Appendix 1

Contrast medium preparation

The contrast agent was reconstituted to a total volume of 3 mL with 0.9% sodium chloride injection. To maintain a constant pressure and prevent microsphere rupture, another injection needle was inserted into the rubber stopper of the vial for ventilation during drug solution preparation and extraction. The vial was then placed flat between the palms and gently rolled for approximately 1 minute to ensure uniform mixing. Vigorous shaking was avoided to prevent microsphere rupture and foam formation. The solution was then diluted with 0.9% sodium chloride injection to a final volume of 20 mL for later use.

Performance of HyCoSy

Before the examination, the results of the vaginal secretion test were reviewed, and the patient's body temperature was checked to confirm it fell within the normal range. The patient was re-informed of the relevant precautions and potential risks of the contrast examination to reduce any anxiety. Thirty minutes before the test, 0.5 mg of atropine was intramuscularly injected into the patient. The patient was directed to empty their bladder and assume the lithotomy position during the HyCoSy procedure.

First, conventional transvaginal ultrasonographic scans were performed to detect any abnormalities in the uterine adnexal or pelvic cavity. The patient's external genitalia and vagina were disinfected, a towel was draped, and the cervix was exposed using a sterile vaginal speculum. A 12-Fr Foley catheter as inserted into the uterine cavity, and the balloon was filled with 1–2 mL of sterile physiological saline to fix the catheter in the uterine cavity. The vaginal speculum was then removed, the balloon was adjusted under ultrasound guidance, and placed in the lower part of the uterine cavity to ensure that there was no cervical leakage and the patient felt comfortable. The volume of the balloon was then recorded.

Next, a transverse section of the uterus was taken for scanning, the spatial relationship between the bilateral uterine horns and ovaries was observed, three-dimensional (3D) ultrasound was initiated, adjusting the sampling frame and volume scanning angle to include both ovaries and the bottom of the uterus as much as possible, and the initial scanning section was determined. Next, the real-time 3D contrast-enhanced ultrasound (CEUS) mode was activated, the contrast agent was slowly pushed into the uterine cavity at a constant speed, and the process of the contrast agent entering the uterine cavity and bilateral fallopian tubes was observed. Attention was paid to the patency of both fallopian tubes, the wrapping of contrast agent around the ovaries, the extent of contrast agent dispersion in the pelvic cavity, and the presence of countercurrent in the myometrium and parauterine venous plexus. Real-time 3D CEUS volume data was simultaneously stored. The two-dimensional dual screen contrast imaging mode was then adjusted, contrast agent was injected continuously and uniformly into the uterine cavity, and the flow trajectory of the contrast agent in the fallopian tubes along both sides of the uterine angle was dynamically tracked in real time. The overflow of contrast agent at the umbrella end, the wrapping of contrast agent around the ovary, and the diffusion of contrast agent in the pelvic cavity were monitored, and dynamic images of the imaging process were stored. The resistance of contrast agent injection, injection volume, and presence or absence of contrast agent intravasation were recorded.

Finally, the balloon was shrunk, and physiological saline was injected into the guide tube to observe the shape of the uterine cavity, and the presence or absence of any space occupying lesion. The balloon was then emptied, and physiological saline was injected, as the tube was removed to observe the lower segment of the uterine cavity and cervical canal. The pain of the participant was recorded in detail during the operation and the injection of the contrast agent, paying attention to whether the participant experienced adverse symptoms and signs such as nausea, vomiting, pelvic infection, and contrast agent intravasation after contrast agent injection. If the participant did not experience any other discomfort, they were observed for 30 minutes before leaving.

Performance of LSC

All participants underwent a LSC examination after HyCoSy to evaluate the patency of their fallopian tubes. Before the LSC examination, each participant was informed of the surgical precautions and potential postoperative risks, and their informed

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consent was obtained. The preoperative examination of each participant was verified, and the preoperative status of each participant was evaluated to ensure they met the surgical requirements.

The LSC examination process was carried out in accordance with the clinical routine operations of the research center. However, during the examination, a laparoscopic methylene blue tubal patency test was performed first to assess the patency of the fallopian tubes, followed by treatment for adhesions, fibroids, etc. LSC was used to observe the shape of the uterus, the course and shape of the fallopian tubes, the bilateral adnexa and their relationship with surrounding tissues, and other pelvic conditions. Methylene blue test solution was slowly injected into the uterine cavity. Under LSC, the filling of methylene blue in the fallopian tubes and the overflow of methylene blue from the fimbria was observed. The patency of the fallopian tubes was evaluated by visual inspection by an experienced obstetrician and gynecologist.

For LSC, the intraoperative video was saved, and screenshots of the fluid infusion results were taken, with the left and right fallopian tubes clearly marked.

Table S1 Diagnostic efficacy of HyCoSy in the evaluation of fallopian tube patency

Evaluation index	Point estimator	95% CI
Accuracy (%)	88.24	83.99, 92.48
Sensitivity (%)	81.44	73.71, 89.18
Specificity (%)	93.55	89.22, 97.87
Positive predictive value (%)	90.80	84.73, 96.88
Negative predictive value (%)	86.57	80.79, 92.34
Positive likelihood ratio	12.62	4.08, 21.17
Negative likelihood ratio	0.20	0.12, 0.28
Kappa	0.76	0.67, 0.85

CI, confidence interval; HyCoSy, hysterosalpingo-contrast sonography.

Table S2 Common AEs (occurrence rate ≥ 5%)

AE	HyCoSy (N=120), n (%)	LSC (N=120), n (%)
Hypogastralgia	53 (44.17)	2 (1.67)
Abdominal pain	23 (19.17)	13 (10.08)
Nausea	4 (3.33)	7 (5.83)
Vaginal bleeding	12 (10.00)	72 (60.00)
Incision site pain	–	39 (40.83)
Contrast intravasation	32(26.67)	–
Anemia	–	26 (21.67)
Hypokalemia	3(2.50)	10 (8.33)
Postoperative infection	2 (1.67)	9 (7.50)

This drug clinical trial used N=120 as the denominator for calculations, following the Safety Analysis Set requirements specified in the trial protocol. AE, adverse events; HyCoSy, Hysterosalpingo-Contrast Sonography; LSC, laparoscopy.

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