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# Diagnostic value of multimodal hysterosalpingo-contrast sonography combined with negative intrauterine contrast-enhanced ultrasound in female infertility

Fen Fu[1†], Yi-Fan Zhu[1†], Yue-Fan Chen[1], Jia-Jing Zhuang[1], Wen-Ting Zheng[1], Rong-Xi Liang[1*] and Qin Ye[1*]

## Abstract

**Objective** To investigate the application value of multimodal hysterosalpingo-contrast sonography (HyCoSy) combined with negative hysterography in assessing tubal patency and diagnosing uterine lesions.

**Methods** A total of 310 infertility patients were selected and examined using multimodal HyCoSy and negative hysterography to evaluate tubal patency and uterine lesions. Based on the doctors' experience levels, they were divided into high seniority group (more than 5 years of experience in Contrast-enhanced ultrasound) and low seniority group (resident doctors). Various ultrasound modes, including two-dimensional contrast-enhanced ultrasound (2D), static three-dimensional contrast-enhanced ultrasound (3D), real-time three-dimensional contrast-enhanced ultrasound (4D), and transvaginal harmonic imaging (HI), were employed for diagnosis. The $\chi 2$ test was used to compare the success rates of tubal patency diagnosis across different mode combinations. The Wilcoxon rank-sum test was used to compare the diagnostic efficiency of tubal patency between high and low seniority groups under multimodal ultrasound.

**Results** A total of 306 patients successfully completed the examinations, with 538 cases of tubal patency and 74 cases of obstruction identified. The combination of multiple modalities significantly improved diagnostic efficiency, with the 4D + 3D + 2D + HI mode demonstrating a significantly higher diagnostic success rate compared to single modes ($P < 0.005$). Negative hysterography showed high consistency with hysteroscopy in diagnosing uterine malformations, with 100% sensitivity and specificity. Additionally, it demonstrated good diagnostic ability for cesarean scar diverticulum, endometrial polyps, submucosal fibroids, and uterine adhesions.

**Conclusion** The combination of multimodal HyCoSy and negative hysterography is a simple, rapid, and accurate examination method suitable for assessing tubal patency and diagnosing uterine lesions.

**Keywords** Infertility, Fallopian tube, Uterus, Multimodal, Contrast-enhanced ultrasound

[†]Fen Fu and Yi-Fan Zhu contributed equally to this work.

*Correspondence:
Rong-Xi Liang
lrx190@163.com
Qin Ye
xhxhyeye@163.com
[1] Department of Ultrasound/Fujian Provincial Institute of Ultrasonic Medicine, Fujian Medical University Union Hospital, No.29 of Xinquan Road, Gulou District, Fuzhou, Fujian Province 350001, China

## Introduction

Infertility refers to a disease in which a fixed spouse, without protective measures, has had normal sexual activity for one year or more but has not yet successfully conceived [1]. Prolonged infertility after marriage can put enormous pressure on patients from various aspects such as family and society [2]. As important participants and builders of modern society, women with infertility face not only physical and mental health issues but also challenges to family harmony [3]. The fallopian tube and the uterus play a crucial role in human reproduction. Factors such as tubal or intrauterine synechiae, endometrial polyps, and congenital uterine malformations (e.g., septate uterus) have led to an increase in the incidence of female infertility [4, 5]. Therefore, determining fallopian tube patency and diagnosing uterine cavity lesions in a simple, quick, and accurate manner is crucial.

Hysterosalpingo-contrast sonography (HyCoSy) is a novel non-invasive infertility examination method developed in recent years. It has shown significant advantages in diagnosing uterine and tubal pathologies, particularly in evaluating tubal patency [6]. During a HyCoSy examination, doctors inject contrast agents into the patient's uterine cavity and fallopian tubes through a catheter to visualize the morphology and position of the uterus and fallopian tubes, detect uterine cavity lesions, and assess tubal patency. As ultrasound technology continues to advance, HyCoSy techniques have been continuously innovated and improved.

The combination of two-dimensional contrast-enhanced ultrasound (2D-HyCoSy), static three-dimensional contrast-enhanced ultrasound (3D-HyCoSy), real-time three-dimensional (four-dimensional) contrast-enhanced ultrasound (4D-HyCoSy), and transvaginal ultrasonography harmonic imaging (TVS, HI) is collectively referred to as multimodal HyCoSy. Negative hysterography involves using a negative contrast agent (saline) to expand the uterine cavity, creating a clear acoustic window, which enhances the display of uterine cavity lesions. Negative hysterography provides highly consistent diagnostic results for endometrial, submucosal fibroids, and uterine adhesions compared to hysteroscopy [7, 8].

We assessed the effectiveness of multimodal HyCoSy in combination with negative intrauterine contrast ultrasound for diagnosing uterine and fallopian tube lesions. Therefore, the objective of this study is to propose a comprehensive and straightforward "single-step" examination method for the complete evaluation of the female reproductive tract environment.

## Materials and methods

### Patients

A total of 310 infertile patients, aged 21–46 (31 ± 5) years old, were admitted to the outpatient department of our hospital from November 2017 to October 2020. Among them, 117 were primary infertile patients and 193 were secondary infertile patients. This study received approval from the hospital ethics committee, and all participants provided informed consent. Patients with abnormal findings on hysterosalpingography voluntarily chose to undergo hysteroscopy. The inclusion criteria were: having a normal sexual life, no use of contraception for over one year, and no endocrine abnormalities. The exclusion criteria included: a history of ectopic pregnancy, vaginal bleeding, acute sexually transmitted diseases, acute/chronic inflammation of the reproductive tract, and fever.

### Instrument and contrast agent

We utilized a GE-VolusonE10 color Doppler ultrasound diagnostic instrument equipped with a transvaginal probe (model RIC59-D, 5.0–9.0 MHz frequency, mechanical index 0.12–0.18). The instrument incorporated coded contrast imaging technology. We employed the positive contrast agent SonoVue (Bracco, Italy) as the microbubble contrast agent, and 0.9% normal saline solution served as the negative intrauterine contrast medium.

### Preparation before operation

Before the procedure, patients were informed about the examination contents and potential complications, and they provided their informed consent. Patients emptied their bladders 30 min before the surgery, and 0.5 mg of atropine was injected intramuscularly to reduce tubal spasms. Patients were then positioned in the lithotomy position. Routine TVS was performed to check for abnormal echoes in the uterus and bilateral adnexal areas. After routine disinfection, a disposable double-cavity water pipe was inserted into the uterine cavity, and 1.5–3 mL of normal saline was injected to fill the balloon and secure the catheter. A 5 mL contrast agent microbubble suspension was prepared by injecting 5 mL of physiological saline into SonoVue and shaking thoroughly. TVS was used to perform transverse scanning of the uterus, initiating the 3D mode. The volume sampling frame was set to maximum, and the probe was adjusted according to the bilateral uterine angle and ovarian position for 3D pre-scanning. Meanwhile, 20 mL of contrast agent suspension was prepared by mixing 17.5 mL of preheated physiological saline (kept in an electric thermostatic water bath at 33 °C) with 2.5 mL of the prepared microbubble suspension and shaking well. Another syringe was filled with 20 mL of physiological saline.

### Procedure and sequence of multimodal contrast-enhanced ultrasound (Fig. 1)

(a) 4D Mode: The 4D collection was initiated, and 10 mL of contrast agent suspension was injected to observe the dispersion of the contrast agent around the ovaries and pelvic cavity.
(b) 3D Mode: The 3D mode was activated, and 10 mL of contrast agent suspension was injected.

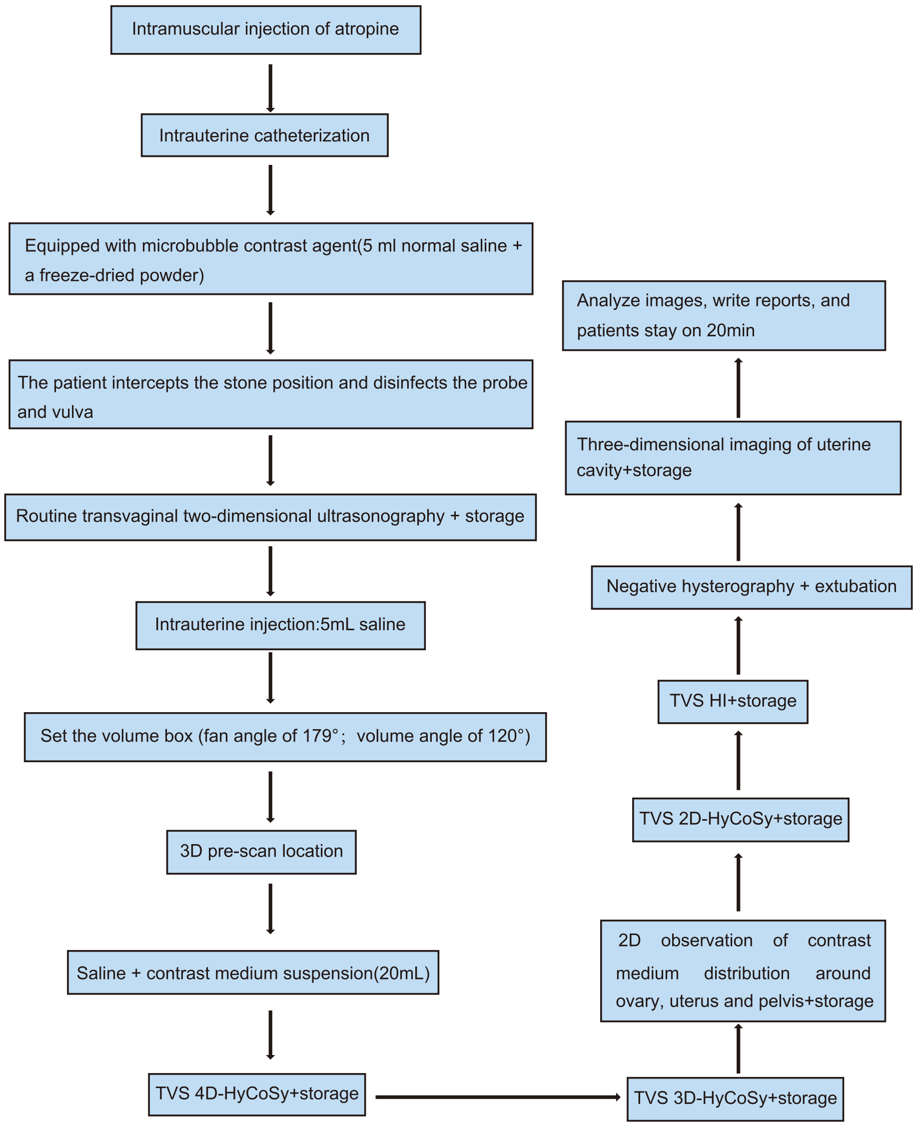


**Fig. 1** Operation flow chart of multimodal transvaginal transvaginal contrast-enhanced ultrasonography combined with negative hysterography

(c) 2D Mode: The 2D contrast mode was initiated. The contrast agent was continuously injected (5–10 mL), and its passage from the uterine cavity through both fallopian tubes and the umbrella end around the ovaries was observed.
(d) HI Mode: All imaging modes were closed, and the contrast agent (5–10 mL) was continuously injected again under dynamic tissue harmonic imaging. Both sides of the fallopian tubes were tracked.
(e) Uterine Cavity Negative Contrast-Enhanced Mode: The water sac was slightly deflated, and the cervical opening plug was gently pulled down. The negative contrast agent was evenly injected through the catheter until the residual SonoVue strong echo signal disappeared. The uterine cavity was then anechoic and filled to maintain an appropriate degree of expansion. Two-dimensional ultrasound was used to continuously scan the uterine cavity in both longitudinal and transverse sections to observe its morphology. The morphology of the uterine cavity, the smoothness of the endometrial surface, and the presence or absence of lesions were fully observed. The normal saline in the water sac was completely drained. As the tube was removed, 0.9% normal saline was rapidly injected, and 3D imaging of the uterus was performed immediately [9].

All ultrasonic image data were stored on the instrument's hard disk for later analysis, and patients were advised to rest and remain under observation for 15–30 min.

### Image analysis and judgment index

Ultrasound physicians were categorized into two groups based on their level of experience: high seniority and low seniority. The high seniority group comprised doctors with more than five years of experience in contrast-enhanced ultrasound (CEUS). The low seniority group consisted of resident doctors who were undergoing standardized training in ultrasound medicine. Both groups independently analyzed the entire research dataset to evaluate fallopian tube patency and identify uterine lesions.

### Evaluation items and criteria of tubal patency and uterine lesions

According to the Standard of Clinical Application Guide of Contrast-enhanced Ultrasonography by the Chinese Medical Association, fallopian tube patency is classified into two types: patency and obstruction [10]. (a) The fallopian tube is deemed "unobstructed" when there is low resistance to the injection of contrast medium, and we observe smooth and natural progression of the fallopian tube, uniform thickness and full development, overflow of contrast medium at the umbrella end, a ring around the ovary, no reflux, and uniform dispersion in the pelvic cavity. (b) The fallopian tube is considered "obstructed" when there is significant resistance to the injection of contrast medium, no or partial development of the fallopian tube, no ring around the ovary, no diffusion of contrast medium in the pelvic cavity, and obvious reflux and counterflow in the uterine wall.

### Statistical methods

Statistical data analysis was performed using SPSS v23.0 (IBM Corp., Armonk, NY). The results of the contrast-enhanced ultrasound of the fallopian tubes were expressed as categorical data. The success rate of contrast-enhanced ultrasound diagnosis of tubal patency in different mode groups was compared using the $\chi2$ test. The diagnostic efficiency of tubal patency between high- and low seniority groups under multimode contrast-enhanced ultrasound was compared using the Wilcoxon rank-sum test.

Negative hysterography considered hysteroscopy results as the "gold standard." The accuracy of negative hysterography in diagnosing uterine lesions, along with sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of common uterine lesions, were calculated.

## Results

### Evaluation of tubal patency by multimodal contrast-enhanced ultrasound

Among the 310 patients with infertility, 4 refused to undergo the examination due to pain. Salpingography was successfully performed in the remaining 306 cases. Of the 612 fallopian tubes assessed, 538 were deemed patent (Figs. 2 and 3), while 74 were found to be obstructed (21 right-sided, 23 left-sided, and 15 bilateral) (Fig. 4). The 15 cases of bilateral obstruction (Fig. 5) were confirmed by laparoscopic surgery. In the unobstructed cases, the 4D mode revealed smooth fallopian tubes with contrast medium ejected from the umbrella end, forming a ring around the ovary and evenly dispersing in the pelvic cavity. The 3D mode showed smooth fallopian tubes, with liquid echoes visible in 2D and HI modes, and low injection pressure of the contrast medium. In the 74 obstructed cases, the 4D, 3D, 2D, and HI modes did not display the fallopian tubes; only a small segment at the proximal end of the fallopian tubes developed, with no contrast medium observed around the obstructed fallopian tubes and pelvis. High injection pressure of the contrast medium and uterine wall counterflow were observed in some patients.

### Comparison of the diagnostic efficiency of multimodal CEUS between the high- and low seniority groups

The diagnostic efficiency of the combination of different modes significantly differed between the high and low seniority groups (high seniority group: $\chi2 = 115.599$, $P = 0.0001, < 0.01$; low seniority group: $\chi2 = 104.614$, $P = 0.0002, < 0.01$). Table 1 compares the diagnostic efficiency of single-mode and multimode joint examinations and verifies the fallopian tube patency examination by ultrasound doctors from different seniority groups. Significant differences were observed between the 4D + 3D + 2D + HI group and the 4D + 3D + 2D, 4D + 3D, and 4D groups. Table 2 compares the diagnostic efficiency of tubal patency between the high and low seniority groups. The results indicate that a greater number of combined modes correspond to higher diagnostic efficiency of tubal patency.

There was no significant difference in the diagnostic efficiency of tubal patency between the high seniority group and the low seniority group when using the combination of different modes ($P = 0.857, > 0.05$). This

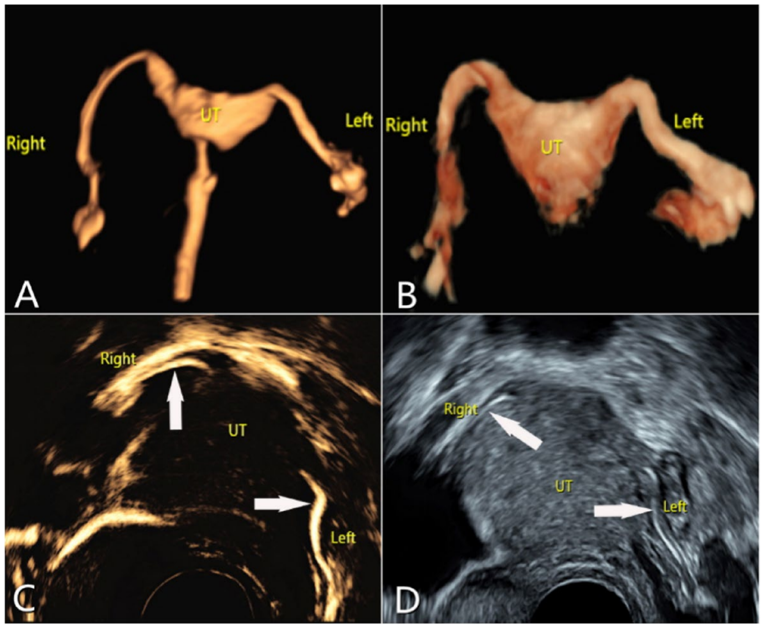


**Fig. 2** Multimodal contrast-enhanced ultrasound of tubal patency of a patient with bilateral tubal patency. TVS 4D-HyCoSy mode (**A**), TVS 3D-HyCoSy mode (**B**), stereoscopic and intuitive display of bilateral fallopian tube patency; TVS 2D-HyCoSy mode (**C**) and TVS HI mode (**D**) can show bilateral tubal patency (shown by arrow) (UT: uterus)

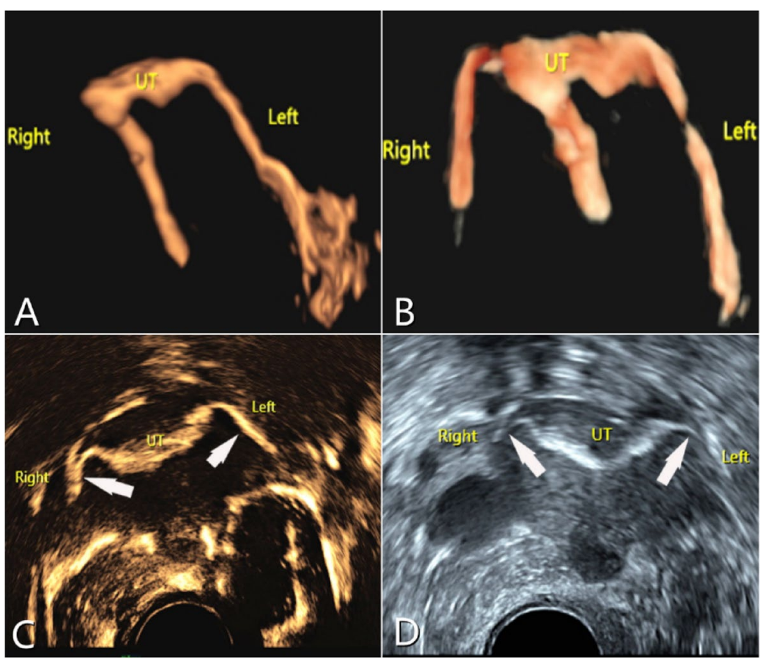


**Fig. 3** Multimodal contrast-enhanced ultrasound of tubal patency of a patient with bilateral tubal patency. TVS 4D-HyCoSy mode (**A**) shows only left fallopian tube patency, right fallopian tube is not developed; TVS 3D-HyCoSy mode (**B**) shows bilateral fallopian tube patency; TVS 2D-HyCoSy mode (**C**) and TVS HI mode (**D**) both show bilateral fallopian tube patency (shown by arrow) (UT: uterus)

indicates that with the use of multimodal contrast-enhanced ultrasound, both groups of ultrasound doctors can effectively diagnose tubal patency.

## Results of negative hysterography in the diagnosis of uterine lesions

Of the 310 patients with infertility, four refused to

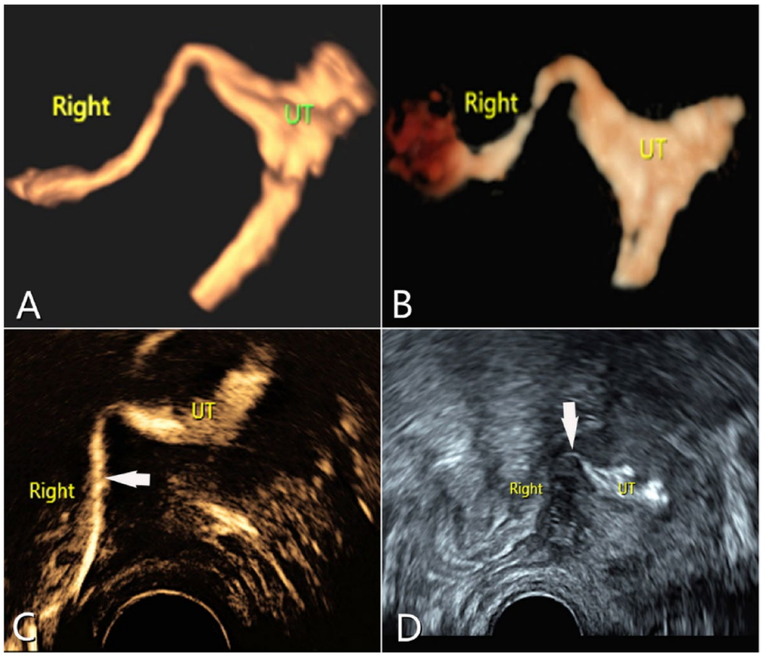


**Fig. 4** Multimodal contrast-enhanced ultrasound of tubal patency of a patient with left fallopian tube obstruction. TVS 4D-HyCoSy mode (**A**), TVS 3D-HyCoSy mode (**B**), TVS 2D-HyCoSy mode (**C**), TVS HI mode (**D**) all show that the right fallopian tube is unobstructed, while the left fallopian tube is blocked (shown by the arrow) (UT: uterus)

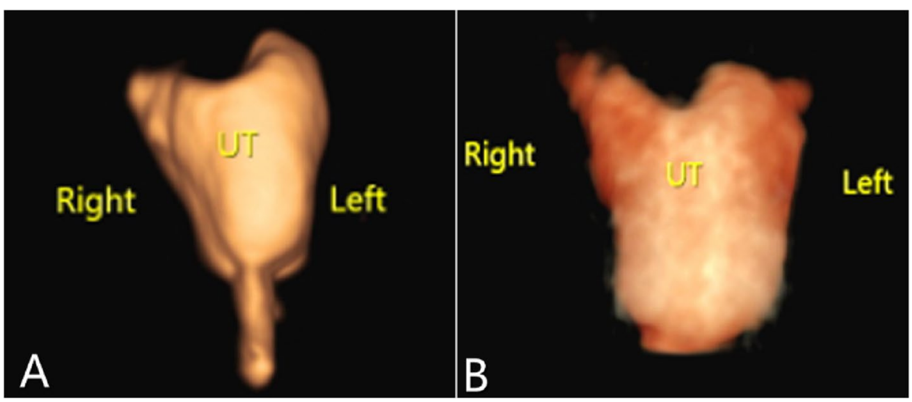


**Fig. 5** Multimodal contrast-enhanced ultrasound of tubal patency of a patient with bilateral fallopian tube obstruction. TVS 4D-HyCoSy mode (**A**); TVS 3D-HyCoSy mode (**B**), both modes show bilateral fallopian tube obstruction (UT: uterus)

undergo the examination due to pain, while the remaining 306 patients successfully underwent negative uterine radiography. A total of 91 cases presented with abnormal uterine cavities, resulting in a detection rate of 29.7% for uterine lesions. Among these, 62 cases were further examined using hysteroscopy. The hysteroscopy results revealed five cases of uterine malformation (one with uterus unicornis and four with uterus septus) (Fig. 6), two cases of scar diverticulum, 27 cases of endometrial polyps (Fig. 7), two cases of submucosal myoma, and 25 cases of uterine adhesion following cesarean section (Fig. 8). When comparing the results of negative hysterography with hysteroscopy, the final diagnosis was consistent in 57 cases, yielding an accuracy rate of 91.94% (57 out of 62). This indicates that negative hysterography was highly accurate for diagnosing uterine lesions. A comparison of the results of negative hysterography and hysteroscopy is presented in Table 3. The results for different types of uterine lesions were statistically analyzed. Compared with hysteroscopy findings, the overall accuracy

**Table 1** Diagnostic efficiency of single-mode and multimodal examination/verification of tubal patency in different seniority groups (section)

| Different mode groups | Diagnostic efficiency of doctors in high seniority group | | Diagnostic efficiency of doctors in low seniority group | |
|---|---|---|---|---|
| | Displayable (+) | Not displayable (-) | Displayable (+) | Not displayable (-) |
| 4D | 530 | 82 | 530 | 82 |
| 4D + 3D | 558 | 54 | 558 | 54 |
| 4D + 3D + 2D | 596 | 16 | 593 | 19 |
| 4D + 3D + 2D + HI | 612 | 0 | 610 | 2 |

**Table 2** Tubal patency diagnostic efficiency between high- and low seniority groups by ultrasound physicians in different mode groups

| Different mode groups | Diagnostic efficiency of doctors in high seniority group | | | Diagnostic efficiency of doctors in low seniority group | | |
|---|---|---|---|---|---|---|
| | 4D + 3D | 4D + 3D + 2D | 4D + 3D + 2D + HI | 4D + 3D | 4D + 3D + 2D | 4D + 3D + 2D + HI |
| 4D | $\chi^2 = 6.485$ $P = 0.014$ | | | $\chi^2 = 6.485$ $P = 0.014$ | | |
| 4D + 3D | | $\chi^2 = 21.880$ $P = 0.0003$ | | | $\chi^2 = 17.845$ $P = 0.0003$ | |
| 4D + 3D + 2D | | | $\chi^2 = 16.212$ $P = 0.0003$ | | | $\chi^2 = 14.002$ $P = 0.0002$ |

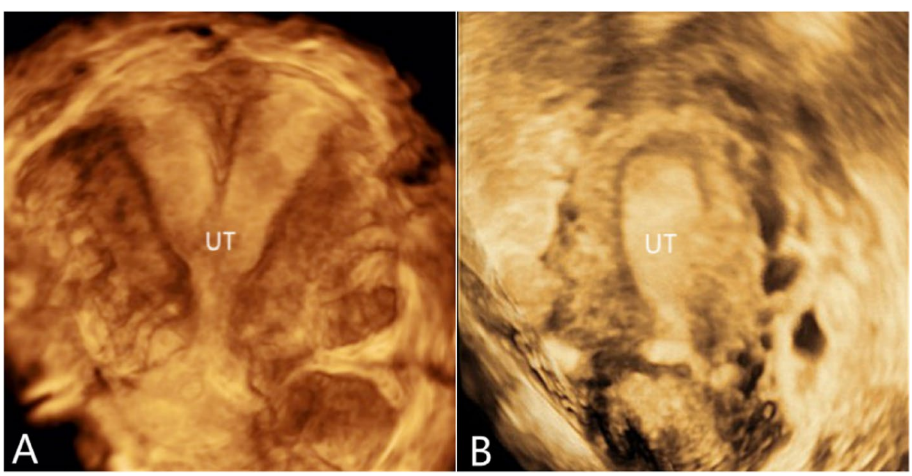


**Fig. 6** Negative hysterography shows uterine malformation. 3D-TVS Render coronal imaging after negative hysterography shows uterus septus (**A**) and uterus unicornis (**B**)

of negative hysterography in diagnosing various uterine lesions was 91.94% (57 out of 62). This included 100% accuracy for uterine deformities (5 out of 5), 98.39% for cesarean section scar diverticulum (61 out of 62), 93.55% for endometrial polyps (58 out of 62), 95.16% for uterine submucous myoma (59 out of 62), and 93.55% for uterine adhesion (58 out of 62). The statistical analysis of various uterine lesions examined using negative hysterography is presented in Table 4.

## Discussion

In this study, we utilized multimodal HyCoSy in combination with negative hysterography to assess tubal patency and detect uterine lesions. This method offers a

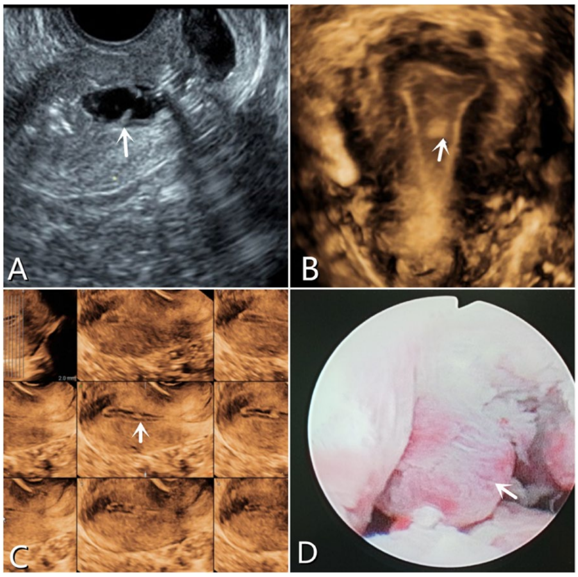


**Fig. 7** Negative hysterography shows endometrial polyps. Negative hysterography showing endometrial polyps (**A**) 3D-TVS Render coronal imaging of the uterus showing endometrial polyps (**B**) uterine tomographic ultrasonography showing endometrial polyps (**C**) hysteroscopic examination of endometrial polyps (**D**)

simple, rapid, and accurate examination technique suitable for grassroots hospitals. The details of each step-by-step mode of CEUS are described below.

### 4D mode

The 4D mode can clearly display three-dimensional images of the fallopian tube and uterine cavity [11]. It shows the patency of each segment of the fallopian tube in real time and directly displays its shape, course, and contrast medium distribution to evaluate patency. It can also display the speed differences in bilateral fallopian tube development, the diffusion of contrast medium around the ovary and pelvis, and allows for frame-by-frame analysis of clear and intuitive images [12, 13].

These images are easily interpreted by clinicians. Consequently, in 4D mode, physicians in both high and low seniority groups demonstrated the same diagnostic efficiency for tubal patency. However, if only 4D mode examination was used, the diagnostic success rate for both seniority groups was 86.7% (530 out of 612).

In 4D mode, it is crucial to control the injection dose of contrast medium suspension to 5–10 mL. In some cases, the contrast medium cannot reach the umbrella end or be ejected, and excessive diffusion into the pelvis may affect subsequent modal examinations. Approximately 13.3% (82 out of 612) of the fallopian tubes in this group could not be diagnosed for the above reasons. Combining 4D mode with other modes can improve the diagnostic success rate.

### 3D mode

The 3D mode enables the three-dimensional reconstruction of the fallopian tube's shape and course, facilitating diagnosis [12–16]. With proficient examination, 3D mode can capture the entire process of the contrast medium flowing through the fallopian tube to the umbrella end, providing clear and intuitive three-dimensional imaging. In 3D mode, the diagnostic efficiency for tubal patency is consistent across high and low seniority groups [17].

However, the success rate of displaying the fallopian tube in 3D mode depends on the scanning angle and phase, which varies with the length and course of the fallopian tube, necessitating higher examiner skill and proficiency.

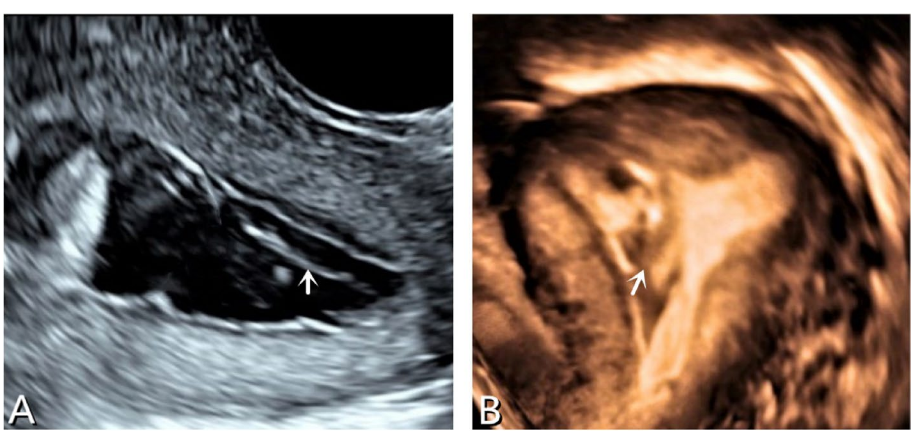


**Fig. 8** Negative hysterography shows uterine adhesion. Negative hysterography showing uterine adhesion (**A**) coronal 3D-TVS Render imaging of the uterus shows uterine adhesion (**B**)

**Table 3** Results comparison between negative hysterography and hysteroscopy in 62 patients (example)

| Negative hysterography | Hysteroscopy | | Total |
|---|---|---|---|
| | + | - | |
| Uterine malformation | | | |
| + | 5 | 0 | 5 |
| - | 0 | 57 | 57 |
| Total | 5 | 57 | 62 |
| Cesarean section scar diverticulum | | | |
| + | 2 | 0 | 2 |
| - | 1 | 59 | 60 |
| Total | 3 | 59 | 62 |
| Intimal polyp | | | |
| + | 25 | 2 | 27 |
| - | 2 | 33 | 35 |
| Total | 27 | 35 | 62 |
| Submucosal myoma | | | |
| + | 1 | 1 | 2 |
| - | 2 | 58 | 60 |
| Total | 3 | 59 | 62 |
| Uterine adhesion | | | |
| + | 24 | 1 | 25 |
| - | 3 | 34 | 37 |
| Total | 27 | 35 | 62 |

If the scanning time is too short, the contrast medium may not fully fill the fallopian tube, resulting in incomplete image acquisition and difficulty in analysis, potentially leading to misjudgments. Additionally, it is challenging to determine the presence of contrast medium around the ovary. If the intubation depth is too deep, the contrast medium may flow along one side of the fallopian tube, hindering the development of the other side. Therefore, it is essential to reposition the balloon and combine other modes to verify the results.

This study demonstrated that 558 fallopian tubes could be successfully diagnosed in both high and low seniority groups using the combined 4D + 3D modes, with a diagnostic success rate of 91.2% (558 out of 612), significantly higher than the single 4D mode group ($P < 0.05$). However, 54 fallopian tubes could not be displayed due to unfavorable scanning angles, intubation depth, prior contrast medium injection, and diffusion into the pelvis. Thus, combining other modes is necessary to improve diagnostic success rates.

#### 2D mode

The modal operation method is flexible and capable of dynamically tracking the entire course of the fallopian tube. It allows for the observation of the contrast medium's ejection at the umbrella end of the fallopian tube, as well as the wrapping of the contrast medium around the ovary and its diffusion in the pelvic cavity. However, it requires higher levels of manipulation and experience. Therefore, this study enhances the 2D modal examination and verification (4D + 3D + 2D group) by combining 4D and 3D modes, thereby improving the success rate of fallopian tube patency diagnosis. This combination can further verify the accuracy and credibility of 4D and 3D mode diagnoses.

The study demonstrated that the success rate of tubal patency diagnosis in the 4D + 3D + 2D group was significantly higher than in the 4D + 3D group in both the high and low seniority groups ($P < 0.01$). The accuracy of diagnoses was further confirmed. However, due to the varying lengths and courses of fallopian tubes, it is challenging to display the entire course of the fallopian tubes on a single scan plane. Sixteen fallopian tubes in the high seniority group were not clear enough to make accurate diagnoses, while 19 fallopian tubes in the low seniority group could not be accurately diagnosed.

#### HI mode

The flow of the contrast medium in the fallopian tube can be clearly and dynamically observed in this mode, allowing for the flexible manipulation of the entire fallopian tube process [18]. Even if part of the fallopian tube is intermittently displayed due to surrounding intestinal gas, the assessment of its patency is not affected. This study demonstrated that the success rate of fallopian tube patency diagnosis in both high- and low seniority groups

**Table 4** Statistical analysis of various uterine lesions examined by negative hysterography

| Classification of uterine lesions | Sensitivity | Specificity | Positive predictive value | Negative predictive value | Accuracy |
|---|---|---|---|---|---|
| Uterine malformation | 100% | 100% | 100% | 100% | 100% |
| Cesarean section scar diverticulum | 66.67% | 100% | 100% | 98.33% | 98.39% |
| Intimal polyp | 92.59% | 94.29% | 92.59% | 94.29% | 93.55% |
| Submucosal myoma | 33.33% | 98.31% | 50% | 96.67% | 95.16% |
| Uterine adhesion | 88.89% | 97.14% | 96% | 91.89% | 93.55% |

was significantly higher than in the 4D + 3D + 2D + HI group ($P < 0.01$). All 612 fallopian tubes in the high seniority group and 610 fallopian tubes in the low seniority group were successfully diagnosed. The diagnosis was less affected by the limitations of single-modal examination, thereby improving accuracy and reliability.

### Negative hysterography

Negative hysterography directly revealed uterine malformations, achieving 100% sensitivity and specificity in diagnosing uterine malformations in this study. The three-dimensional reconstruction showed a semi-circular, irregular localized eminence. However, one case of scar diverticulum post-cesarean section was missed due to the diverticulum being small and fissure-shaped, with water sac occlusion affecting the imaging. Additionally, the presence of large endometrial polyps complicated the case, leading the focus to be solely on the polyps and the small diverticulum being overlooked during examination, resulting in a missed diagnosis. When the uterine cavity was filled with negative contrast media, the contrast between the endometrium and the lesions was enhanced, making endometrial polyps and submucosal myomas clearly visible. These lesions appeared as irregular, round, or nodular localized protuberances or depressions in the uterine cavity [19].

In this study, two cases of endometrial polyps were missed because the polyps were small and located in the lower segment of the uterus, and the water sac occlusion affected the imaging. Additionally, two cases were misdiagnosed: one as submucosal myoma due to a large polyp and the other as uterine adhesion. Two cases of submucosal leiomyomas were also misdiagnosed. All missed cases involved endometrial polyps with submucosal myomas, but only the endometrial polyps were misdiagnosed as submucosal myomas.

In negative hysterography, the sonogram of endometrial polyps is similar to that of submucosal myoma, making them easy to misdiagnose, especially when both lesions coexist. To improve the detection rate of endometrial polyps, the examination should be conducted 3–5 days after menstruation. During the early stages of hyperplasia, the endometrium is hypoechoic and thin, allowing polyps to be easily detected. In the secretory phase, the endometrial echo is slightly enhanced and thicker, which can be confused with intimal hyperplasia. Notably, when a submucosal myoma undergoes ischemic degeneration, the local echo can become highly echogenic, resembling intimal polyps and leading to misdiagnosis [19, 20].

Through comparative examinations with hysteroscopy, it was found that lesions missed by negative hysterography were often small, had uneven intima thickness, and were obscured by the water sac in the uterine cavity. To avoid missing small lesions, it is crucial to observe the middle and lower segments of the uterus (where the balloon is positioned) at the end of the examination while removing the tube and simultaneously injecting normal saline. In this study, three cases of uterine adhesion were missed, and two cases were misdiagnosed due to a thin endometrium with only slight adhesions. The adhesion bands were fine and filamentous, making them difficult to detect on 3D-TVS coronal images, resulting in missed diagnoses. One case involved adhesion with multiple endometrial polyps, where only the polyps were diagnosed without detecting the uterine adhesion. Negative hysterography can not only improve the detection rate of uterine lesions but also re-evaluate tubal patency by observing the fluid flow in the uterine cavity and fallopian tubes [21–23]. Continuous fluid flow in the fallopian tube is strong evidence of patency. The patency can be assessed by observing the fluid flow from the uterine corners into both fallopian tubes. If the injection speed is consistent, the fallopian tube walls are smooth, the lumen thickness is uniform, and the tube direction is straight, the liquid will pass smoothly and flow quickly through the cavity. Conversely, if the fallopian tube walls are irregular due to inflammation, and the tube is slender, unevenly thick, stiff, or twisted, the liquid's flow slows down. If the proximal end of the fallopian tube is blocked, fluid will accumulate at the corresponding uterine corners without entering the fallopian tube, potentially causing eddy currents.

In this study, we found that step-by-step multimodal HyCoSy combined with negative hysterography enhanced diagnostic efficiency, with higher efficiency observed with more joint modes. When a 4D diagnosis is confirmed, modal verification can be added to improve diagnostic accuracy. In cases of uncertainty, multiple modes can be combined for diagnosis and verification.

Negative hysterography not only significantly improves the detection rate of uterine lesions but also re-evaluates tubal patency by observing fluid flow in the uterine cavity and fallopian tubes, as well as fluid overflow in the pelvic cavity.

Using consistent evaluation criteria, senior and junior doctors analyzed the data and images of the same cases, achieving results consistent with those of experienced doctors. This demonstrates that multimodal HyCoSy has high consistency in evaluating tubal patency and, when combined with negative hysterography, provides high accuracy in diagnosing uterine lesions. The combination of multimodal transvaginal contrast-enhanced ultrasonography and negative hysterography is practical, accurate, and easy to popularize and apply for diagnosing female infertility.

### Limitations of this study

This study had a small sample size, which introduced a certain degree of bias. Due to the risks associated with radiation exposure, only a few patients underwent X-ray Lipiodol radiography, and few patients underwent tubal patency examinations. Consequently, comparative studies on other tubal patency examination methods were limited. Additionally, contrast-enhanced ultrasound procedures were performed exclusively by senior doctors, making it impossible to compare diagnoses between high and low seniority groups.

## Conclusion

In this study, we demonstrated that step-by-step multimodal HyCoSy combined with negative hysterography significantly enhances diagnostic efficiency. The use of multiple imaging modalities provides a more comprehensive and accurate evaluation of reproductive anatomy. Our findings show consistent diagnostic outcomes across physicians with varying levels of experience, highlighting the user-friendliness and effectiveness of this combined approach.

In conclusion, the integration of multimodal HyCoSy and negative hysterography is a simple, rapid, and accurate method for assessing tubal patency and diagnosing uterine lesions, making it a valuable tool for clinicians in reproductive health.

### Abbreviations

| | |
|---|---|
| HyCoSy | Hysterosalpingo-Contrast Sonography |
| 2D-HyCoSy | Two-dimensional Hysterosalpingo-Contrast Sonography |
| 3D-HyCoSy | Static Three-dimensional Hysterosalpingo-Contrast Sonography |
| 4D-HyCoSy | Real-time Three-dimensional (Four-dimensional) Hysterosalpingo-Contrast Sonography |
| TVS | Transvaginal Ultrasonography |
| HI | Harmonic Imaging |
| CEUS | Contrast-Enhanced Ultrasound |

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### Authors' contributions

Author Contribution Notes: Qin Ye*, Fen Fu, conceived and designed the study; Qin Ye, Rong-Xi Liang, Fen Fu and Yi-Fan Zhu performed the examinations; Yue-Fan Chen and Jia-Jing Zhuang collected the data. All authors analyzed the data and were involved in writing the manuscript.

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### Data availability

The datasets generated and/or analysed during the current study are not publicly available due to their involvement in an ongoing research project, but are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

All subjects signed informed consent prior to the examination. The retrospective study was approved by the Ethical Evaluation Committee of Fujian Medical University Union Hospital in China (Approval number: 2020KY024; Date: 20 March 2020).

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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