

Ultrasonic Assessment of Infertility

Ikuko Honda

Department of Obstetrics and Gynecology, Division of Infertility, Yamachika Memorial General Hospital, Odawara, Japan

Correspondence: Ikuko Honda

Department of Obstetrics and Gynecology, Division of Infertility, Yamachika Memorial General Hospital,
3-19-14 Koyawata, Odawara City, Kanagawa, Japan 256-0815

Telephone: 81-465-47-7151, FAX: 81-465-47-8178, E-mail: yamachika_hospital@yahoo.co.jp

Abstract: Ultrasonographic changes of endometrium, ovaries and salpinges detected by realtime B-mode and 3D ultrasound indicate effective treatment of infertility not only in the Assisted Reproduction Technology (ART) but also in the conventional management where essential plan for the therapy is indicated. The articles concerning ultrasonographic assessments of fecundity and infertility are reviewed. Although the ART has become ordinary treatment of infertility, the pregnancy rate is yet unsatisfactory and it is important to select cycles where favorable conception is predicted in order to increase pregnancy rate in ART. Although antral follicle count (AFC) is the index for predicting ovarian response to human menopausal gonadotropin (hMG) stimulation, it is able to select stimulation options for a poor responder of low AFC value. Since three-dimensional (3D) ultrasonography with power Doppler angiography (PDA) indicates an organ blood flow as a whole, they are important tools for the evaluation of perfusion. The assessment of perifollicular blood flow in preovulatory follicles may be useful for predicting conception and cancelling *in vitro* fertilization (IVF) in poor vascularity cycle. Since rich endometrial vascularity assessed by 2D and 3D flow signs and the low impedance to flow suggests well perfused endometrium and the high receptivity in the embryo transfer (ET), and vice versa, it would be recommended to limit the number of embryos for the ET in the high receptivity, while to cryopreserve the embryo in case of bad flow signs and wait the cycle of favorable perfusion in order to successfully transfer the embryo.

Key words: Ultrasound, infertility, ART, uterus, ovary, blood flow, sonosalpingography, pulsed Doppler, color Doppler, 3D power Doppler.

INTRODUCTION

Ignited by the birth of the child by world's first IVF-ET in 1978, infertility examination and treatment has accomplished remarkable development. The advance of treatments was given the great benefit by the development of ultrasound equipments, and the sonographic examination is indispensable for the infertile treatment.

The highly precise diagnosis of realtime transvaginal scan (TVS) introduced from early 1980s was attained because of a close approach of TVS probe to the pelvic organs and cul-de-

sac, and due to the capability to select options of suitable infertility treatment.

As compared with endocrinologic markers, the great advantage of an ultrasonography is the realtime evaluation of a menstrual cycle in every changing moment. The ultrasonic examination enables to measure the follicle diameter and endometrial thickness, which evaluates the fecundity function by using blood-flow assessment and the combined 3D and blood-flow investigation.

Gynecological infertility treatment also included male infertility after a success of ICSI-ET in 1992. The pregnancy by ART has increased since a success in 1983 and the rate increased to 65 newborn infants in Japan in 2003. Although the ART is the ordinary infertile treatment, the pregnancy rate cannot be satisfied. Aiming at the improvement in a pregnancy rates, it is one of the important matters to select the cycles which conception is expectable.

UTERINE FACTOR (ENDOMETRIUM)

Various ultrasonographic indicators have been investigated for evaluation of endometrial receptivity in spontaneous and stimulated cycles, including endometrial thickness, endometrial pattern, uterine artery and endometrial blood flow.

Since the endometrium is instantaneously measurable by transvaginal ultrasonography, a lot of papers concerning in the prognostic value of endometrial thickness and texture have been reported. Endometrial thickness and uterine blood flow had a high negative predictive value and sensitivity, but low positive predictive value and specificity as prognostic indicators for implantation.¹ There was no difference between conception and non-conception cycles in endometrial thickness ranges. A preovulatory endometrial thickness of < 9 mm related to an elevated miscarriage rate in IVF cycles and no relationships were found among endometrial appearance.² The uterine receptivity prior to embryonic transfer (ET) was discussed in available imaging modalities;³ where the endometrial pattern with an outer hyperechogenic and inner hypoechogenic layer

on the day of oocyte retrieval had predictive value on IVF treatment. Homogenous and hyperechogenic sonographic endometrial pattern had a predictive value of 100% for a nonconceptional cycle, whereas multilayered endometrium was visualized in conception cycles.⁴

Goswamy *et al* (1988)⁵ presented a hypothesis with Doppler ultrasound in the studies of unsuccessful IVF patients that poor uterine perfusion is a cause of failure of implantation of embryos; it would follow that poor uterine perfusion is a cause of infertility, although the concentration for investigation had to be set to the ascending branch of the uterine artery by transabdominal scanning.

Uterine arterial blood flow was lower in clomiphene citrate stimulated cycles during the periovulatory period than those in the spontaneous menstrual cycles,⁶ and also demonstrated that uterine vascular impedance during the day of ovulation was lower in the conception cycles, while there were no differences between conception and nonconception cycles in the luteal phase.⁷ Accordingly, increased endometrial perfusion may be mandatory for successful conception on the periovulatory period. The investigation on a downstream blood vessel has come to be acquired by development of equipment. A significantly lower uterine arterial PI and higher diastolic blood flow were reported in the subendometrial radial arteries shortly after ovulation in conception cycles.⁸ Also, the endometrial tissue blood flow (ETBF) measured by the hysteroscopic laser blood-flowmetry revealed a pregnancy-predictive value in spontaneous cycles prior to IVF/ICSI programs.⁹

Recently, pulsed Doppler and three-dimensional color and power Doppler studies have been applied to evaluate endometrial receptivity by the uterine and endometrial blood flow status.

Ng *et al*¹⁰ showed that endometrial and subendometrial blood flow measured by 3D-power Doppler angiography with virtual organ computer-aided analysis (VOCAL) and shell-imaging in the stimulated cycles at the time of oocyte retrieval were significantly lower than in the natural cycle in the same patients undergoing IVF treatment. They could not demonstrate any correlation between uterine Doppler flow indices concentrated on ascending main branch of uterine artery and endometrial as well as subendometrial blood flow. Then, they showed that implantation and pregnancy rates were higher in patients without endometrial and subendometrial blood flow on the day of oocyte retrieval than those with blood flow.¹¹

Raine-Fenning *et al*¹² reported that the endometrial and subendometrial vascularity increased during the proliferative phase, a peak value is reached for three days before ovulation, and the lowest value for after-ovulation five days; uterine receptiveness estimated by a blood flow is influenced by the inspection time and the inspection region (Figs 1A to D). Therefore, it is appropriate to study the endometrial receptivity

in the day of ET as discussed in following reports when it is intended to improve the pregnancy rate of the ART.

There were no pregnancies in high impedance of uterine arterial flow expressed by high pulsatility index (PI) (3+) group, where the embryo is cryopreserved until the uterus is more receptive, and reduce multiple pregnancy by indicating the limited number of embryos if the uterus is the most receptive.¹³

Endometrial perfusion measured by uterine, radial, spiral and ovarian arteries presents accurate assay of uterine receptivity used to predict implantation success rate, to reveal unexplained infertility problems, and to select patients for correction of endometrial perfusion abnormalities.¹⁴

Transvaginal color Doppler showed low resistance index (RI) in subendometrial flow, and favorable flow index by 3D power Doppler histogram in the patients who became pregnant. The high degree of endometrial perfusion shown by color Doppler ultrasonography and high flow index on 3D power Doppler histograms on the day of embryo transfer can indicate a more favorable endometrial milieu for successful IVF.¹⁵ Mean uterine arterial RI and PI values were significantly lower in the pregnant than in the nonpregnant group before ET.¹⁶

In all of the reports, sufficient endometrial perfusion determined by ultrasonic color Doppler, power Doppler and pulsed Doppler at the day of ET immediately before the procedure indicated high endometrial receptivity and improved pregnancy rate. On the contrary, low perfusion of the uterus or endometrium resulted in low pregnancy after the ET. The significance of ultrasonic determination of uterine and endometrial perfusion was approved in the ART.

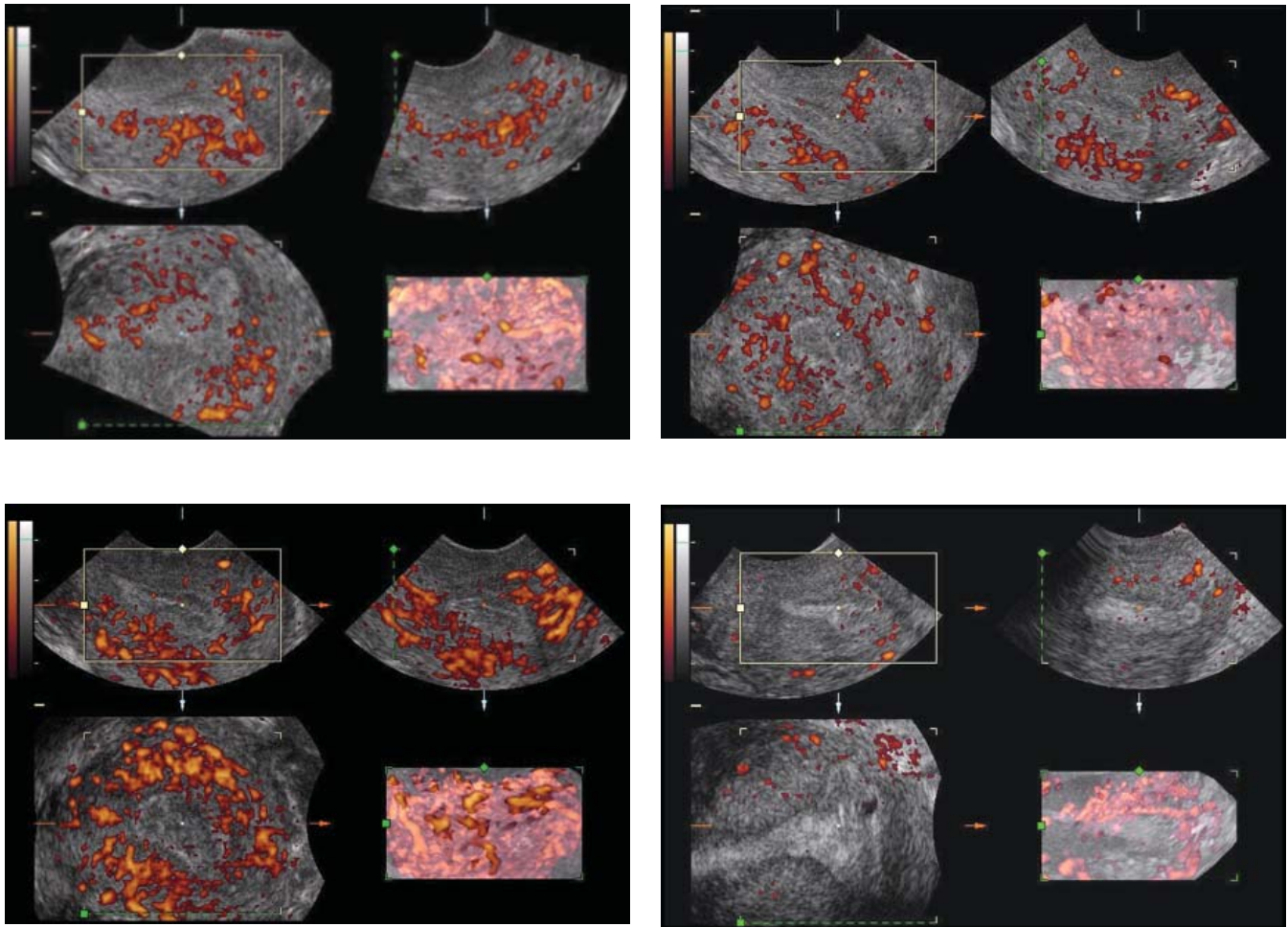
Although the advanced cryopreservation technology has enabled the acquisition of more embryos according to the clinical request to improve the pregnancy rate, it may be unnecessary to transfer the embryo into the uterus of unsuitable condition for the implantation. In the endocrinology of stimulated cycles also, high serum estradiol concentration also has an adverse effect on the success of IVF treatment.¹⁷

OVARIAN FACTORS

Ovarian Reserve

Assessment of ovarian reserve is valuable for selection of cycles and stimulation protocols on ART treatments. Endocrinologic markers have been used to evaluate ovarian reserve, variations basal FSH values were not reflect ovarian response to gonadotropin.¹⁸

There is no matter in a patient's age being a clearly stronger predictor. Chronological age is more strongly associated with follicle count than with FSH, inhibin B, E, or with measures of the ovary or follicles.¹⁹ Chronological age, ovarian volume, FSH and inhibin B were each significantly associated with antral follicle count (AFC).²⁰ The AFC is reported the benefit to predict ovarian response and to reduce cancellation cycles.²¹



Figs 1A to D: The changes of endometrial vascularity with 3D power Doppler angiography: The patient received clomiphene citrate-hMG for ovarian stimulation. The images demonstrate the longitudinal, transverse and coronal views in the upper left, upper right and lower left, respectively. The lower right images show the compounded 3D results of vascularity. (A) menstrual day 10th, (B) Day 12th, (C) Day 14th (oocyte retrieval), and (D) Day 16th (ET)

Total AFC was significantly different among 4 age groups (< or = 30, 31 to 35, 36 to 40, and > or = 41 years); Total ovarian vascularization index (VI) and vascularization flow index (VFI) were significantly lower in women aged > or = 41 years; AFC had the best correlation with the age of women; and ovarian stromal blood flow was significantly reduced in women aged > or = 41 years.²²

A more exact value of the AFC was acquired when counted identifying by our 3D ultrasonography. The number of follicles >12 mm on the day of oocyte retrieval correlated significantly with AFC using 3D as compared with 2D ultrasonography. Inversion rendering, which is one of the imaging methods and extracted low echoic parts in the ovary selectively, was possible to visualize the location and the size of antral follicles in the ovaries (Fig. 2). Although the AFC obtained by 3D

ultrasonography is useful for predicting the number of preovulatory follicles, it has not resulted in qualitative evaluation of oocytes in the preovulatory follicles.

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is not only important as diagnosis of an ovulation disorder but also it is important as a predictor of OHSS to gonadotropins in infertility treatments, and long-term health of the patients.

Diagnostic criteria, defined by Japan Society of Obstetrics and Gynecology, were menstrual irregularity, serum LH elevation, and a characteristic ultrasonic image.²³ PCO morphology by ultrasound was not in NIH criteria is included in 2003 Rotterdam criteria.²⁴ It is more than eight discrete of < 10 mm diameter in

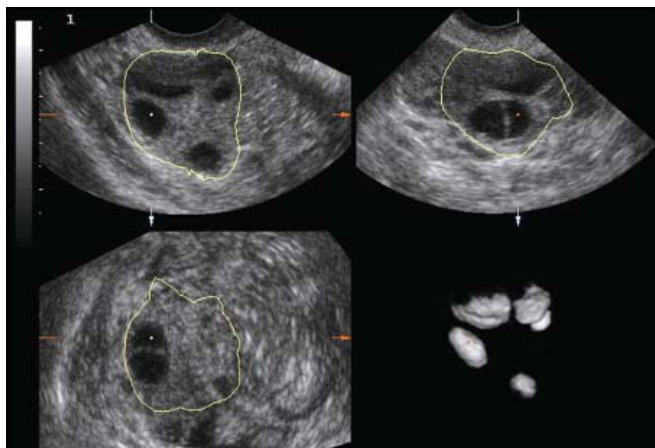


Fig. 2: 3D antral follicle count and inversion rendering: Each follicle can be identified in three-dimensional multiplane images, where a small point shows the same follicle. Inversion rendering can be seen in the lower right image, where it is possible to visualize the location of antral follicles in the ovaries by rotating 360 degrees

one plain of the ovary, usually arrayed around, but sometimes scattered throughout, an enlarged, hyperechogenic, central stroma, by transabdominal ultrasonography.²⁵ The number of small cysts in one ovary is 10.6 ± 2.1 in PCOS patients, it is significantly higher compared with 3.5 ± 2.1 in normal control.²⁶ They also demonstrated that an increase of the number of small cysts is related to the frequency of menstrual disturbances.²⁷

There are two types of PCO as reflecting histopathological differences.²⁸ One is small cysts distributed in the peripheral ovary and another is small cysts scattered through the entire ovaries, and the difference was demonstrated in LH/FSH ratio²⁹ or Doppler evaluation between two types of PCO.³⁰ The PCO follicles were aspirated under the guidance of transvaginal ultrasound in anovulatory patients with PCO, the ovulation rates after the procedure were 87.5% per patients in PCOD and 100% per patients in PCO, and pregnancy rate was 50%.³¹

Endometriosis

The 2,080 patients were subjected to diagnostic laparoscopy in our studies, and 1,263 cases diagnosed as the endometriosis by the revised classification of American Fertility Society were confirmed by the direct laparoscopic visualization or the biopsy. Of 1,263 patients were having endometriosis, 587 (44.3%) were stage I, 348 (38.0%) were stage II, 184 (15.9%) were stage III and 144 (11.8%) were stage IV.³²

As strong association exists between infertility and endometriosis,^{32,33} it is very important to diagnose correctly and to treat endometriosis in infertile patients. The most significant mechanism causing infertility in moderate to severe endometrioses can be the adhesion between bilateral ovaries

with the cul-de-sac and the formation of endometrioma. Although there are few reports of the ultrasonographic diagnosis of adnexal adhesion,³⁴ an endometriosis stage III or IV can be diagnosed with the characteristic position of the ovary which adhered with posterior uterine wall, where endometrioma is found in a retroflexed uterus at the site of uterosacral ligaments, blebs are formed around the ovary and uterus. It is very hard to induce pregnancy by conventional infertile therapy, when the endometriosis cysts diameter exceeds 40 mm in the ovaries in our study.³²

The infiltration of endometriosis to uterosacral ligaments is diagnosed by transrectal approach. The infiltrated uterosacral ligaments were thick and irregular image in the transrectal ultrasonography. Clinical symptoms were parallel to the thickness of infiltrated uterosacral ligaments.³⁵

Ultrasound-guided transvaginal ethanol sclerotherapy has been performed for ovarian endometriotic cysts, and there was no adverse effect on reproductive capability in subfertile women after the procedure.³⁶

Monitoring Follicles

In work-up infertility and ovulation induction, follicular size measured by transvaginal ultrasonography frequently used as indicators of oocyte maturity. It is valuable to predict the time of ovulation, the number of aspirated oocyte and occurrence of OHSS. Although a positive relationship was described between follicular sizes and the presence of metaphase II oocytes,³⁷ the size of preovulatory follicles stimulated with the hMG distributed in wide ranges.³⁸ Monitoring of preovulatory follicles with follicle diameters has limitation to predict oocyte quality. Although the follicle growth pattern may be a predictive indicator of the oocyte quality,³⁹ it is difficult to identify each follicle changes in multiple ovarian follicle growth induced by the hMG stimulation. It will be useful to exclude the nonconception cycle, if qualitative evaluation of the ovum in ovarian follicle is attained before oocyte retrieval. The image of an intrafollicular cumulus oophorus was discussed,⁴⁰ while it may be difficult to analyze the oocyte image in the stable cumulus in preovulatory follicles.

The perifollicular blood flow characteristics measured by color Doppler images related to the intrafollicular oxygen content and VEGF concentration, and oocytes from severely hypoxic follicles were associated with high frequencies of abnormalities in the organization of the chromosomes on the metaphase spindle,⁴¹ presented that the best predictors of IVF outcome are the ovarian flow index (FI) using 3D ultrasound and power Doppler angiography (PDA) on the hCG day and the transfer of grade 1 embryos.⁴² 3D ultrasonography with PDA which evaluates an ovary blood flow as a whole is not able to separate stromal blood flow and perifollicular blood flow by angiogenesis.

There are reports on the relation between successfully induced ovulation or pregnancy and the change of ovarian blood flow. The ovarian perfusion changed with the successful ovulation induction after the hCG administration. The V_{\max} increased and flow index reduced in the ovarian artery from 15 minutes after hCG injection in the case of ovulation, while nonovulatory cycle demonstrated no significant change.⁴³

Intraovarian blood flow impedance should be low before the periovulatory phase of a natural cycle to achieve pregnancy, because PI values determined by transvaginal Doppler flow study severely decreased in pregnant cycles.⁴⁴

In addition, ovarian follicular vascularity was studied and many significant results were obtained. High follicular vascularity was associated with increased pregnancy rate, and there was a potential link between follicle vascularity and implantation potential.⁴⁵

The chance of achieving pregnancy predicted by uterine artery Doppler and perifollicular blood flow in women whose PI values were higher than 3.26 and 1.08 was very low, with a sensitivity of 1.00 and specificity of 0.59 and 0.82, respectively. The data provided evidence for an association between utero-ovarian perfusion and reproductive outcome following IVF treatment.⁴⁶ The ovarian volume, follicular volume, vascularization index, flow index and vascularization-flow index were significantly greater in the pregnant group. 3D ultrasonography and power Doppler angiography allow for an easier ovarian assessment in IVF cycles.⁴⁷

The total antral follicle number achieved the best predictive value for favorable IVF outcome; 3D ultrasound facilitated determination of the antral follicle number, ovarian volume, evaluation of the ovarian stroma and intensity of ovarian stromal blood flow.⁴⁸

Sufficient utero-ovarian perfusion facilitated the favorable outcome of IVF and ET in the studies carried out with 2D color Doppler, pulsed Doppler, 3D color Doppler and 3D power Doppler histogram analysis.

Luteal Function

The relation of blood flow indices in the corpus luteum measured by transvaginal color Doppler ultrasonography and hormone profiles were studied; the velocity and the impedance indices of the blood flow were both associated with the P/E_2 ratio in spontaneous and clomiphene citrate cycles, while the blood flow indices and the P/E_2 ratio were not correlated in controlled ovarian hyperstimulation cycles.⁴⁹

TUBAL FACTORS

The oviduct is playing sperm transportation, capture of an ovum, the place of fertilization, growth of a fertilized ovum, and a role of embryo transportation.

Salpingitis

It is hard to visualize the peritubal adhesion, also the intratubal adhesion due to inflammation by ultrasonography. As the grade of hydrosalpinx progresses, the images of salpinx can be identified.

For the patients with obstructed salpinx, IVF is in the tendency to be chosen rather than laparoscopic tubal plasty, but when the patient with hydrosalpinges can also expect postoperative pregnancy, it is required to diagnose whether there is any indication of salpingostomy.

Although the salpingoscopy can observe the grade of mucosal damage, the state of a muscle layer is unknown. Laparoscopy-guided intrapelvic sonography with a high-frequency, realtime and miniature transducer may be useful in the assessment of tubal texture. The muscle and mucosal layers could not be distinguished in subject of hydrosalpinx.⁵⁰ Although there were small number of examination, the propriety of tubal plasty may be judged by evaluating the function of an oviduct.

Sonosalpingography

Evaluation of tubal patency is one of the basic examinations in infertile patients, as 35% of female infertility is due to tubal factors, which decide subsequent management of infertility. Laparoscopic chromopertubation is the gold standard in the diagnosis of tubal patency, but laparoscopy requires hospitalization and general anesthesia. Then, X-ray hysterosalpingography (HSG) is carried out as daily work-up; however, HSG has a risk of allergic reactions and exposure to radiation.

Transvaginal color Doppler and sonosalpingography are highly accurate diagnostic tools not only for the test of tubal patency, but also for preoperative evaluation of the uterine cavity defects. Removing the intrauterine septum in the patients suffering from infertility and recurrent pregnancy wastage is beneficial.⁵¹

We compared the tubal patency between the sonosalpingography by using color Doppler ultrasonography with contrast medium and chromopertubation at the laparoscopy on 134 salpinges. The concordance rate was 89.6%, and the sensitivity and the specificity were 90.1% and 84.6%, respectively.

Although it is difficult to diagnose the site of stenosis or occlusion exactly, sonosalpingography can visualize not only the patency of both tubes but also the distribution of the contrast medium around the ovaries simultaneously.

Since two-dimensional hysterosalpingo-contrast sonography was impossible to visualize the entire salpinx, three-dimensional hysterosalpingo-contrast sonography was evaluated.⁵² Unlike laparoscopic chromopertubation performed under general anesthesia, HSG and sonosalpingography have

a pitfall conducted to the false diagnosis owing to spasm of uterotubal junction, the position of catheter in uterine cavity. Of 166 examples with bilateral occlusions on HSG, 36 examples were confirmed as unilateral occlusion and 29 examples were bilateral patency by laparoscopic chromopertubation in our study evaluated the possibility of three-dimensional hysterosalpingo-contrast sonography (3D-HyCoSy). The assessment of tubal patency by 3D-HyCoSy was compared with chromopertubation at the diagnostic laparoscopy. The sensitivity of 3D-HyCoSy for detecting tubal patency was 100% with a specificity of 67%, respectively. The concordance rate was 91%.⁵³

Peritoneal Sperm Recovery Test (PSRT)

Sperm transport to the site of fertilization is one of the important problems for fertility. Sperm recovery from the peritoneal fluid is an absolute evidence of successful sperm transport to the site of fertilization. The difference was seen by the pregnancy rate in the patient by whom sperm transportation is not seen even if tubal patency is maintained. In our study, 1,124 patients were followed up at least one year after laparoscopy. In the motile sperm recovery group, a pregnancy rate was 32.6%, whereas it was 11.6% in the negative sperm recovery group, which was significantly the lowest. It is evident that the deficiency of sperm transport is one of the serious causes of infertility diagnosed by PSRT. There was a positive relationship between the movement of the sperm suspension after AIH observed by transvaginal ultrasonography and the results of PSRT at laparoscopy.⁵⁴ The patients with active movement of sperm suspension in the uterine cavity were recovered motile sperm in PSRT significantly. The uterine activity may play a key role for sperm migration from the uterine cavity into the oviducts (Fig. 3). The uterine hyperperistalsis demonstrated by transvaginal ultrasonography would be the mechanical cause of endometriosis rather than retrograde menstruation, and dysperistalsis in the late follicular phase of patients with endometriosis may compromise rapid sperm transport.⁵⁵

EMBRYO TRANSFER PROCEDURE IN THE ART

Embryo transfer (ET) is one of the important procedures in assisted reproduction technology (ART) programs. A failure of transfer technique decreased the pregnancy rate in the past.⁵⁶ The ET guided by a transabdominal ultrasound has been generally employed for the catheter introduction. However, transabdominal ultrasonography forces a full bladder upon patients and it is difficult to demonstrate the uterine cavity in hyper-retroflexed cases with inappropriate bladder volume. Transvaginal ultrasound-guided ET improved significantly both implantation and pregnancy rates compared with the clinical manipulation,⁵⁷ although the TVS ultrasound guide is technically complicated.

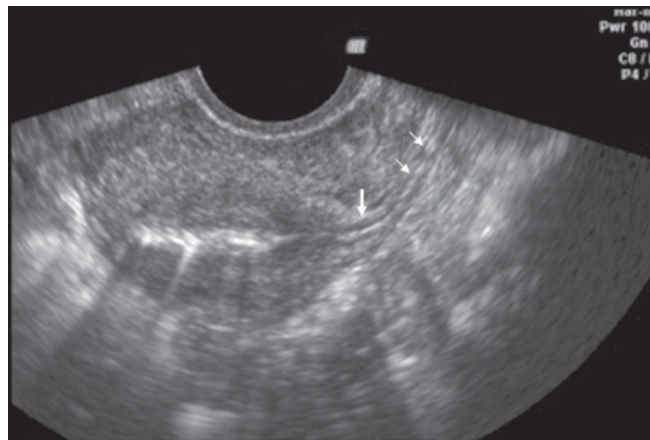


Fig. 3: Transverse view shows the transport of AIH sperm suspension by uterine peristalsis. Tubal passage is demonstrated by tracing of sperm suspension immediately after AIH. Big arrow shows the left interstitial portion and small arrows show the isthmus portion of salpinx

An ET is performed when various reproductive conditions are appropriate.

The endometrial receptivity is estimated on the day of ET by the uterine arterial and endometrial blood flow determined by transvaginal 2D and 3D color Doppler and 3D power Doppler histogram, estimating endometrial receptivity. Ovarian antral follicles are counted, and also perifollicular blood flow is studied by the ultrasound techniques. The ET is carried out if the endometrial receptivity is sufficient and the number of transferred embryos is limited in case of high endometrial receptivity in order to prevent multiple pregnancies. On the contrary, the ET is postponed if the endometrial receptivity determined by its perfusion is poor and insufficient, where the embryo is cryopreserved until a suitable cycle of sufficiently high receptivity.

CONCLUSIONS

Advanced ultrasonic diagnosis including B-mode, harmonic image, B-flow, pulsed Doppler indices, color and power Doppler flow mapping, sonosalpingography, sonocontrast-hysterosalpingography, 3D and 4D ultrasound in transvaginal scan are common techniques in the researches as well as in the clinical management of fertility and sterility, particularly in the ART, e.g. the most effective ET to increase pregnancy and reduce multiple pregnancy is achieved by ultrasonic diagnosis of utero-ovarian perfusion, oocytes are retrieved by ultrasonically guided transvaginal puncture after ultrasonic ovarian follicle monitoring. Uterine anomalies are diagnosed with 3D ultrasound in order to structural uterine disorders as an treatment of sterility. Hence, ultrasound techniques are indispensable in every occasion of sterility diagnosis and treatment.

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