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Therapeutic effect of human umbilical cord mesenchymal stem cells on tubal factor infertility using a chronic salpingitis murine model

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Abstract

Background The study was conducted to evaluate the application of human umbilical cord mesenchymal stem cells (hUCMSCs) in the treatment of tubal factor infertility (TFI) caused by *Chlamydia trachomatis*, and investigate their effect on fertility in animal models of chronic salpingitis.

Methods In this study, we investigated the therapy effects of the transplantation of hUCMSCs in tubal factor infertility using a chronic salpingitis murine model which induced *Chlamydia trachomatis*. Twenty rats were divided into two groups: control group ($n=10$) and treatment group ($n=10$). hUCMSCs were given to mice after exposure to *C. trachomatis* for 4 weeks. After treatment for 4 weeks, five mice were randomly selected from each of the two groups to sacrifice and we examined the organ morphology and pathology, inflammatory cytokines, proliferation, and apoptosis in fallopian tube (FT).The remaining five mice from each group were caged 2:1 with male mice for another 4 weeks, the numbers of pregnant mice and the mean number of pups in the different groups were enumerated and calculated.

Results Intravaginal inoculation of hUCMSCs alleviated hydrosalpinx of the oviduct. EdU-labeled hUCMSCs are located at the interstitial site of the fallopian tube. Macrophage (F4/80) infiltration was significantly reduced in the treatment group compared with the control group and expression levels of the anti-inflammatory cytokine IL10 were increased after hUCMSCs treatment. Furthermore, mRNA and protein expression levels of PCNA and Caspase-3 were increased and decreased, respectively, in the hUCMSCs' treatment group compared with the control. Moreover, hUCMSCs' transplantation improved murine fertility.

Conclusions Anti-inflammatory and anti-apoptotic effects of hUCMSCs may play an important role in TFI. Our data suggest that hUCMSCs' transplantation contributed to the repair of tubal injury and improvement of fertility, providing a basis for assessing the contribution of stem cells in the oviduct for direct repair of the tube to assist reproduction.

Keywords Human umbilical cord mesenchymal stem cells · Tubal factor infertility · Anti-inflammatory · Anti-apoptotic

Abbreviations

hUCMSCs	Human umbilical cord mesenchymal stem cells
MSCs	Mesenchymal stem cells
TFI	Tubal factor infertility
FT	Fallopian tube
IL-10	Interleukin-10
PCNA	Proliferating cell nuclear antigen
Caspase-3	Cysteine-containing aspartate-specific protease

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Introduction

The fallopian tube (FT) plays a key role in reproductive functions, such as sperm transport and capacitation, ova retrieval and transport, fertilization, and embryo nourishment and transport. Tubal factor infertility (TFI) accounts for 36% of female infertility cases. Genital infection by *Chlamydia trachomatis* causes severe complications,

including pelvic inflammatory disease (PID), ectopic pregnancy and TFI [1]. Chronic salpingitis caused by *C. trachomatis* can damage the structure of the FT and cause distal tube obstruction and hydrosalpinx, all of which are causes of infertility. At present, laparoscopic surgery and antibiotics are the main treatment for TFI. However, the occurrence of tubal reattachment and blockage of the FTs after surgery can cause recurrence of infertility. Additionally, the long-term use of antibiotics has side effects and can lead to antibiotic abuse. Moreover, emerging antibiotic-resistant strains [2] and absence of a vaccine has led to the requirement for new therapeutic strategies to repair the reproduction function of the FT, thereby alleviating TFI.

Mesenchymal stem cells (MSCs) are pluripotent stem cells which are able to differentiate into various cell types. MSCs and tissue-resident macrophages, which supply tissue-reconstructing cells and remove cell debris from inflammation sites, respectively, are essential for injured tissue restoration and regeneration [3–5]. MSCs also exert potent immunosuppressive and anti-inflammatory effects in cell-based therapies [6, 7] for disorders such as inflammatory bowel disease [8], atopic dermatitis [9] and rheumatoid arthritis [10]. MSCs modulate their anti-inflammatory effects in multiple ways in response to different tissue microenvironments following injury [11]. Several groups have reported that direct cell-to-cell contact and paracrine action by soluble factors are crucial for the immunomodulatory ability of MSCs [12, 13].

Stem cell transplantation has opened up a new way for preserving or recovering the damaged fertility. The application of stem cells transplantation is often associated with controversies, such as safety, source, and their poor survival time in vivo. Umbilical cord is a rich source of MSCs [14]; these cells are easily isolated and acquired through a painless donation procedure that is not ethically controversial [15]. Moreover, hUCMSCs have a higher proliferative potency and lower immunogenicity, which shows many advantages over other MSCs [16]. Although its exact protective role in the injured tissues is still obscure, more and more studies have implied that it could inhibit cell apoptosis by secreting growth factors.

Recent studies have shown MSC therapeutic potency in animal models of thin endometrium [17] and ovarian failure [18]. However, the therapeutic efficacy and mechanisms by which hUCMSCs act on TFI have not been determined. This has led us to explore further the capacity of hUCMSCs as a therapeutic target for chronic salpingitis in mice. Our present study aimed to: (1) investigate whether hUCMSCs' transplantation can alleviate chronic salpingitis; (2) explore the underlying mechanisms responsible for these effects; and (3) investigate whether hUCMSCs' transplantation can improve fertility.

Materials and methods

hUCMSCs' isolation and identification

Fresh hUC samples were obtained from the International Stem Cell Joint Research Center of Boyalife Stem Cell Technology Co., Ltd., Jiangsu province. After full-term delivery with written consent of the mother. hUCMSCs were prepared and verified as described previously [19].

Before in vivo experiments, cells were characterized for their capability to differentiate toward adipocytes and osteoblasts, as previously reported. Their mesenchymal phenotype was also assessed by fluorescence-activated cell sorting (FACS).

Labeling and tracing of hUCMSCs

HUCMSCs (third passage) were collected using 0.25% EDTA and cultured at a density of $5 \times 10^3/cm^2$ in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). After 24 h, 5-ethynyl-2′-deoxyuridine (EdU; Cell-Light™ EdU Apollo™ DNA in vivo Kit, Bo Rui Inc., Guangzhou, China) was added to the medium at a concentration of 10 μM. After 48 h, the cells were washed twice with phosphate-buffered saline (PBS). Approximately 1×10^6 EdU-labeled hUCMSCs were subsequently utilized for injection. After transplantation, EdU-labeled hUCMSCs were traced in the FT tissues using immunofluorescence staining according to the manufacturer's instructions. FT tissues were fixed with methanol, dewaxed and then incubated in 0.5% Triton® X-100 in PBS at room temperature. The tissues were then incubated with Apollo reaction cocktail and counterstained with Hoechst 33,324 (both for 30 min at room temperature in the dark), prior to imaging under a fluorescence microscope(ECLIPSE TE2000-E, Nikon, Tokyo, Japan) at ×400 magnifications, as previously described [20].

Model *Chlamydia trachomatis* MoPn strain

MoPn strain was purchased from American ATCC. McCoy cells were provided by laboratory of Guangdong Dermatology and Venereology Center and preserved for passage. The MoPn strain was infected with McCoy cells and cultured in RPMI 1640 medium containing 10% FBS. After culturing for 48–72 h, the majority of McCoy cells were collected and observed to be infected under the microscope. The cells were then centrifuged at 500×*g* for 10 min at 4 °C to obtain the supernatants, and were centrifuged

at 30,000×*g* for 45 min at 4 °C to collect the precipitate, both of which were stored at − 80 °C in sucrose potassium glutamate (SPG).

Establishment and treatment of the murine chronic salpingitis model

Twenty female and five male, 6–8-week-old C3H/HeN mice were included in the study [average body weight of 16–18 g; Beijing Vital-China Laboratory Animal Technology Co., Ltd.; Production Animal Permit No. SCXY (Beijing) 2016-0011]. Feeding was conducted using the SPF barrier system in appropriate facilities [Certificate number: SYXK (Guangdong) 2012-0083]. Mice were infected intravaginally with 50 µl of 1×10^7 IFU per mouse with MoPn chlamydial strain while under the intraperitoneal injection anesthetic of ketamine (100 mg/kg; Futian Pharmaceutical Co., Ltd.) and xylazine (4 mg/kg; Sigma, USA).

Mice were divided into two groups ($n = 10$/group): the PBS control group and the hUCMSCs' treatment group. To evaluate therapeutic efficacy, treatment with hUCMSCs was initiated 4 weeks post-infection. Mice in the treatment and control groups received either 50 µl of 1×10^6 EdU-labeled hUCMSCs or 50 µl of PBS for single transplantation, respectively. After treatment for 4 weeks, five mice were randomly selected from each of the two groups to sacrifice and the remaining five mice from each group were caged 2:1 with male mice for 4 weeks (Fig. 1).

Histopathologic evaluation

Murine FT tissue specimens from both groups were fixed in 4% formaldehyde, embedded in paraffin, sectioned (4–10 mm) onto slides, and stained with hematoxylin and eosin (H&E). Tissue sections were then immersed in xylene (10 min, twice) and rehydrated in a decreasing ethanol series diluted in distilled water (100%, 100%, 95%, 95%, and 75% for 0 and 3 min each). Tissue sections were then rinsed in deionized water, stained in hematoxylin for 1 min, rinsed in deionized water, and stained in eosin for 15 s. After the color reaction, sections were dehydrated through an ethanol series in xylene. Oviduct morphology was evaluated and compared between groups. Photographs were taken using an optical microscope (ECLIPSE 80i, Nikon, Tokyo, Japan) at ×200 magnification.

Immunofluorescence

Immunofluorescence was performed to assess EdU-positive hUCMSCs and infiltrated F4/80-positive macrophages within the tubal tissues. Tissue sections were incubated overnight with primary antibodies at 4 °C, followed by the appropriate horseradish peroxidase-conjugated secondary antibodies for 30 min. Immunoreactive areas were visualized following exposure to diaminobenzidine (Dako) solution for 20 min. Tissues were then reacted, counterstained and visualized as described in the previous section (Labeling and tracing of hUCMSCs). Photographs were imaged under

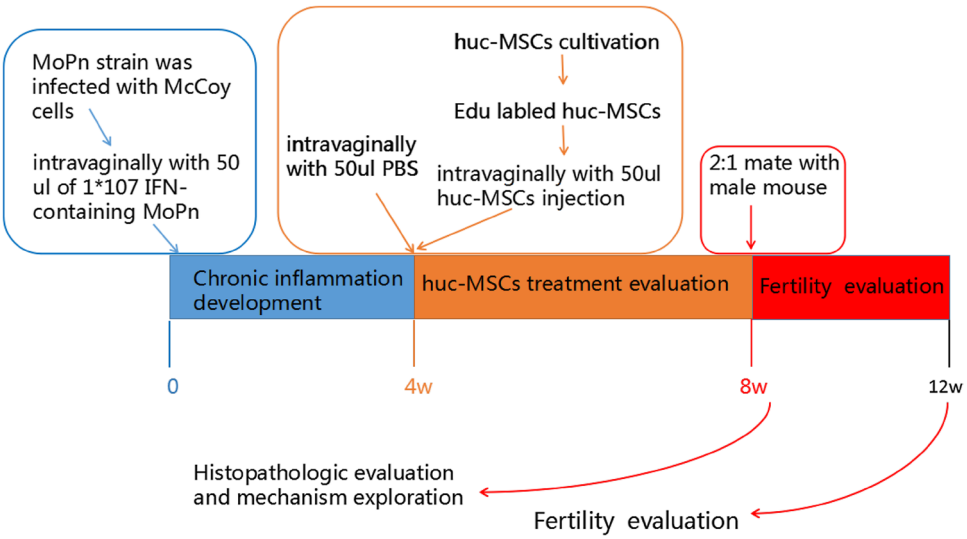

Fig. 1 Experimental schedule. Mice were acclimatized for a minimum of 7 days prior to experiments. Mice were then intravaginally injected with 50 µl of 1×10^7 IFN-containing MoPn to simulate chronic inflammation of the oviduct. Four weeks later, animals were intravaginally injected with PBS (control group) or hUCMSCs (treatment group). After a 4-week treatment period, five mice were randomly selected from each group for sacrifice and the remaining five were caged 2:1 with male mice for 4 weeks. The results were evaluated 4 weeks after therapy (8 weeks from experimental initiation). Fertility was evaluated at week 12 of experimental initiation

a fluorescence microscope (ECLIPSE TE2000-E, Nikon, Tokyo, Japan) at ×600 magnifications.

Enzyme-linked immunosorbent assay analysis

Blood was collected from the mice in both groups after 4 weeks. Serum samples were assayed for interleukin-10 (IL-10) production with an IL-10 enzyme-linked immunosorbent assay (ELISA) quantification kit (Santiago, CA, USA) according to the manufacturer's recommendations.

Total RNA extraction and quantitative RT-PCR

Gene expression analysis was determined by quantitative real-time PCR (qRT-PCR) using the SYBR Green Master and a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) on a One-Step Plus instrument using standard protocols. The results were analyzed using the $2^{-\Delta\Delta CT}$ method with normalization against GAPDH expression ($n = 5$/group).

Total RNA was isolated from murine FT tissues using Trizol reagent (Invitrogen) and RNA (1 μg) was reverse-transcribed using the AMV First Strand cDNA Synthesis Kit (Invitrogen), following the manufacturer's instructions, yielding the complementary DNA (cDNA) template. The cDNA was then amplified by PCR using the primer sequences (Lan Tun, Shanghai, China) shown in Table 1.

Immunohistochemistry

Immunohistochemistry was performed to assess proliferating cell nuclear antigen (PCNA) and cysteine-containing aspartate-specific protease (Caspase-3) according to the manufacturer's procedures. After fixation in 4% paraformaldehyde, oviducts were embedded in paraffin and 6-mm serial sections obtained. Sections were deparaffinized in xylene, rehydrated through a series of ethanol washes, and rinsed in water. Endogenous peroxidase activity was blocked by incubating sections in 0.3% H_2O_2 in methanol for 40 min at room temperature. Slides were blocked for 1 h in phosphate-buffered saline (PBS) supplemented with 10% normal goat serum. Expressions of PCNA and caspase-3 proteins were performed by incubating sections of mice oviduct with either rabbit polyclonal antibodies against PCNA and caspase-3 overnight at 4 °C. Sections were incubated with 1:3000 horseradish peroxidase-conjugated goat anti-rabbit IgG in 10% goat serum for 1 h at room temperature. Sections were then briefly counterstained (10 s) with hematoxylin solution (Sigma, USA), and examined using a digital image-capture system (ECLIPSE 80i, Nikon, Tokyo, Japan) at ×200 and ×400 magnifications.

Effect of hUCMSCs' transplantation on murine fertility

To determine the influence of intravaginal hUCMSCs' treatment on murine fertility, five mice from each group were paired with proven C3H/HeN male mice 4 weeks post-treatment. Pregnancy was confirmed by monitoring of a vaginal suppository in the first 3 days and weight gain for approximately 4 weeks. A consistent 3-day weight gain of > 30 g is affirmative of pregnancy in mice and was considered evidence of pregnancy. The numbers of pregnant mice and the mean number of pups in the different groups were enumerated and calculated.

Statistical analysis

All data are expressed as mean values ± standard deviation (SD). Statistical analyses of the data were performed using GraphPad Prism software version 5.0 (GraphPad Software, San Diego, CA, USA). A two-tailed unpaired Student *t* test and the Chi-square test were used to analyze data between groups. Values of $P < 0.05$ were considered to indicate statistically significant differences.

Results

hUCMSCs ameliorate the macroscopic appearance and morphological features of the FT

The macroscopic appearance of the FT showed severe mucous congestion accompanied by tubal edema and loss of ciliated epithelial cells (arrows) in the control group, whilst the FT in the treatment group appeared normal, without swelling.

Table 1 Primer sequences of murine target genes

Genes	Primer sequence (5′–3′)		Amplicon size (bp)
PCNA	F:GCAGGCAGTATCACTCATTGT	R:GGAAAAGAAGGTGCTCATGT	100
Caspase-3	F:GGCACATTTCCAGGACTGA	R:TAATGAGGGCAAGACGTGTAC	133
GAPDH	F:GGCCTCCAAGGAGTAAGAAA	R:GCCCCTCCTGTTATTATGG	141

Histological evaluation of the PBS control group HE-stained FT sections revealed massive inflammatory cell infiltration, FT mucosal fold flattening and reduction, an enlarged FT lumen, hydrosalpinx, and loosened tissue. After hUCMSC treatment, the FT wall structure was clear and the lumen was smooth, with increased cilia and multi-branching (Fig. 2).

The EdU-labeling efficiency of hUCMSCs for immunofluorescence

Incubation of hUCMSCs with EdU (10 μmol/L) for 48 h resulted in a 45.53% labeling efficiency. Fluorescence microscopy revealed that the EdU-positive cell nuclei were intensely red, with counterstained Hoechst 33,324 (blue fluorescence) also observed in the nuclei (Fig. 3A).

hUCMSCs therapy located Ct-induced chronic salpingitis and downregulated infiltrating macrophages (F4/80)

EdU-labeled hUCMSCs were transplanted into the mice via intravaginal injection. hUCMSCs' location in the FT tissue was examined by immunofluorescence 4 weeks post-transplantation. Microscopic evaluation of the FT sections revealed high F4/80-positive macrophage infiltration in the control group, with labeled EdU-positive cells predominantly distributed around the interstitial area of the FT tissue. Interestingly, hUCMSCs' treatment markedly down-regulated F4/80-positive macrophage infiltration (Fig. 3A).

hUCMSCs increase serum IL-10 expression levels

To verify the effect of hUCMSCs on the production of inflammatory cytokines closely associated with chronic salpingitis pathogenesis, serum levels of the anti-inflammatory marker, IL-10, were determined using ELISA. Serum levels of IL-10 were significantly increased in the hUCMSCs' treatment group compared with the control group ($p = 0.001$) (Fig. 4A).

hUCMSCs promote proliferation and inhibit apoptosis of FT cells in vivo

Chlamydia trachomatis-induced chronic salpingitis is associated with FT cell apoptosis. Therefore, the effect of hUCMSCs on FT cell proliferation and apoptosis was analyzed by determining gene expression levels of PCNA and caspase 3. Our results showed that PCNA and caspase 3 expression levels were lower and higher, respectively, in the chronic salpingitis group 4 weeks after *Chlamydia trachomatis* exposure. Interestingly, expression of PCNA was increased ($P = 0.031$) and caspase 3 was decreased ($P = 0.019$) after hUCMSCs' transplantation ($P < 0.05$)

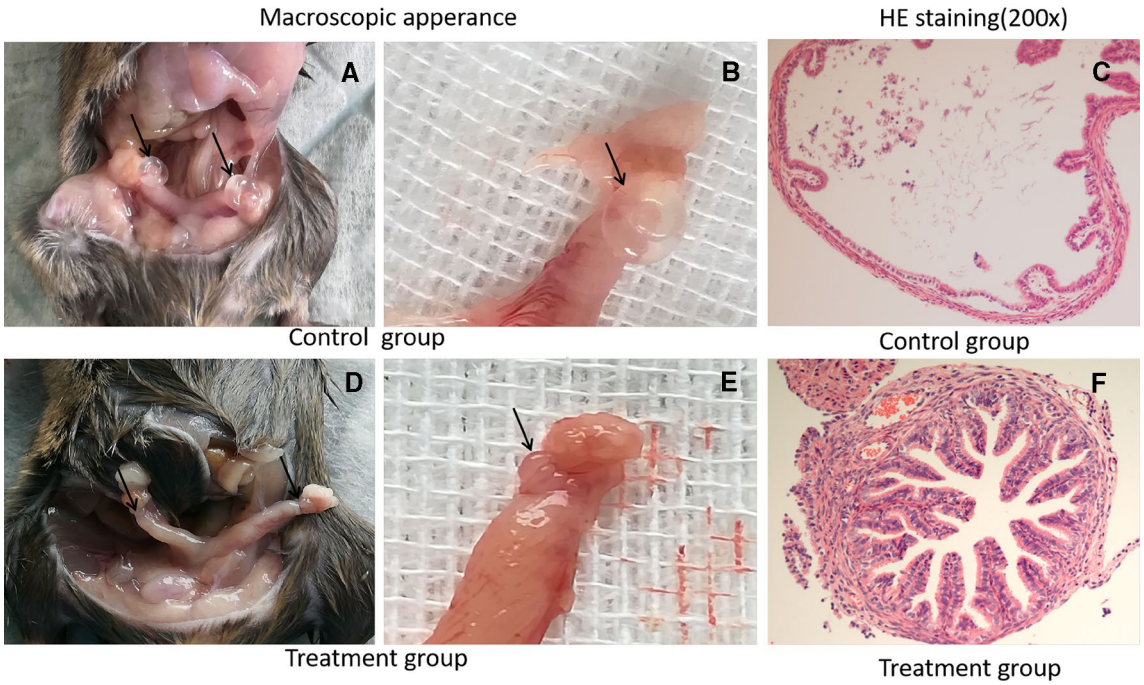

Fig. 2 Macroscopic appearance and changes and H&E staining (×200) of FTs in the control group (**A**–**C**) and treatment group (**D**–**F**) [the fallopian tube (arrows)]

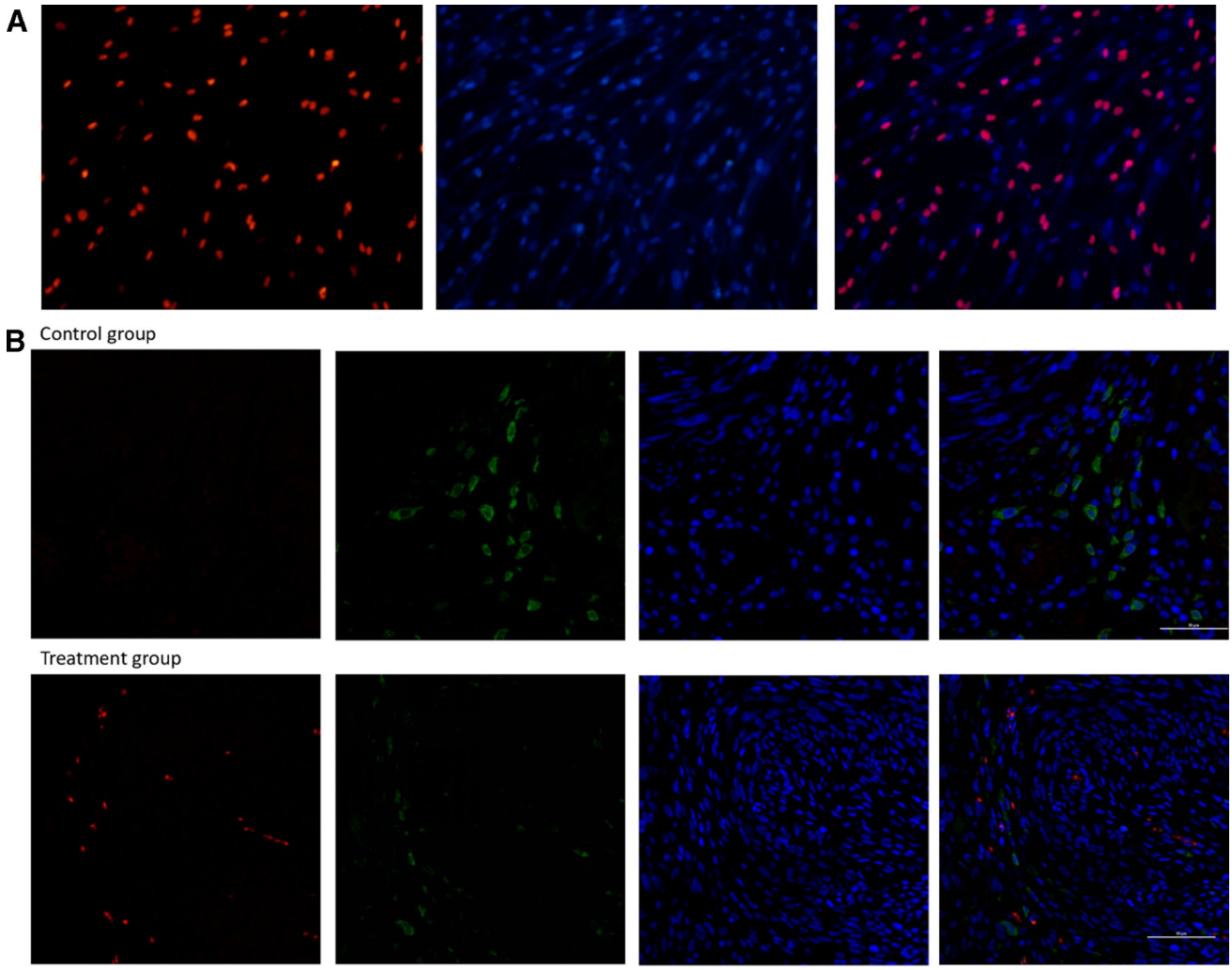

Fig. 3 Immunofluorescence of EdU-labeled hUCMSCs. EdU-positive cell nuclei are shown by intense red fluorescence; Hoechst 33,324 staining was observed in the nuclei (blue fluorescence). Nucleus showing EdU and Hoechst 33,324 staining (×200 magnification) (**A**). Immunofluorescence of FT tissue. EdU-positive cell nuclei are shown by intense red fluorescence; Hoechst 33,324 staining was observed in the nuclei (blue fluorescence). F4/80 immunofluorescence staining is shown in the cell membrane (green fluorescence) of FT tissue in both control and treatment group mice (×600 magnification) (**B**)

(Fig. 4B, C). Immunohistochemical staining of PCNA and caspase 3 was also carried out. FT tissue from the hUCMSCs' treatment group had increased numbers of PCNA-positive cells and decreased numbers of caspase-3-positive cells compared with the chronic salpingitis control group (Fig. 4D).

hUCMSCs' transplantation improved murine fertility

In the hUCMSCs treatment group, 100% (5/5) of mice became pregnant with a mean litter size of 5.4 ± 1.95 kits. However, only a 20% pregnancy rate was observed in the control group. This difference in pregnancy rate was significant between the two groups (Tables 2 and 3, $P < 0.01$).

Discussion

The FT bears the role of sperm activation, ova retrieval and transport, and early embryo development. Consequently, integrity of the FT epithelial fold microvilli is crucial because these structures function to increase the cell reception surface area and act as cell stimuli [21]. Our study showed that in the untreated control group, FT mucous membranes disappeared, cilia were removed, microvilli were reduced, and chronic inflammation occurred following increased inflammatory macrophage infiltration; all resulting in failure of sperm transport and sperm and egg combination. Furthermore, these structural and functional changes in the FT can eventually lead to TFI.

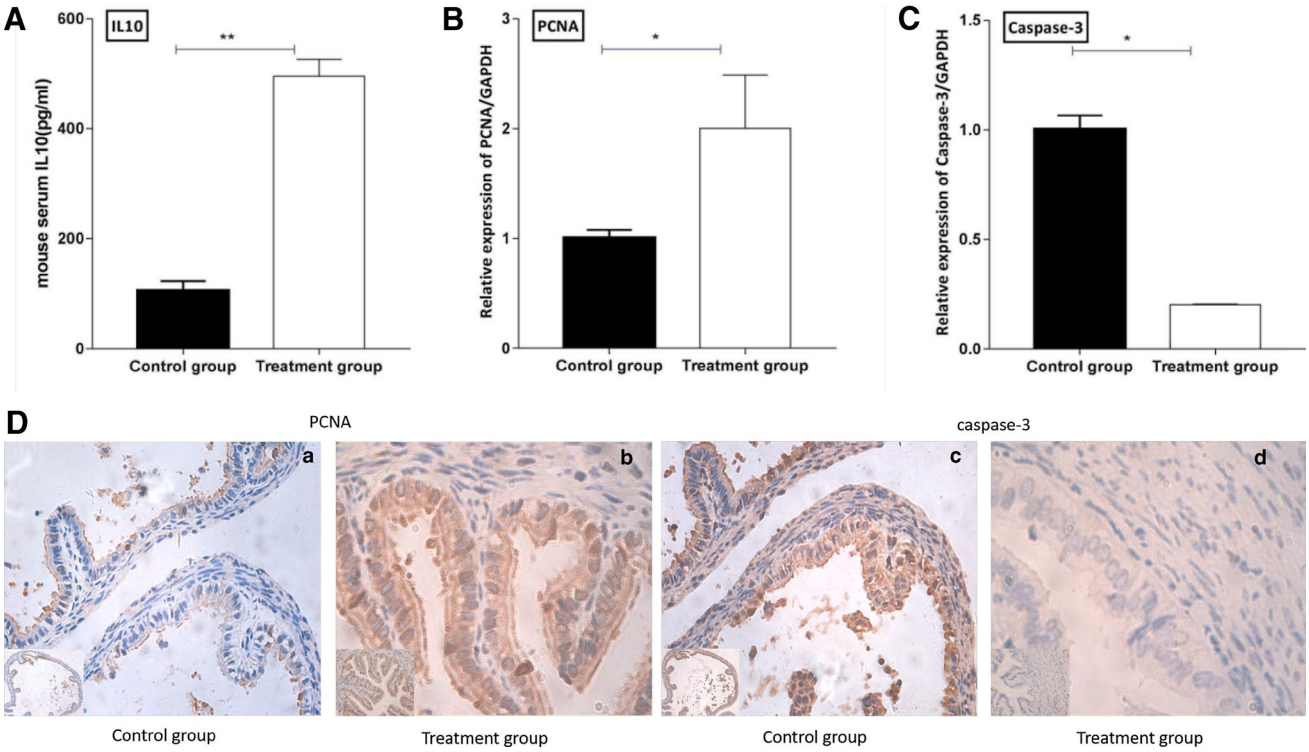

Fig. 4 Expression of inflammatory cytokines and changes of FT cell proliferation and apoptosis after hUCMSC injection. Expression levels of serum IL-10 were determined by ELISA. $*P < 0.05$ versus PBS control group (**A**). Expression levels of PCNA and caspase-3 in the FT tissues were determined by qRT-PCR. $*P < 0.05$ versus the PBS control group (**B**, **C**). Changes of FT cell proliferation and apoptosis after hUCMSC injection. Representative images of PCNA (**a**, **b**) and caspase-3 (**c**, **d**) staining in FT tissue sections in the control and treatment groups (×400 magnification; insets ×200) (**D**)

Table 2 Litter size of the mice in each group

	1	2	3	4	5
Control group	0	2	0	0	0
Treatment group	6	3	4	6	8

Table 3 Pregnancy rate and litter size in each group

	Pregnancy rate (%)	Litter size (*n*)	*p*
Control group	20	0.4 ± 0.89	0.001
Treatment group	100	5.4 ± 1.95	

Many studies report MSCs' homing and the beneficial effects of stem cell-based therapy for inflammatory immune diseases. Cho et al. [22] found that the preferential shift of the macrophage phenotype from M1 to M2 may be related to the immune-modulating characteristics of MSCs that contribute to cardiac repair. According to Wise et al. [23], MSCs can home to injured kidneys and promote repair, which may be mediated by their ability to promote M2 macrophage polarization. Kim et al. [9] found that production of PGE2 by MSCs and subsequent production of IL-10 are required to reduce the severity of colitis. However, very few reports exist relating to hUCMSCs transplantation as a TFI treatment, particularly in a *Chlamydia trachomatis*-induced chronic salpingitis mouse model. Moreover, the mechanism of action underlying this remains unclear.

We have previously demonstrated that efficient hUCMSC-labeling can be achieved with incubation in 10-μmol/L EdU medium for 48 h; without affecting their biological characteristics such as proliferation and apoptosis. In the present study, 4 weeks after local vaginal transplantation of hUCMSCs, EdU-positive hUCMSCs were found to be localized in the FT mesenchyme. This is consistent with the finding [20] that EdU can be used as a long-term marker to show MSCs migration-distribution in animal tissues. One study has shown that exogenous transplanted MSCs home to damaged tissues where they contribute to repair through release of trophic factors rather than by engraftment [24]. Other clinical and scientific evidence indicates that MSCs and myeloid cells home to sites of tissue damage and incorporate into various organs—transdifferentiating into the cells of the new tissue in which they reside [25]. However, the exact mechanism of MSCs homing remains unknown. Our

results support that local vaginal perfusions provided direct contact of hUCMSCs with the injured tubal epithelium. The injured tubal epithelium likely secretes chemotactic factors and adhesion molecules to attract the hUCMSCs to colonize and play a role in tissue repair. Alternatively, the hUCMSCs may incorporate into the tissue and transdifferentiate into cells of the new tissue, from which they then mobilize or regulate the residing local MSCs to further contribute to regeneration of the tubal epithelium; as described in other organ injury models [26].

After the migration of MSCs to sites of injury, they can produce anti-inflammatory cytokines and possess an immunomodulatory property. A recent study demonstrated that MSCs influence the process of inflammation by immunomodulating the expression of inflammatory cytokines from a variety of immune cells, including macrophages [23]. Macrophages are central mediators of the inflammatory response, contributing both to the initiation and resolution of inflammation, especially in chronic inflammation. Our results show that hUCMSCs accelerated reconstruction of the FT by reducing the infiltration of macrophages (F4/80). IL-10 is a multifunctional negative regulator with anti-inflammatory effects that is produced mainly by activated macrophages. Previous research indicates that MSCs can upregulate anti-inflammatory cytokines, such as IL-4 and IL-10, in animal disease models [27]. Our results show that hUCMSCs' transplantation increased expression of the anti-inflammatory cytokine IL-10. The concentration of IL-10 in the treatment group was significantly higher than that in the control group ($P=0.001$).

PCNA serves as a key factor in many essential cellular processes, such as DNA-replication, -repair and -damage protection, cell cycle control, and cell survival [28, 29]. MSCs have the ability to induce DNA formation and repair and undergo asymmetrical division whilst retaining the template DNA [30]. The pro-apoptotic protein caspase-3 has been shown to play an important role in apoptosis by removing inhibition to mediate feedback amplification. Moreover, MSCs are resistant to apoptotic activation following induction of DNA damage, probably through reducing activation of proapoptotic factors and increasing expression of anti-apoptotic proteins [31]. Therefore, we examined PCNA and caspase-3 expression by qRT-PCR and immunohistochemistry to investigate the anti-apoptosis effect of hUCMSCs' transplantation in mice with chronic salpingitis. Our results showed that PCNA expression was approximately two-fold higher in the treatment compared to the control group ($P=0.031$). Moreover, caspase-3 expression was significantly lower in the treatment group compared with the control group ($P=0.019$). These results support direct vaginal local infusion of hUCMSCs into the injured FT, which likely promote mucosal cell regeneration by promoting epithelial cell proliferation and inhibiting cell apoptosis.

In mice treated with hUCMSCs, 100% became pregnant within 8 weeks, with a mean litter size of 5.4 ± 1.95. This was a significant improvement in the overall fertility and this pregnancy rate was in agreement with previous reports of 100% [32]. However, only 20% of mice in the control group became pregnant with a smaller mean litter size of 0.4 ± 0.89. This difference may be due to the use of a chlamydial infection in this experiment that results in a persistent immune response different to bacterial infections. In fact, Chlamydial infection can produce chlamydia heat shock protein, making it difficult to remove persistent local infection of the FT, resulting in a series of complications such as tubal edema, adhesion and obstruction, and eventually TFI. In the previous study [32], New Zealand rabbits were infected with *Escherichia coli* suspension into the uterine cavity to establish the model. Chronic salpingitis caused by bacteria is predominantly the result of delayed acute infection, and it is relatively more prone to early infertility. To our knowledge, there are few reports showing that hUCMSCs' transplantation can improve TFI, especially in a *Chlamydia trachomatis*-induced chronic salpingitis murine model.

In conclusion, our study indicates that hUCMSCs' therapy improves epithelial cells via a secretary function, including increased secretion of IL-10. A possible mechanism could be that hUCMSCs home to the damaged FTs where they exert anti-inflammatory and anti-apoptotic properties to accelerate reconstitution of the oviduct structure and thus improve the fertility of mice. These findings provide a good basis to identify new treatments for patients with TFI. To apply hUCMSCs more effectively in the clinical setting, more studies on the mechanism-of-action of hUCMSCs should be performed.

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Author contributions XM-L, TL, and XR-T conceived and designed the experiments. WJ-L, XM-L, TL, and XR-T performed the experiments. WJ-L and XR-T analyzed the data. WJ-L, XM-L, and TL contributed reagents/materials/analysis tools. WJ-L and XR-T wrote the paper. All authors read and approved the final manuscript. Ethics approval and consent to participate.This study was approved by the Ethics Committee of the the Third Affiliated Hospital of Sun Yat-sen University and run in accordance with the guidelines of the Helsinki Declaration. All participants provided written informed consent to participate in this study. All of the hUCMSCs in our study were manufactured and provided by the International Stem Cell Joint Research Center of Boyalife Stem Cell Technology Co., Ltd., Jiangsu province of China. All female and male C3H/HeN mice were were provided by the Beijing Vital-China Laboratory Animal Technology Co., Ltd.; (qualification no. SCXY (Beijing) 2016 - 0011). The Institute of Vaccines at the Third Affiliated Hospital of Sun Yat-sen University of the People's Republic of China approved the animal experimental protocol. Animals

were used in accordance with the Animal Care and Use Committee of Sun Yat-sen University.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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