We would like to express our sincere thanks to the reviewers for the constructive and positive comments. We have revised the manuscript, and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point-by-point responses to the reviewers' comments are listed.

Replies to Reviewer 1 (03727922)

In the methods:

1 - Inform the precise date and year of the Ethical approval.

Answer: We have added the precise date and year of the Ethical approval on page 8, as "on July 20th, 2015".

2. I suggest you to perform other control group - with only the ALF, because you done only two groups the case and the sham.

Answer: We indeed performed a control group with only ALF induction. There was no significant difference in survival time between the control group and the sham group, demonstrating that the jugular vein catheterization did not affect the survival time of animals.

3. It is necessary to discribe better the surgical procedure and the intraportally stem cell administration.

Answer: We have added the detailed descriptions of surgical procedure and the intraportal transplantation on page 8, as "the animals with right lateral position were sterilized on their jugular skin. Then, we made an incision along the medial margin of the sternocleidomastoid followed by exposing and separating the external jugular vein. After ligating the distal end of the vessel with sutures, the vessel was inserted into a double channel catheter (7Fr). Finally, the wound was closed in layers, a sterile dressing was carried out." and "Specifically, after locating the portal vein, a puncture needle (18G) pierced the portal vein slowly under B-ultrasound guidance. When a free flow of blood appeared in the needle, PKH26- MenSCs were infused through into the portal vein."

Discussion:

1. Need to clarify the benefits of your study to the clinical practice and if will able to perform in clinical practice and translate to the medicine.

Answer: We have added relevant content on page 15, as "ALF is a life-threatening liver disease with extremely high mortality due to lack of effective treatment methods. However, few preclinical studies have been reported on the therapeutic effect of MenSCs in treating ALF in large animal models, although MenSCs have shown promising properties in tissue regeneration. Hence, our research provided feasibility and preliminary basis for future exploration and clinical application. Since the clinical trials of MenSCs are a drop in the bucket, there is still a long way to go whether they will be able to perform in clinical practice and translate to the medicine. We need to take into account their in-vivo survival time, long-term safety, standard collection procedure, and heterogeneity derived from diverse donors before MenSC transplantation may be a

clinical strategy for treating ALF".

2. Very important to better discribe the limitations of your study.
Answer: We have added the limitations of our study on page 15, as "The current study has some limitations. First, we produced the ALF porcine model by D-gal. The model focused on drug-induced liver failure and cannot reflect the diversity of causes for liver failure in human patients. Furthermore, the survival time was short in our study, so we did not implement further experiments to confirm the in-vivo differentiation of MenSCs. The therapeutic dose, the quality of MenSCs, the delivery route and the timing of grafting may lead to the result. ".

Replies to Reviewer 2 (00503536)
Major points:
1. As shown in Fig 5, survival time of MenSCs in vivo is short (2 days). What do the authors think the mechanism for the short survival? If the in vivo survival could be longer, the therapeutic effect of MenSCs would be better.
Answer: Our group also feels sorry for this result that the transplanted MenSCS survived short time in vivo. So far, few studies have been reported on the therapeutic effect of MenSCs in treating ALF in large animal model. Although several researches indicate that MenSCs are rapidly evolving, it is not yet determined how long MenSCs can survive in foreign bodies. What's more, the high-quality and high consistency of MenSCs are still scarce because there are no golden standardization and ideal molecular markers to verify them. We think the therapeutic dose, the quality of MenSCs, the delivery route and the timing of grafting may lead to the result.

2. The mechanism of the effect of injected MenSCs is unclear. Do the injected MenSCs produce human albumin, which can be measured in the blood?
Answer: Some studies have demonstrated that MenSCs can express mature hepatocyte markers, such as albumin and CK18 in vivo at gene level from liver tissues at 1 week after cell-based therapy in small animal models. Seldom researches reported that albumin can be detected from the blood, maybe due to low concentration in blood.

3. Are there any interactions between injected MenSCs and pig's hepatocytes?
Answer: MenSCs are adult stem cells, which have a high level of immune privilege, so no obvious immune rejection response were reported. As a most commonly used type of MSC for cell therapy, human bone marrow mesenchymal stem cell (BMMSCs) have shown promising effects in several pre-clinical models. Based on these reported results, MenSCs may possess similar characteristics. Since this is not the purpose of our study and there is little relevant research on it, we guess that the injected MenSCs may interaction with pig's hepatocytes via paracrine mediators. They may regenerate injured liver by cell fusion. Furthermore, they may promote liver stem cells to differentiate into hepatocytes or expand the residual hepatocyte population to obtain sufficient numbers and quality.

4. Do the injected MenSCs produce proteins that regulate immunity of pigs or protect pig's hepatocytes?

Answer: MenSCs may play an immunomodulatory role through producing inhibitory cytokines to inhibit dendritic cells, B lymphocyte cells, T lymphocyte cells, mixed lymphocyte reaction, and induce the development of regulatory T cells. However, the research is still in its infancy. So, to optimize MenSC transplantation therapy, the mechanism of immunological regulation need to be further explored.

5. Dose response effects of injected MenSCs should be shown in order to prove the therapeutic effects.

Answer: Indeed, it is necessary to evaluate dose response effects, since the dose is an important factor of influencing therapeutic efficacy. However, it is not easy to culture a large quantity of stem cells in large animal models. The therapeutic doses have been reported between 10^6/kg and 10^7/kg. In our study, the dose of 2.5×10^6/kg was used, and whether higher dosage of MenSCs could achieve better efficacy need to be further studied. High dose of transplanted stem cells have been reported to cause organ embolism.

Minor points:

1. How many cells can be harvested from menstrual blood cells? The authors should add the data.

Answer: We have added the data on page 6, as "About one million primary cells were isolated from 5ml menstrual blood".