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Original Article

Intrauterine injection of bioengineered hydrogel loaded exosomes derived from HUCM stem cells and spermidine prominently augments the pregnancy rate in thin endometrium rats

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ABSTRACT

The endometrium is essential to the development of embryos and pregnancy. Human umbilical cord mesenchymal stem cells (HUCMSCs) are promising stem cell sources. HUCMSCs self-renew quickly and are painless to collect. Spermidine is an inherent polyamine needed for cellular and molecular processes that regulate physiology and function. HUCMSCs and spermidine (SN) may heal intrauterine adhesions. HUCMSCs were investigated for endometrial repair in rats. Composite hydrogels are used for medical exosome implantation, including their materials, properties, and embedding procedures. This study examined whether bioengineered hydrogel-loaded exosomes from HUCMSCs and spermidine prenatally improved conception rates in mice with poor endometrial lining. The data show that HUCMSC and SN provide a good experimental base for HUCMSC safety and intrauterine treatment in rats. Western blots, exosome structural analysis, pregnancy outcomes, flow cytometry, H&E staining, immunohistochemistry, and immunofluorescence labelling found and recovered the aberrant area. HUCM-derived stem cells and spermidine-derived exosomes biophysically match. These traits strengthen and prolong endometrial function. Pregnant rats with HUCMSC and SN had thinner endometrium. Hydrogel-incorporated HEHUCMSC and SN exosomes may improve IUI in rats with thin endometrium.

1. Introduction

Exosomes generated from human gingival MSCs and human placental MSCs were cultured on silk hydrogel sponge as a platform for sustained local drug delivery [1]. When exosomes, polymers, and crosslinkers are combined, gelation can be triggered. The method was described in detail by Qin et al. [2] and it involved the use of thiolated hyaluronic acid (HA), gelatin, and heparin. Polyethylene glycol diacrylate (PEGDA) was used as a gelation agent, and exosomes isolated from bone marrow stem cells were incorporated into the polymer [3]. Hydrogels are similar to biological soft tissues in that they can absorb and hold water, expand without breaking down, and maintain part of their original suppleness. Hydrogel's low surface tension and adhesion qualities allow for effective diffusion and retention of contents [4]. Hydrogels are highly hydrated three-dimensional networks of natural or synthetic polymers. Chemical bonding, electrostatic interactions, and physical entanglement all play a role in keeping the compositions' outer and inner structures in place.

For this reason, they have found applications as artificial organs, wound dressings, and biosensors [5] due to their adaptability, receptivity to stimuli, and material exchanging capacity. Some biocompatible hydrogels have the potential for regulated drug delivery due to their large molecular volume, high charge density, and muco-adhesive properties [6]. Intrauterine adhesion (IUA), also known as Asherman's syndrome, is a condition that typically manifests after an intrauterine surgery or infection and is associated with menstrual abnormalities, infertility, and abnormal

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placental development. Asherman's syndrome, or intrauterine adhesion (IUA), creates uterine scar tissue. When the endometrium is injured, the uterine walls combine, forming these adhesions. After dilatation and curettage (D&C), cesarean sections, or other uterine cavity procedures that tear the lining, this syndrome typically occurs. These pathways may function differently in IUA development according on the first endometrial injury kind and degree. These mechanisms must be understood to prevent, diagnose, and treat intrauterine adhesions. Common clinical prophylactic methods for IUA include using barriers to keep the uterine wall segregated after surgery and using oestrogen or other drugs to encourage endometrial growth. However, clinical research have shown that oral medications have minimal efficacy and harmful consequences. Many in-situ delivery systems have been employed to increase drug absorption, as mentioned by Alvarez-Lorenzo et al. [7]. Normal uterine growth and the presence of healthy embryos both increase the odds of a successful pregnancy. Several factors, including an increase in abortions induced by medicine, operational operations on the uterine cavity, and functional anomalies in endometrial stem/progenitor cells, have been linked to a reduction in endometrial thickness to the typical 7 mm. The work of Caserta et al. [8] is cited. Low implantation rates have been linked to an anomaly in endometrial development, which may explain why women with a thin endometrium have trouble getting pregnant. It takes time for people with a thin endometrium to regain their previous endometrial thickness [9]. Stem cells have demonstrated promising outcomes in the therapy of tissue injury. Due to their high quality, extensive availability, great proliferative potential, and low immunogenicity, mesenchymal stem cells derived from human umbilical cords (UCMSCs) are now the cells of choice for gene/cell therapy. Site-specific chemicals released by mesenchymal stem cells (MSCs) in response to the local microenvironment at the site of damage perform multiple critical reparative roles [10]. Endometrial receptivity (ER) is the short window of opportunity for embryo implantation [11]. The endometrium, blastocyst, and synchronised communication between the mother's tissues and the embryo's are all necessary for successful implantation [12]. Mesenchymal stem cells (MSCs) are a type of adult stem cell with the ability to self-renew and specialise into a variety of cell types. Zhang et al. [1] revealed that MSCs are present in the endometrium, suggesting that their absence may contribute to the onset of IUA. In vitro fertilization and embryo transfer patients benefit from enhanced endometrial receptivity and endometrial microcirculation, which in turn raises the clinical pregnancy rate. The regenerative effects of bone marrow stromal cells (BMSCs) on rabbits with injured endometrium were recently discovered in our lab's work [13]. We evaluated the effectiveness of HUCMSCs in restoring uterine function using a rat model of thin endometrial injury. Mesenchymal stem/stromal cells (MSCs) were first discovered in the bone marrow by Alexander Friedenstein. MSCs are multifunctional adult nonhematopoietic cells. The molecules CD14, CD34, and CD45 are not expressed by mesoderm-derived MSCs, whereas CD73, CD90, and CD105 are [14]. Exosomes generated from mesenchymal stem cells (MSCs) are biologically active in a similar way to MSCs [15], packaging and transporting active biomolecules such peptides, proteins, and RNA species to the sick cells/tissues. Exosomes, one of the three forms of EVs, can be anywhere from 30 to 150 nm in size. The other two populations are larger than 100 nm and consist of microvesicles/shedding particles and dead corpses, respectively. Late endosomes, sometimes called multivesicular bodies (MVBs) [16], are where exosomes are made. Intralumenal vesicles (ILVs) arise in the luminal region of MVBs. Polysaccharides (such as chitosan, hyaluronan, and alginate) and proteins (such as fibrin, fibroin, collagen, and gelatin) are just some of the natural elements that can be used to create hydro gels [3]. Paracrine signaling can occur through various mechanisms, including as the direct release of proteins or the release of microvesicles capable of carrying proteins, messenger RNA (mRNA), and microRNA (miRNA) [17]. Natural hydrogels are preferable for bone repair since they are biodegradable and interact with cells appropriately. The advantages of exosomes are well-documented, but there is concern that a therapeutic dose of exosomes, especially when injected systemically, could pose more hazards than benefits. However, the high expense of manufacturing techniques that maintain the homogeneity and purity of nanoscale biomaterials presents challenges for exosome purification and mass production [18]. To avoid being destroyed by the host immune system, exosome delivery is preferable. The present study aims to examine the currently available exosome extraction methods with the purpose of therapeutic use, and recent significant results addressing the medical application of MSC-derived exosomes in regenerative medicine in both animal models and clinical studies. We used a rat model of thin endometrial damage to assess the efficacy of human HUCMSCs in promoting endometrial healing. In rats, HUCMSC and SN provide a robust experimental platform for safety and intrauterine therapy. Western blots, exosome structural analysis, pregnancy outcomes, flow cytometry, H&E staining, immunohistochemistry, and immunofluorescence labeling identified the aberrant area. HUCM-derived stem cells and spermidine-derived exosomes match biophysically. These features boost and prolong endometrial function. HUCMSC and SN pregnant rats exhibited thinner endometrium. In rats with thin endometrium, hydrogel-incorporated HEHUCMSC and SN exosomes might enhance IUI.

2. Methodology

2.1. Experimental methods and animals

Animals used in this investigation were cared for and used in accordance with the ethical standards outlined in the Guide for the Care and Use of Laboratory Animals. Female Sprague-Dawley rats reaching sexual maturity at 8 weeks of age (220–250 g) were acquired and approved by Changchun Institute of Veterinary Medicine, China. Rats' estrous cycles were studied using vaginal smear, and experimental models were built during the diestrus phase. Based on our team's prior studies, an IUIH (Poly-ε-L-lysine (EPL)-Intra uterine injection hydrogel) rat model was developed. Rats ($n = 36$) were split into three groups: sham operation (sham, $n = 12$), in which the animals underwent laparotomy but were not treated; IUIH model (IUIH, $n = 12$), in which the rats were induced to develop intrauterine adhesions; Injecting 0.2 ml of phosphate-buffered saline (PBS) into each horn of the uterus, and then 2 weeks later performing a relaparotomy and HEHU The uterine tissues of 18 rats ($n = 6$ per group) were collected for further analysis after they were slaughtered during the estrous phase four weeks after treatments [19].

2.2. Isolation & hydrogel loaded exosomes derived from HUCMSCs and SN

Cells were collected after 48 h of culture in the presence of varied concentrations of HEHUCMSC & SN (100 μg/ml) in serum-free culture media after fibroblasts were serum-starved for 24 h. Purification and expansion of human glandular and stromal cell cultures Women's Hospital at Zhejiang University in China recruited 20 women, all of whom were between the ages of 21 and 48 and had regular menstrual cycles (21–35 days). They haven't started hormone therapy 3 months before to the scheduled operation. During the infertility evaluation, hysteroscopy and endometrial biopsies were performed on all of the patients. The

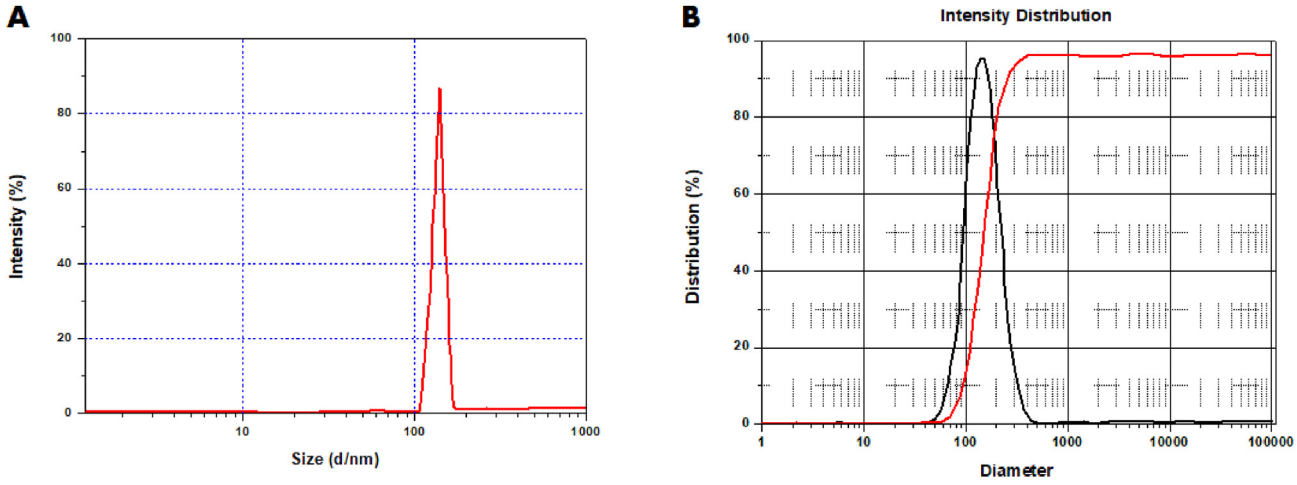

Fig. 1. A) Particle size distribution by DLS analysis of exosomes with hydrogel B) Confirmation of hydrogel loaded exosome size by DLS.

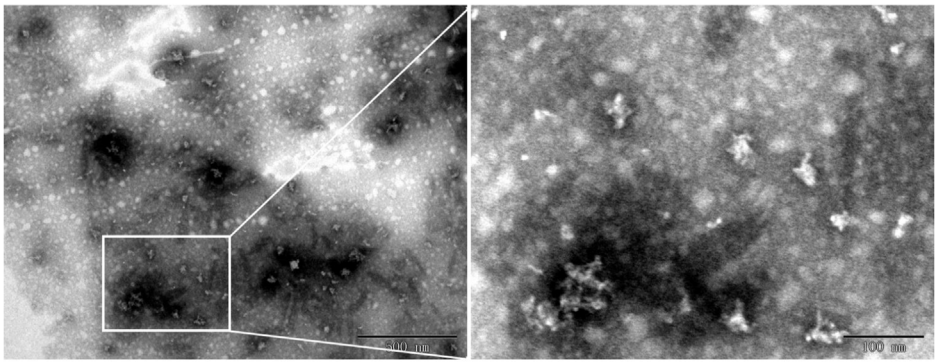

Fig. 2. Scanning Electron Microscopic evaluation of hydrogel loaded Exosomes.

endometrium was cultured to get stromal and glandular cells. At 37° Celsius in a humidified 5% CO_2 atmosphere, both sets of cells were grown in DMEM/F12 media (Hyclone, Waltham, US) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Cells were employed for an EdU/Transwell/Western blot experiment after being co-cultured with HP-MSCs for 24, 48, and 72 h. Isolation of HUCMSCs and SN. Human umbilical cords were collected at the above-mentioned hospital, after routine cesarean deliveries. In a nutshell, the epithelium of the umbilical cord was scraped off, the arteries were dissected apart, and the tissue that remained was sliced into pieces that were just 1 mm^3 in volume. The culture media was added to the 10-cm pans after the tissue pieces were tiled on them. Expression of CD73, CD44, CD105, CD34, CD45, CD31, and HLA-DR on F5 cells was analyzed using fluorescent-activated cell sorting (FACS) to determine the phenotype of UC-MSCs [10].

2.3. Characterization

Following the manufacturer's instructions, we measured the size distribution of exosomes with DLS on a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Transmission electron microscopy (TEM) was used to analyse the separated exosomes for their morphology. To sum up, the samples were negatively stained with 1% phosphotungstic acid after being placed on an electron transparent test support (EM grid).

2.4. Western blot analysis

The contralateral side of the uterus (n = 6 per group) was homogenised in RIPA lysis solution (Solarbio) containing 1 mM PMSF. The concentration of the extract was determined by centrifuging it at 12,000 rpm for 5 min and then analysing the supernatant using a BCA protein assay kit. Electrophoretically, a 4–20% polyacrylamide gradient gel was used to separate the 30 g of extracted protein. Milk at 5% in TBST was used to inhibit nonspecific binding for 1 h. Primary antibodies were used to detect the following proteins: rabbit anti-Alix, rabbit anti-CD63, mouse anti-GAPDH, rabbit anti-VEGF (1:1000; Santa Cruz), mouse anti-LIF, rabbit anti–Tubulin (1:1000; Antibody Revolution), and mouse antiintegrin-3. After that, the membranes were treated with a fluorescently labelled IRDye 800 secondary antibody (1:3000; Rockland) of the matching species for 2 h at room temperature in the dark. The LICOR Odyssey (Lincoln, NE) was used to take digital pictures of the protein bands. ImageJ software (NIH, Bethesda, MD, USA) was used to measure the grey value of the protein band to determine the relative expression. The levels of -Tubulin were utilised as a reference. At least three separate trials were conducted.

2.5. Analysis of endometrial epithelila cells morphology

Treatment of EECs with TGF-1 for 24 and 48 h at 10 μg/ml, 10 μg/ml, 50 μg/ml, and 100 μg/ml induced H_2O_2 HEHUCMSC and SN,

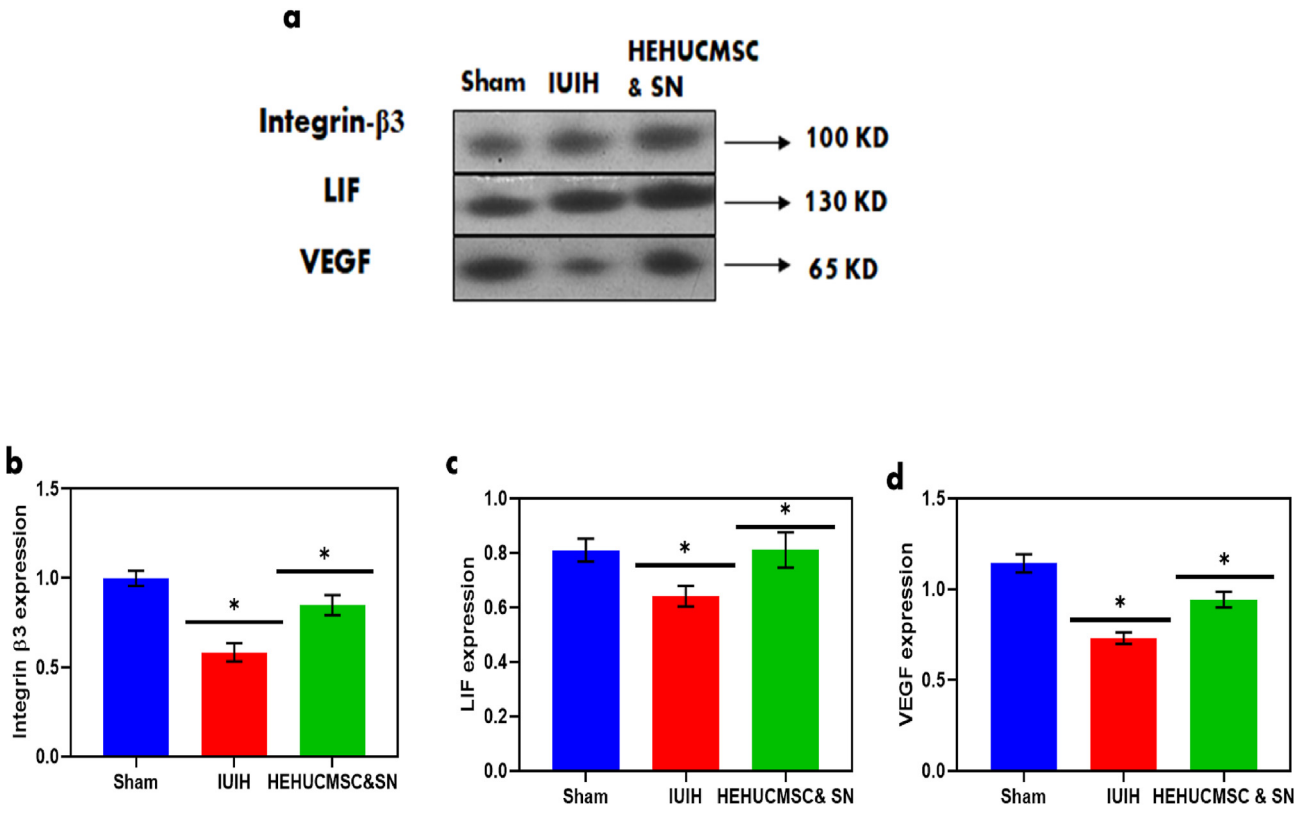

Fig. 3. a) Western blot analysis of endometrium related proteins b) Histogram reveals the expressions of integrin-β3 c) LIF d) VEGF. Data were expressed in mean standard deviations and significant $p < 0.05$.

respectively. Alterations in cell morphology and signs of apoptosis were identified. After 24 h of exposure to 90 μg/ml TGF-1, EECs were treated with varying doses of Exo (0 μg/ml, 25 μg/ml, 50 μg/ml, and 100 μg/ml) to determine its influence on EMT.

2.6. In vivo tracking internalization of exosomes

DIR (DIR; Molecular Probes) was used to label HEHUCMSC and SN-Exos following the manufacturer's instructions. An intravenous injection of a complete dye combination into regular mice served as a negative control, while labelled exosomes that dye mixture (not include exosomes), and PBS were injected into the tail veins of Balb/c mice with a 1.5 × 2 cm dorsal wound. Fluorescence pictures of exosome distribution were obtained using 740 nm excitation and 790 nm emissions filters on Day 1, Day 3, Day 7, and Day 21 after mice were anaesthetized for observation under a bioluminescence system.

2.7. Cytometry analysis

EECs were gathered, washed, and re-suspended in 200 μl of binding buffer after being treated with varying doses of H_2O_2 & HEHUCMSC& SN for 24 and 48 h, respectively. The EECs were subsequently incubated with Annexin V-FITC for 15 min at room temperature, followed by 30 min in the dark with PI at 4° Celsius. Finally, flow cytometry was used to study apoptosis. According to researchers [20].

2.8. Functional test

Researchers have looked into how HEHUCMSC and SN -exo affect fertility in terms of outcomes like decreased time to conceive, improved pregnancy rates, and enhanced embryo implantation. Beginning four weeks after treatment, female rats from every one of the three groups (n = 6 per group) cohabited with viable males in a 1:1 ratio for a total of eight weeks. Vaginal smear tests were performed on female rats every morning for 8 weeks, from the beginning of mating till the discovery of sperm. The first day of gestation, or GD0, was officially recognised. Female rat body weight was measured before mating, at GD4, GD8, GD10, and GD14 to find the optimal time to prevent reproductive problems after mating. The gestation period was calculated based on the timing of mating and fluctuations in body weight. Pregnant rats were murdered 15 days later to determine the number of embryos. The uterine horns were both cut off. The number of gestating rats and embryo implants was counted after 4 and 8 weeks of reproduction to calculate the pregnancy rate. Time to conceive is defined as the amount of time it takes from the end of therapy (four weeks) to the detection of pregnancy (twelve weeks). We assessed the gestation period and the number of embryos implanted after an initial 8-week reproductive cycle.

2.9. Histology analysis

Six uteruses from each treatment group were preserved in 4% paraformaldehyde and embedded in paraffin according to the procedures outlined in the journal Reproductive Science. Paraffin slices were cut at a thickness of 4–6 m, and H&E and trichrome stains were applied in accordance with established protocols. The mean area % of collagen fibre, number of glands, and endometrial thickness were all determined using Image Pro Plus software (version 6.0). Under a light microscope, the morphological alterations were seen. The average number of glands per HPF was calculated, and the horizontal separation across luminal interfaces

and the middle muscle layer on each HE-stained slice was defined as the endometrial thickness. The extent of endometrial fibrosis was determined by selecting four high-power fields (HPF) from each Masson-stained slide and calculating the ratio of fibrotic areas to total endometrial areas (excluding the uterine cavity) in Image Pro Plus 6.0. We determined typical proportions for the whole group.

2.9.1. Statistical analysis

The average and standard deviation (SD) for continuous variables were plotted. One-way analysis of variance followed by Tukey's multiple comparisons test was used to determine statistical significance between the groups. Prism (GraphPad, San Diego, USA) was used to analyse all of the data, and when a difference had a probability (p) value of less than 0.05, or 0.01, it was considered to be statistically significant.

3. Result and discussion

3.1. Characterization

The spherical shape of MSC-derived exosomes was shown by Transmission Electron Microscopy and hydrogel loading as determined by Characterization of the exosome from HUCMSCs and SN structure. Dynamic light scattering (DLS) utilising Zetasizer software (7.11 version) was used to analyse the size distribution of the isolated exosome nanoparticles. Both isolated exosomes showed hydrogel of similar size, perhaps around 100 nm, in a DLS analysis. The vesicle size distribution was confirmed using DLS (Fig. 1A and B). Exosome presence was characterised using TEM, DLS, and another conformational test. Fig. 2 displays the results of a Scanning Electron Microscopy analysis of Exosomes that have been placed onto hydrogel. Content of exosomes isolated using ultracentrifugation

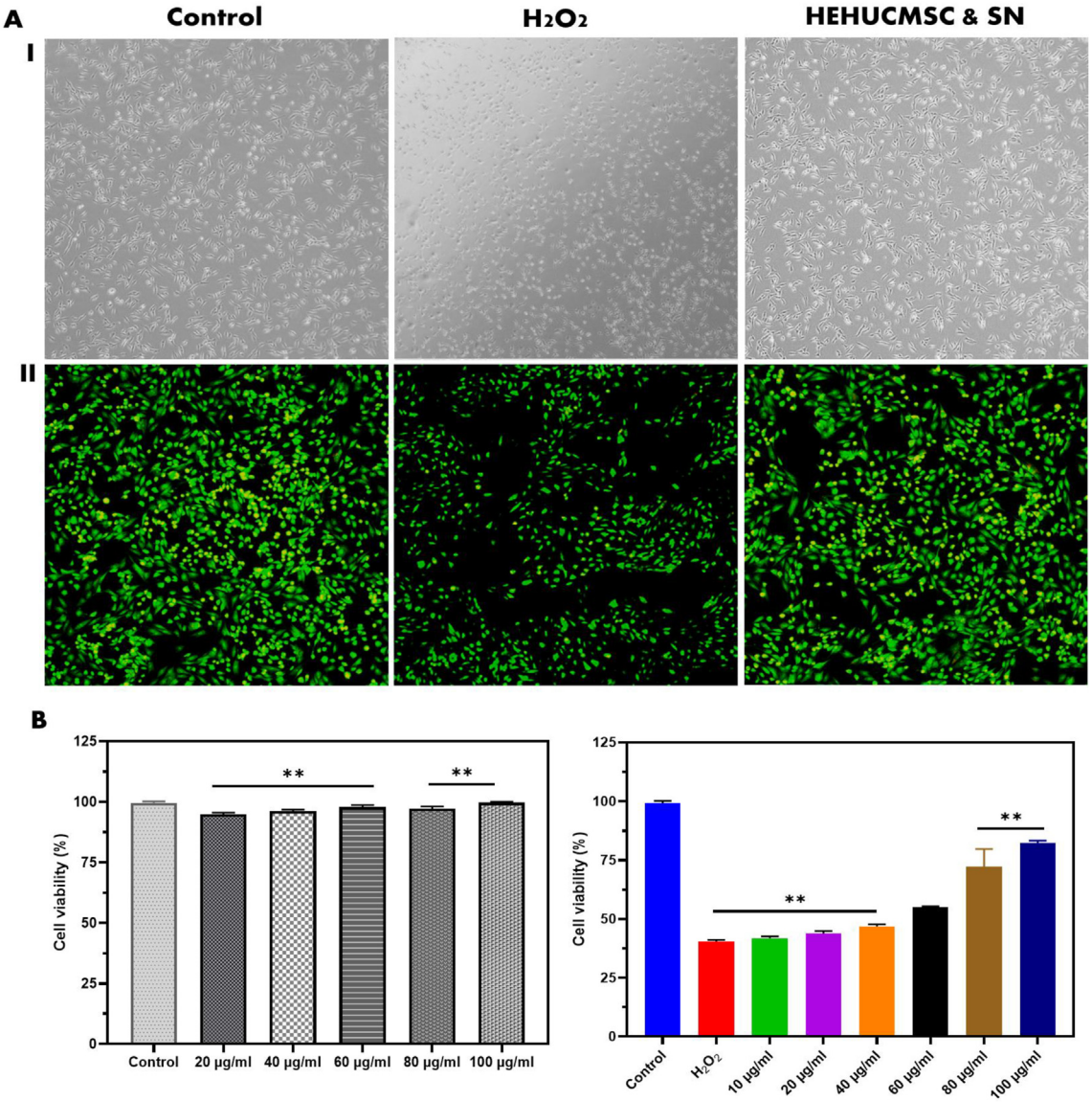

Fig. 4. Morphology of endometrial epithelial cells, identification and post induction structural changes among treated groups of Phase contrast (I) and Fluorescent microscopic (II) view at 4× magnification at 200 µm (A). Cell viability (%) statistically significance $p < 0.01$ (B).

was comparable to that of a commercial kit. Both methods of isolation produced spherical exosomes, as seen in electron microscopy, however the density of exosomes appeared higher in the ultracentrifugation method than in the commercial kit isolation method [21].

3.2. Western blot analysis

Compared to the IUIH group, which was similar to the sham group, the HEHUCMSC & SN group showed significantly greater expression of integrin-β3, LIF, and VEGF protein. Our results showed that VEGF expression was higher in the HEHUCMSC & SN group than in the IUIH group. Proteins associated with the endometrium were tested by Western blotting at the same time. Protein expression histograms (Fig. 3) show that integrin-β3, LIF, and VEGF are all present. This finding is consistent with the findings of the recent study by Kilic et al. [22], who demonstrated that ADSC therapy increased VEGF expression. Endometrial VEGF expression was reported to be reduced following intrauterine implantation of ADSCs by Hunter et al. [23], although this discovery is at odds with the current one.

3.3. Culture of endometrial epithelial cells (EECs)

EECs were studied for their cell morphology. On day three after inoculation, cells began to stick to the culture flask, and after 24 h, some EECs adhered to the culture flask in the shape of uneven triangles. Phase contrast and fluorescence microscopy were used to demonstrate differences in the structure of endometrial epithelial cells between the treated groups after induction (Fig. 4: I & II). The cells seemed round or elliptical, with well-defined borders and abundant cytoplasm. The cell's nucleus can be found near its epicentre. The cells were packed in like bricks in a wall [13].

3.4. Internalization of exosomes

Researchers have found that exosomes can infiltrate target cells and alter their behaviour biologically. Therefore, we looked at whether spermidine and exosomes from human umbilical cord stem cells had any effect. Cellular uptake of H_2O_2 & HEHUCMSC & SN hydrogel laden exosomes was assessed by fluorescence microscopy in our *In vitro* tracking experiment after incubation with labelled exosomes for 24 h. H_2O_2 and HEHUCMSC& SN exosomes were Dil and DAPI dye-labelled for 24 h (Exosome (+)) and compared to cells cultured with dye alone (Exosome (−)) (Fig. 5). This study supports the hypothesis that fibroblasts are capable of internalising ASCs-Exos because it shows that exosomes can enter the cytoplasm of fibroblasts and are primarily localised to the perinuclear area. Numerous reports have highlighted the importance of exosomes, which originate in stem cells, in facilitating tissue healing. Umbilical cord mesenchymal stem cell exosomes stimulate collagen synthesis, while iPS-Exos (human induced pluripotent stem cell exosomes) positively affect endothelial cell

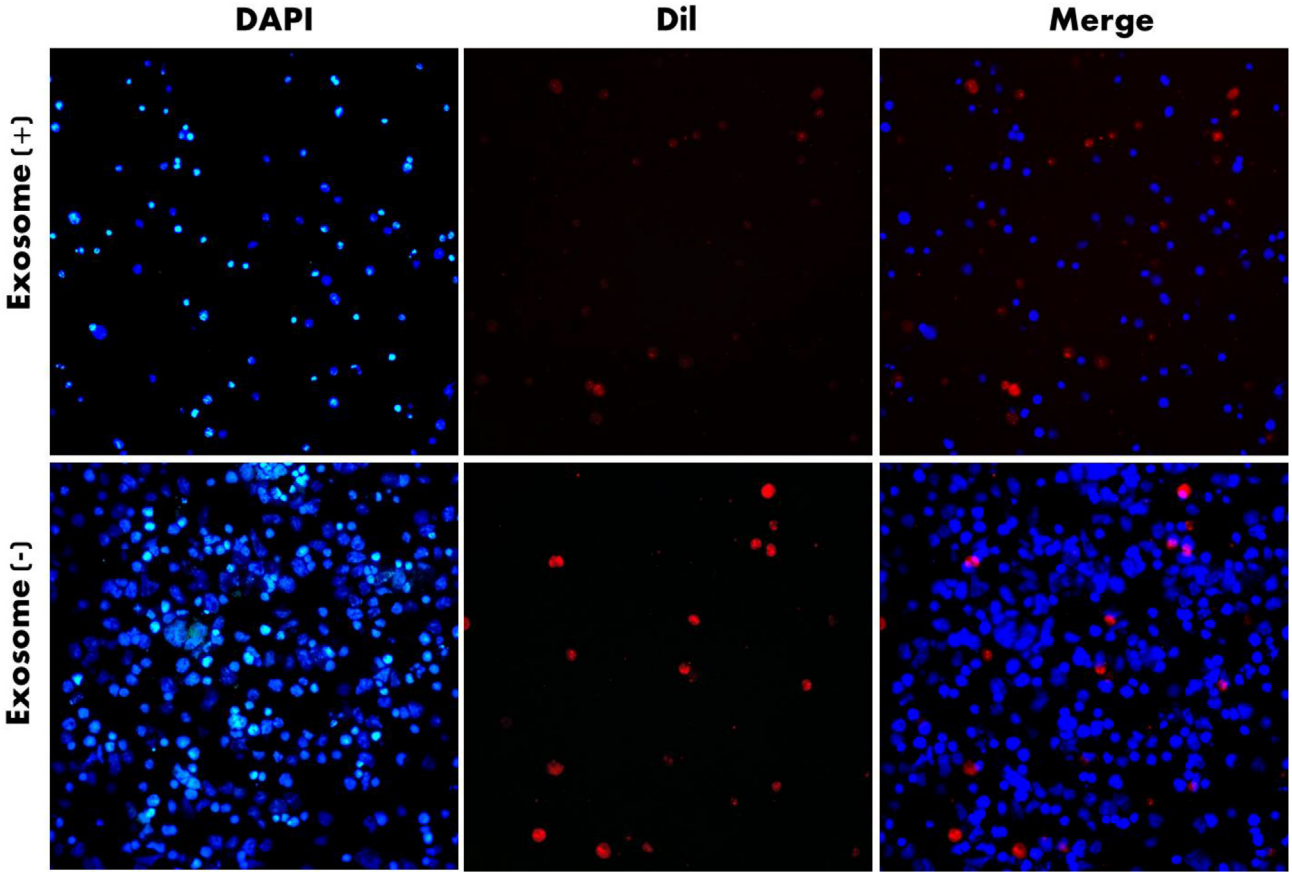

Fig. 5. Cellular internalization of HEHUCMSC and SN exosomes with dye (Dil) labelled for 24 h (Exosome (+)) and cells incubated with dye without exosomes as negative (Exosome (−))10 × , scale bar 100 μm.

migration, proliferation, and tube formation38. Exosomes are thought to have this effect because they contain several RNAs and proteins that are important to fibroblast activity [24].

3.5. Evaluation of flow cytometry

The apoptosis of H_2O_2 and HEHUCMSC& SN was measured with flow cytometry. Early and late apoptosis (Fig. 6) rates were considerably higher than the control group ($P < 0.05$) after treatment with 100 μg/mL TGF-1 for 24 h, as indicated in Fig. 6. The apoptotic rates of the H_2O_2 and HEHUCMSC& SN groups at 24 and 48 h post-treatment were compared to those of the Sham group and the IUIH group at the same time ($P < 0.05$ for each comparison). The late apoptosis rate was considerably higher than control ($P < 0.05$) after treatment with 60 g/mL or 110 ng/mL TGF-1 for 48 h, as reported by yao et al., 2019. Therapy with TGF- 1 (60 ng/mL and 110 ng/ml) for 48 h resulted in a considerably increased early apoptotic rate compared to therapy for 24 h. After 24 h of treatment, 60 ng/mL TGF-1 was found to strongly trigger apoptosis in EECs. Therefore, it was discovered that 60 ng/ml TGF-1 for 24 h is the best induction concentration and time [25].

3.6. Pregnancy outcomes

Rats with IUIH have their pregnancy rates, embryo implant rates, and conception times compared across multiple treatments. Pregnancy results at day 15 between the sham, IUIH, and HEHUCMSC & SN groups according to embryo insertion sites on the uterine horns are shown in Fig. 7. At 8 weeks post-treatment, there was no statistically significant difference in the aggregate pregnancy rate between the three groups. Compared to the sham group, the number of embryos implanted was lower in the IUIH group

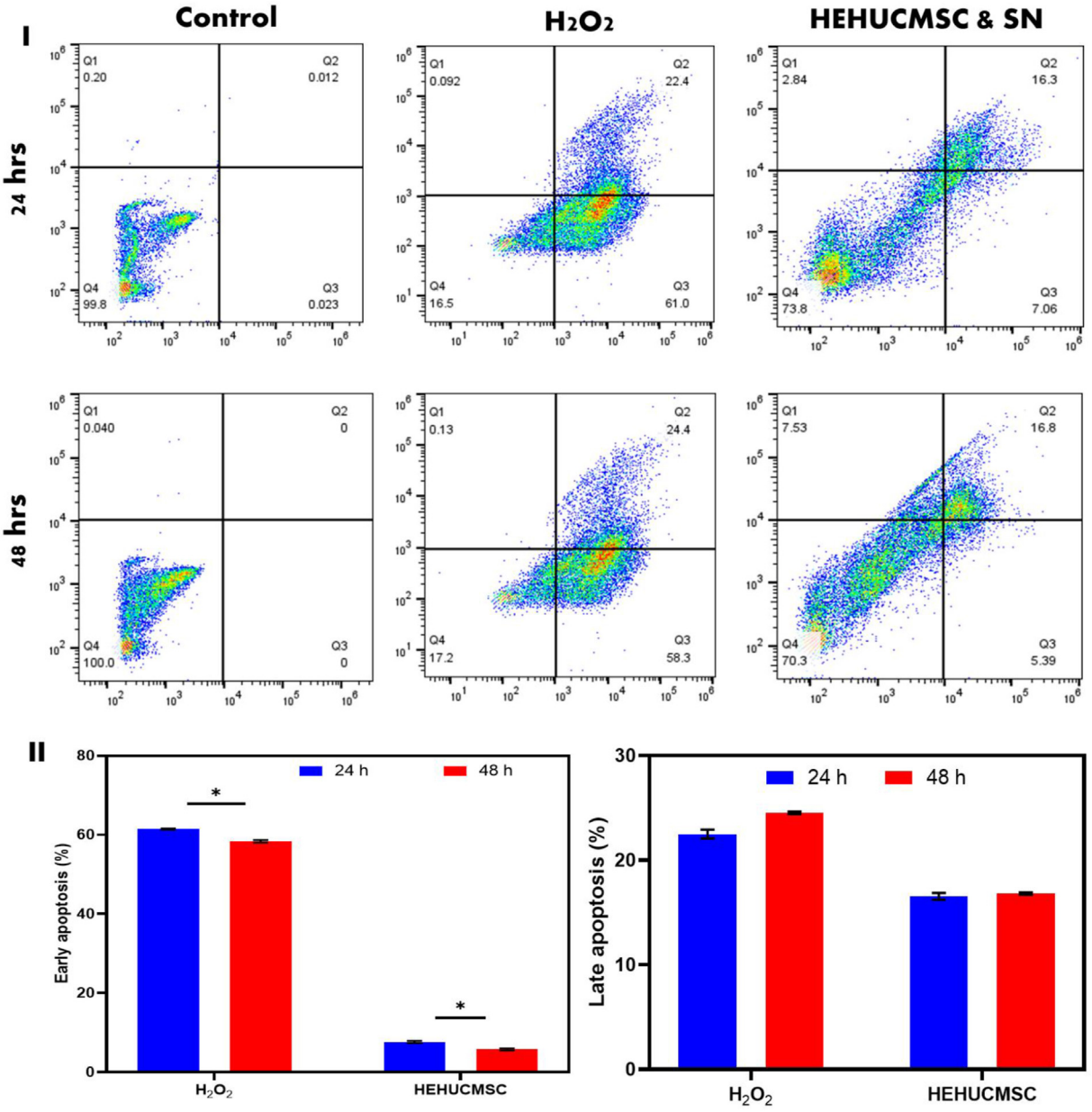

Fig. 6. Comparison of apoptotic rates between groups under intervention of different groups $P < 0.05$, compared to control group at the same time point; $P < 0.05$, compared to H_2O_2 at the same time point $P < 0.05$, compared HEHUCMSC & SN between 24 h and 48 h at the same concentration.

(*p < 0.05). In contrast, the number of embryos implanted was substantially higher in the HEHUCMSC & SN group than in the IUIH group (*p < 0.05). Additionally, we assessed the impact of reproduction on fertility across demographic groups. Eight weeks following treatment, researchers reviewed data on the total amount of inserted embryos and the length of time it took mice to conceive. Percentages are displayed, and significance at the p < 0.05 level is indicated (Fig. 7: II). When compared to controls, however, there was actually not a significant difference in the time it took to conceive after ADSC-exo treatment (p > 0.05) [12]. The adhesion level is also correlated with the endometrial thickness and volume. Most research have focused on vascular endothelial growth factor (VEGF) as a regulator of endometrial angiogenesis, and some have shown that thin endometrium affects VEGF expression. The endometrium is damaged and becomes less receptive if adhesions are widespread and severe [26]. We used H&E and Masson staining to evaluate the endometrium's histological healing following ADSC-exo therapy. It agrees with previous research that has used MSCs to heal endometrial damage. Based on these findings, it was hypothesised that instead of clonally expanding, transplanted cells emit paracrine substances that aid in endometrial regeneration [22].

3.7. Histological examination analysis

3.7.1. The H&E data

The administration of HEHUCMSC and SN led to an enlargement of the uterine cavity, a restoration of the endometrial surface characterized by an elevation in columnar epithelial cells, and a rise in the number of endometrial glands (Fig. 8: I). A mean standard

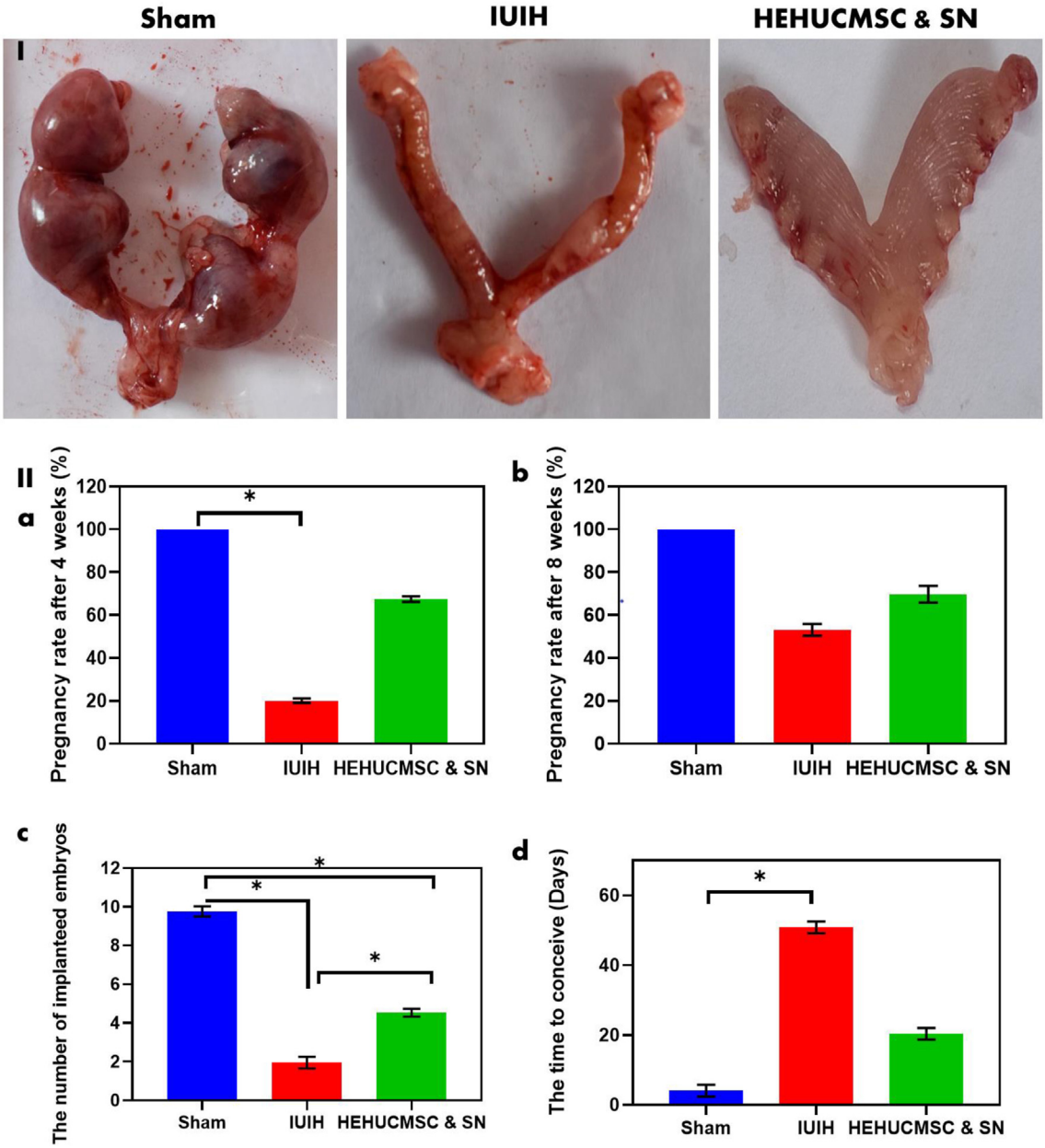

Fig. 7. Pregnancy outcomes in different groups. (I) Embryo implantation sites on the uterine horns of sham, IUIH, and HEHUCMSC & SN groups at the fifteenth day of pregnancy. (II) Reflection on fertility of different groups. (a) The pregnancy rate after 4 weeks of treatment (b) The pregnancy rate after 8 weeks of treatment (c) The number of implanted embryos (d) The time to conceive at 8 weeks after treatment. Data are represented as percentage or mean ± SEM; and significant to *p < 0.05.

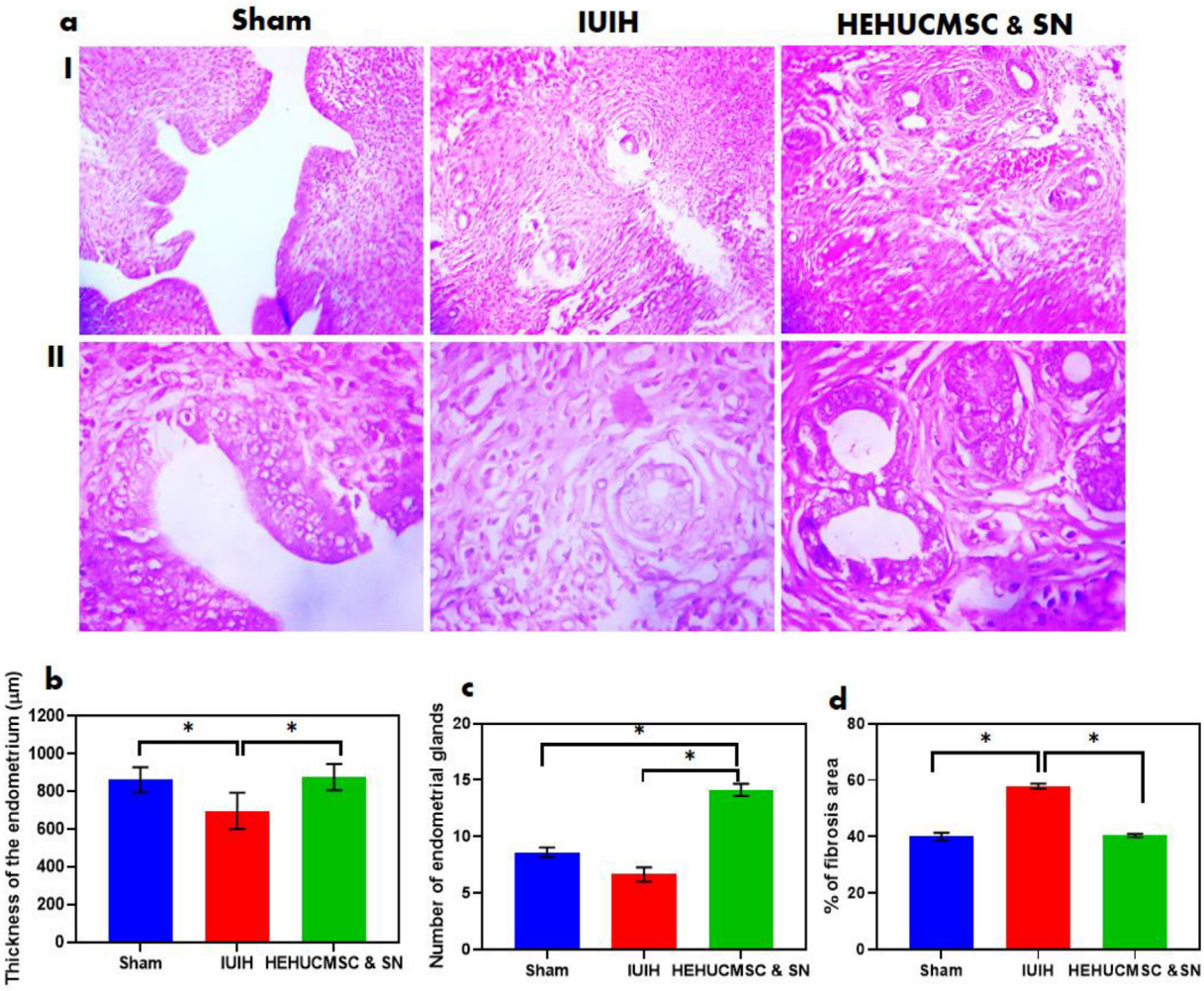

Fig. 8. H&E staining of the uterine tissue in three groups (a). (I) H&E staining of the uterine tissue and its magnifications (II). The uterine cavity of the Sham group was open, the endometrial surface was covered with simple high columnar epithelial cells, The uterine cavity of IUIH group narrowed, and in HEHUCMSC & SN groups the uterine cavity enlarged. The results of histological examination analysis (b) Endometrial thickness analysis (c) Number of endometrial glands (d) percentage of fibrosis area. Data are presented as mean ± SEM and are significantly analyzed *$p < 0.05$.

analysis (Fig. 8: II) was conducted on the histological data to assess the endometrial thickness, the quantity of endometrial glands, and the proportion of fibrosis region. Beyond uterine cavity enlargement, HUCMSCs and spermidine's effects on endometrial regeneration, stem cell fate, angiogenesis, immunomodulation, exosome-mediated effects, ECM remodeling, and molecular changes can help explain the treatment's effects on the uterine microenvironment and its ability to promote tissue repair and pregnancy. These details can help interpret results from studies on these drugs' efficacy and mechanisms. The endometrial surface of the sham group had been covered with basic high columnar epithelial cells, and the sham group's endometrial glands and tiny blood vessels were concentrated in the submucosa and basal layer, as shown with H&E staining. The uterine cavity and glands beneath the epithelial layer shrank in the IUIH group four weeks after induction. The mean endometrial thickness for the three groups was 738.29 ± 18.16 mm, 526.90 ± 79.68 mm, and 782.45 ± 43.40 mm. The thickness of the participants in the sham and ADSC-exo therapy groups was greater than that of the IUA group. 8.17 ± 0.47, 4.96 ± 1.25, and 12.04 ± 1.69 endometrial glands were counted in the three groups, respectively. Compared to the IUA group, the sham group and the ADSC exosome therapy group had significantly *$p < 0.05$ higher numbers [12].

4. Conclusion

Exosomes isolated from HUCMSCs and spermidine were used to successfully construct biopolymer based hydrogels. This hydrogel shows promise as a medication delivery technology for improving rat endometrial thickness and conception rates. Characterization of exosomes was performed using Treatment with injectable hydrogel for thin endometrial regeneration has been shown to be effective through embryonic implantation. The biological compatibility of HUCM stem cell and spermidine-derived exosomes has been extensively studied in various aspects, including endometrium epithelial morphology, exosome internalization, flow cytometric analysis, the results of pregnancy in an intrauterine embryonic assay, and immunohistochemistry. These structures possess biophysical and rheological characteristics that preserve their form and enhance their practicality within the uterine cavity.

Ethical statement

The study was approved by Experimental Animal Ethics Committee of School of Basic Medicine of Jilin University No. 2022472.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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