

Original Article

Transplantation induced pluripotent stem cells *in situ* improves fertility outcome impaired by intrauterine adhesions in mice

Jie Zhang^{1,2*}, Nin Li^{3*}, Sifeng Chen⁴, Mo Chen¹, Bin Li¹, Xiaobo Li⁴, Xiaoyan Zhang¹, Congjian Xu¹, Lei Yuan¹, Liangqing Yao¹

¹Department of Gynecology, Obstetrics and Gynecology Hospital, Fudan University, Shanghai 200011, P. R. China;

²Department of OB/GYN, Minhang District Central Hospital, Shanghai, P. R. China; ³Department of Gynecology, Nanning Maternal and Child Health Hospital, Nanning, Guangxi Province, P. R. China; ⁴Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Fudan University, Shanghai, P. R. China. *Co-first authors.

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Abstract: Intrauterine adhesion (IUA) is a common cause for secondary infertility. However, traditional treatment nowadays can only restore the shape of the uterine cavity but not the inadequate endometrium. In this study, we investigated the therapeutic potentials of induced pluripotent stem cells (iPSCs) to treat IUA in a mouse model and further explore the role of heme-oxygenase-1 (HO-1) gene in exerting such effects. Dual injury of lipopolysaccharides and mechanical injury was first applied to establish an IUA model and different cells were then infused *in situ* into the mice uterus after modeling, including PBS, MEF, iPS, iPS-ho1siRNA and iPS-CT siRNA. Subsequent fertility outcomes were observed 28 d after treatment, so were the degree of tissue edema and inflammation 24 h and 5 d after treatment. Results showed that transplantation iPSCs *in situ* could significantly increase pregnancy rate and fetus number when compared with controls (PBS and MEF). Pathological studies also revealed that iPSCs could significantly ameliorated tissue edema and inflammation with the proof of having higher number of nucleus but less neutrophil than control groups 24 h after treatment. Furthermore, iPSCs were detected in the impaired endometrium by immunofluorescence staining 5 d after treatment. This, coupled with the increased level of ER expression and number of endometrial gland, strongly indicated the functional improvement of impaired endometrium. However, when knocking down HO-1 expression, the effect of iPSCs was significantly weakened. In conclusion, our results indicated that iPSCs might be a pragmatic solution for the treatment of IUA and HO-1 gene play an important role in regulating the therapeutic effect of iPSCs.

Keywords: Induced pluripotent stem cells (iPSCs), intrauterine adhesion (IUA), lipopolysaccharides (LPS), heme-oxygenase-1 (HO-1), transplantation

Introduction

Over the last decade, both delay of childbearing and fertility problems have become increasingly common among women in the world. Infertility, which has been recognized as a public health issue worldwide by the World Health Organization (WHO), has a global prevalence of 9% [1]. Primary infertility is defined as the inability to bear any children while secondary infertility is the inability to become pregnant after previously conceiving, whether or not the first pregnancy came to full term. Despite being common, infertility is often experienced as a lonely road for affected couples.

Even though technological advances, including artificial assisted technology, have provided many more options available to individuals experiencing fertility problems, there still remain some diseases and conditions untreatable with the drugs. And among those unsolved clinical issues, intrauterine adhesion (IUA) or Asherman's syndrome is regarded as a major limiting factor [2]. IUA is a syndrome characterized by adhesion and/or fibrosis within the uterine cavity due to trauma of the basalis layer of endometrium [3]. With the popularization of hysteroscopy and surgery, the incidence of IUA has increased and become the second most common cause of female secondary infertility [4, 5].

However, clinical treatment nowadays for IUA can only restore the shape of the uterine cavity, but it remains difficult to repair uterine physiological function, especially the biological function of inadequate endometrium. Therefore, the rates of cure and pregnancy are relatively low, particularly in moderate and severe IUA.

The identification of stem cells has shed light on those unsolvable issues in reproductive medicine with the hope of differentiating stem cells into different cell types to treat incurable diseases. Thereafter, a new discipline, termed regenerative medicine has been established. Since 2006, researchers have identified somatic stem cell *in situ* of uterine endometrium [6]. And later, Nagori CB et al [7] successfully treated a patient with severe Asherman's syndrome using autologous stem cells isolated from her own bone marrow, suggesting their possible role in regenerating and reconstructing the endometrial tissue in cases of dysfunctional or atrophic endometrium.

Progress has been made with the new development of induced pluripotent stem cells (iPSCs) by Yamanaka and Takahashi [8]. Derived from somatic cells reprogramming via overexpression of exogenous transcription factors, iPSCs can theoretically be differentiated to any mature cell type derived from the three primary germ layers. This, coupled with their autologous, non-controversial origins, means that iPSCs could be used to study a wide range of conditions. A Pilot study using iPSCs to treat age-related macular degeneration has commenced and is now in full swing in Japan [9].

However, to the best of our knowledge, there has been no documentation of iPSCs in treating IUA up till now, partially due to the lack of appropriate animal models or limited knowledge of iPSCs. Considering the great potentiality and advantages iPSCs brings, it is now a particularly opportune moment for us to explore its role in treating IUA and subsequent infertility, especially on long-term outcome of pregnancy rate.

In the present study, a female mouse model of IUA was first successfully established with dual injury of lipopolysaccharide (LPS) injection and mechanical injury of endometrium. Then, the mice-derived iPSCs were transplanted *in situ* to

evaluate its therapeutic effect in the animal model. And the role of heme-oxygenase-1 (HO-1), an important gene regulating oxidative stress and inflammation [10], which might be responsible for such therapeutic effect of iPSCs was further explored. The purpose of this study is to investigate whether transplantation iPSCs *in situ* could improve adhesion of IUA and increases subsequent pregnancy rate, which might provide new strategies to the treatment of IUA -a frequently occurring, but currently unsolved, clinical problem.

Materials and methods

Animals

8-week-old female C57 (RT1u haplotype) mice were purchased from Slaccas, Inc. (Shanghai, China). The animals were allowed to acclimate for 1 week and were maintained at a room temperature of $22 \pm 2^{\circ}\text{C}$ on a 12 h light/dark cycle with free access to food and water. For estrous cycle studies, vaginal smears were obtained daily between 8:00 and 10:00 AM. Only mice with four consecutive 4-day estrus cycles were selected. All housing facilities and experimental protocols were in accordance with the Ethical Committee for the Use of Laboratory Animals of Fudan University and complied with the "Guide for the Care and Use of Laboratory Animals" from NIH. This study was approved by the institutional ethics review board of Obstetrics and Gynecology Hospital, Fudan University.

iPSCs culture

Murine iPSCs were generated from mouse embryo fibroblasts (MEF) derived from 13.5-day-old embryos of C57BL/6 mice. The iPSCs were reprogrammed by the transduction of retroviral vectors encoding 4 transcription factors, Oct-4, Sox2, c-Myc, and Klf4, as described previously, with brief modifications [8].

Suppression of HO-1 with siRNA transfection

To down-regulate HO-1 (*ho-1*) mRNA expression in iPSCs, a siRNA specific for *ho-1* (*ho-1* siRNA) and a non-coding control siRNA (CTsiRNA; Shanghai GenePharma Co., Ltd., Shanghai, China) were transfected using LipofectamineTM 2000 transfection reagent (Invitrogen, Carlsbad, CA) as previous report [11]. Briefly, two mil-

IPSCs improve fertility in mice with IUA

lion cells were transfected with 100 nM siRNA (final concentration) according to the manufacturer's protocol. Twenty-four hours later, cells were harvested, purified, stained and used for injection into mice and gene expression monitored by real-time PCR. The result showed that the HO1 expression was successfully inhibited by siRNA transfection (data not shown).

Mouse model for IUA

After 1-week-acclimation, mice were anesthetized with pentobarbital (60 mg/kg, intraperitoneally) and operated on through a longitudinal incision. After laparotomy, the uterine horns were carefully exposed and wrapped by a piece of sodden gauze to prevent desiccation. Lipopolysaccharides (LPS, Sigma-Aldrich) was intrauterine infused at a dose of 1.0 mg/kg body weight using a 27G needle from the tip of left uterine horn and the left uterus was carefully scratched back and forth with the needle for 1 min, while leaving the right one intact as self control. The whole surgical procedure was sterile and visualized under stereo microscope (Leica, M320, Germany).

A preliminary study was carried out to verify the validity of the model. Fifteen mice were randomly divided into three groups. Mice in group A (n=5) and group B (n=5) were sacrificed at 24 h and 5 d respectively after surgery, in order to investigate the degree of acute and chronic inflammation on endometrium. Mice in group C (n=5) were mated with male mice twenty hours later from the surgery and sacrificed 28 d post-operatively. Pregnancy rate in each uterine horn was observed and compared.

Transplantation iPSCs in mouse model of IUA

The IUA model was set up with dual injury according to procedures mentioned before. Thirty minutes after LPS injection and mechanical injuring, one hundred mice were randomly assigned to 5 groups and injected *in situ* into the left uterine horns with a 27G needle with the following suspensions, including: 1) PBS group: 0.02 ml PBS, 2) MEF group: 6.0×10^4 MEFs in 0.02 ml PBS, 3) iPS group: 6.0×10^4 iPS cells in 0.02 ml PBS, 4) iPS-*ho1*siRNA group: 6.0×10^4 iPS cells transfected with *ho1*siRNA in 0.02 ml PBS, 5) iPS-CTsiRNA group: 6.0×10^4 iPS cells transfected with CT siRNA in 0.02 ml PBS. Cells in each group were stained with (red

fluorochrome, Sigma-Aldrich) for further tracing before *in vivo* experiments. The abdomen of mice was then closed as usual.

Five mice from each group were sacrificed at various times following treatment (24 h, 5 d), in order to evaluate the change of acute and chronic inflammation on endometrium. The uteri were excised and HE/immunostaining were carried out for histology study.

The rest mice (n=10 in each group) were mated with male mice to investigate the subsequent pregnancy rate after iPSCs treatment. Male mice were exchanged among cages every 2 days in order to exclude the effect of male infertility. Mice were euthanized 28 d after treatment. Pregnancy rate was observed and the number of fetus in each uterine horn was counted and compared among groups.

Hematoxylin- and eosin staining

The uterine tissues were fixed in 4% formalin for 24 h, embedded in paraffin and serial 4 mm sections were obtained from each block. Hematoxylin- and eosin staining was routinely performed for those mice 24 h after treatment. On each hematoxylin- and eosin-stained slice, four high-power fields were selected, the number of nucleus and neutrophils per high-power field was counted, and the means were calculated.

Immunohistochemistry

Uterine tissues from 5 d after treatment were stained for estrogen receptor (ER) using two steps method as described previously [12]. The rabbit polyclonal antibody against ER (Abcam, Cambridge, UK; Cat. No: ab130088), diluted to 1:100 was used as a primary antibody. For antigen retrieval, the slides were heated at 98°C in an EDTA buffer (pH 9.0) for a total of 30 minutes and cooled naturally to the room temperature. Endogenous peroxidase was blocked using 3% H₂O₂ in methanol for 10 min prior to washing with phosphate-buffered saline (PBS). Sections were then incubated with the primary antibody overnight at 4°C. The slides were rinsed and incubated with horseradish peroxidase-labeled secondary anti-rabbit antibody detection reagent (Shanghai BioTech Company, Ltd., Shanghai, China) at room temperature for 30 min. The bound antibody complexes were

stained for 3-5 minutes or until appropriate for microscopic examination with diaminobenzidine and then counterstained with hematoxylin and mounted.

The immunoreactivity staining was characterized quantitatively using a semi-quantitative scoring system, as described previously [12]. The number and intensity of positive cells was quantified using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville MD, USA) in a blinded-manner. A series of five random images on several sections were captured to obtain a mean value.

Immunofluorescence

The freshly harvested uterine tissues from 5 d after treatment were embedded with O.C.T. and were sectioned using a cryostat to 6 μ m. The cryosections were then stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Molecular Probes, Inc, Eugene, OR) for 5 min, washed with PBS, and analyzed using a fluorescence Microscope 710 (Carl Zeiss) to tracing the location of infused PKH26 labeling cells.

Statistical analysis

All data were presented as means \pm SD. The present study compared continuous variables using a t-test or analysis of variance (ANOVA) and used non-parametric tests (Mann-Whitney), when appropriate, to compare differences. $P < 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Endometrial morphology after LPS and mechanical injury in the mouse IUA model

The number of nucleus and neutrophils per high-power field were counted and compared between the left and right uterine horn 24 h after injury. The group of injured left horns had fewer nucleus, but more neutrophils than the right control ones ($P < 0.05$, **Figure 1A**). The diluted intercellular space and increased number of neutrophils indicated endometrial tissue edema and inflammatory response resulted from LPS and mechanical injury.

Estrogen-mediated proliferation in endometrium is important in ovum implantation and successful pregnancy. The immunoreactivity of ER and number of endometrial glands were calculated 5 d after injury. Histological findings showed that the expression of ER was significantly reduced in the group of injured left horns, as well as the number of endometrial glands ($P < 0.05$, **Figure 1B**), which demonstrated the occurrence of tissue edema and loss of functional layers possibly resulted from chronic inflammation.

After 28 days mating with male mice, all female mice were sacrificed. Gross observation showed that the number of fetus was significantly reduced in the injured left horn when compared with the right one ($P < 0.05$, **Figure 1C**). Moreover, in the injured horn, the intact structure was completely destroyed and intra-uterine adhesion seemed to occur (**Figure 1C**). This, coupled with the findings mentioned before illustrated the successful establishment of IUA model in our study.

Infusing iPSCs in situ improves fertility outcome in the mouse IUA model

To examine the potential role of iPSCs in treating IUA, different treatment strategies were applied into 5 groups and the following pregnancy rate after 28 d mating with male mice was observed. As shown in **Figure 2**, iPSCs group had the highest pregnancy rate when compared with the control group of PBS and MEF ($P < 0.05$). Further gross observation of each pregnant horns revealed that the iPSCs treated horns conceived as much fetuses as that in the intact right ones whereas fetuses were significantly reduced in MEF or PBS treated horns (**Figure 2A, 2B**, $P < 0.05$).

However, when knocking down HO-1 expression, the iPSCs could no longer retrieve the biological function of injured endometrium, resulting in poor fertility outcome of less implanted fetuses than that in the control group (iPSC-TsRNA group).

Detection and localization of infused iPSCs in mice endometrium

To confirm whether iPSCs can migrate and localized into the impaired endometrium, cells were labeled with PKH26 before infusing into

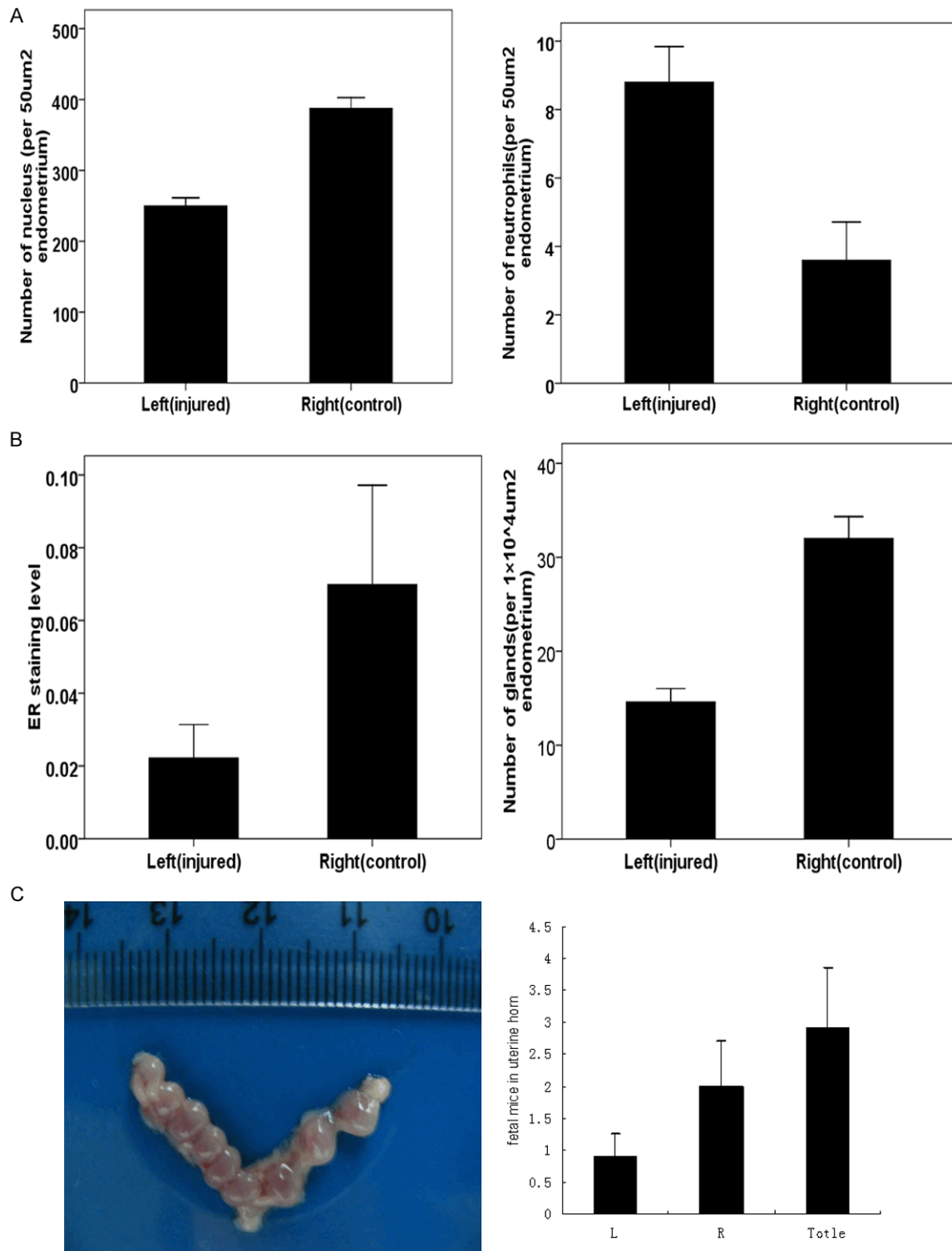


Figure 1. Comparison of histological changes between the injured left horns and the right control ones in mouse IUA model. A. Comparison of numbers of nucleus and neutrophils 24 h after surgery (n=5, P<0.05); B. Comparison of ER expression and number of endometrial glands 5 d after surgery (n=5, P<0.05); C. Comparison of fetus numbers 28 d after surgery (n=5, P<0.05).

the uterine cavity. Red fluorescent labeling cells were observed in the endometrium of

iPSCs, iPS-ho1siRNA and iPS-CTsiRNA group, but not control 5 d after treatment (**Figure 3A**).

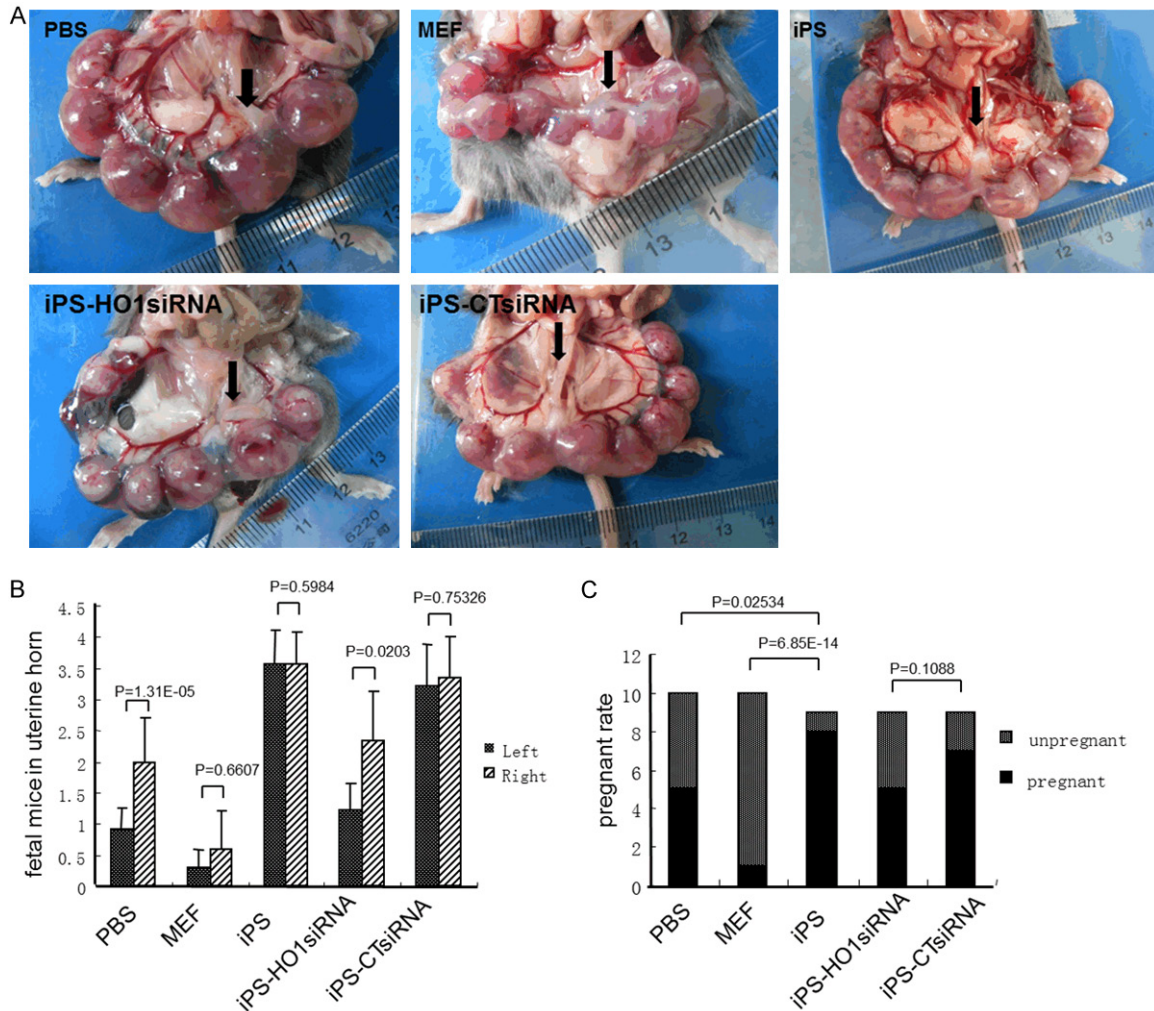


Figure 2. Comparison of pregnancy rate and fetus number in different groups 28 d after treatment. A. The implantation sites of fetus in each uterine horn were exposed 28 d after treatment. Black arrow showed the location of cervix. B. Comparison of fetus number in both injured left horn and intact right control in each group. C. Comparison of pregnancy rate in each group. Data are presented as Mean \pm S.D. PBS: C57 mice injected with PBS. MEF: C57 mice injected with mouse embryonic fibroblasts, nourishing cells for iPSCs. iPS: C57 mice injected with iPSCs. iPS-HO1 siRNA: C57 mice injected with iPSCs successfully transfected with *ho1* siRNA. iPS-CTsiRNA: C57 mice injected with iPSCs transfected with noncoding control siRNA.

This result revealed that iPSCs could still retain in the uterus and even more localized in the impaired endometrium 5 d after being infused into the uterine cavity. Therefore, it was likely that the localized iPSCs might exert the therapeutic role in regenerating and reconstructing the inadequate endometrium and improving the subsequent fertility outcome.

Further quantitative analysis showed that the fluorescent intensity was significantly reduced in iPS-ho1siRNA group compared with iPS-CTsiRNA group ($P<0.05$, **Figure 3B**), suggesting the fundamental role of HO1 gene in regulating receptivity of iPSCs.

Infusing iPSCs in situ alleviates tissue edema and inflammation of the impaired endometrium

To explore the effect of iPSCs transplantation on the impaired endometrium, we calculated the number of nucleus and neutrophils per 50 μm^2 of the endometrium 24 h after treatment, as well as the expression of ER and the number of glands 5 d after treatment, in order to evaluate the degree of tissue edema and inflammatory response.

Tissue edema and inflammation was significantly ameliorated in iPSCs group with the

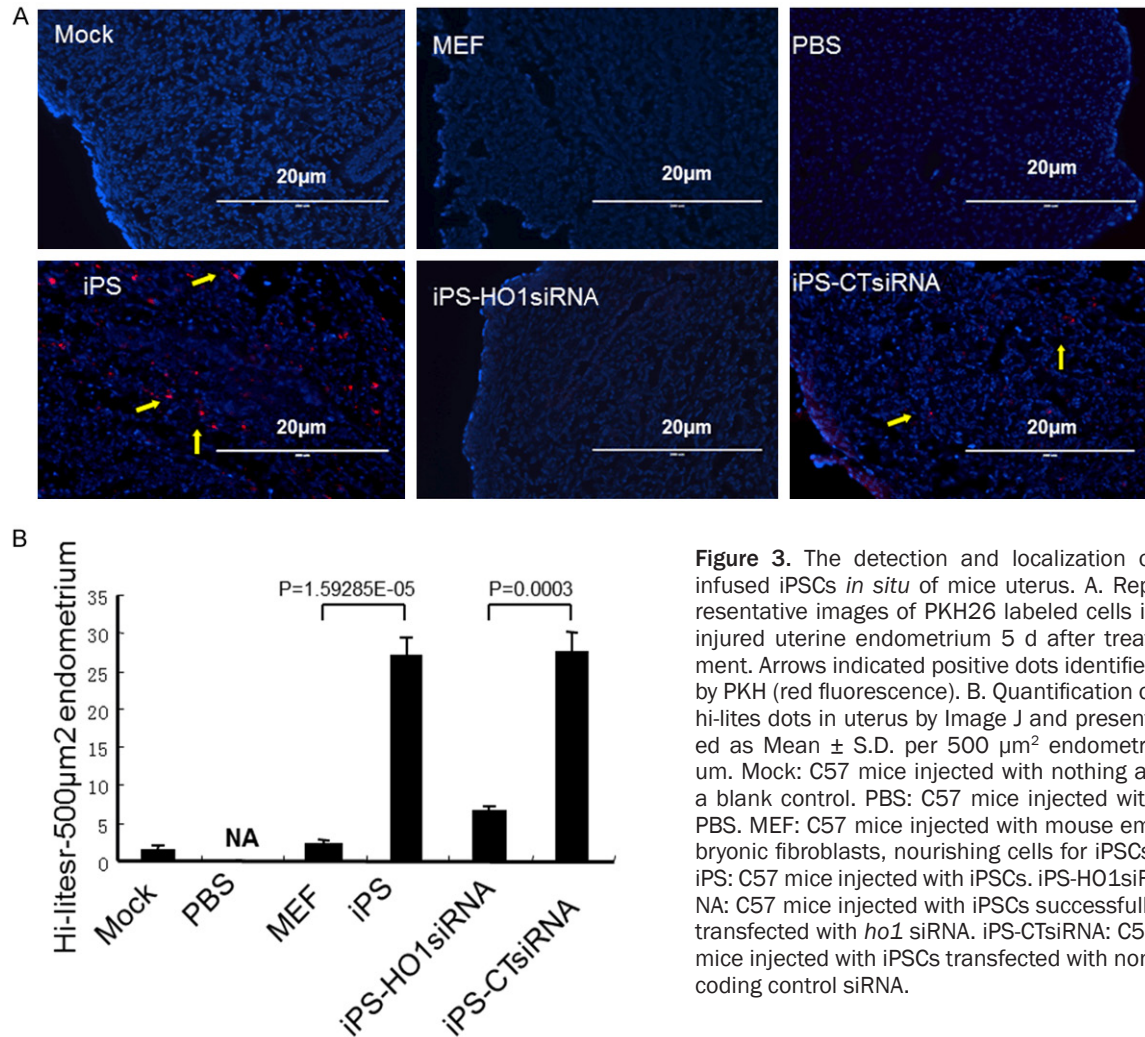


Figure 3. The detection and localization of infused iPSCs *in situ* of mice uterus. **A.** Representative images of PKH26 labeled cells in injured uterine endometrium 5 d after treatment. Arrows indicated positive dots identified by PKH (red fluorescence). **B.** Quantification of hi-lites dots in uterus by Image J and presented as Mean \pm S.D. per 500 μm^2 endometrium. Mock: C57 mice injected with nothing as a blank control. PBS: C57 mice injected with PBS. MEF: C57 mice injected with mouse embryonic fibroblasts, nourishing cells for iPSCs. iPS: C57 mice injected with iPSCs. iPS-HO1siRNA: C57 mice injected with iPSCs successfully transfected with *ho1* siRNA. iPS-CTsiRNA: C57 mice injected with iPSCs transfected with non-coding control siRNA.

proof of having higher number of nucleus but less neutrophils than control groups (PBS and MEF) 24 h after treatment ($P < 0.05$, **Figure 4A, 4B**). Moreover, the expression of ER and the number of glands of the endometrium were significantly increased 5 d after treatment in iPSCs group ($P < 0.05$, **Figure 4C, 4D**), indicating the functional improvement of the impaired endometrium.

However, when knocking down HO-1 expression, the effect of iPSCs on improving the injured endometrium was significantly weakened. The endometrium in iPS-*ho1*siRNA group exhibited less nucleus but more neutrophils 24 h after treatment ($P < 0.05$, **Figure 4A, 4B**), as well as lower level of ER expression and the number of glands 5 d after treatment when compared with the control group ($P < 0.05$, **Figure 4C, 4D**). This, along with the previous

findings in effective treatment by iPSCs, strongly demonstrated the important role of HO1 gene in exerting therapeutic effects of iPSCs.

Discussion

It has been more than a century since Heinrich Fritsch [13] first described a case of posttraumatic intrauterine adhesion. Comprising a condition involving partial replacement of endometrial surfaces with fibrotic tissue, IUA could result in irregular bleeding ranging from hypomenorrhea to amenorrhea, infertility, and pregnancy loss [14]. The prevalence of IUA was increased rapidly in the last decade and came up to highly as 22% in infertile women, possibly due to the popularization of medical abortion and increasing incidence of genital tuberculosis and puerperal infection in different countries [3]. Accordingly, how to deal with IUA and

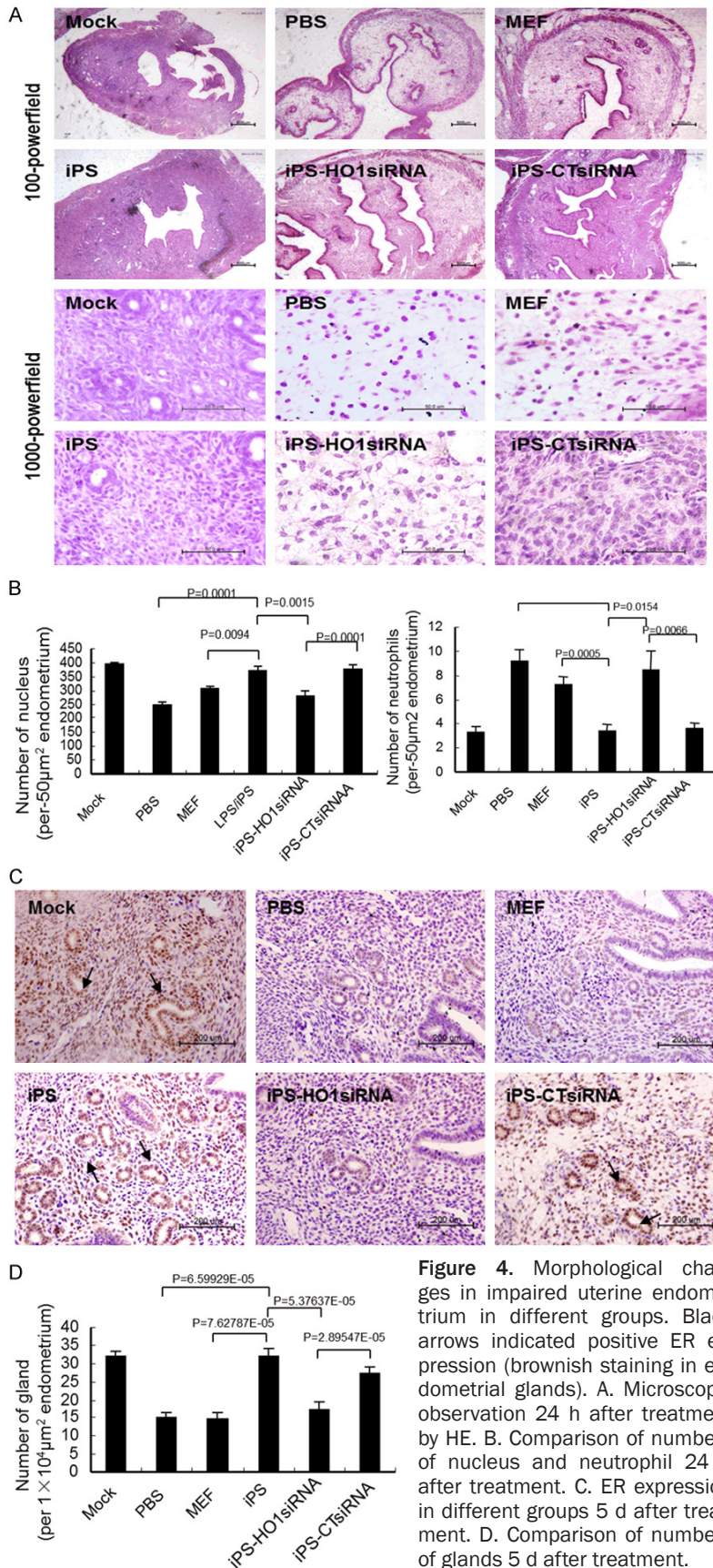


Figure 4. Morphological changes in impaired uterine endometrium in different groups. Black arrows indicated positive ER expression (brownish staining in endometrial glands). A. Microscopic observation 24 h after treatment by HE. B. Comparison of numbers of nucleus and neutrophil 24 h after treatment. C. ER expression in different groups 5 d after treatment. D. Comparison of numbers of glands 5 d after treatment.

improve fertility outcome is gaining great momentum nowadays.

One of the difficulties in doing research on IUA may lie in the shortage of an appropriate animal model of IUA. Trauma, infection, endometrial repair disorder and low estrogen levels are often regarded as four contributions relating to IUA and among them, trauma and infection are the most common causes [15-18].

In this study, we successfully set up a mouse model of IUA with dual injury of LPS injection and mechanical injury, which mimicking the etiological factors of trauma and infection to a large extent. As the major structural component of gram-negative bacteria's cell wall, LPS has many deleterious effects and plays a significant role in a number of disease processes by increasing inflammatory cytokine release [19, 20]. In the present study, we used LPS as an inflammatory-inducing factor and direct curettage as mechanical injury. Results showed that severe acute and chronic inflammatory response occurred after dual injury with the phenomena of tissue edema and functional loss of ER expression of the injured endometrium. The reduced pregnancy rate, as well as the destroyed intact structure of the injured uterine horn 28 d after dual injury further demonstrated the formation of intrauterine adhesion. Although researchers

once reported the establishment of a female rabbit model of IUA with dual infection [5], yet it does not take much insight to note that our mouse IUA model with self-control method (left injured horn VS right intact horn) was more convenient to model and could exclude individual heterogeneity during the whole experimental process.

As we known, the ultimate goal for treating IUA is to repair the biological function of the impaired endometrium. However, conventional treatments using hysteroscopy or hormonal therapy could not reconstruct the scarred uterus so effectively because of the shortage of naïve tissues [21], which might remain as another difficulty in IUA treatment. These clinical observations prompted us to investigate therapeutic strategies for this issue. Stem cell therapies are a promising alternative because stem cells can be differentiated to be functionally equivalent to the endogenous cell types they are intended to support.

In the present study, mice-derived iPSCs were injected in situ into the uterine cavity of the IUA model. Results showed that the pregnancy rate, as well as the number of fetus, was significantly increased after iPSCs treatment. Pathological studies further demonstrated the alleviation of tissue edema and inflammation 24 h after treatment. The increased expression of ER 5 d after treatment may be a sign of functional recovery of the impaired endometrium as ER is the main mediator to introduce the proliferation effect of estrogen on endometrium [4, 22], which could explain the agitational consequence in the subsequent improved fertility outcome.

As one kind of stem cells, iPSCs have several potential advantages over other cell types in that they do not require the use of embryonic cells and have minimal immunogenicity due to their autologous origins. While the application of iPSCs is widely popularized in DM, such as type 1 diabetes mellitus, Parkinson's disease, spinal cord injury, chronic liver disorders, and many other degenerative diseases [23], its usage in treating IUA is still fragmentary. Our study was a preliminary attempt to apply iPSCs in this field and the result strongly indicated the therapeutic role of iPSCs in treating IUA as we expected. Although we detected cells labeling red fluorescence in the impaired endometrium

after iPSCs treatment, it was still difficult to determine whether the LPS and curettage-induced damage provided a portal of entry for iPSCs into the residual endometrium and differentiated into hormonal responsive cells or whether iPSCs only acted as an initiator of endogenous stem cell-based tissue repair, stimulating previously refractory resident stem/progenitor cells to generate a thicker endometrium [24, 25].

In the present study, we not only showed the effectiveness of iPSCs on subsequent fertility outcome, but also explored the possible mechanisms underlying such effects. Catalyzing the rate-limiting step in oxidative degradation of heme to biliverdin, HO-1 is an antioxidant enzyme which could protect tissue from inflammation injury [26]. Numerous studies have revealed that HO-1 played a protective role in metabolic disease [27], diabetes [28], ischemia-reperfusion lung injury [29] and other inflammatory disease [30]. In accordance with the previous studies, our data also indicated the essential role of HO-1 in the therapeutic effect of iPSCs. Downregulation of HO-1 expression could largely inhibit the implantation of iPSCs into the impaired endometrium and attenuate the therapeutic effect of iPSCs.

To the best of our knowledge, this is the first attempt to apply iPSCs in treating IUA in vivo. The encouraging results of improved pregnancy rates and functional recovery of scarred uterus may provide us a new insight into the treatment of IUA and expand future clinical applicability. However, how to effectively direct differentiating iPSCs to hormonal responsive endometrial cells and the underlying mechanism of HO-1 in regulating the therapeutic effect of iPSCs still await in depth investigation in the future.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Lei Yuan and Liangqing Yao, Department of Gynecology, Obstetrics and Gynecology Hospital, Fudan University, 419 Fangxie Road, Shanghai 200011, P. R. China. Tel: +86-138-1670-7003; E-mail: ylronda@163.com (LY); Tel: +86-139-1664-4444; E-mail: yaoliangqing@163.com (LQY)

References

- [1] Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod* 2007; 22: 1506-1112.
- [2] Simón C. Somatic stem cells and tissue engineering shed light on unsolved clinical issues in reproductive medicine: in stem cells we trust. *Fertil Steril* 2012; 98: 1-2.
- [3] Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome—one century later. *Fertil Steril* 2008; 89: 759-779.
- [4] Johary J, Xue M, Zhu X, Xu D, Velu PP. The Efficacy of estrogen therapy in patients with intrauterine adhesions: systematic review. *J Minim Invasive Gynecol* 2014; 21: 44-54.
- [5] Liu F, Zhu ZJ, Li P, He YL. Creation of a female rabbit model for intrauterine adhesions using mechanical and infectious injury. *J Surg Res* 2013; 183: 296-303.
- [6] Chan RW, Gargett CE. Identification of label-retaining cells in mouse endometrium. *Stem Cells* 2006; 24: 1529-1538.
- [7] Nagori CB, Panchal SY, Patel H. Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. *J Hum Reprod Sci* 2011; 4: 43-48.
- [8] Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* 2007; 1: 39-49.
- [9] D.Castelvecchi. Nature newsblog [Internet]: nature [cited 2014]. Available from: <http://blogs.nature.com/news/2013/07/japan-to-start-stem-cell-study-on-humans.html>.
- [10] Wang X, Quinn PJ. Lipopolysaccharide: Biosynthetic pathway and structure modification. *Prog Lipid Res* 2010; 49: 97-107.
- [11] Ning Y, Riggins RB, Mulla JE, Chung H, Zwart A, Clarke R. IFN γ restores breast cancer sensitivity to fulvestrant by regulating STAT1, IFN regulatory factor 1, NF- κ B, BCL2 family members, and signaling to caspase-dependent apoptosis. *Mol Cancer Ther* 2010; 9: 1274-1285.
- [12] Yuan L, Liu X. Platelets are associated with xenograft tumor growth and the clinical malignancy of ovarian cancer through an angiogenesis-dependent mechanism. *Mol Med Rep* 2015; 11: 2449-2458.
- [13] Fritsch H. Ein Fall von volligen Schwund der Gebärmutterhöhle nach Auskratzung. *Zentralbl Gynaekol* 1894; 18: 1337-1342.
- [14] Panayiotides I, Weyers S, Bosteels J, Van Herendaal B. Intrauterine adhesions (IUA): has there been progress in understanding and treatment over the last 20 years? *Gynecol Surg* 2009; 6: 197-211.
- [15] Roge P, Cravello L, D'Ercole C, Brousse M, Boubli L, Blanc B. Intrauterine adhesions and fertility: results of hysteroscopic treatment. *Gynaecol Endosc* 1997; 6: 225-228.
- [16] Taylor PJ, Cumming DC, Hill PJ. Significance of intrauterine adhesions detected hysteroscopically in eumenorrheic infertile women and role of antecedent curettage in their formation. *Am J Obstet Gynecol* 1981; 139: 239.
- [17] Gupta N, Sharma JB, Mittal S, Singh N, Misra R, Kukreja M. Genital tuberculosis in Indian infertility patients. *Int J Gynaecol Obstet* 2007; 97: 135-138.
- [18] Al-Inany H. Intrauterine adhesions. An update. *Acta Obstet Gynecol Scand* 2001; 80: 986-993.
- [19] Arima K, Nasu K, Narahara H, Fujisawa K, Matsui N, Miyakawa I. Effects of lipopolysaccharide and cytokines on production of RANTES by cultured human endometrial stromal cells. *Mol Hum Reprod* 2000; 6: 246-51.
- [20] Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010; 140: 805-820.
- [21] Lin N, Li X, Song T, Wang J, Meng K, Yang J, Hou X, Dai J, Hu Y. The effect of collagen-binding vascular endothelial growth factor on the remodeling of scarred rat uterus following full-thickness injury. *Biomaterials* 2012; 33: 1801-1807.
- [22] Tas M, Kutuk MS, Serin IS, Ozgun MT, Oner G, Ozturk F. Comparison of antiproliferative effects of metformine and progesterone on estrogen-induced endometrial hyperplasia in rats. *Gynecol Endocrinol* 2013; 29: 311-314.
- [23] Inoue H, Nagata N, Kurokawa H, Yamanaka S. iPS cells: a game changer for future medicine. *EMBO J* 2014; 33: 409-417.
- [24] Dong F, Caplan AI. Cell transplantation as an initiator of endogenous stem cell-based tissue repair. *Curr Opin Organ Transplant* 2012; 17: 670-674.
- [25] Gargett CE, Ye L. Endometrial reconstruction from stem cells. *Fertil Steril* 2012; 98: 11-20.
- [26] Barbagallo I, Galvano F, Frigiola A, Cappello F, Riccioni G, Murabito P, D'Orazio N, Torella M, Gazzolo D, Li Volti G. Potential therapeutic ef-

- fects of natural heme oxygenase-1 inducers in cardiovascular diseases. *Antioxid Redox Signal* 2013; 18: 507-521.
- [27] Son Y, Lee JH, Chung HT, Pae HO. Therapeutic roles of heme oxygenase-1 in metabolic diseases: curcumin and resveratrol analogues as possible inducers of heme oxygenase-1. *Oxid Med Cell Longev* 2013; 2013: 639541.
- [28] Zhao Y, Zhang L, Qiao Y, Zhou X, Wu G, Wang L, Peng Y, Dong X, Huang H, Si L, Zhang X, Zhang L, Li J, Wang W, Zhou L, Gao X. Heme oxygenase-1 prevents cardiac dysfunction in streptozotocin-diabetic mice by reducing inflammation, oxidative stress, apoptosis and enhancing autophagy. *PLoS One* 2013; 8: e75927.
- [29] Wu SY, Li MH, Ko FC, Wu GC, Huang KL, Chu SJ. Protective effect of hypercapnic acidosis in ischemia-reperfusion lung injury is attributable to upregulation of heme oxygenase-1. *PLoS One* 2013; 8: e74742.
- [30] Uddin MJ, Joe Y, Zheng M, Blackshear PJ, Ryter SW, Park JW, Chung HT. A functional link between hemeoxygenase-1 and tristetrapiroline in the anti-inflammatory effects of nicotine. *Free Radic Biol Med* 2013; 65: 1331-1339.