Please resolve all issues in the manuscript based on the peer review report and make a point-by-point response to each of the issues raised in the peer review report. Note, authors must resolve all issues in the manuscript that are raised in the peer-review report(s) and provide point-by-point responses to each of the issues raised in the peer-review report(s); these are listed below for your convenience:

Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision
Specific Comments to Authors: The authors report in the present manuscript that a human amniotic fluid stem cell transplantation can mitigate diabetic bladder dysfunction similar to insulin therapy in type 2 diabetic rats. There are multiple concerns in their approach. The authors seem to confuse terminology between stem cell transplantation with stem cell therapy.

ANS:
Thank you for the advice. We have revised the stem cell transplantation to stem cell therapy to prevent from confusion.

We have also revised the manuscript title to "Human amniotic fluid stem cell therapy can help to regain bladder function similar to insulin treatment in type 2 diabetic rats".

The authors also confuse a hyperglycemia induction with a T2D model. A T2D model follows insulin resistance development, chronic glucose, and lipid metabolism changes, multi*organ deterioration, and, finally, beta-cell exhausting. However, beyond these misconceptions, the work is very interesting, but it needs major revision.

ANS:

We are sorry for the confusion on the hyperglycemia induction with a T2D model. We have revised in the first paragraph of Discussion in page 17 to "A systemic review demonstrated that ~~normalizing blood glucose levels by~~ insulin treatment starting early after STZ injection could prevent bladder hypertrophy…".

We have also added "…could cause insulin resistance development, chronic glucose and lipid metabolism changes, multi-organ deterioration, and, finally, beta-cell exhausting..." in the first paragraph of page 21 (marked in red).

Introduction This section is good, but the authors must incorporate information about the model used. SZT-induced hyperglycemia is through an oxidative stress mechanism that affects multiple tissues included innervation, ganglia, and urinary tree. The antioxidant defense at the median- and long-time participates in recovering tissue functions, even structural changes. Although the SZT is a very used model, it is also very common that the mechanism of hyperglycemia and injury be obviated. The cells themselves can recover from the damage caused and are often attributed to the treatment.

ANS:

We thank reviewer for the advice.

We have followed the reviewer's advice to incorporate the information about the SZT-induced DM model in Introduction in the last paragraph of page 6, "STZ-induced hyperglycemia like-DM may act through an oxidative stress mechanism that affects multiple tissues including innervation, ganglia, and urinary tree, and there are reduced nerve growth factor (NGF) levels in the bladder and dorsal root ganglia of lumbar spine which are associated with

voiding dysfunction caused by defects in A-delta and C fiber bladder afferent nerves[6, 7]. The antioxidant defense at the median- and long-time can participate in recovering tissue functions, even structural changes." (marked in red).

Materials and Methods Feeding with a high-fat diet is unusual in humans. Hypercaloric diets are based on simple sugars or a high-carbohydrate diet. The time-induction with an HFD is short; therefore, the authors do not observe changes in any measured parameter compared to the control group. It is also relevant to inform the nutrient content of the control diet.

ANS:

We thank reviewer for the comments.

Indeed, a high-fat diet is unusual in humans. However, in animal study, some will use high-fat diet to create a similar situation to human.

Regarding the time-induction with an HFD, we followed the method by Srinivasan et al. which described "the feeding of HFD for 4 weeks produced a significant increase in body weight, total fat pad weight, basal/fasting plasma glucose, insulin, basal triglyceride (TG) and total cholesterol (TC) levels…". We have added this reference as reference 13 after "Group 2, high-fat diet (HFD, D12492, Research Diets, New Brunswick, NJ, USA)[13] containing 60% fat, 20% protein and 20% carbohydrate (Kcal)" in the Animal model section of MATERIALS AND METHODS in page 7 (marked in red).

Regarding the nutrient content of the control diet, we have mentioned "normal-diet control (control) with rodent chow diet (labdiet 5001, Richmond, IN, USA) containing 13.6% fat, 28.9% protein and 57.5% carbohydrate (Kcal); Group 2, high-fat diet (HFD, D12492, Research Diets, New Brunswick, NJ, USA)[13] containing 60% fat, 20% protein and 20% carbohydrate (Kcal)" in the

Animal model section of MATERIALS AND METHODS in page 7 (marked in red).

The authors say, "a single intraperitoneal dose of 35 mg/kg STZ, dissolved in 0.1 M citrate buffer with pH 4.5 to induce experimental DM, which resembles the condition of human type 2 DM". So, the authors must show insulin resistance, dyslipidemia, glucose intolerance, hyperinsulinemia, and classical deteriorate tissues metabolism that are T2D features, otherwise, they should mention it as hyperglycemia like-diabetes.

ANS:

We agree with the reviewer, and we think it is better to mention as "hyperglycemia like-diabetes". We have revised STZ-induced DM to "STZ-induced hyperglycemia like-DM" in the first paragraph of MATERIALS AND METHODS in page 7 and in every condition mentioning "STZ-induced" (marked in red).

The insulin administration was insufficient, thereby the results and assumptions are wrong. The authors forget that rats have nocturnal activity, thereby insulin must be administered between 18 - 21 hrs, even if the insulin has a prolonged effect, such as glargine.

ANS:

In the second paragraph of "Induction of hyperglycemia like-DM" in page 9, we mentioned "insulin injection at a fixed time (9:00 AM) every day".

We agree with the reviewer that insulin must be administered between 18 - 21 hrs. However, we followed the methods by Mohammad Ishraq Zafar et al. In their manuscript, the rats received detemir and glargine insulin at a fixed time (10 AM) daily for 4 weeks.

Also, we followed the methods by Mohammad Ishraq Zafar et al. [Ref 17, Zafar MI et al, J Diabetes Res 2014] that DM rats were administered with long-acting glargine insulin (LANTUS®, Sanofi-Aventis, Germany) at a dose of 3U/day subcutaneously, and the dose was later adjusted according to the glycemic level.

Please, define CGRP, MafA, PDX-1, etc., before abbreviating.

ANS:

Calcitonin gene-related peptide (CGRP), Maf family of transcription factors (MafA) and pancreatic-duodenal homeobox-1 (PDX-1) have been defined in MATERIALS AND METHODS in the second paragraph of page 8 (marked in red).

If the aim was to demonstrate that human amniotic fluid stem cell transplantation can mitigate diabetic bladder dysfunction similar to insulin therapy. Why do authors present pancreatic effects? It seems like two different works.

ANS:

We are sorry for misleading the readers.

We have revised in the second paragraph of page 7, "The present study aims to investigate the effect of hAFSCs therapy and whether the therapeutic effect could be similar to insulin treatment using a type 2 DM rat model." (marked in red).

The reason why we examined the pancreatic effects is to understand if hAFSCs therapy is similar to insulin treatment to improve the pancreatic

function. Our revised statistical results showed that insulin is better than hAFSCs therapy to improve area of reactive beta cell and average area of islets. However, both insulin and hAFSCs can improve the immunoreactivities of PDX-1 compared with DM rats.

The statistical used to evaluate the effect of hAFSCs among the groups, seems incorrect. Chi-Square test is used to compare 2 variables (non-parametric), in this case, seems to be better to use a Kruskal-Wallis test. Also, a one-way ANOVA does not offer sufficient information, it is recommendable a two-way ANOVA.

ANS:

We thank reviewer for the advice.

We have followed the reviewer's instruction and change all statistical methods in the revised manuscript. In Statistical analysis in page 14, we have mentioned "Data were analyzed with Prism 5 (GraphPad Software Inc., San Diego, CA, USA) and expressed as median with first and third quartile for continuous variables. First, two-way analysis of variance was used for analysis. Then, Kruskal-Wallis test with posthoc Bonferroni test was performed for intergroup analysis. Mann-Whitney U test was used for the comparison between 4 weeks and 12 weeks. Probability values of < 0.05 were considered statistically significant. The statistical review of the study was performed by a biomedical statistician." (marked in red).

Result and Discussion Based on the changes made in the methodology, the result and discussion section must be rewritten, focusing to answer and discussing the work hypothesis.

ANS:

We have followed reviewer's advice and revise the sections of Result and Discussion based on the changes made in the methodology (marked in red).

We have also discussed the work hypothesis based on the revised statistical results (marked in red).

Reviewer #2:
Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (High priority)
Specific Comments to Authors: I congratulate the authors for this experimental study.The present results show that, similar to insulin treatment, hAFSCs transplantation can improve STZ-induced diabetic bladder dysfunction and have a protective effect on pancreatic beta cells in type 2 DM rats.

ANS:

We thank reviewer for the kindness to accept our manuscript. Thank you!

4 LANGUAGE POLISHING REQUIREMENTS FOR REVISED MANUSCRIPTS SUBMITTED BY AUTHORS WHO ARE NON-NATIVE SPEAKERS OF ENGLISH

As the revision process results in changes to the content of the manuscript, language problems may exist in the revised manuscript. Thus, it is necessary to perform further language polishing that will ensure all grammatical, syntactical, formatting and other related errors be resolved, so

that the revised manuscript will meet the publication requirement (Grade A).

ANS:

We have asked a native English-speaking expert to revise and edit our manuscript and a new language certificate is provided along with the manuscript.