To the Editorial Team of *World Journal of Stem Cells*,

Re: Manuscript: "Rational use of mesenchymal stem cells in the treatment of autism spectrum disorders" We thank the editor and reviewers for their efforts on our manuscript. A point-by-point 'responses to the reviewers' comments is shown below and highlighted in the revision draft with ***bold italic font***:

Reviewer #1 (Reviewer's code: 02567328): Please the legend of Figure 1 is too long and looks like an abstract. Please modify.

Response: Thanks for the comment. Based on your suggestion, the legend of Figure 1 has been changed as: "***Genetic and environmental risk factors for autism spectrum disorders (ASD). Genetic risk factors for ASD including: important candidate genes, immune-related genes (such as, MHC), epigenetics, and family history of autoimmune disease. Prenatal infection (MIA), maternal exposure to drugs, prenatal stress, advanced parental age, zinc deficiency and abnormal melatonin synthesis are important environmental risk factors for ASD. ASD children exhibit social communication deficits and repetitive behavior. Brain dysfunction and physiological abnormalities are observed in ASD patients and animal models.***"

Reviewer #2 (Reviewer's code: 03197771):

1. Although the review is well structured and easy to read some room for improvement was detected. For example, original references and details of the NurOwn product and the BTBR original description of the BTBR strain as an ASD animal model (see cite below) would be desirable. Wahlsten D, Metten P, Crabbe JC. Survey of 21 inbred mouse strains in two laboratories reveals that BTBR T/+ tf/tf has severely reduced hippocampal commissure and absent corpus callosum. Brain Res. 2003 May 2;971(1):47-54. PubMed PMID: 12691836.

Response: Thanks for the suggestions.

Original references (yellow color highlighted) and details of the NurOwn product were added into the section "2.4 Pre-clinical and clinical evidence for MSC therapy in ASD" Paragraph 1: "*This study demonstrated NurOwn®* ***[175]*** *are superior to MSCs without* ***induced*** *neurotrophic factors in several aspects.* ***In particular, NurOwn® contains 2 and 5 fold levels of BDNF and glial cell-derived neurotrophic factor (GDNF) respectively, compared to MSCs from the same donor*** ***[176]***."

Description of BTBR mouse strain and one recent study of the effects of MSC-exo

on BTBR mouse were included into the section "2.4 Pre-clinical and clinical evidence for MSC therapy in ASD" Paragraph 1: "***A widely accepted mouse model of ASD is the BTBR T+, tf/J (Black and Tan Brachyury, BTBR) inbred mouse strain, which display autistic-like behavior and neuroanatomical abnormalities, including absence of corpous callosum and reduced hippocampal commissure, analogous to the core endophenotype of autism*** *[170-172]****."***

"***Exosomes derived from MSCs (MSC-exo) serve as the main mediators of the therapeutic effect of MSC, with an involvement in repairing damaged tissues, suppressing inflammatory responses and modulating the immune system*** *[177, 178]****. Their potential as a surrogate of therapeutic MSCs has been widely explored. Recently, it has been shown that BTBR mice treated with MSC-exo via intranasal administration present with significant behavioral improvements in social interaction and ultrasonic communication and reduced repetitive behavior. Interestingly, BTBR mothers that were treated with MSC-exo showed improvements in maternal behaviors such as pup retrieval behavior*** *[179]****."***

In addition, another cell therapy study on VPA model was added into section "2.4 Pre-clinical and clinical evidence for MSC therapy in ASD" Paragraph 1. "***In addition, it has been demonstrated that by promoting the maturation of newly formed neurons in the granular cell layer of the dentate gyrus, MSC transplantation restores post-developmental hippocampal neurogenesis in VPA-exposed mice*** *[169]****. This is associated with improvements in cognitive and social behavior 2 weeks after transplantation of the MSCs and thus may be related to the modulation of hippocampal neurogenesis*** *[169]****."***

2. Also, the clinical trial evidence for the use of MSC in the treatment of ASD should be more exhaustive and updated. For example, the following reference, among other, should have been included: Chez M, Lepage C, Parise C, Dang-Chu A, Hankins A, Carroll M. Safety and Observations from a Placebo-Controlled, Crossover Study to Assess Use of Autologous Umbilical Cord Blood Stem Cells to Improve Symptoms in Children with Autism. Stem Cells Transl Med. 2018 Apr;7(4):333-341. doi: 10.1002/sctm.17-0042.

Response: Thanks for such valuable suggestions. The most updated clinical trial from Chez et al. was added into section "2.4 Pre-clinical and clinical evidence for MSC therapy in ASD", at the end of Paragraph 2. "***Recently, the first randomized, doule-blinded, placebo-controlled clinical trail provided the further evidence that AUCB was safe, but there was minimal clinical efficacy compared to the findings of the previous open-label trial*** *[186]****. 29 ASD children 2-6 years of age were infused with either AUCB or placebo, and evaluated at baseline, 12 and 24 weeks*** *[186]****. This study suggested that infusion of AUCB was no serious adverse events for the treatment of ASD and potentially had an impact on***

socialization for children with ASD."

Even though there are **19** clinical trial studies of stem cell therapy on ASD across the globe according to clinical trials.gov, only **6** articles related with MSC clinical trials on ASD have been published. One more follow-up study from Dawson's research team and a small pilot clinical trial was involved into section "2.4 Pre-clinical and clinical evidence for MSC therapy in ASD", Paragraph 2. Thus we totally reported 6 clinical trials in the revision draft: "***Another small pilot open label study recently investigated the clinical benefits of bone marrow aspirate concentrate (BMAC) stem cell with intrathecal transplantation in 10 ASD children (4-12 years of age) [183]. The maximal effect of cell therapy was observed within the first 12 months following the treatment. Interestingly they also found that improvement decreased as the age of ASD child increased [183]. However, there was no control group and the number of subjects in this study was quite small.***"
"***Dawson's research team [185] performed a secondary follow up study and reported changes in electroencephalography (EEG) spectral power by 12-months post-treatment of AUCB on ASD children. Baseline posterior EEG beta power was positively associated with an improvement in social communication symptoms in ASD children, suggesting the EEG may be a useful biomarker to predict outcome of clinical trials for ASD.***"

3. In general, it is recommended to authors an update of the cited references, previous reviews included, to improve the quality of the review. For example, link of immune defects related to ASD is nicely covered by the following review: Estes ML, McAllister AK. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. Nat Rev Neurosci. 2015 Aug;16(8):469-86. doi:10.1038/nrn3978. And in the case of reviews of the use of MSC on the immune system, the selection of reference papers goes up to year 2011 (references 134-136), when more updated reviews are available. Latest cite for the effects of TLRs on immunomodulation by MSCs dates of 2010 (see reference 143).

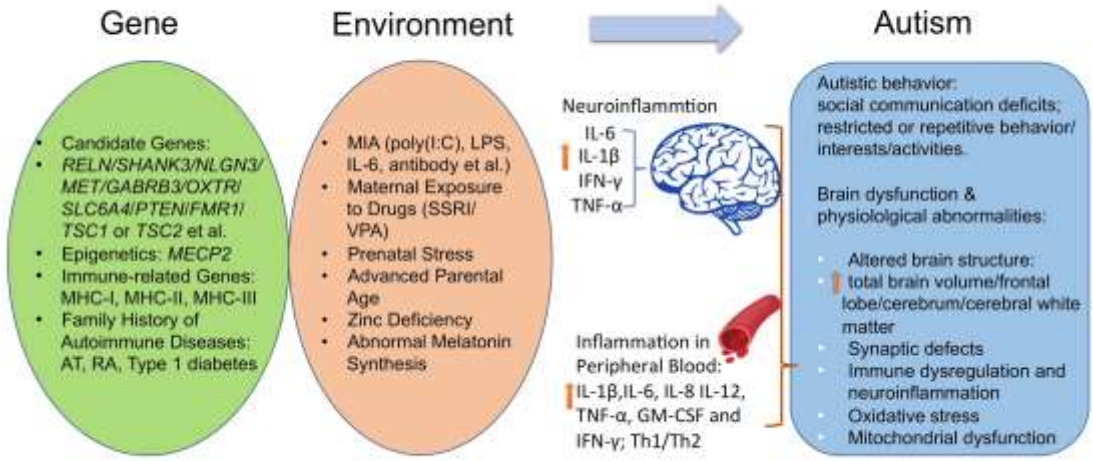
Response: Thanks. The review from Estes ML, McAllister AK was added into the '**Conclusion'** part, reference 189. The other references are updated respectively: reference 142-146; reference 155-156.

4. Please add appropriate references to the following statement: "MSCs are capable of crossing the blood brain-barrier and migrating to sites of tissue injury and inflammation" on pag. 15. Add cites to the following statement: "Several proof-of-concept clinical studies have shown the safety and efficacy of MSCs treatment in autistic patients" on page 19.

Response: Thanks. The references were added. See reference 148,149; and

references 180,181,184 and 190.

5. It is suggested that numberings in Figure 1 are substituted by bullets to avoid readers' confusion by linking numberings in the different lists presented. The overlap in Venn diagrams does not seem appropriate as no factor is included in the overlapping section.
Response: We agree with this and Figure 1 has been revised based on your suggestion.



6. Please review the text for some orthographic mistakes, such as "lable" for: label, "pretreatments" for: pretreated, "Zinc" in a context that should be lower case…etc

Response: Sorry for the typo. We checked through the whole draft carefully and changed typo.

Reviewer #3 (Reviewer's code: 03671529):

A small drawback is that the authors cite references for interpretation those works which demonstrated the efficacy of MSCs influence immune processes and inflammation. There are a number of studies that show the rapid elimination of transplanted MSCs and the absence of any effect from their administration, as well as in vitro.

Response: Thanks for the suggestions. Even though many studies have reported that MSCs could replace dysfunction cells and migrate to sites of injury to

interact with inflammatory cells, MSCs may not have a long lifespan after administration. Also, there are other issues, such as the best administration routes of MSCs or *in vivo* function of MSCs, remain uncertain. We include these concerns into section **2.1 MSCs**, the first Paragraph: "***MSCs are relatively easy to isolate and expand in culture and capable of self-renewal and differentiation, making them a promising treatment option for a variety of clinical conditions. Although the multipotency of MSCs is demonstrated in vitro [129], this is still not definite in vivo. Till now, it is also still unclear whether MSCs isolated from different tissue sources have similar therapeutic potentials [130]. Furthermore, it is uncertain whether systematic delivery (i.e. intravenous) of MSCs is sufficient to reach the brain as compared to direct implantation of MSCs [131, 132]. Though intranasal application of cells provides an alternative, non-invasive method to deliver MSCs directly into the CNS [133]. At present, neither intravenous or direct injection of MSCs have been able to yield consistent clinical results, since infused cells exhibit limited survival and transient functionality in host tissues [134-136].***"