your review.
Yours Sincerely