# OPEN PEER REVIEW REPORT 1

**Reviewer:** Aldo Calliari, Veterinary School-UdelaR, Uruguay.

**Comments to authors:**

Firstly, the manuscript's title is need to be reviewed. I would suggest "Adipose-Derived Stem Cells Modified by BDNF Gene Rescues Erectile Dysfunction after Cavernous Nerve Injury", otherwise the original title state that is the cavernous nerve injury that has erectile dysfunction.

I found several concerns all through the manuscript and I will no point out one by one all them; instead I will focus the main ones:

1- The MS is plenty of confusing or misleading statements. This partially due to the misuse of the English, the ambiguous use of the nomenclature ("ADSC alone" and "ADSC-GFP" are the same?; "dorsal nerves" and "Cavernous nerves" are the same?; usage of initials without previous definition, etc.). Several text edition mistakes such as wrong cap letters usage, wrong spacing usage, etc.

2- In the Abstract and also in the discussion there are references to experiments never described in M&M or Results: "Toluidine blue staining of cavernous nerves", "masson's trichrome staining of corpus cavernosum"; also the statement "The smooth muscle of corpus cavernosum was significantly preserved in BCNI with ADSCs and ADSCs infected with lenti-rBDNF groups compared with BCNI group" is impossible to hold since there are no specific experiments addressing this point.

3- The section "Preparation of rat ADSCs" (M&M) must be briefly explained, including cell purification and culture and identity and source of markers to characterize ADSCs.

4- Erectile function assessment: What are the units used to express the ICP? How was calculated the mean ICP value? The graphs displaying ICPs (Figure 2) are poor quality and axis labels cannot be read.

5- The nNOS Staining results and Analysis are highly unsatisfactory: the quality of the image is very bad and axonal fibers are hardly visible; what is the red staining? One suppose that the green staining corresponds to BDNF; is it correct? What is the rationale behind the BDNF staining? To identify the local source of BDNF, perhaps? Connective components of nerves seems to be more strongly labeled with anti BDNF than the nervous fibers; should this be expected? All these points needs to be discussed. Anyway, after a detailed study of the images I cannot appreciate any substantial difference among the four experimental groups so, this raise the question about how was the quantification done (Figure 3b)? Another important issue that must be clarified is the identity of the nerve immunostained region: was it proximal or distal to the crush? This point is relevant since the interpretation of the results would depend on the chosen region.

6- Quantification of nNOS protein. Despite that the authors do not explicit the rationale behind the nNOS protein quantification, this reviewer assume this is a way to link putative functional improvement to nNOS supply recovery to cavernous muscle by nerve fibers. However, the results are presented in a so confusing way that precludes any rational interpretation of the data: the quantification behind the claim "decreased expression of nNOS was observed in the BCNI group than the control group ($P < 0.05$, Figure 4)" and "There was significantly increased expression of nNOS in the ADSC rBDNF and ADSC GFP groups compared with the BCNI group ($P < 0.05$)", must be described in M&M and incorporate to Figure 4. What does "A", "B","C" and "D" means in Figure 4a and 4b? What is trying to depict the bar plot of figure 4b?; Why the corresponding figure legend state that the graph is the "Densimetric analysis to β-actin of nNOS"?.

Overall, and probably most important, I consider that author's claim "we have demonstrated that ADSC

rBDNF group resulted in better functional and histological preservation in ED after cavernous nerve injury than ADSC GFP group" cannot be hold since the experimental evidence they show is still weak. ADSC GFP cells also produce an apparent functional recovery and it is unclear, whether or not the treatment with ADSC BDNF achieve nerve regeneration. This point is relevant and needs to be addressed because real functional recovery rely on nerve repair and any observed functional recovery without nerve repair would be temporal and reversible.