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Original Research

Hydrogen Sulfide Promotes Functional Endometrium Recovery via Regulating Pyroptosis in Severe Endometrial Injury: A Prospective Laboratory Based Randomized Control Trial

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Abstract

Background: Severe endometrial injury constitutes a significant risk to female fertility, often leading to the development of intrauterine adhesions. Pyroptosis, a form of programmed cell death associated with inflammation, is initiated by the cleavage of gasdermin family proteins by caspase, which has been implicated in endometrial injury. In recent years, hydrogen sulfide (H_2S), a gaseous signaling molecule, exerts significant regulatory effects on pyroptosis in diverse pathological processes. The aim of this study is to elucidate the role of H_2S in facilitating functional recovery of the endometrium following injury in a murine model. **Methods**: A prospective laboratory based randomized control trial was performed to evaluate the protective role of H_2S in endometrial injury. Ethanol-induced endometrial injury mouse models were established and H_2S donor sodium hydrosulfide (NaHS) was randomly administered to the injured mice. Western blot analysis was conducted to assess changes in the expression of endogenous H_2S metabolism and pyroptosis-related markers and histological analysis (Hematoxylin & Eosin and Masson staining) was employed to examine alterations in endometrial morphology. Finally, a fertility test was performed to evaluate the restoration of the uterine function. **Results**: Following endometrial injury, the expression of key endogenous H_2S enzymes-cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST)-is significantly reduced, while the levels of pyroptosis-related proteins are elevated ($p < 0.05$). However, treatment with H_2S resulted in an increase in the expression of endogenous H_2S enzymes and a decrease in pyroptosis-associated proteins compared to the Model group ($p < 0.05$). Moreover, endometrial morphology and embryo count showed the most pronounced improvement in the H_2S treatment group ($p < 0.05$). **Conclusions**: This study confirms the therapeutic efficacy of H_2S in facilitating the recovery of injured endometrium and revealed its potential therapeutic mechanism, offering a promising therapeutic avenue for patients with severe endometrial injury.

Keywords: endometrial injury; hydrogen sulfide; intrauterine adhesions; pyroptosis

1. Introduction

With the rise in uterine surgeries, endometrial injury has become a common cause of intrauterine adhesions (IUA), resulting from impaired repair of the endometrial basal layer. During this process, damage to the basal lamina is usually irreversible and accompanied by fibrosis, which led to irregular bleeding, hypomenorrhea, secondary dysmenorrhea and amenorrhea, reduced fertility and premature birth [1]. Transcervical resection of uterine adhesions is the standard clinical treatment for IUA, but it is often associated with poor reproductive outcomes post-surgery [2]. Both intravenous and intrauterine administration of stem cells have proven effective in treating endometrial dysfunction, as evidenced by preclinical and clinical studies. The efficacy of cell transplantation for uterine repair, particularly in the context of IUA, has been shown to have variable results, with only a small percentage of transplanted cells successfully engrafting in the uterus [3]. Consequently, there is a pressing need for innovative therapeutic strategies to expedite endometrial repair and enhance its receptivity.

Hydrogen sulfide (H_2S), a gaseous signaling molecule, is involved in multiple pathophysiological processes, including inflammation, oxidative stress, abnormal angiogenesis and cell death [4].

Emerging evidence suggests that H_2S may offer therapeutic benefits in the management of various female reproductive disorders, including endometriosis, intrauterine adhesions, and preeclampsia [5–7]. Xia *et al.* [7] reported that H_2S has demonstrated the potential to mitigate adhesion formation after uterine horn post-operative model. How-

ever, the detailed molecular healing mechanism of H_2S in endometrial injury has not been reported to date.

Pyroptosis, a type of inflammatory cell death, can play an important role in cell and tissue damage, which is involved in the pathogenesis of multiple endometrial diseases [8]. Pyroptosis, a highly inflammatory form of cell death, is triggered by the cleavage of gasdermin proteins, resulting in the formation of pores in the cell membrane. This process leads to the release of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and interleukin-18 (IL-18), which are implicated in the development and progression of endometriosis and endometrial cancer [9,10]. In light of the increasing interest in targeted therapy and immunotherapy, it is imperative to explore the potential therapeutic value of modulating pyroptosis in the treatment of endometrial diseases [10].

Endometrial cells employ various adaptive strategies, such as the inhibition of pyroptosis, to ensure their survival and function within the demanding uterine environment. While significant progress has been made in understanding the role of pyroptosis in endometriosis and endometrial cancer, the precise mechanisms that regulate this process are still not fully elucidated in endometrial injury. Our group recently reported on the regulatory effects of H_2S on pyroptosis in brain injury, suggesting that there is a mutual relationship between hydrogen sulfide and pyroptosis [11–13]. However, to date, the mechanisms regulating the interplay between hydrogen sulfide and pyroptosis in the endometrial injury are still poorly understood. Building on these studies, we hypothesize that pyroptosis and endogenous H_2S metabolism are involved in the molecular mechanism of endometrial injury. Thus, we sought to investigate the potential therapeutic benefits of exogenous H_2S in a mouse model of endometrial injury and to elucidate the underlying molecular protective mechanisms.

2. Materials and Methods

2.1 Animals

Adult female ICR mice (specific pathogen free [SPF] grade), aged 6–8 weeks and weighing 20–25 g, were obtained from the Laboratory Animal Center at Soochow University. The mice were housed under controlled conditions of 50% humidity, 25 °C, and a 12-hour light/dark cycle with ad libitum access to food and water. Mice were randomly assigned to experimental groups using a computer-generated random number table. All animal procedures were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Prior to experimentation, the protocols were reviewed and approved by the Soochow University Animal Use and Care Committee (reference number: SYXK (Su) 2021-0065).

2.2 Groups and Treatments

A prospective laboratory based randomized control trial was performed to evaluate the protective role of H_2S in endometrial injury. Mice were coded with computer-generated random numbers prior to the experiment, and randomly divided into a sham operation group (Sham group), endometrial injury group (Model group), and sodium hydrosulfide (NaHS) treatment after injury group (Model+NaHS group). It is important to ensure that reducing the number of animals used is balanced against any additional suffering that might be caused by their repeated use according to the welfare of animals. Therefore, we choose n = 6/group.

Mice in the control group underwent a sham laparotomy and were fed a standard laboratory diet. Uterine tissue samples were harvested concurrently with the experimental groups. 95% ethanol (1590100500, Merck KGaA, Shanghai, China) was used to establish the mouse endometrial injury model, and a visual representation of the established endometrial injury model is provided in Fig. 1 [14,15]. Mice were anesthetized using an isoflurane gas anesthesia machine (VetFlo-1205SP, Kent Scientific Corporation, Torrington, CT, USA) first with 2–3% isoflurane and then with 1.5–2% isoflurane to maintain the anesthetic effect, and allow the mice to breathe spontaneously during the procedure. After the mouse were anesthetized, the mouse abdominal mouse hair was removed with a shaver, and the mouse abdomen was sterilized with 75% alcohol, and then the animals were immobilized, and then, in the middle of the rectus abdominis muscle along the lower abdomen, the abdominal wall was carefully incised in layers, and the sterile gauze pads were placed around the incision, to expose the pink Y-ring. Gauze was placed around the incision to expose the pink Y-shaped uterus. The uterine horns were relocated out of the abdominal cavity with sterile ophthalmic forceps, after which the right uterus of the mouse was gently clamped at the pro-ovarian end and the pro-cervical end with sterile forceps to form a closed uterine cavity. A 1 mL insulin needle aspirating 95% ethanol was inserted from the proximal cervical opening, and 95% ethanol was slowly injected until the uterus filled up and turned white (about 20 µL) and kept for 1 min to induce endometrial injury in mouse, and then the uterine cavity and the abdominal cavity were rinsed with physiological saline in each layer three times to remove the residual ethanol. Meanwhile, the left uterus was kept intact.

After establishing the mouse endometrial injury model, the model group (Model group) was given 200 µL of phosphate-buffered saline (PBS) (C0221A, Beyotime, Shanghai, China) tail vein injection immediately after suturing the abdominal cavity. The dosage of NaHS (161527, Sigma-Aldrich, Shanghai, China) is based on previous literature reports [7,13,16]. For the NaHS treatment group (Model+NaHS group), mice were immediately administered 200 µL of 1.2 mg/kg NaHS via tail vein injection

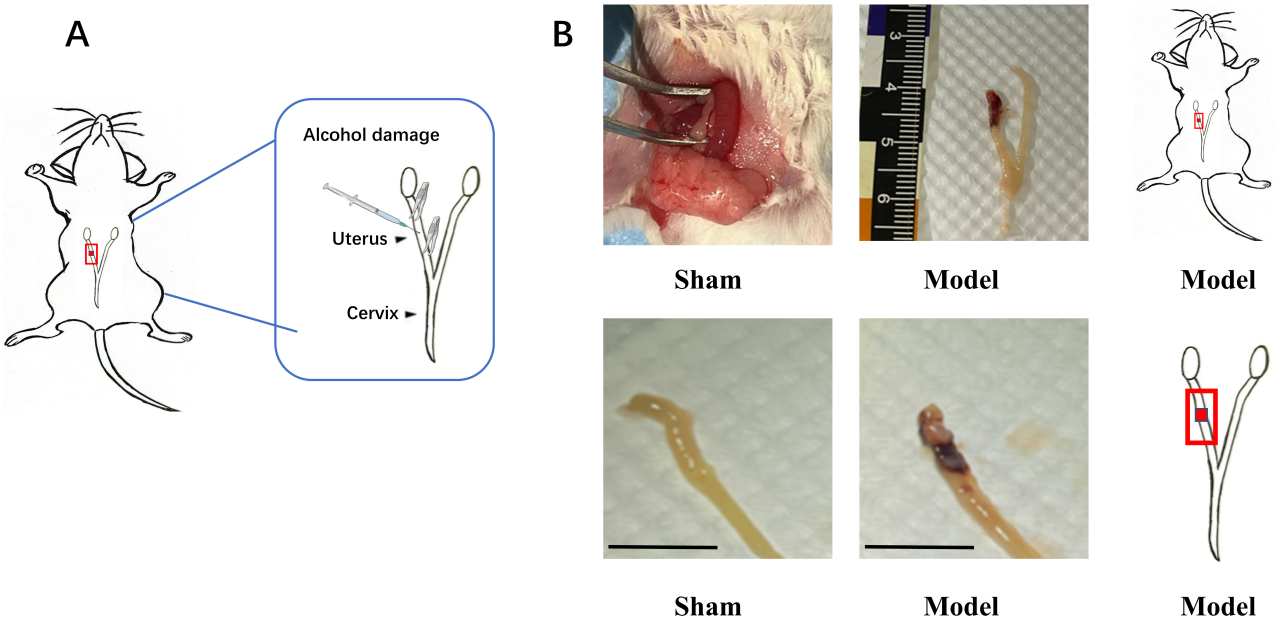

Fig. 1. Schematic representation of the mouse endometrial injury model and the morphology of uterus after injury. (A) Schematic illustration of the experimental procedure for inducing endometrial injury in mice by intrauterine injection of 95% ethanol for a duration of 20 seconds. (B) The morphology of the uterus was observed under visible light and schematic of the endometrial injury site. Scale bar = 1 cm. The schematic illustration of mice model is created by PowerPoint (21.0, Microsoft Inc., Washington, USA) using pictures drawn by our team.

post-surgery. The sham operation group was given 200 µL of PBS via tail vein injection. After completion of the surgery, the mice were placed on a heating pad at 37 °C. After the mice were awakened, they were returned to the cage. Mice in all three groups were euthanized after one estrous cycle (5 d), and the right uterus was removed and placed in a refrigerator at –80 °C for subsequent processing. Female mouse used for fertility tests were modeled at the same time and were allowed to recover for 15 d for subsequent experiments. After these experiments, the mice were euthanized using carbon dioxide inhalation. The mice were placed in a suitable euthanasia chamber and exposed to a slow flow of 100% CO_2 gas that displaces the chamber's air volume in 30–70 seconds. Once the mice became unresponsive and ceased breathing, death was confirmed by palpating for a heartbeat and checking for a corneal reflex. The deceased animals were then disposed of according to institutional guidelines.

2.3 Western Blot Analysis

Fresh uterine tissues were homogenized on ice in radio immunoprecipitation assay (RIPA) buffer (P0013B, Beyotime, Shanghai, China) supplemented with phenylmethylsulfonyl fluoride (PMSF, ST506, Beyotime, Shanghai, China) using an ultrasonic histocytometer (JY99-IIDN, Scientz Biotechnology, Ningbo, Zhejiang, China) to obtain a tissue lysate. The tissue homogenate was centrifuged at 14,000 rpm for 25 minutes at 4 °C, and the supernatant was collected. The protein concentration in different samples was detected by Nanodrop-2000 ultra-micro spectrophotometer (Thermo Fisher, Shanghai, China), and a mixture of RIPA and PMSF was used for leveling to adjust the total concentration of the samples to 20 µg/µL. 5× sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) protein sampling buffer was added, and the samples were heated in a metal bath heated to 100 °C for 3 min, and then vortexed to make a homogeneous mixture. 20 µg of total protein was separated by 10% SDS-PAGE and transferred to a pre-wetted polyvinylidene difluoride (PVDF) membrane (ISEQ15150, Merck, Shanghai, China). Membranes were incubated in a blocking solution containing 5% bovine serum albumin (BSA) (B6917, Merck, Shanghai, China) in Tris-buffered saline (TBST) for two hours, and then the membranes were sequentially incubated with primary and secondary antibodies. Finally, the immunoreactive bands were detected using an enhanced chemiluminescence system (ChemiScope 6000 Touch, Clinx Science Instruments, Shanghai, China). Protein band intensities were quantified using ImageJ software (1.54, National Institutes of Health, Bethesda, MD, USA) and expressed as the ratio of the target protein to the housekeeping protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Detailed information on all antibodies used in this study is provided in **Supplementary Table 1**.

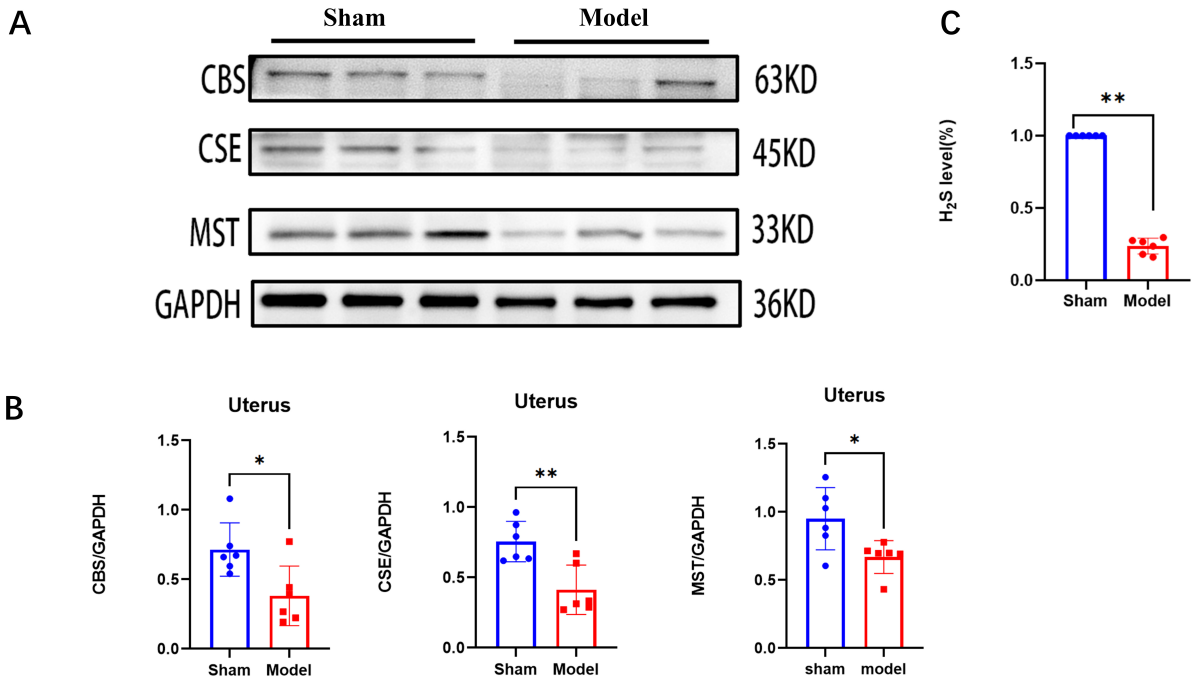

Fig. 2. The changes of the level of endogenous hydrogen sulfide (H_2S) and H_2S-synthesizing enzymes after endometrial injury. (A) Representative Western blot images illustrating the protein expression levels of cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), mercaptopyruvate sulfurtransferase (MST), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (B) The bar chart shows the relative expression levels of CBS, CSE, and MST normalized to GAPDH. CBS, CSE and MST significantly decreased after endometrial injury in the model group compared with the sham groups. GAPDH was used as a loading control for western blotting. (C) Micro sulfide ion electrode was used to detect the release of H_2S in the uterus after injury. The amount of H_2S in the uterus after injury decreased significantly when compared with the sham group. Bars represent the mean $\pm$ SEM (*$p < 0.05$, **$p < 0.01$; n = 6 per group). SEM, standard error of the mean.

2.4 In Vivo H_2S Release in Uterus Tissue

To quantify H_2S levels in uterine tissue, we employed a previously described method using Micro Sulfide Ion Electrode (LIS-146AGSCM, Lazar Research Laboratories Inc., Los Angeles, CA, USA) [13]. Before data acquisition, the probe was calibrated using a standard curve generated with NaHS solutions ranging from 10^{-2} to 10^{-6} M, prepared in an antioxidant buffer containing 250 g sodium salicylate (S3007, Merck, Shanghai, China), 65 g ascorbic acid (PHR1008, Merck, Shanghai, China), and 85 g sodium hydroxide (NaOH) (655104, Merck, Shanghai, China) per liter of distilled water. Subsequently, the probe was carefully inserted into 2 mm below the tissue aqueous solution and the value of the probe was monitored and recorded for each experimental group. The H_2S level (%) = [(value at Sham group – value at Model group or Model+NaHS group)/value at Sham group] $\times$ 100%.

2.5 Histological Analysis

The dehydrated uteri were oriented and embedded in optimal cutting temperature compound (OCT). Subsequently, the uteri were sectioned longitudinally at a thickness of 10 µm. The uterine morphology was observed with a microscope (DS-Ri2, Nikon Instruments Inc, Shanghai, China). The thickness of endometrial and the number of endometrial glands in each uterine section was calculated using Image J software (1.54, National Institutes of Health, Bethesda, MD, USA) with hematoxylin-eosin (HE) staining (C0105M, Beyotime, Shanghai, China). Masson's trichrome staining was used to assess endometrial fibrosis. The staining procedure was carried out according to the manufacturer's instructions for the Masson staining kit (G1346; Solarbio, Beijing, China). Gland number, endometrial thickness, and fibrotic area were quantified in at least 10 random images per slide using ImageJ software. These photographs were processed using Photograph software (22.0, Adobe Inc., San Jose, CA, USA).

2.6 Pregnancy Function Test

Fertility was assessed in female mouse after three estrous cycles, approximately 15 d after modeling, according to the grouping in Method 2.2. Female mice (n = 6) in each group were housed in a 2:1 ratio with sexually mature male on the day of estrus. Female mice were observed to develop vaginal plugs marked for 0.5 d. These mice were then isolated and euthanized at 10 d after the development of vaginal plugs, and data on the embryo count were recorded.

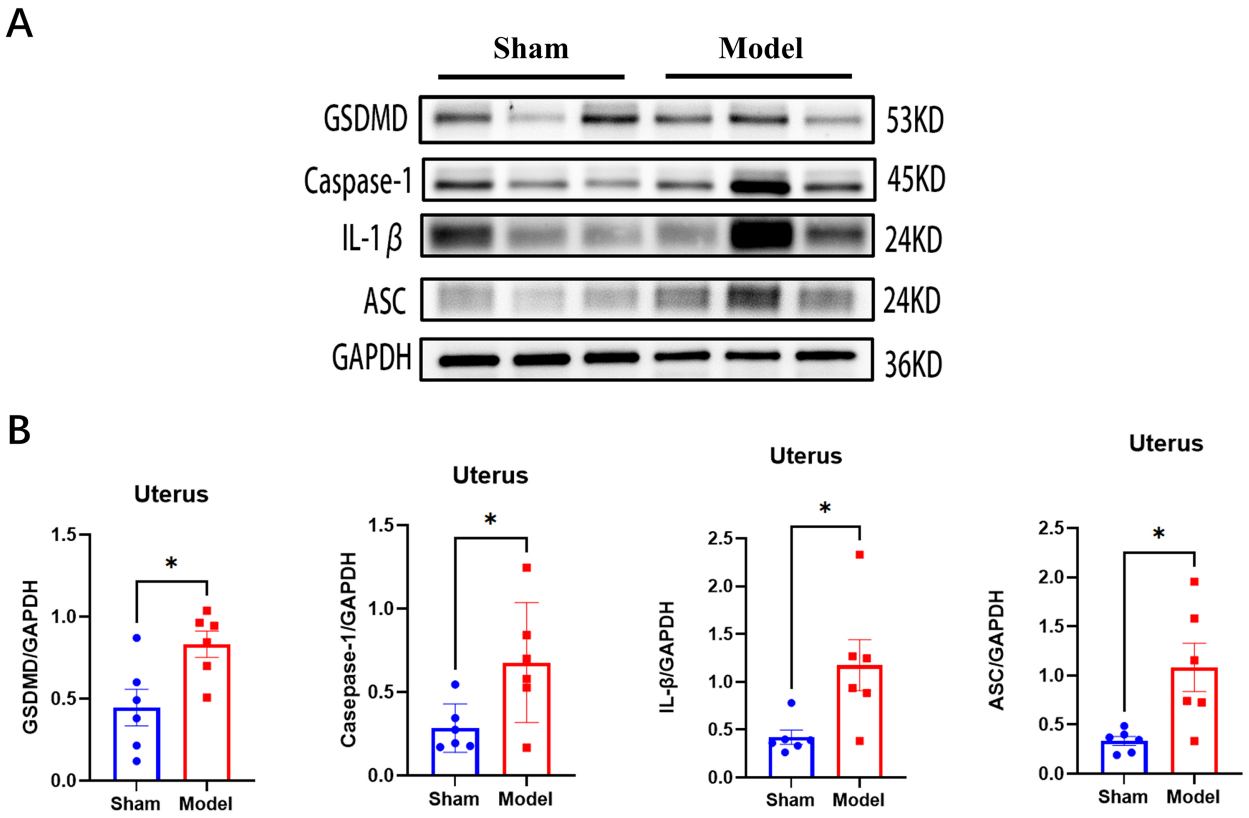

Fig. 3. The changes of pyroptosis-related proteins after endometrial injury. (A) Representative Western blot images illustrating the protein expression levels of GSDMD, Caspase-1, IL-1β, ASC and GAPDH. (B) The bar chart shows the relative expression levels of GSDMD, Caspase-1, IL-1β, ASC relative to GAPDH. GSDMD, Caspase-1, IL-1β, ASC significantly increased after endometrial injury in the model group compared with the sham groups. GAPDH was used as a loading control for western blotting. Bars represent the mean $\pm$ SEM (*$p < 0.05$; n = 6 per group). GSDMD, gasdermin D; ASC, apoptosis associated speck-like protein containing a caspase recruitment domain (CARD); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1β, interleukin-1β.

2.7 Statistical Analysis

The data obtained in the study were statistically analyzed using GraphPad Prism (9.0, Dotmatics Inc., Boston, MA, USA). All data were presented as mean $\pm$ standard error of the mean (SEM). One-way analysis of variance (ANOVA) was conducted to determine statistically significant differences between the groups. A Shapiro-Wilk test was conducted to determine if the data was normally distributed. Dunnett's T3 test was employed to compare groups with unequal variances, with statistical significance defined as $p < 0.05$.

3. Results

3.1 Ethanol Induce Severe Damage to the Morphology of Endometrium

An *in vivo* model of endometrial damage was established. Briefly, 95% ethanol was injected into the uterine horn to induce endometrial injury, with the severity of injury depending on the duration of ethanol exposure. The uteri of sham-operated mice exhibited normal histological features. In contrast, the uteri of the model group displayed significant pathological changes, including tissue degeneration, reduced tissue elasticity, fluid accumulation, and bleeding (Fig. 1).

3.2 The Expression of H_2S Synthase and Level of H_2S after Endometrial Injury

H_2S has been implicated in the regulation and therapeutic applications in the treatment of female reproductive disorders. Cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and mercaptopyruvate sulfurtransferase (MST) were found to produce endogenous H_2S in the uterus [4,17,18]. To validate the expression of endogenous H_2S synthase, we examined the protein levels of CBS, CSE, and MST in sham and model groups using Western blot analysis. In the uterus surrounding the injury, CBS, CSE and MST decreased significantly in the model group compared with the sham groups (CBS or MST, $p < 0.05$; CSE, $p < 0.01$). We observed a similar trend in endogenous H_2S levels and H_2S synthase expression in the injured uterine tissue ($p < 0.01$) (Fig. 2).

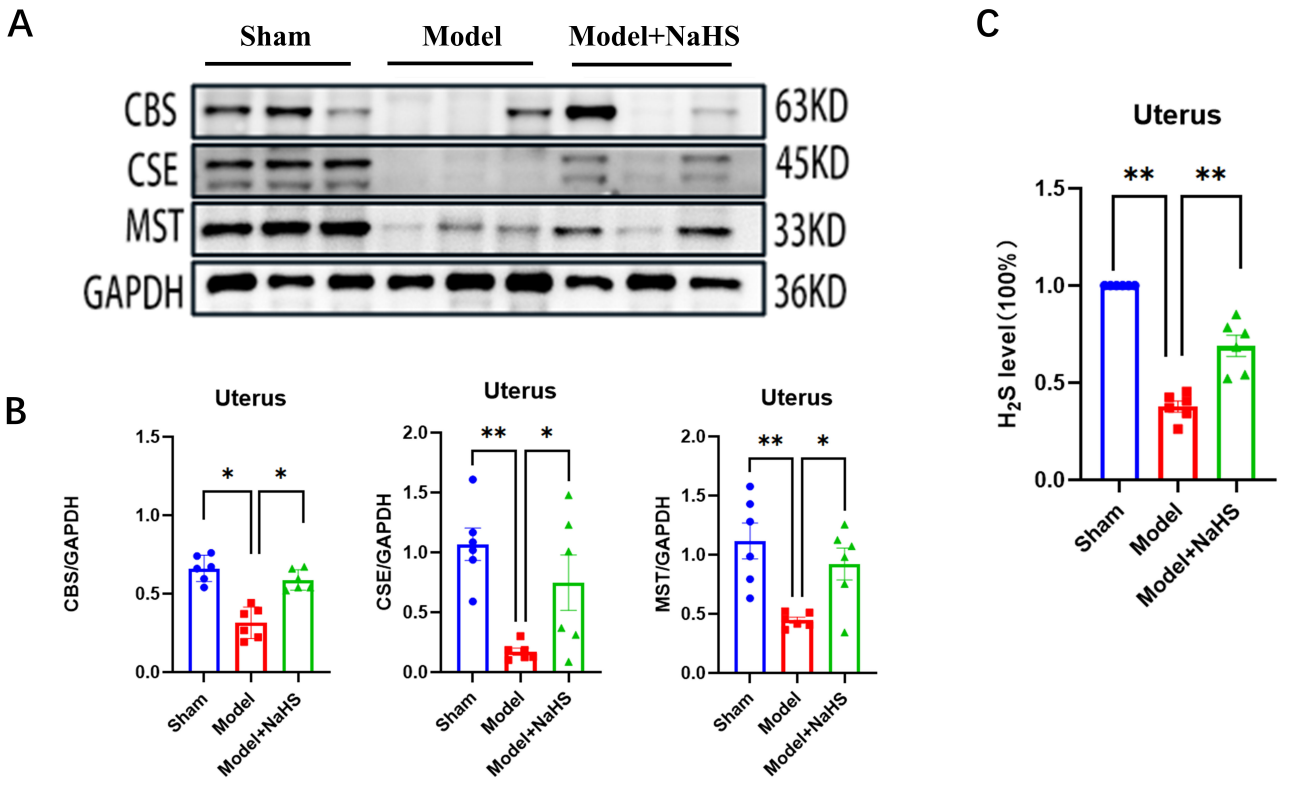

Fig. 4. Treatment with NaHS ameliorated decrease in the expression of endogenous H_2S enzyme and H_2S levels induced by EI. (A) Representative western blot analysis illustrating the altered expression of CBS, CSE, and MST proteins in the injured uterus of the Sham group, Model group, and Model+NaHS group, respectively. (B) The levels of CBS, CSE, and MST in the injured uterine tissue from different groups were quantified using ImageJ software. (C) The level of H_2S was detected by micro sulfide ion electrode. GAPDH was used as a loading control for western blotting. Bars represent the mean ± SEM (*$p < 0.05$, **$p < 0.01$; n = 6 per group). NaHS, sodium hydrosulfide; EI, endometrial injury.

3.3 The Changes in Uterine Pyroptosis Following Endometrial Injury

Pyroptosis, a proinflammatory cell death pathway, plays a crucial role in the onset and progression of endometrial injury. To validate the occurrence of pyroptosis, we detected gasdermin D (GSDMD), Caspase-1, IL-1β, and apoptosis associated speck-like protein containing a caspase recruitment domain (CARD) (ASC) expression in sham and model groups. In the uterus surrounding the injury, a significant upregulation of GSDMD, Caspase-1, IL-1β, and ASC protein expression was observed in the model group compared to the sham group ($p < 0.05$), suggesting that pyroptosis was involved in the pathophysiological processes of endometrial injury (Fig. 3).

3.4 Treatment with NaHS Reversed Endometrial Injury (EI)-induced Decrease of H_2S Expression and Increase of Pyroptosis

To investigate the effect of H_2S on pyroptosis triggered by endometrial injury, we analyzed the expression of pyroptosis-related proteins following the injury. Firstly, we observed that exogenous H_2S treatment reversed the decreased expression of H_2S production-related proteins (CBS, CSE and MST) and the level of endogenous levels ($p < 0.05$) (Fig. 4). Then, we innovatively found that pyroptosis-related proteins (GSDMD, Caspase-1, IL-1β and ASC) significantly decreased after H_2S treatment ($p < 0.01$) (Fig. 5). These results indicate that treatment with NaHS inhibits EI-induced decrease of H_2S and increase of pyroptosis.

3.5 Exogenous H_2S Reduced the Endometrial Damage after Injury

HE staining revealed disrupted uterine cavity morphology, thinner endometrium, and decreased glandular density. Compared with the model group, H_2S groups exhibited increased endometrial thickness ($p < 0.05$) and glandular density ($p < 0.01$) (Fig. 6). Masson's trichrome staining revealed a significant increase in fibrosis area in the control group post-injury. However, the H_2S groups demonstrated the most substantial reduction in fibrosis area ($p < 0.001$) (Fig. 7).

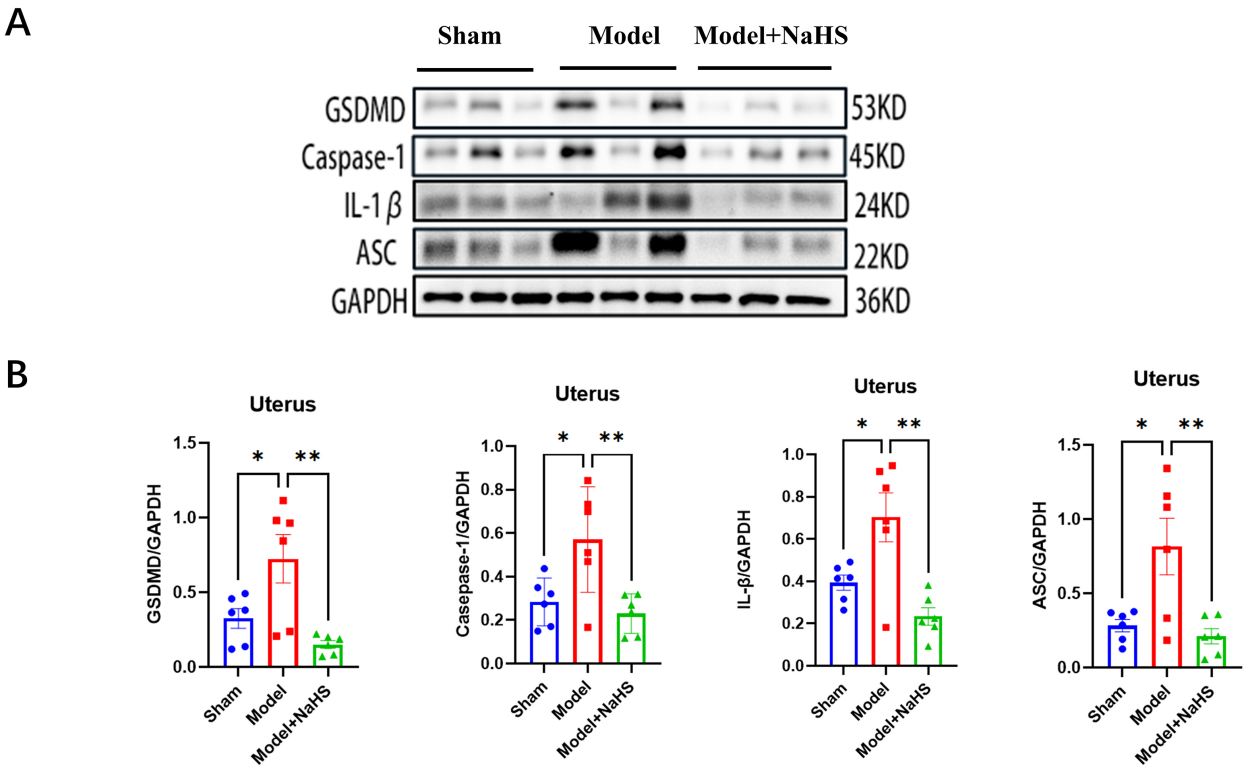

Fig. 5. Exogenous H_2S inhibits the increase for pyroptosis. (A) Representative western blot analysis illustrating the altered expression of GSDMD, Caspase-1, ASC, and IL-1β proteins in the injured uterus of the Sham, Model, and Model+NaHS groups, respectively. (B) The levels of GSDMD, Caspase-1, ASC, and IL-1β in the injured uterine tissue from different groups were quantified using ImageJ software. GAPDH was used as a loading control for western blotting. Bars represent the mean $\pm$ SEM (*$p < 0.05$, **$p < 0.01$; n = 6 per group).

3.6 Exogenous H_2S Improves Pregnancy Outcome after Endometrial Injury

Treatment with exogenous H_2S can significantly improve uterus damage in female mice after injury. To further assess the impact of exogenous H_2S on endometrial function and recovery, fertility tests were performed. To evaluate the functionality of the damaged uterus, female mice with unilateral uterine damage were mated with sexually mature males during estrus following the H_2S treatment period. On the eighth day after the vaginal plug appeared, the distribution of embryos within the uterine horn was analyzed to evaluate the effect of exogenous H_2S on fertility. Compared to the Model group, H_2S treatment had more embryos in the Model+NaHS group ($p < 0.01$) (Fig. 8).

4. Discussion

In an attempt to understand the role of hydrogen sulfide metabolism and pyroptosis in the endometrial injury, a murine model of endometrial injury was established by the intrauterine administration of 95% ethanol. Our study provides novel evidence for the downregulation of endogenous H_2S-synthesizing enzymes, such as cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate transferase (MST), in the injured mouse uterus. A similar trend was observed in the decreased levels of endogenous H_2S and the downregulation of H_2S-synthesizing enzymes in the injured uterine tissue. Meanwhile, the pyroptosis-related proteins (GSDMD, ASC, and Caspase-1) increased in the uterus after endometrial injury. Importantly, H_2S can effectively re-establish the balance and stability of the uterine tissue microenvironment, improve the pathological changes of uterine tissue, reduce fibrosis, and improve the reproductive ability of mice. The molecular mechanism underlying the protective effects of H_2S may be attributed to the downregulation of pyroptosis, providing a robust experimental basis for its therapeutic potential in endometrial injury.

A good and receptive endometrium provides an essential internal environment for embryo implantation and is the prerequisite and guarantee for the achievement of pregnancy. Endometrial injury plays a crucial role in causing infertility and also leads to a lot of gynecological diseases, such as intrauterine adhesions (IUA) and thin endometrium, which can directly lead to amenorrhea, infertility, abortion or other serious symptoms [19]. Approximately 90% of patients with endometrial injury develop IUA, a pathological condition characterized by the formation of endometrial fibrosis. Persistent inflammation was

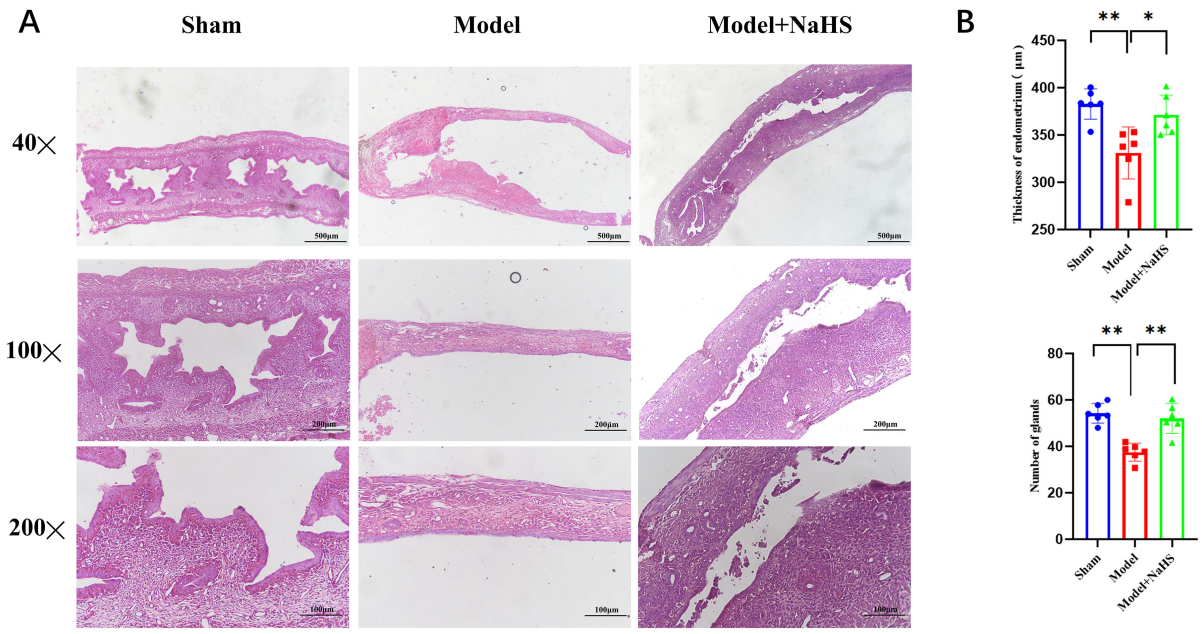

Fig. 6. The endometrial morphology was evaluated by HE staining after H_2S treatment. (A) Hematoxylin-eosin (HE) staining of mice uterine tissue. (B) Histogram depicting the distribution of endometrial thickness and gland number across different experimental groups. Bars represent the mean ± SEM (*$p < 0.05$, **$p < 0.01$; n = 6 per group). Scale bar: 40×, 500 μm; 100×, 200 μm; 200×, 100 μm.

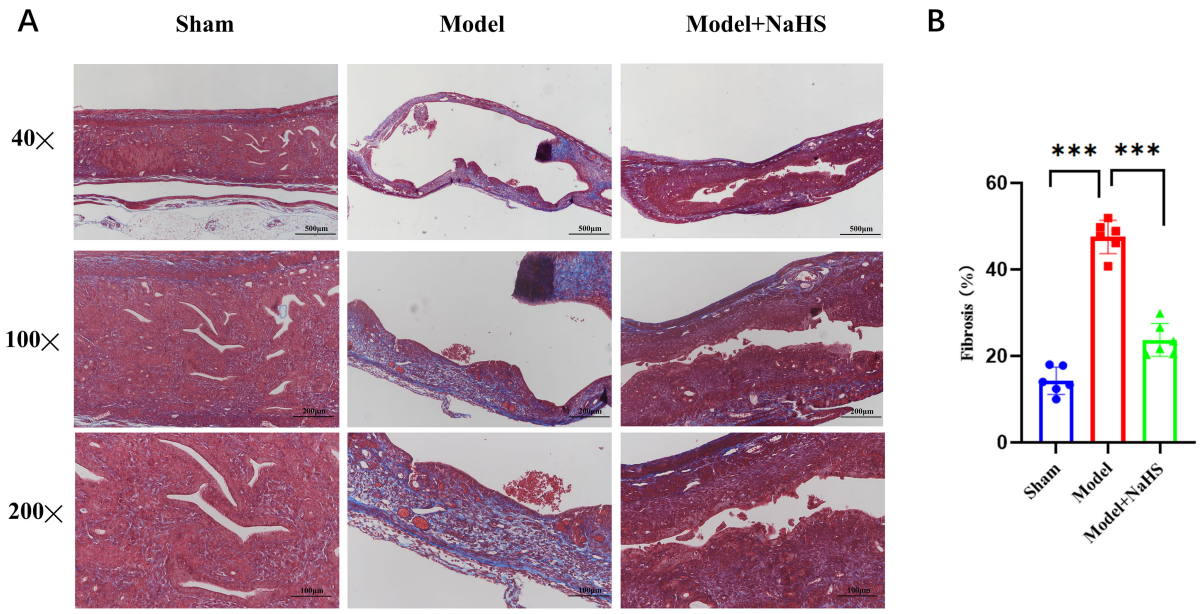

Fig. 7. The endometrial fibrosis was evaluated by Masson staining after H_2S treatment. Masson's trichrome staining was used to assess endometrial fibrosis. (A) Masson stain showed fibrotic tissue (stained pale blue) in the endometrium. (B) Statistical analysis was conducted on the percentage of endometrial fibrosis area in each group. Bars represent the mean ± SEM (***$p < 0.001$; n = 6 per group). Scale bar: 40×, 500 μm; 100×, 200 μm; 200×, 100 μm.

evident in the endometrium of IUA patients, and the degree of endometrial fibrosis was positively correlated with the severity of inflammation [20]. The endometrium is composed of two layers: the functional layer, which undergoes cyclical changes and is shed during menstruation and postpartum, and the basal layer, which remains intact and

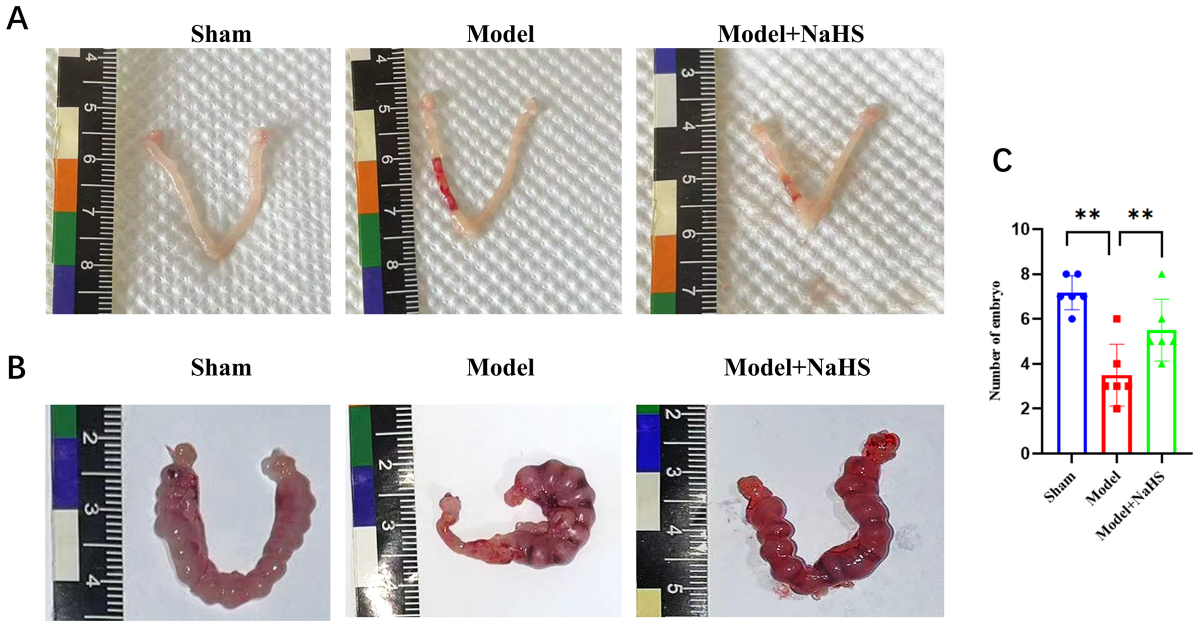

Fig. 8. H_2S treatment improve the mice fertility. Two weeks following the endometrial injury model, female mice were mated with males to record and analyze the number of embryos in each pregnancy. (A) The protective role of H_2S in uterine after injury in each group. (B) The protective role of H_2S in the mice fertility. (C) Bars represent the mean $\pm$ SEM (**$p < 0.01$; n = 6 per group) of embryo number in the control group, Model group, and Model+NaHS group.

serves as a regenerative source for the functional layer. Recently, pyroptosis was reported to play an important role in the endometrium-related disease, such as endometriosis [21]. Pyroptosis is initiated by inflammasomes, which active caspases-1, cleave gasdermin D (GSDMD) and then leading to the release of inflammatory cytokines [22]. GSDMD, caspase-1, and IL-1β expression in endometrium were consistent with fibrosis levels of patients with endometriosis and inhibiting pyroptosis can significantly reduce the degree of endometrial fibrosis [23–25]. Endometrial injury can also induce chronic pelvic inflammation and tissue fibrosis. However, the role of pyroptosis in endometrial injury has not been reported to date. We have examined the expression of pyroptosis-related proteins and found that GSDMD, Caspase-1, IL-1β and ASC significantly increased in the uterus surrounding the injury in the model group compared with the sham groups, suggesting that pyroptosis may be involved in endometrial injury and a potential therapeutic target in the treatment of severe endometrial injury.

The treatment of severe endometrial injury induced intrauterine adhesion aims to promote endometrial repair, prevent adhesion recurrence and restore the fertility. Current treatments include surgical treatment (hysteroscopic adhesiolysis or transcervical resection of adhesion (TCRA)), pharmacotherapy, and stem cell therapy [1]. However, surgical treatment is limited to physically separating the adhesions and may inadvertently cause further damage to the remaining endometrial tissue [26]. Infection-induced inflammation results in the damage of endometrial stem cells, compromising their regenerative capacity and hindering the repair of the uterine cavity [27]. Therefore, pharmacologic treatment is the priority in treatment of severe endometrial injury. Hydrogen sulfide (H_2S), as an endogenous gasotransmitter, is widely distributed in many tissues and organs of the human body and contributes to the modulation of a myriad of biological signaling pathway. Endogenous H_2S is predominantly synthesized by three key enzymes: CBS, CSE, and MST. Studies have shown endogenous H_2S generation systems have been found in female reproductive system and dysregulation of the endogenous H_2S is involved in various pathophysiological processes in reproductive disease [4]. We have investigated the expression of endogenous H_2S synthase and found that CBS, CSE and MST significantly decreased in the uterus surrounding the injury compared with the sham groups. A similar trend was observed in the endogenous H_2S levels and the expression of H_2S-synthesizing enzymes in the uterine tissue surrounding the injury. These results suggest that endogenous H_2S is involved in regulating the uterine microenvironment. To determine the effect of maintaining hydrogen sulfide homeostasis on endometrial damage, we observed the effects on H_2S metabolism and cell pyroptosis after injury by administering exogenous hydrogen sulfide. Our results showed that exogenous H_2S restored the hydrogen sulfide homeostasis after endometrial damage, suggesting that exogenous H_2S has a protective role in endometrial injury. Our group previously shown that H_2S significantly inhibited traumatic brain injury-induced neuronal pyroptosis, suggesting that there is a mutual relationship be-

tween hydrogen sulfide and pyroptosis [13]. In this study, we have confirmed that pyroptosis is significantly upregulated after endometrial injury. To further verify whether exogenous hydrogen sulfide regulates the occurrence of pyroptosis after endometrial injury, we examined the expression of GSDMD, Caspase-1, IL-1β and ASC proteins in the model group after exogenous H_2S treatment. We innovatively found that pyroptosis-related proteins (GSDMD, Caspase-1, IL-1β and ASC) significantly decreased after H_2S treatment. These findings suggest that exogenous H_2S can suppress the upregulation of pyroptosis induced by endometrial injury.

Persistent chronic inflammation can lead to fibrosis, making endometrial recovery challenging [14,28]. Disruption of the endometrial repair process can result in abnormal fibrosis, leading to uterine cavity occlusion and subsequent infertility. The endometrium plays a vital role in determining uterine receptivity, and a thin endometrium may compromise successful embryo implantation [29]. To further evaluate the effect of exogenous H_2S on the endometrium function after injury, HE staining and Masson's trichrome staining were used to quantify the thickness of endometrium, the number of glands, and the area of fibrosis. We found that exogenous H_2S showed an increase in endometrial thickness and glands number. Compared to the control group, the fibrosis area was significantly increased after injury in the model group, while the fibrosis area in the H_2S groups showed the greatest reduction in endometrial fibrosis area by Masson's trichrome staining. These results suggested that H_2S may be a key factor in the induction of tissue-specific repair processes. Our results also demonstrate that exogenous H_2S can improve fertility efficiency and yield the good effect in injured endometrium in mice, suggesting that treatment with H_2S can enhance fertility and promote the recovery of uterine function after endometrial injuries. While H_2S has shown promise as a potential treatment for infertility, it is not currently used in clinical practice. More research is needed to determine the optimal dose and delivery method of H_2S for fertility treatment. Additionally, the safety of H_2S for long-term use needs to be further investigated. Caspase-1 can also initiate apoptosis in cells that lack GSDMD [30]. Based on current results, it is difficult to rule out the role of apoptosis in endometrial injury. The relationship between different death pathways in endometrial injury should be investigated in the future research.

5. Conclusions

In conclusion, our study demonstrated that endogenous H_2S metabolism and pyroptosis is involved in the pathological process of endometrial injury. This dual action of reducing inflammation and fibrosis while modulating pyroptosis pathways highlights the therapeutic potential of exogenous H_2S in enhancing fertility and treating uterine disorders.

Availability of Data and Materials

All data points generated or analyzed during this study are included in this article, and no further underlying data is necessary to reproduce the results.

Author Contributions

HZ, LYT, MYZ and HYS designed the research study. HYS, MYX, MCX, JX, JN, LZ and XHY performed the research and analyzed the data. All authors are credited with the editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experiments performed in this study have been carried out according to the rules in the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health Animal Use and Care and Animal Research. The present study was approved by the Ethics Committee of Soochow University (SUDA20240911A04). The mice used in the experiment were obtained from the Experimental Animal Laboratory of Soochow University (SYXK (Su) 2021-0065). Informed consent was N/A.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG26554.

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