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Morphological Changes and Expression of Cytokine After Local Endometrial Injury in a Mouse Model

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Abstract

Objective: To establish a mouse model for endometrial injury and determine the underlying mechanism regarding its favorable effect on embryo implantation. **Study Design:** Female Kunming mice were randomly allocated into 4 groups: group I, normal control; group II, injury procedure control; and group III and group IV, the mice being scratched with a blunt syringe on the right uterine horn or both, respectively. All the mice were mated with the males during the next estrus phase. The number of implanted embryos on each side of uterus was calculated on day 8 of pregnancy. The endometrial samples were taken on day 4 of pregnancy, and the local morphological changes and cytokine expressions were examined. **Results:** Compared to group II, our results showed that in group IV (1) there were significantly higher numbers of implanted embryos, (2) the endometrial glands and vasculatures in stroma were obviously increased and the pinopodes were abundant and well developed, and (3) the local levels of cytokines leukemia inhibitory factor (LIF) and oncostatin M (OSM) messenger RNA and protein expression were significantly increased. **Conclusions:** Local mechanical injury on mouse uteri enhanced endometrial receptivity and improved embryo implantation, which were correlated with the characteristic changes in endometrial morphology and the upregulation of *LIF* and *OSM* gene and protein expression.

Keywords

local endometrial injury, endometrial receptivity, leukemia inhibitory factor, oncostatin M, mouse model

Introduction

Implantation is a multistage process involving blastocyst apposition and attachment to the uterine endometrium and subsequent invasion of the trophoblast into the stroma of the uterine wall. It has been estimated that about 75% of concepti are lost before or at the time of implantation.[1] Implantation failure may be attributed to a problem with the embryo or with the environment in which the embryo tries to implant. However, during an in vitro fertilization/embryo transfer (IVF-ET) cycle, even if good-quality embryos (good shape and cell numbers) are transferred to a normal uterus, repeated implantation failure (RIF) may occur.[2] Inadequate uterine receptivity was blamed to account for approximately two-thirds of implantation failures.[3] To improve the endometrial receptivity, various strategies have been suggested, including hysteroscopic correction of cavity pathologies, myomectomy, treatment of thin endometrium, immunotherapy, and endometrial stimulation by biopsy.[4-7] Among these solutions, local endometrial injury has been recognized as a promising option.

In the last decade, a number of clinical reports have confirmed the favorable effect of local endometrial injury on clinical pregnant rates in couples with unexplained infertility or experiencing RIF after IVF-ET.[8-14] In 2003, Barash et al first reported that endometrial biopsies before IVF treatment cycle can double the rates of implantation, clinical pregnancies, and live births.[8] Recently, a systematic review and meta-analysis of studies comparing the efficacy of endometrial injury versus no intervention in women with RIF after IVF-ET showed that inducing injury was 70% more likely to result in a clinical pregnancy as opposed to no treatment.[15] It was postulated that local mechanical injury can induce inflammatory response and angiogenesis reaction, which facilitate the preparation of the endometrium for implantation and subsequent pregnancy outcome.[13] However, the exact mechanism by which the local injury improves pregnancy remains to be investigated.

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Although a number of studies have shown a significant correlation between the incidence of local endometrial injury and the improved pregnancy, the merits of endometrial injury on clinical outcomes in IVF clinics remain controversial. For example, Baum et al did not find any benefit from local injury to the endometrium in women with a high number of RIF.[16] Due to the limitations of human endometrium sampling and lack of suitable animal models for endometrial injury, the efforts on the exploration of endometrial injury and repair mechanism, as well as its clinical treatment evaluation, are being limited. Therefore, there are merits for both research and clinical application to establish efficient animal models for endometrial injury.

As early as 1907, Loeb reported that scratching the guinea pig uterus provoked decidualization during the secretory phase of the estrous cycle and resulted in improved receptivity of the uterus to implantation.[17] The similar effect was also observed by injecting oil into the endometrial cavity in mice.[18] In the current study, we used simple curettage by a blunt syringe to construct a model of endometrial injury in mice. Through this model, we further determined the underlying mechanism for the improved embryo implantation in terms of morphology and cytokine expression. The changes in the endometrial morphology and pinopodes ultrastructure were observed using hematoxylin and eosin (H&E) staining and scanning electron microscopy (SEM), respectively. The levels of local cytokines leukemia inhibitory factor (LIF) and oncostatin M (OSM) messenger RNA (mRNA) and protein expression were detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blot analysis, respectively.

Materials and Methods

Animals

Adult female virgin mice of the Kunming strain (weighing 25-30 g) and fertile males of the same strain (weighing 30-35 g) were purchased from the Medical Experimental Animals of Hubei Province (Wuhan, China) for studies approved by the Animal Care and Use Committee of Tongji Medical College (Certificate No. 00021013). Mice were housed in vivarium cages under standard conditions (22°C and on a 12-h light–dark cycle) and provided food and water ad libitum. Estrus was identified by daily vaginal smear. The study was approved by the local ethics committee.

Experimental Design

Female Kunming mice were randomly allocated into 4 groups at the estrus phase, which was identified by daily vaginal smear. The normal control group (group I, n = 20) was composed of untreated mice that were naturally mated during their estrus phase. The injury procedure control group (group II, n = 20) just had abdominal opening alone. Unilateral injury procedure was performed in 1 uterus and another uterus remained normal to serve as a self-control (group III, n = 20). Bilateral injury procedure in both uteri was performed (group IV, n = 20). The local injury to the endometrium was done by the following procedures: first opening the abdomen and exposing the uteri after being anesthetized and then using the blunt syringe inserted into the uterine lumen and reached at 0.5 cm for endometrial scratching at 3 fixed sites of the uterus (one near the uterus horn at 1 cm, another at the middle of the uterus, and last at the bottom of the uterus). At the next estrus phase, 2 females and 1 male were housed in each cage at night. The next morning in which a vaginal plug was found was designated D1 of pregnancy. The pregnant mice were killed by cervical dislocation at noon on the eighth day of pregnancy, and the uteri were excised immediately. The uteri were photographed, and the number of implantation sites was determined.

Tissue Collection and Histological Analysis

The mice were killed by cervical dislocation on the fourth day of pregnancy and the whole uteri were collected promptly. Part of the excised uteri was rapidly fixed in 10% formalin, embedded in paraffin, and cut into 5-μm sections. The H&E staining was used for histological evaluation according to the criteria of Noyes et al.[19] The remaining endometrial tissues were stored at −80°C and used for the detection of LIF and OSM mRNA and protein expression.

Scanning Electron Microscopy

Three pregnant mice on the fourth day of pregnancy in groups I, II, and IV, respectively, were used for SEM to detect the uterine morphology. Mouse uteri were excised and fixed immediately in 2.5% glutaraldehyde. The fixed samples were washed 3 times (15 minutes each) with 0.1 mol/L phosphate-buffered saline and fixed for the second time in 1% osmium tetroxide for 1 hour at room temperature. Subsequently, the samples were dehydrated using increasing concentrations of alcohol (50%, 70%, 80%, 90%, and 95%) and isopentyl acetate (15 minutes each), then dried in freezing conditions and coated with gold. Images of the surface structure of the treated samples were acquired using a scanning electron microscope (VEGA3LMU; TESCAN, Czech Republic).

Analysis of Gene Expression by Real-Time RT-PCR

To assay the gene expression of LIF and OSM in the endometrium during the implantation window after local mechanical injury, the pregnant mice were killed on the fourth day of pregnancy and the uterine endometrial tissues were collected. Total RNA was extracted from frozen endometrial tissues with Trizol reagent (Ambion, Quantity, Grand Island, New York) in accordance with the manufacturer's instructions. The complementary DNA (cDNA) templates for PCR analysis were synthesized from the total RNA in accordance with the instruction of the first-strand cDNA synthesis kit (Fermentas, Toronto, Canada). Quantitative PCR was conducted using SYBR Green PCR Master Mix Reagent (SYBR Premix Ex Taq kit, Cat. DRR041A; TaKaRa Clontech, Mountain View, California). The PCR reaction mixes for each standard and sample were

Table 1. Primer Sequences for Real-Time PCR.

Genes	Primer Sequence	
LIF	Forward	5′-GGCAACCTCATGAACCAGAT-3′
	Reverse	5′-ACCATCCGATACAGCTCCAC-3′
OSM	Forward	5′-ACGGTCCACTACAACACCAGTA-3′
	Reverse	5′-TGGAGCCATCGTCCCATTC-3′
β-*Actin*	Forward	5′-GTCCCTCACCCTCCCAAAAG-3′
	Reverse	5′-GCTGCCTCAACACCTCAACCC-3′

Abbreviations: LIF, leukemia inhibitory factor; OSM, oncostatin M; PCR, polymerase chain reaction.

prepared in separate tubes, using Sybergreen II (TaKaRa Clontech), primers (synthesized by Invitrogen), and cDNA. All samples were a 20-μL aliquot of each reaction mix and were transferred to a well of a MicroAmp optical 96-well reaction plate (MX3000P; Stratagene, Santa Clara, California) to perform reactions. The reaction conditions were as follows: 95°C for 10 minutes; 40 cycles of 95°C for 10 seconds, 58°C for 20 seconds and 72°C for 15 seconds; and melting curve from 60°C to 95°C, increasing in increments of 0.5°C every 5 seconds. The primer sequences are shown in Table 1. Relative mRNA expression was calculated by the $2^{-\Delta\Delta CT}$ method, as described previously.[20]

Western Blot Analysis

To assay the levels of LIF and OSM proteins in the endometrium during the implantation window after local mechanical injury, the pregnant mice were killed on the fourth day of pregnancy and the uterine endometrial tissues were collected. Proteins were prepared from frozen uterine tissues by homogenization and lysis in extraction buffer (100 μg/mL PMSF and RIPA). Proteins and prestained molecular weight markers were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by transfer onto nitrocellulose membranes. The membranes were blocked with 5% fat-free powdered milk in TBST (10 mmol/L Tris, 150 mmol/L NaCl, and 0.05% Tween-20, pH 8.0) for 1 hour at room temperature and then incubated overnight in 1% TBST at 4°C with LIF purified goat polyclonal antibody (sc-1336; Santa Cruz Biotechnology, Inc, Dallas, Texas) or OSM mouse-antigoat polyclonal antibody (AF-495-NA; Santa Cruz Biotechnology, Inc) diluted in 1: 500. Following 3 washes with TBST, blots were incubated with the appropriate peroxidase-conjugated secondary antibody at dilution of 1:1000 at 37°C for 1 hour. After final washing with TBST, immunoreactive bands were visualized by enhanced chemiluminescence in accordance with the manufacturer's protocol. To evaluate the expression of LIF or OSM in tissues, the bands were scanned using a GS-800 scanning densitometer (BioRad, Hercules, California). The intensity of each protein band was quantified with BioRad Quantity One software analysis system (Biorad GelDoc XR; BioRad). The relative level of LIF or OSM was expressed as the intensity ratio of LIF or OSM to β-actin, which was calculated by the formula: LIF or OSM/β-actin = intensity of LIF or OSM band/intensity of β-actin.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism5 software (GraphPad, San Diego, California). Descriptive statistical analysis was performed initially to examine the distribution of data. Differences in the number of implanted embryos and the expression of LIF and OSM protein were analyzed using the unpaired t test. The differences in the expression levels of LIF and OSM mRNA were determined by Mann-Whitney U test. A value of $P < .05$ was considered statistically significant.

Results

Effects of Local Endometrial Injury on Embryo Implantation

The implantation sites were determined on day 8 of pregnancy (Figure 1A-D). Compared with group I (n = 14; 13.21 ± 0.70), the mean number of implanted embryos on both sides of the uteri was significantly decreased in group II (n = 13; 11.15 ± 2.19; Figure 2A; $P < .01$) and group III (n = 14; 11.64 ± 2.24; Figure 2B; $P < .05$) but was significantly increased in group IV (n = 13; 15.08 ± 2.43; Figure 2C; $P < .05$). Compared with group II (n = 13; 5.54 ± 1.05), the mean number of implanted embryos on the right side of the uterus was significantly increased in group III (n = 14; 7.50 ± 1.83; Figure 2D; $P < .01$). However, there was no significant difference in the total number of implanted embryos on both sides of the uteri between group II and group III (11.15 ± 2.19 vs 11.64 ± 2.24; Figure 2D; $P > .05$). As a self-control, the mean number of implanted embryos on the right side of the uterus (7.50 ± 1.83) in group III was significantly higher than that on the left side of the uterus (4.14 ± 1.51; Figure 2D; $P < .01$). The mean number of implanted embryos on each side of the uteri and the total number in group IV (n = 13) were significantly higher than those in group II (right side: 7.69 ± 2.69 vs 5.54 ± 1.05, $P < .05$; left side: 7.38 ± 1.81 vs 5.62 ± 1.98, $P < .05$; and both sides: 15.08 ± 2.43 vs 11.15 ± 2.19, $P < .01$, respectively; Figure 2E). Moreover, compared with group III (n = 14), the mean number of implanted embryos on both sides and the left side of the uterus was significantly increased in group IV (n = 13; both sides: 15.08 ± 2.43 vs 11.64 ± 2.24, $P < .01$; left side: 7.38 ± 1.81 vs 4.14 ± 1.51, $P < .01$, respectively; Figure 2F). However, the mean number of implanted embryos on the right side of the uterus had no significant difference between group III and group IV (7.50 ± 1.83 vs 7.69 ± 2.69; $P > .05$; Figure 2F). These results indicated that local mechanical injury on mouse uterus can improve the embryo implantation, and the mechanical injury on both uteri presented an optimal model better than that on one side of the uteri.

Impact of Local Endometrial Injury on Endometrial Morphology at the Duration of Implantation Window

As the results previously indicated that bilateral injury on mice uteri significantly increased the competence of embryo implantation, we then detected its effect on uterine morphology

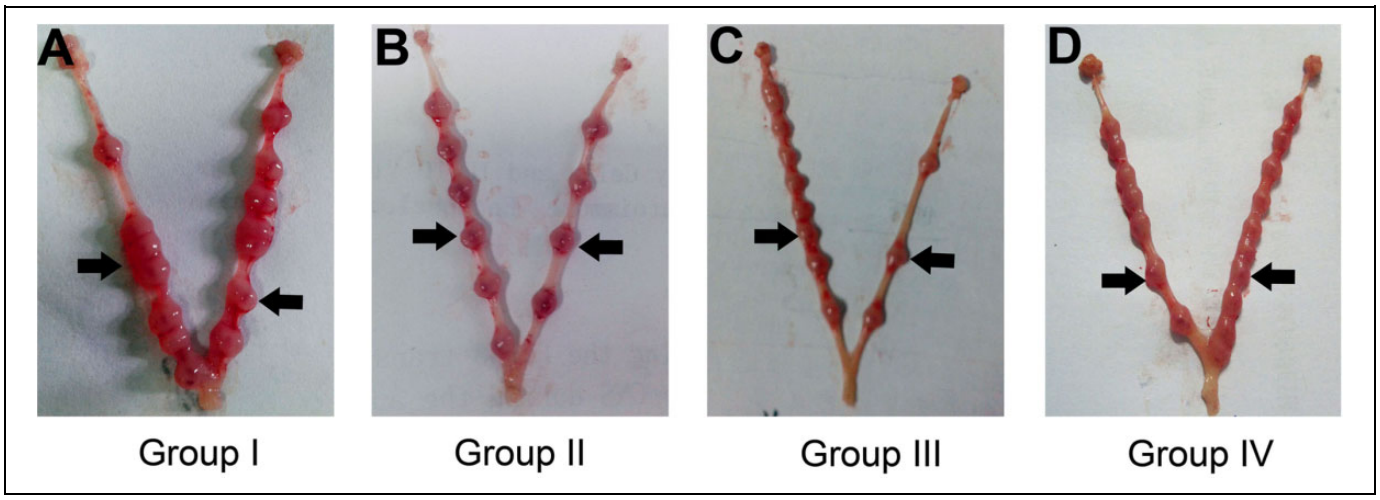

Figure 1. Representative image of implanted embryos in mouse uteri on the day 8 after coitus in each group. Group I (normal control; A), group II (injury procedure control; B), group III (the mice were scratched with a blunt syringe on the right side of uterus and the left side served as a self-control; C), and group IV (the mice were scratched with the blunt syringe on both sides of uteri; D). Arrows indicate implanted embryos. (The color version of this figure is available in the online version at http://rs.sagepub.com/.)

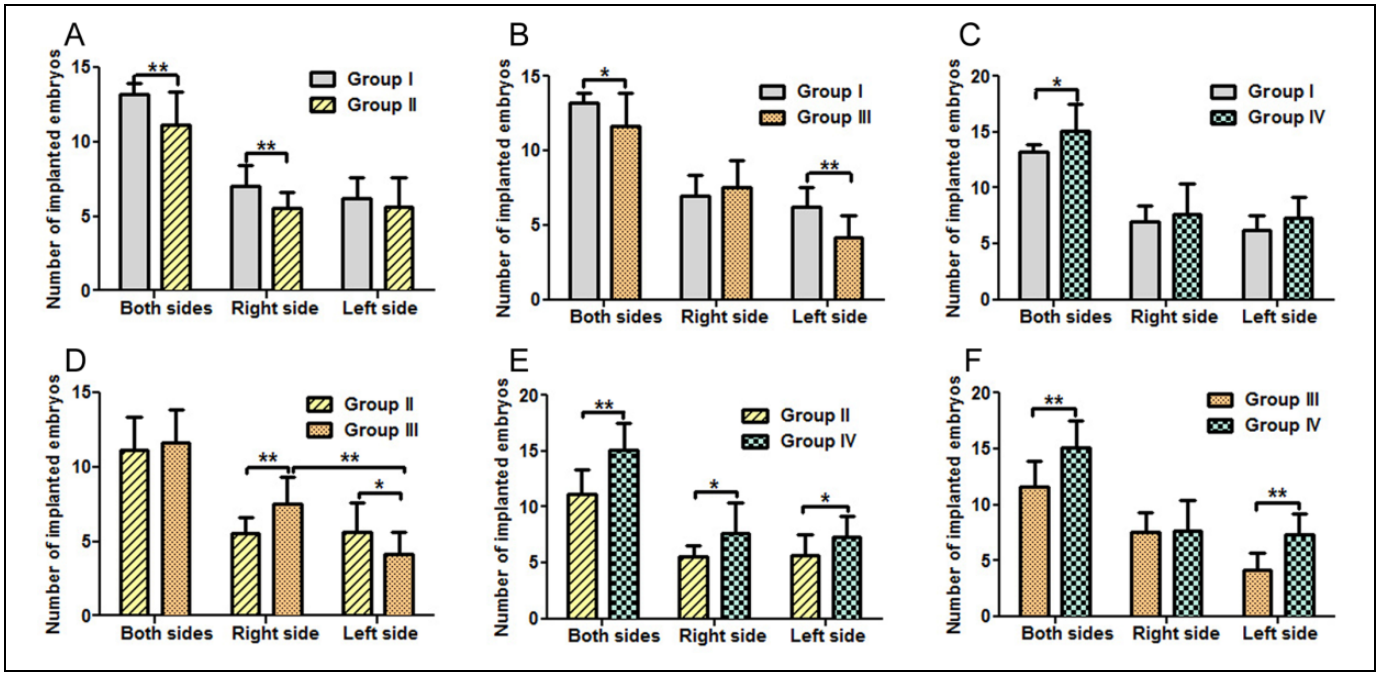

Figure 2. Comparison of the number of implanted embryos. Compared with group I (n = 14), the number of implanted embryos on both sides of the uteri was significantly decreased in groups II (n = 13; A; $P < .01$) and III (n = 14; B; $P < .05$) but was significantly increased in group IV (n = 13; C; $P < .05$). Compared with group II, the number of implanted embryos on the right side of the uterus was significantly increased in group III (D; $P < .01$). As a self-control, the number of implanted embryos on the right side of the uterus in group III was significantly higher than that on the left side of the uterus (D; $P < .01$). The number of implanted embryos on each side of the uteri and the total number in group IV were significantly higher than those in group II (right side: $P < .05$; left side: $P < .05$; and both sides: $P < .01$, respectively; E). Moreover, compared with group III, the number of implanted embryos on both sides and the left side of the uterus were significantly increased in group IV (both sides: $P < .01$; left side: $P < .01$, respectively; F). Data are expressed as mean $\pm$ standard deviation (SD). *$P < .05$, **$P < .01$.

as an indication of endometrial receptivity. By H&E staining on D4 of gestation, group I and group IV showed a closed lumen, while group II displayed an open lumen (Figure 3A-C). Moreover, the mouse uteri in group I and group IV showed scattered stroma cells, abundant glands, and increased vasculatures in the stroma as compared to those in group II (Figure 3D-F). Observation by SEM revealed that the luminal epithelial cells were covered by fully developed pinopodes in group I and group IV (Figure 3G and I), whereas the luminal epithelial cell surface was covered by the regressing pinopodes in group II (Figure 3H).

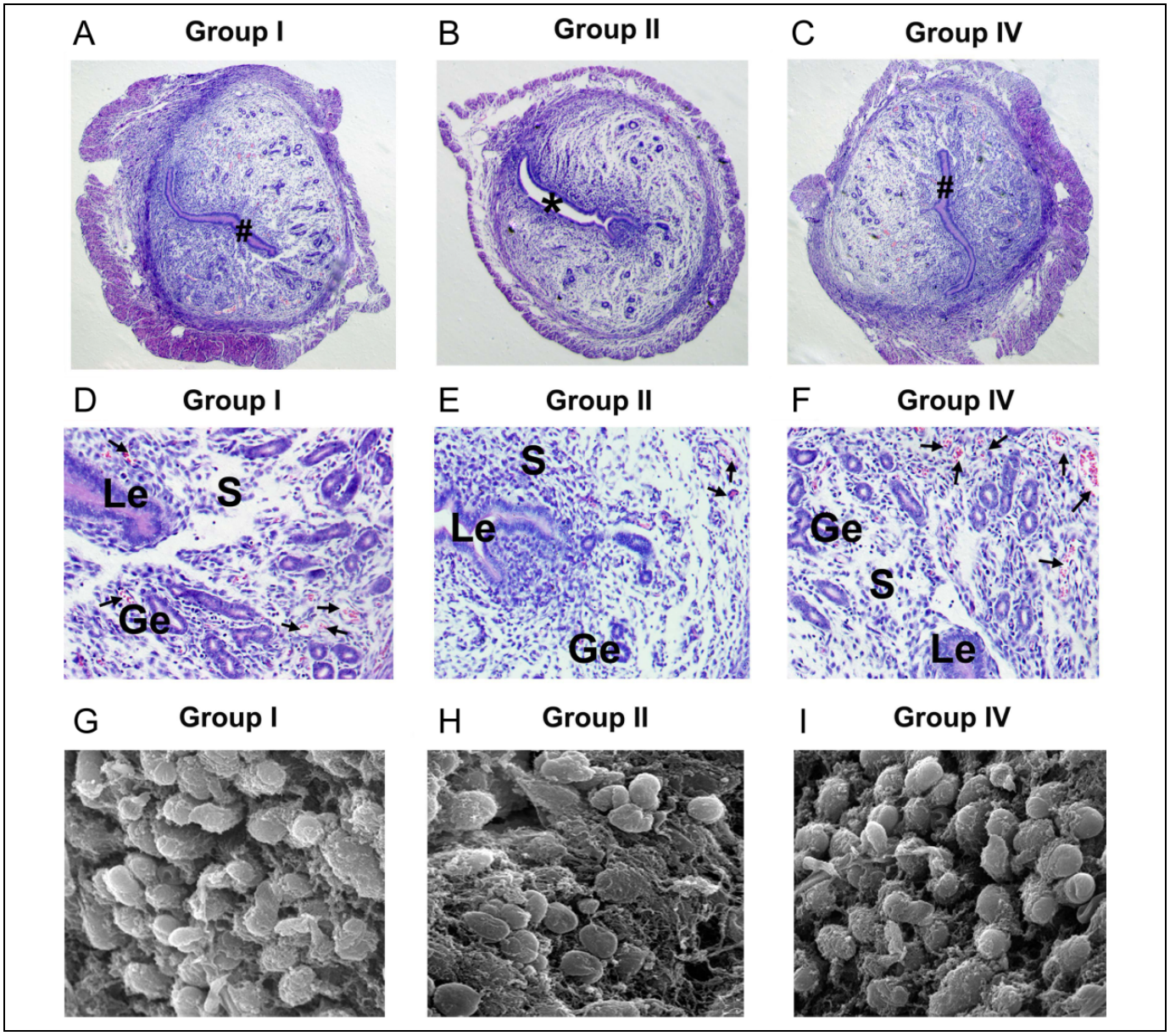

Figure 3. Impact of local endometrial injury on endometrial morphology at the duration of implantation window. A-F, Representative morphology of uteri on day 4 in pregnant mice detected by hematoxylin and eosin (H&E) staining. Groups I and IV showed a closed lumen (A and C), while group II displayed an open lumen (B). Moreover, the mouse uteri in groups I and IV showed scattered stroma cells, abundant glands, and increased vasculatures in the stroma when compared to those in group II (D-F). Magnification: ×100 (A-C); ×200 (D-F). G-I, Representative scanning electron microscopy (SEM) of the lumen of uteri on day 4 in pregnant mice. The luminal epithelial cells were covered by fully developed pinopodes in group I and group IV (G and I), while the luminal epithelial cell surface showed regressing pinopodes in group II, in which cell bulging decreased. Representative photograph of 3 tissues. Magnification: ×2000. Ge indicates glandular epithelium; Le, lumen epithelium; S, stromal cell; #, closed uterine lumen; *, opened uterine lumen; →, vascular.

These results together demonstrated that the mice after local endometrial injury had enhanced receptivity for embryo implantation at the duration of implantation window.

Impact of Local Endometrial Injury on the Expression of LIF and OSM mRNA and Protein

Leukemia inhibitory factor is one of the most vital cytokines influencing the endometrial receptivity for embryo implantation by regulating the function of trophoblasts and vascular formation of placenta. Its absence leads to implantation failure in mice. The OSM promotes angiogenesis, which is a highly regulated process that is essential in embryogenesis, and normal physiological growth and tissue repair. Compared to group II, the levels of both LIF and OSM mRNA expression were significantly increased in group IV (Figure 4A and B; LIF: $P < .01$ [group I vs group IV]; $P < .01$ [group II vs group IV] and OSM: $P < .01$ [group II vs group IV]; $P < .05$ [group I vs group II]).

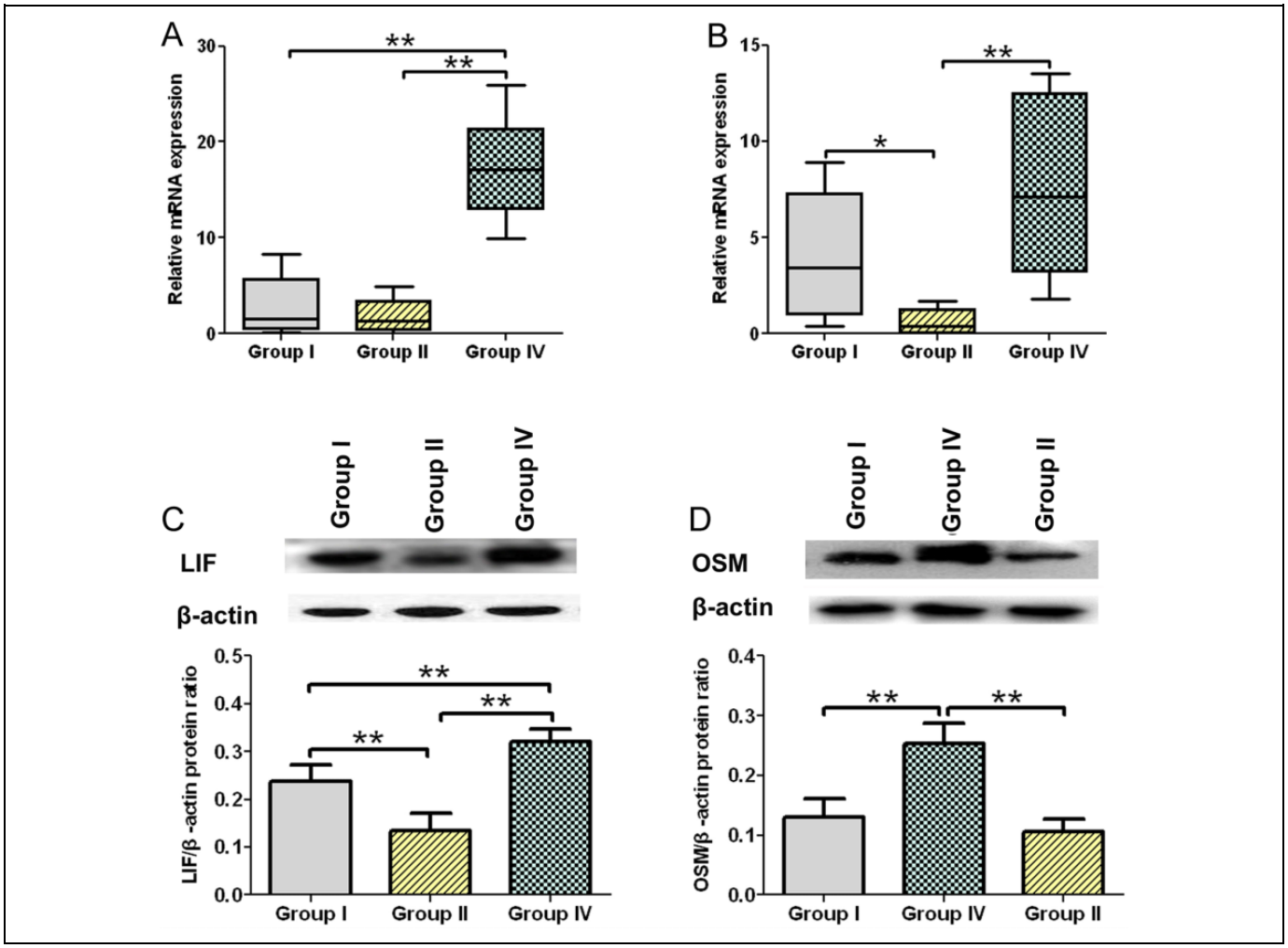

Figure 4. Comparison of expression levels of LIF/OSM mRNA and protein. The mice endometrial samples were taken at day 4 of pregnancy. A and B, Comparison of LIF and OSM mRNA expressions. Using quantitative reverse transcription polymerase chain reaction (qRT-PCR) method, the levels of LIF and OSM mRNA expression were detected and significantly increased in mice after local mechanical injury in group IV (n = 7 each group). The bars in the box plots represent the median; whiskers, the highest and lowest values; the boxes, the interquartile ranges. C and D, Representative images of Western blot and comparison of protein levels for LIF and OSM. The protein levels of LIF and OSM were significantly increased in mice after local mechanical injury in group IV (n = 7 each group). The relative level of LIF or OSM was expressed as the intensity ratio of LIF or OSM and β-actin, which was calculated by the formula: LIF or OSM/β-actin = intensity of LIF or OSM band/intensity of β-actin. Data are expressed as mean ± standard deviation (SD). *$P < .05$, **$P < .01$. LIF indicates leukemia inhibitory factor; mRNA, messenger RNA; OSM, oncostatin M.

Moreover, LIF and OSM protein can be detected in all groups. Compared to groups I and II, the protein expression levels of LIF and OSM were also significantly increased in group IV (Figure 4C and D; LIF: $P < .01$ [group I vs group IV]; $P < .01$ [group II vs group IV]; $P < .01$ [group I vs group II] and OSM: $P < .01$ [group I vs group IV]; $P < .01$ [group II vs group IV]).

Discussion

Our findings in this study suggest that the simple curettage to the mouse uterus with the blunt syringe at the estrus cycle could promote implantation efficacy. Using this model, we demonstrate that the injury-induced improved implantation in mice results from the improved endometrial receptivity, which is characterized by 2 findings: local morphological changes as the hyperplasia of endometrial glands, increased vasculatures and well-developed pinopodes, and upregulation of local cytokines as LIF and OSM at the duration of implantation window.

The questions regarding the underlying mechanism of the procedure remain unanswered. Some animal models have been developed and supported the effects of mechanical manipulation on reproduction. In1907, Loeb initially reported that injury caused by scratching the guinea pig uterus during the progestational phase of the estrous cycle provoked a rapid growth of endometrial cells, which were similar to the decidual cells.[17] Other forms of local trauma were the administration of intrauterine oil or air injection and suturing the uterine horn, which

can induce the decidual formation in pseudo-pregnant mice.[18,21] Based on these early observations in rodents, one possible explanation was made that the local injury to the endometrium induced proper decidualization for implantation competency.[9-11]

In this study, we used the blunt syringe to scratch the mouse endometrium to set up the animal model for local endometrial injury. The model was successful, which was confirmed by the following evidence: (1) in group III, the number of implanted embryos on the injured side of the uteri (the right side) was significantly higher than that on the un-injured side (the left side) and was also higher than that in group II (as injury procedure control). (2) Especially, when both uteri were injured in group IV, the number of implanted embryos on each side and both of the uteri together was significantly increased as compared to group II. Since the bilateral injury procedure on mouse uteri in group IV had the consistent results regarding the number of increased embryo implantation not only on each side of uteri but also on both sides, it was used for the subsequent research on determining the underlying mechanism for the improved embryo implantation. Through this model, we found that the improved embryo implantation was due in part to the enhanced endometrial receptivity.

Endometrial receptivity is also defined as the window of implantation (WOI), which is maintained only for a limited time period. In human, the uterus becomes receptive during the mid-secretory phase, which spans 7 to 10 days after ovulation. In mice, the estrous cycle is relatively short (4 days). The mouse uterus is receptive on day 4 of pregnancy or pseudopregnancy, whereas it is prereceptive on days 1 to 3 and by the afternoon of day 5 it becomes nonreceptive toimplantation.[22] During the process of embryo implantation in mammals, many characteristic changes in morphology or physical signals occur in the uterus that are indicative of the uteri becoming receptive for embryo implantation. These morphological changes during the receptive period include the transformation of the fibroblast-like endometrial stromal cells into larger and rounded decidual cells (decidualization), the growth and development of secretory glandules, as well as the gradual loss of uterine epithelial cell polarity and the formation of large apical protrusions (pinopodes) on the luminal epithelium.[23-26]

Endometrial glands play a key role in the maintenance of the zygote in the preimplantation and the regulation of placental development in many domestic species.[27] It is believed that the carbohydrate- and lipid-rich secretions represent an important source of nutrients during the first trimester. The secretions also contain a variety of growth factors that may regulate placental morphogenesis. Normal pregnancy needs the support of the endometrial glands. Our results demonstrate that the bilateral injury procedure on mouse uteri can induce the hyperplasia of endometrial glands as compared to the control (group II).

Pinopodes are ectoplasmic protrusions of endometrial epithelial cells that have been suggested as a major morphological marker of endometrial receptivity.[28] Their appearance suggests an open window period favoring implantation.[29-31] It was reported that pinopodes were present on the surface of the human endometrium for 48 hours or up to 7 days during the WOI.[32] The decreased or dissynchronous appearance of pinopodes was related to impaired fertility.[33,34] During the WOI, pinopodes absorb the intraluminal macromolecules and fluid, an event that is coordinated with generalized stromal edema to induce the closure of the lumen.[35] From the results of H&E staining on D4, we found that the uterine lumen in group IV was in the closed state as the same as those in group I, while those in group II was open. The closure of the lumen assists the contact of the embryo with the epithelium and positioning of pinopodes, which has better adhesion competence to the embryo than the microvilli, thereby promoting embryo implantation. The previous morphological findings indicated that local endometrial injury improved the uterine receptivity at the duration of WOI by inducing the hyperplasia of endometrial glands and the formation and development of the pinopodes. This might be one of the reasons why the local endometrial injury enhanced the embryo implantation in mice.

Apart from physical signals, many different molecules have also been implicated as chemical signals for embryo implantation. LIF, a pleiotropic cytokine of IL-6 family, is one of the most vital cytokines shown to be critical for implantation in mice.[36] It plays an important role in regulating the function of trophoblast and vascular formation of placenta. The LIF mRNA and protein are maximally expressed in the murine endometrial glandular epithelium just prior to blastocyst implantation. Blastocysts failed to adhere to the endometrial luminal epithelium in LIF gene knockout female mice. After LIF was given into the uterine cavity of these mice, the implantation rate was significantly increased.[37] In human endometrium, LIF and its receptor are expressed throughout the menstrual cycle with a striking increase during the peri-implantationphase.[38] However, in infertile women, LIF expression on the endometrium during the period of receptivity was significantly decreased when compared to that of normal fertile women.[34,39,40] Moreover, LIF deficiency is noted in women with unexplained recurrent abortions.[41] Therefore, its high endometrial production in the mid-late secretory phase stresses its important role in human embryo implantation. In the present study, we found that the mRNA and protein expression levels of LIF during the WOI were significantly increased in group IV when compared to group I and group II. During healing of the endometrial injury, the increased production of LIF could facilitate implantation.

Angiogenesis, the formation of blood vessels from preexisting vessels, is a highly regulated process that is essential in embryogenesis and normal physiologic growth and repair.[42] It also involves in the establishment of a placenta by supplying blood and nutrition to the fetus. In this study, we found an increased vasculature formation in group IV as compared to that in group II. On the one hand, the increased angiogenesis might be beneficial to the trauma repair in the endometrium, just like the endometrial repair after menses. On the other hand, it might lead to sufficient blood supply and nutrition to the embryo to sustain the pregnancy.

The OSM, another gp130 ligand, is produced mainly by activated T lymphocytes, monocytes, and macrophages and is

involved in the regulation of inflammation, tissue remodeling, and cell growth. In particular, OSM plays an important role in angiogenesis, which has been shown to upregulate the expression of the major angiogenic factor—vascular endothelial growth factor in different cell types and tissues.[42] In this study, we also found that the mRNA and protein expression levels of OSM were significantly increased in group IV when compared to group II. These results indicated that local endometrial injury can upregulate the OSM expression during the WOI, which facilitated the angiogenesis in mouse endometrium and subsequently improved the embryo implantation. Taken together, the observations indicate that local endometrial injury provoked the compensative production of such cytokines as LIF and OSM, which can improve the endometrial receptivity and help tissue repair as well. This might be another reason why the local endometrial injury enhanced the embryo implantation.

Apart from the structural alterations as seen in our findings, other functional alterations may also involve in the enhancement of endometrial receptivity and embryo implantation, which include injury-derived endometrial inflammatory reaction, wound healing, immune cell recruitment, neoangiogenesis, and upregulation of a wide variety of gene expression.[43-45] All these events in response to injury can benefit embryo implantation.

Although several evidence-based studies have concluded a beneficial effect of endometrial injury on the improvement in embryo implantation and clinical pregnancy rates in women with failed IVF,[11,14,15,46,47] contradictory results still exist. For example, a randomized controlled trial (RCT) by Karimzade et al shows that endometrial injury on the day of oocyte retrieval has a negative impact on implantation in IVF cycle.[48] Endometrial injury cannot improve implantation of frozen-thawed embryos.[49] Moreover, a recent RCT shows that no significant improvement in pregnancy rates was observed after endometrial injury in unselected subfertile women undergoing IVF treatment.[50] These contradictions might result from no standard operation practice on the criteria for the selection of patients (all IVF women or only patients with RIF), the optimal timing (proliferative phase, secretory phase, or oocyte retrieval cycle), the optimal number of procedures (single vs multiply curettage), and injury approach (using hysteroscopy vs a Pipelle catheter) required for the endometrial injury to exert its maximal effect. As a result, more evidence from well-designed trials is needed.

Conclusion

To clarify the possible mechanism by which local endometrial injury improve endometrial receptivity thereby increasing embryo implantation rates, we established a mouse model and demonstrated that local endometrial injury generated by the blunt syringe on the mouse uteri can increase the uterine receptivity by provoking characteristic changes in endometrial morphology and inducing upregulation of LIF and OSM gene and protein expression. All these events are essential for the transition of the endometrium from nonreceptive to the receptive stage.

Authors' Note

Xiao-Hui Zhang and Zhao-Zhao Liu share first authorship of this article. They contributed equally to this work.

Declaration of Conflicting Interests

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