SRTM Student Research in Translational Medicine

Original Article Doi: 10.22037/srtm.v7.51463

An Experimental Murine Model of Asherman's Syndrome: Characterization of Endometrial Fibrosis and Functional Assessment of Fertility Using a Novel Anesthesia Approach

Kimiya Rashidan[1], Ashkan Rasouli-Saravani[1], Malaksima Ayadilord[1], Amirmohammad Mohammadiara[1], Nariman Mosaffa[1], Hossein Aminianfar[2], Seyed Mahmoud Hashemi[1*]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

ARTICLE INFO
Date Submitted: 12 January 2026
Date Accepted: 18 February 2026

KEYWORDS
Asherman's syndrome; Intrauterine adhesion; Mouse model; Fibrosis; Fertility; Anesthesia

***CORRESPONDING AUTHOR**
Seyed Mahmoud Hashemi

Email: smmhashemi@sbmu.ac.ir
0000-0003-1389-5803

ABSTRACT

Background and Aim: Intrauterine adhesion (IUA), or Asherman's syndrome, is a significant gynecological disorder characterized by fibrous scar tissue formation within the uterine cavity. It commonly results from endometrial trauma following surgical procedures or infection and is a major cause of menstrual abnormalities, including hypomenorrhea or amenorrhea, as well as infertility. Progress in developing effective therapies has been limited by the lack of well- characterized preclinical models that adequately assess both structural damage and fertility outcomes. This study aimed to establish and characterize a reproducible mouse model of IUA that recapitulates both the pathological and functional features of the human disease.

Methods: Female NMRI mice underwent mechanical endometrial injury via uterine scraping. A balanced anesthetic protocol consisting of ketamine/ xylazine induction followed by isoflurane maintenance was used to ensure stable, long-duration anesthesia with minimal distress. Histological, molecular, and fertility assessments were performed to evaluate endometrial damage and reproductive function.

Results: Histopathological analysis demonstrated a significant reduction in endometrial thickness ($p = 0.008$) and gland number ($p = 0.024$), accompanied by extensive fibrosis ($p = 0.016$), as evidenced by H&E and Masson's trichrome staining. Molecular analyses revealed marked upregulation of type I collagen (Col-1) ($p = 0.048$) and α-smooth muscle actin (α-SMA) ($p = 0.033$), confirming a fibrotic phenotype. Functionally, fertility was severely impaired, as indicated by a substantial decrease in the number of implanted fetuses ($p = 0.011$) and the live birth rate ($p = 0.004$) in the IUA group compared with controls.

Conclusion: This murine model reproduces the structural, molecular, and functional abnormalities associated with IUA and provides a robust platform for investigating endometrial fibrosis and evaluating novel therapeutic strategies.

INTRODUCTION

Intrauterine adhesion (IUA), also known as Asherman's syndrome, is an acquired gynecological disease characterized by the formation of fibrous scar tissue within the uterine cavity (1). This condition significantly impairs female reproductive health, leading to clinical manifestations that range from menstrual disturbances, such as hypomenorrhea and amenorrhea, to recurrent pregnancy loss and infertility (2). IUA most commonly develops as a consequence of trauma to the basal layer of the endometrium, which can occur during procedures like dilatation and curettage, myomectomy, or cesarean section, as well as following uterine infections (3). The conventional treatment for IUA is hysteroscopic transcervical resection of adhesions (TCRA) (4). However, this procedure is invasive, technically demanding, and is associated with high rates of adhesion

Please Cite This Paper As:
Rashidan K, Rasouli-Saravani A, Ayadilord M, Mohammadiara A, Mosaffa N, Aminianfar H, Hashemi SM. An Experimental Murine Model of Asherman's Syndrome: Characterization of Endometrial Fibrosis and Functional Assessment of Fertility Using a Novel Anesthesia Approach.. Stud Res Transl Med. 2025;7:e51463.

reformation, with pregnancy rates post-surgery often remaining low (5, 6). Given these limitations, there is an urgent clinical need to explore novel therapeutic strategies, such as stem cell- based therapies or new pharmacological agents, to effectively prevent fibrosis and regenerate the endometrium.

The development of such treatments is critically dependent on the availability of valid preclinical animal models that can accurately replicate the pathophysiology of human IUA (7). To date, several animal models have been established (8-10). Experimental models of IUA are generally divided into three categories: mechanical, chemical, and combination approaches. Mechanical models involve thermal injury, electrocoagulation, or endometrial damage using a curette or blade in nonpregnant rats, as well as surgical abortion followed by curettage in pregnant rats. Chemical models are established using agents such as 95% ethanol, phenol mucilage, or trichloroacetic acid. Combination models apply two or more insults simultaneously, most commonly mechanical curettage together with lipopolysaccharide (LPS) administration (7). A significant shortcoming of many existing IUA models is their focus on histological and molecular changes, often neglecting the comprehensive assessment of fertility post-induction (11). Since impaired fertility is the ultimate functional consequence of IUA, its evaluation is essential for a model to be considered clinically relevant.

Furthermore, the surgical induction of IUA is a serious and invasive procedure (7) that can cause significant pain and distress to the animal if ethical considerations are not meticulously addressed. The mechanical scraping or chemical injury required to damage the endometrium is inherently painful, and a robust and well- defined perioperative care protocol is a scientific and ethical necessity. Therefore, optimizing the entire procedure, from pre-operative preparation to the anesthetic regimen, is not just a matter of animal welfare but is also crucial for ensuring the reproducibility of the model by minimizing confounding variables like stress and inflammation. Establishing a detailed, step-by-step protocol that includes anesthesia, surgical technique, and post- operative monitoring, and even standardized procedures for assessing outcomes like mating and fertility is essential for creating a truly reliable research platform.

To address these gaps, the objective of this study was to establish and comprehensively characterize a reproducible, ethically optimized murine model of Asherman's syndrome using a balanced anesthesia approach with ketamine/ xylazine and Isoflurane. We developed an ethical surgical protocol with a focus on animal welfare and established the model by evaluating a full spectrum of outcomes. This included histopathological analysis of endometrial atrophy and fibrosis, molecular quantification of key pro-fibrotic markers (COL-1 and α-SMA), and, most critically, a functional assessment of fertility through a controlled mating study. This well- characterized model provides a robust platform for future preclinical testing of novel therapeutic interventions for IUA.

MATERIALS and METHODS

Animals and Experimental Design

In this experimental study, a total of 32 adult- NMRI mice (22 female and 10 male) (8-10 weeks old; 25-30 g) were obtained from Royan Institute, Tehran, Iran. For histopathological analysis, ten female mice were allocated into two groups, including an intrauterine adhesion (IUA) group (n = 5) and a sham control group (n = 5). In the sham group, mice underwent the same surgical procedures, including anesthesia and uterine manipulation, without induction of mechanical endometrial injury.

For the assessment of pregnancy outcomes, six mice from each group were mated with male mice, and those that became pregnant were subsequently included in further analyses.

All animals were prepared and experimental procedures were performed in the accredited animal facility of the Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, in accordance with institutional animal care and use guidelines. Animals were housed under controlled conditions (22 ± 2 °C; 12-h light/dark cycle) with ad libitum access to standard chow and water. A one- week adaptation period was allowed before any experimental procedures began. At the end of the experiments, animals were humanely euthanized according to institutional ethical protocols, and carcasses were processed using an approved disposal system (burbibg machine).

To minimize potential confounders, only mice in the diestrus phase were included, and all animals were housed under identical environmental conditions. Surgical procedures and outcome assessments were performed using standardized protocols by the same operator and at fixed time points. The order of treatments and measurements, as well as cage location, were not randomized and were therefore not specifically controlled.

All animal experiments were approved by the Ethics Committee of the School of Medicine, Shahid Beheshti University of Medical Sciences, under the ethics code IR.SBMU.AEC.1403.039, and were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals in Scientific Procedures, approved by the National Committee for Ethics in Biomedical Research.

Anesthetic Protocol & Surgical Preparation Procedures

To minimize hormonal variability, the estrous cycle of each mouse was determined by daily vaginal cytology for three consecutive days. Only mice identified to be in the diestrus phase were included in the surgical procedures. Food was withdrawn 4-6 hours before surgery. Each mouse was weighed to ensure accurate drug dosage. The surgical site on the lower abdomen was shaved and aseptically prepared by scrubbing with povidone- iodine solution followed by 70% ethanol. A sterile ophthalmic ointment (e.g., Vitamin A Ointment**)** was applied to the eyes to prevent corneal dryness. Post- operative analgesics were not administered, as analgesic agents such as carprofen may interfere with the inflammatory response and compromise the proper establishment of the IUA model.

Anesthesia was induced with an intraperitoneal (IP) injection of a ketamine (Bremer Pharma, Germany) (80 mg / kg) and xylazine (Alfasan, Netherlands) (10 mg / kg) cocktail. Once a surgical plane of anesthesia was confirmed by loss of the pedal reflex, mice were maintained under anesthesia using 4% isoflurane (Terrell isoflurane USP liquid for inhalation, USA) delivered via a nose cone.

Induction of Intrauterine Adhesions (IUA)

Each anesthetized mouse was placed in a supine position on a sterile surgical field. A 1-1.5 cm midline laparotomy was performed to expose the uterine horns. To induce injury, the luminal endometrium of both horns was gently but thoroughly scraped using the beveled tip of a 26-gauge insulin syringe needle until mild, uniform bleeding was observed along the horn, indicating sufficient trauma to the basal endometrial layer. Care was taken to avoid perforation. The uterine horns were then repositioned into the abdominal cavity. The abdominal wall and skin were closed in two separate layers using 5-0 absorbable sutures (Supa, Iran). A thin layer of topical antibiotic ointment (Tetracycline) (Kimia Biotechnology Company, Iran) was applied to the incision. Mice were monitored on a warming pad until full recovery (Figure 1).

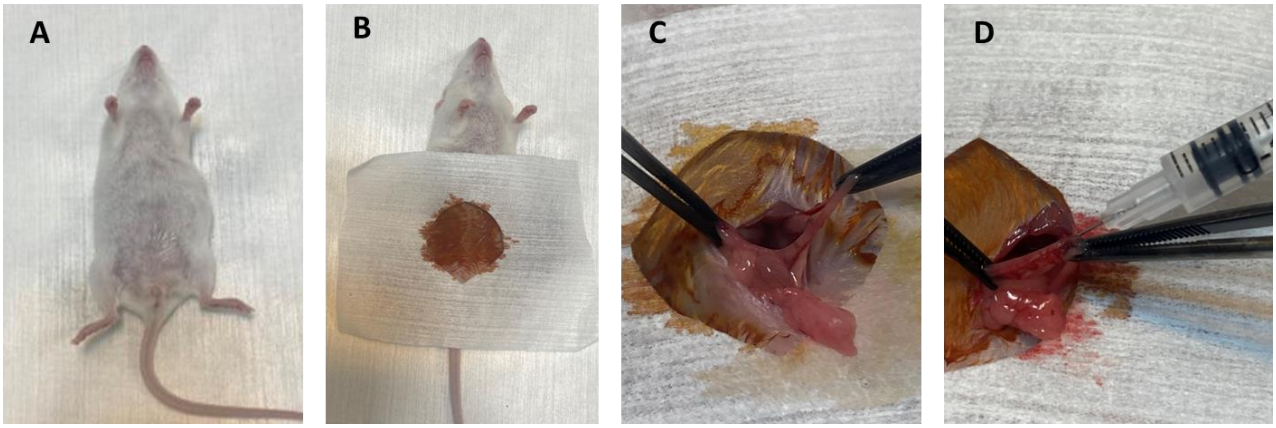

FIGURE 1. Steps of the IUA model induction

A) The mouse was anesthetized and positioned in the supine position. B) Povidone- iodine was applied to the abdominal area for disinfection. C) A midline incision was made, and the uterine horns were exposed. D) The interior walls of the uterus were gently scraped with an insulin syringe until visible bleeding was observed. IUA: intrauterine adhesions.

Tissue Collection and Processing

Five days post-surgery, mice in the pathological analysis cohort were euthanized by CO_2 inhalation. The uterine horns were excised and dissected free of adipose tissue. The right horn from each mouse was fixed in 10% neutral buffered formalin for 24 hours for histological analysis, while the left horn was snap- frozen in liquid nitrogen and stored at -80 °C for subsequent RNA isolation.

Histopathological Analysis

Staining was performed as previously described (12). Briefly, formalin- fixed tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of 4 µm thickness were cut and mounted on glass slides. For evaluation of general endometrial morphology, sections were stained with H&E (Merck, Darmstadt, Germany), and endometrial thickness (µm) as well as the number of endometrial glands per high-power field (HPF) were measured. To assess fibrosis, additional sections were stained using Masson's Trichrome (Merck, Darmstadt, Germany). All slides were imaged with a light microscope. For quantitative analysis, four random high-power fields (40×) from three non-consecutive sections per mouse were analyzed using ImageJ software (v1.52, NIH, USA), and the fibrotic area was calculated as the percentage of blue-stained collagen relative to the total endometrial stromal area.

Quantitative Real-Time PCR (qPCR)

Total RNA was extracted from frozen uterine tissue using an RNA isolation kit (Anacell, Iran). RNA concentration and

purity were determined using a NanoDrop spectrophotometer (ND-ONE; Thermo Fisher Scientific, USA). One microgram of total RNA was reverse-transcribed into complementary DNA (cDNA) using a reverse transcription kit (Anacell, Iran).

qPCR was performed using a SYBR Green master mix (Amplicon, Denmark) on a StepOnePlus™ Real- Time PCR System (Thermo Fisher Scientific, USA). The relative mRNA expression levels of COL-1 and α-SMA were quantified. Primer sequences were designed using Primer-BLAST and are listed in Supplementary Table 1. Gene expression was normalized to the housekeeping gene β-actin, and relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ method with REST software (v2.0.13, Qiagen, Germany). All reactions were run in triplicate.

Table1. Quantitative real- time PCR primers sequences

Gene (Symbol)	NCBI Accession No.	Primer Sequence (5′-3′)	Amplicon Size (bp)
Actb	NM_007393.5	Forward: GCTACAGCTTCACCACCACA Reverse: AAGGAAGGCTGGAAAAGAGC	208
Col1a1	NM_007742.4	Forward: GGCTCCTGCTCCTCTTAG Reverse: ACAGTCCAGTTCTTCATTGC	194
Acta2	NM_007392.3	Forward: TCCAGCCATCTTTCATTGG Reverse: TCGTCGTATTCCTGTTTGC	307

Abbreviations: β-ACT, β-actin; COL1, collagen type I; α-SMA, alpha-smooth muscle actin.

Determination of Pregnancy Potential

Two weeks after surgery, the female mice designated for fertility pregnancy evaluation were co-housed with proven fertile males at a 1:1 female-to-male ratio. According to the presence of a vaginal plug, fertilization was measured 12 hours after proximity and co-caging with a male mouse. The following day, at 12 noon, was recorded as gestational day 0/5. Plug- positive females were then separated and housed individually.

Assessment of Fetal Implantation Number

Four out of six mice in the IUA group and all six mice in the sham group became pregnant. On gestational day 10, two pregnant mice from the IUA group and three pregnant mice from the sham group were anesthetized, and a laparotomy was performed to determine the number of implantation sites (fetuses) in each uterine horn.

Assessment of Live Birth Rate

The remaining pregnant mice were allowed to continue their pregnancy to term. The live birth rate was evaluated.

Statistical Analysis

All quantitative data are presented as the mean ± standard error of the mean (SEM), in accordance with recommended reporting guidelines. Data distribution was assessed using the Shapiro- Wilk normality test. Statistical analyses were performed using GraphPad Prism software (version 10.6.0; GraphPad Software, San Diego, CA, USA). Comparisons between the control and IUA groups were made using an unpaired, two-tailed Student's *t*-test for normally distributed data. A *P*-value of less than 0.05 was considered statistically significant. Statistical significance is indicated as follows: $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

RESULTS

Establishment of IUA Model Leads to Endometrial Atrophy and Glandular Reduction

Anesthesia was maintained throughout the surgical procedure, and all mice recovered from anesthesia approximately 30 minutes after completion of the operation. To confirm the successful establishment of IUA model, we first evaluated uterine histopathology using H&E staining. Compared to the control group, which displayed a thick, healthy endometrium with abundant glands, the IUA group exhibited significant endometrial atrophy. Specifically, the endometrial thickness was markedly reduced in the IUA group compared to the control group ;(138.4 ± 24.93 µm in IUA group vs. 270.2 ± 34.44 µm in the sham group, $p = 0.008$). Furthermore, the number of endometrial glands per high-power field was significantly lower in the IUA group versus the control (14.67 ± 3.21 vs 6 ± 2.64 per HPF, $p = 0.024$), indicating impaired endometrial structure (Figure 2 A-D).

Mechanical Injury Induces Endometrial Fibrosis

We next assessed the degree of fibrosis, a key pathological feature of IUA. Masson's trichrome staining revealed extensive collagen deposition (stained blue) throughout the endometrial stroma in the IUA group, which was minimal in the control group. Quantitative analysis confirmed this observation, showing that the fibrotic area as a percentage of total endometrial tissue was significantly higher in the IUA group than in the control group (72.51 ± 13.06 % in the IUA group vs. 32.05 ± 10.58% in the control group, $p = 0.016$) (Figure 2 E and F).

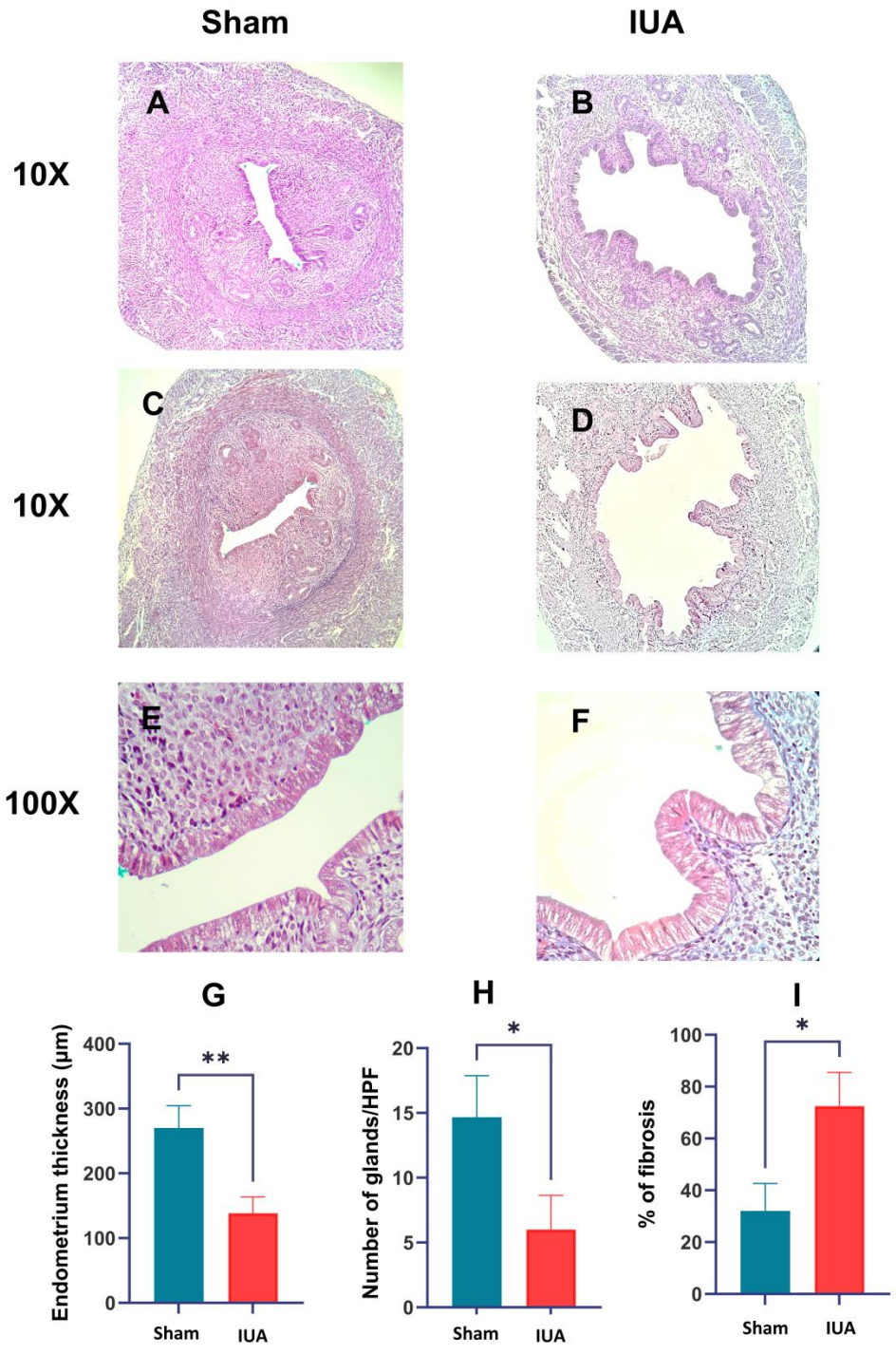

FIGURE 2. Histopathological analysis of the uterus in sham and IUA mice

(A, B) H&E- stained sections of the sham and IUA groups, respectively. (C-F) Masson's trichrome staining of the sham (C, E) and IUA (D, F) groups. (G) Quantification of endometrial thickness, showing a significant reduction in the IUA group ($p < 0.01$). (H) Number of glands per HPF, significantly decreased in the IUA group ($p < 0.05$). (I) Percentage of fibrosis significantly increased in the IUA group ($p < 0.05$). Comparisons between groups were performed using Student's t-test. *$P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)*.

Upregulation of Pro-fibrotic Gene Expression in the IUA Model

To investigate the molecular mechanisms underlying the observed fibrosis, we performed qPCR to analyze the expression of key pro-fibrotic markers. As shown in Figure 3, the relative mRNA expression of α-Smooth Muscle Actin (α-SMA), a marker for myofibroblast differentiation, was significantly upregulated in the uterine tissue of the IUA

group compared to the control group (p = 0.033). Similarly, the expression of type I Collagen (COL-1), a potent pro-fibrotic cytokine, was also significantly elevated in the IUA group (p = 0.048) (Figure 3). These results suggest the activation of a fibrotic cascade at the molecular level.

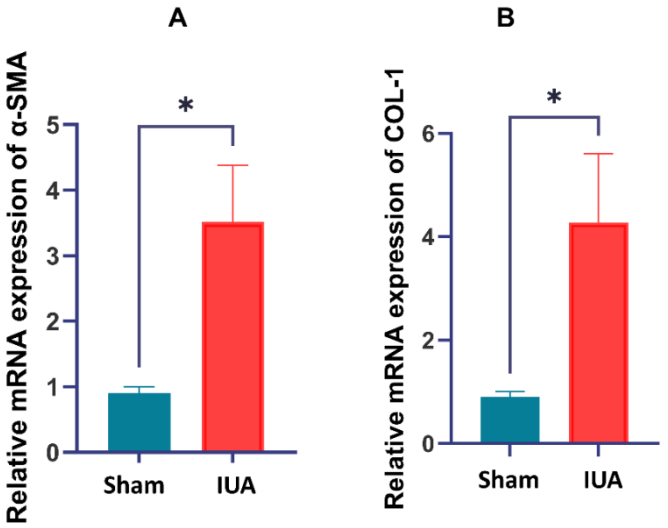

Figure 3. Relative mRNA expression of fibrotic genes in sham and IUA groups.

(A) Relative expression of α-SMA normalized to β-actin. (B) Relative expression of COL1 normalized to β-actin. Gene expression was normalized to the housekeeping gene β-actin, and relative levels were calculated using the 2^-ΔΔCt method. All reactions were performed in triplicate, and statistical differences between groups were assessed using Student's t-test. P < 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

The IUA Model Results in Impaired Fertility

Finally, to determine whether the observed pathological changes resulted in functional impairment, fertility was evaluated in the IUA and control groups. After co-housing with fertile males, plug- positive females were monitored, and on gestational day 10, a laparotomy was performed to quantify fetal implantation sites in each uterine horn. The IUA group exhibited a markedly reduced number of implantation sites compared with the control group; (5 ± 1 in the IUA group vs.10.33 ± 1.52 in the sham group (p = 0.011). In the remaining pregnant mice that were allowed to carry their pregnancies to term, the IUA group also showed a substantially lower live birth rate, along with reduced litter size and poorer neonatal outcomes, compared with the control group (4 ± 1 in the IUA group vs. 9 ± 1 in the sham group, p = 0.004). Together, these findings demonstrate that injury- induced endometrial fibrosis severely compromises both uterine receptivity and overall fertility potential (Figure 4).

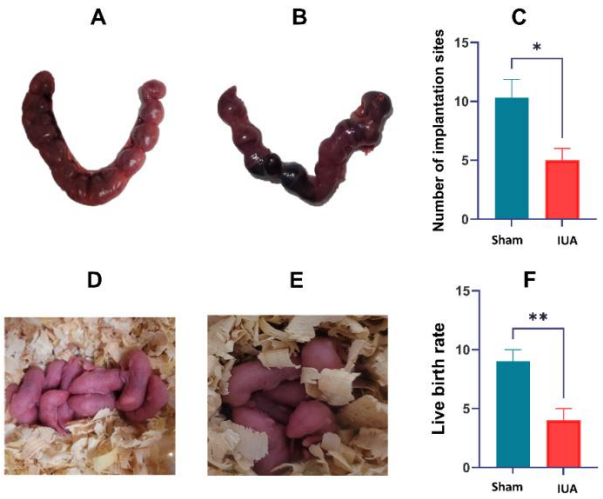

Figure 4. Functional assessment of the uterus in sham and IUA groups.

(A) Implantation sites in a sham uterus. (B) Implantation sites in an IUA uterus.(C) Quantification of implantation sites, showing a significant reduction in the IUA group (p < 0.05). (D) Newly born pups from a sham mouse. (E) Newly born pups from an IUA mouse. (F) Comparison of live birth rates, demonstrating a significant decrease in the IUA group (p < 0.01). All analyses were performed using Student's t-test. P < 0.05 (*), P < 0.01 (**), and P < 0.001 (***).

DISCUSSION

IUA remains a major clinical challenge and a serious threat to women's fertility and reproductive health worldwide (13). Under normal conditions, the basal layer of the endometrium has a strong ability to proliferate and repair. However, intrauterine procedures such as curettage can damage this basal layer, and sometimes even the myometrium. When such injury occurs alongside inflammation-induced fibrous exudation, excessive fibroblast proliferation, and scar- driven repair, the normal regeneration of uterine glands, blood vessels, and epithelial cells is disrupted. These combined factors ultimately lead to the development of intrauterine adhesions (14). In parallel, increased expression of the fibrosis markers COL1 and α-SMA, as observed in patient samples, is a characteristic feature of IUA (15). While impaired fertility is a serious outcome of IUA, one important limitation of several earlier IUA models is that fertility outcomes were not thoroughly evaluated (11). Assessing reproductive performance is essential for determining whether an experimental model truly captures the functional impact of the condition (11). Therefore, a comprehensive animal model of IUA should recapitulate all key pathological features of the disease.

As mentioned earlier, experimental IUA models are categorized as mechanical (curettage or thermal / electrocoagulation injury), chemical (intrauterine administration of fibrogenic agents such as ethanol or

trichloroacetic acid), or combined approaches (most commonly mechanical injury with lipopolysaccharide to augment inflammation and fibrosis) (7, 16). Mechanical induction of IUA offers several advantages over chemical, infectious, or thermal models. Clinically, most cases of IUA arise from mechanical trauma to the endometrium, such as curettage, making this approach highly translational (8). The method allows precise control over the location and extent of injury while minimizing systemic inflammatory effects that may confound immunological or antifibrotic analyses. Unlike chemical or infectious models, it does not introduce exogenous agents that could artificially amplify inflammatory pathways or interfere with therapeutic interventions. Importantly, mechanical injury reliably reproduces key histopathological features of human IUA, including glandular loss and collagen deposition, while preserving uterine structure sufficiently to permit fertility assessment. Together, these features make mechanical induction a reproducible and clinically relevant model for evaluating antifibrotic therapies.

Although 95% ethanol perfusion reliably induces endometrial thinning, gland loss, fibrosis, and impaired fertility, chemically induced uterine damage is uncommon in clinical settings and may not fully recapitulate the pathogenesis of human IUA. Therefore, while suitable for evaluating anti- adhesion biomaterials or stem cell–based therapies, chemical models are less appropriate for mechanistic studies, further supporting the translational relevance of mechanical injury models (8).

We successfully established a mouse model of intrauterine adhesions that closely mimics the mentioned clinical features, including fibrosis and decreased fertility. Our findings demonstrate that mechanical endometrial injury in mice leads to pronounced histological fibrosis and significant upregulation of key fibrotic markers, including COL-1 and α-SMA. These results are consistent with previous reports showing increased mRNA expression of these fibrotic genes following mechanical uterine damage (17). This mechanical injury markedly disrupted uterine architecture, resulting in a reduced number of endometrial glands, decreased endometrial thickness, and a significant expansion of the fibrotic area, as similarly reported in previous studies (17, 18). Importantly, the inclusion of fertility assessment enhances the translational relevance of this model by demonstrating the functional consequences of IUA. In our study, IUA induction resulted in a significant reduction in both the number of implantation sites and the number of live- born pups. Similarly, previous studies evaluating fertility outcomes in murine models have consistently reported a decreased number of implanted embryos following IUA induction (19).

Anesthesia is essential for reducing stress during invasive procedures, yet it can also disrupt normal physiology and produce unwanted side effects. In animal experiments, such changes may compromise animal welfare and potentially influence experimental outcomes (20). Although ketamine–xylazine is routinely used as a standard anesthetic combination in mice, it is known to cause significant adverse effects, including bradycardia and hypoxia (21). The resulting reduction in oxygen levels compromises animal safety and has been associated with a dose-dependent increase in mortality (22). Using a combination of anesthetic agents to reach an appropriate anesthetic plane can improve induction, recovery, and overall safety compared with relying on a single drug. However, despite being standard in other species, premedication before inhalant anesthesia is rarely used in mice (23). A recent study in C57BL/6J mice demonstrated that balanced anesthesia improves the induction phase and reduces the required dose of isoflurane. Pre-medication with xylazine significantly decreased behavioral stress during induction and lowered the minimum alveolar concentration (MAC) of isoflurane without compromising physiological stability. These findings support the use of xylazine prior to isoflurane induction in healthy mice, particularly when procedures are performed under 100% oxygen and minimal blood loss is anticipated (23).

In our study, we employed a triple combination of ketamine, xylazine, and isoflurane, which ensured stable anesthesia throughout surgery and minimized animal discomfort. This optimized anesthetic protocol strengthens the reliability of our IUA model and provides a robust platform for investigating underlying mechanisms as well as evaluating antifibrotic or regenerative strategies.

CONCLUSION

This study establishes an optimized and well- characterized mouse model of intrauterine adhesions, exhibiting marked endometrial fibrosis and impaired fertility. We also introduce a novel balanced anesthesia approach using a combination of isoflurane with ketamine- xylazine to ensure a stable anesthetic plane and improved procedural safety. This model provides a robust platform for the preclinical evaluation of therapeutic strategies aimed at restoring uterine structure and function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

Our research was funded by the National Institute for Medical Research Development Grant No.4040201.

AI USE DECLARATION

The authors declare that no Artificial Intelligence (AI) tools or Large Language Models were used in the preparation of this manuscript. All content was produced solely by the authors, who take full responsibility for its accuracy and integrity.

ACKNOWLEDGEMENTS

This research was funded by the National Institute for Medical Research Development (NIMAD) (Grant No. 4040201). The funding agency had no role in the study design; data collection, analysis, or interpretation; manuscript preparation; or the decision to submit the manuscript for publication.

The authors declare that no artificial intelligence tools were used at any stage of the study or during the preparation, writing, editing, or revision of the manuscript, including translation, grammar checking, or language editing.

AUTHOR CONTRIBUTIONS

Kimiya Rashidan had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Kimiya Rashidan; Seyed Mahmoud Hashemi.

Methodology and investigation (data acquisition): Kimiya Rashidan; Ashkan Rasouli-Saravani; Malaksima Ayadilord; Hossein Aminianfar.

Data analysis and interpretation: Kimiya Rashidan; Nariman Mosaffa.

Drafting of the manuscript: Kimiya Rashidan, Ashkan Rasouli-Saravani; Malaksima Ayadilord; Nariman Mosaffa; Hossein Aminianfar; Seyed Mahmoud Hashemi.

Critical revision of the manuscript for important intellectual content: Ashkan Rasouli-Saravani; Malaksima Ayadilord; Nariman Mosaffa; Hossein Aminianfar; Seyed Mahmoud Hashemi.

Statistical analysis: Kimiya Rashidan; Nariman Mosaffa.

Supervision and project administration: Seyed Mahmoud Hashemi.

All authors contributed substantially to the work, reviewed and approved the final manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

DATA AVAILABILITY

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

REFERENCES

1. Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome--one century later. Fertil Steril. 2008 Apr;89(4):759–79.

2. Hooker AB, de Leeuw R, van de Ven PM, Bakkum EA, Thurkow AL, Vogel NEA, et al. Prevalence of intrauterine adhesions after the application of hyaluronic acid gel after dilatation and curettage in women with at least one previous curettage: short-term outcomes of a multicenter, prospective randomized controlled trial. Fertil Steril. 2017 May;107(5):1223-1231.e3.

3. Toma LM, Socolov D, Matei D, Anton S, Balan R, Anton E, et al. Intrauterine Adhesions and Asherman Syndrome: A Retrospective Dive into Predictive Risk Factors, Diagnosis, and Surgical Perspectives. Diagnostics. 2025;15(8).

4. Myers EM, Hurst BS. Comprehensive management of severe Asherman syndrome and amenorrhea. Fertil Steril. 2012 Jan;97(1):160–4.

5. Capella-Allouc S, Morsad F, Rongières-Bertrand C, Taylor S, Fernandez H. Hysteroscopic treatment of severe Asherman's syndrome and subsequent fertility. Hum Reprod. 1999 May;14(5):1230–3.

6. Liu L, Yang H, Guo Y, Yang G, Chen Y. The impact of chronic endometritis on endometrial fibrosis and reproductive prognosis in patients with moderate and severe intrauterine adhesions: a prospective cohort study. Fertil Steril. 2019 May;111(5):1002-1010.e2.

7. Xi J, Pan Y, Jin C, Liu J, Cheng J, Xu B. Evaluation of different rat models intrauterine adhesion models and improvement of the technique for their establishment. Exp Anim. 2023 May 17;72(2):274–84.

8. Liu PC, Song YT, Zhao LM, Jiang YL, Hu JG, Dong L, et al. Establishment and comparison of different procedures for modeling intrauterine adhesion in rats: A preliminary study. Heliyon. 2024 Feb 15;10(3):e25365.

9. Liu D, Yuan L, Xu F, Ma Y, Zhang H, Jin Y, et al. Interleukin-33 promotes intrauterine adhesion formation in mice through the mitogen-activated protein kinase signaling pathway. Communications Biology. 2024 Aug 20;7(1):1022.

10. Xu XX, Cao LB, Wang Z, Xu Z, Zhang BQ, Wu SL, et al. Creation of a rabbit model for intrauterine adhesions using electrothermal injury. J Zhejiang Univ Sci B. 2018 May;19(5):383–9.

11. Feng Q, Gao B, Zhao X, Huang H, Yi S, Zou L, et al. Establishment of an animal model of intrauterine adhesions after surgical abortion and curettage in pregnant rats. Ann Transl Med. 2020 Feb;8(4):56.

12. Rasouli-Saravani A, Ghanbarian H, Ramezani-Tehrani F, Mosaffa N, Ghaffari-Khaligh S, Ghambari-Mohammadi N, et al. Human amniotic fluidic derived-extracellular vesicles enriched by miR-29 ameliorate endometrial fibrosis and promote repair of damaged endometrium in an experimental model of intrauterine adhesion. Biochem Biophys Res Commun. 2026 Jan 29;806:153344.

13. Liu L, Chen G, Chen T, Shi W, Hu H, Song K, et al. si-SNHG5-FOXF2 inhibits TGF-β1-induced fibrosis in human primary endometrial stromal cells by the Wnt/β-catenin signalling pathway. Stem Cell Res Ther. 2020 Nov 11;11(1):479.

14. Xu C, Bao M, Fan X, Huang J, Zhu C, Xia W. EndMT: New findings on the origin of myofibroblasts in endometrial fibrosis of intrauterine adhesions. Reprod Biol Endocrinol. 2022 Jan 7;20(1):9.

15. Yang J, Ren L, Gu X, Wang L, Cui J. Microfibrillar-associated protein 5 promotes TGF-β1-induced endometrial epithelial fibrosis in intrauterine adhesion. Biol Reprod. 2025 Oct 27;ioaf242.

16. Liu F, Zhu ZJ, Li P, He YL. Creation of a female rabbit model for intrauterine adhesions using mechanical and infectious injury. J Surg Res. 2013 Jul;183(1):296–303.

17. Guo LP, Chen LM, Chen F, Jiang NH, Sui L. Smad signaling coincides with epithelial-mesenchymal transition in a rat model of intrauterine adhesion. Am J Transl Res. 2019;11(8):4726–37.

18. Yang H, Zhang W, Fang J, Tang Z, Zhou Y, Hu H, et al. Intrauterine infusion of platelet-rich plasma improves fibrosis by transforming growth factor beta 1/Smad pathway in a rat intrauterine adhesion model. Reprod Biol. 2024 Jun;24(2):100882.

19. Li B, Zhang Q, Sun J, Lai D. Human amniotic epithelial cells improve fertility in an intrauterine adhesion mouse model. Stem Cell Res Ther. 2019 Aug 14;10(1):257.

20. Virgilio T, Latino I, Pizzichetti C, Chahine K, Capucetti A, Detotto C, et al. Impact of prolonged isoflurane or ketamine–xylazine anesthesia with or without buprenorphine and oxygen on mouse vitals and immune responses. Lab Animal. 2025 Oct 1;54(10):270–7.

21. Blevins CE, Celeste NA, Marx JO. Effects of Oxygen Supplementation on Injectable and Inhalant Anesthesia in C57BL/6 Mice. J Am Assoc Lab Anim Sci. 2021 May 1;60(3):289–97.

22. Mechelinck M, Kupp C, Krüger JC, Habigt MA, Helmedag MJ, Tolba RH, et al. Oxygen inhalation improves postoperative survival in ketamine-xylazine anaesthetised rats: An observational study. PLoS One. 2019;14(12):e0226430.

23. David EM, Pacharinsak C, Jampachaisri K, Hagan L, Marx JO. Use of Ketamine or Xylazine to Provide Balanced Anesthesia with Isoflurane in C57BL/6J Mice. J Am Assoc Lab Anim Sci. 2022 Sep 1;61(5):457–67.