Abdominal Ultrasound-Guided Embryo Transfer Improves Clinical Pregnancy Rates After In Vitro Fertilization: Experiences from 330 Clinical Investigations

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Purpose: To investigate the effect of ultrasound-guided embryo transfer on the rate of implantation and clinical pregnancy.

Methods: A prospective randomized trial was performed to compare ultrasound-guided embryo transfer with the traditional method. A total of 330 patients were randomly divided into two groups on the day of embryo transfer. For the cases ($n = 178$), ultrasound-guided was used; controls ($n = 152$) was performed using routine methods.

Results: The rate of implantation and clinical pregnancy for the cases (19.6 and 37.1%, respectively) was significantly higher than for the controls (12.6 and 25%, respectively; $p < 0.05$).

Conclusion: Ultrasound-guided embryo transfer can significantly increase the rate of implantation and clinical pregnancy, and should be recommended as a routine procedure in the process of in vitro fertilization and embryo transfer (IVF-ET).

KEY WORDS: Implantation rate; pregnancy rate; in vitro fertilization; ultrasound-guided embryo transfer.

INTRODUCTION

Embryo transfer is the final step in the process of in vitro fertilization and embryo transfer (IVF-ET). It has been reported that about 85% of transferred embryos fail to implant. Thirty percent of failed implantations can be attributed to poor techniques of embryo transfer. Embryo transfer, therefore, is a vital step in IVF (1–3). Recently, ultrasound-guided embryo transfer has been reported to improve the

clinical pregnancy rate after IVF. For example, the clinical pregnancy rate among those undergoing embryo transfer with ultrasound guidance has been reported in the range of 38.4–50%, considerably higher than the 25.4–33.7%, reported for those embryo transfer was not assisted by ultrasound (4–8). Conversely, one study did not show any statistical difference in the pregnancy rate between the ultrasound-guided group (26.0%) and the non-ultrasound-guided group (22.5%) (9). The aim of the present prospective study was to compare the effects of ultrasound-guided embryo transfer and traditional methods on the clinical pregnancy rate after IVF.

MATERIALS AND METHODS

Subjects

Infertile patients (28–41 years old) undergoing treatment with IVF-ET, including intracytoplasmic

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sperm injection (ICSI) in the Center of Reproductive Medicine, Peking University Shenzhen Hospital, were recruited into a 2-year study from June 2001 to June 2003. Ethical clearance for the survey was received from the Ethics Committee, Peking University Shenzhen Hospital. Written consent was obtained from patients prior to participating in the investigation.

A total of 330 patients were randomly divided into two groups on the day of transfer of fresh embryo, i.e. the cases who underwent abdominal ultrasound-guidance and the controls who did not. Randomization was conducted by a nurse who was not involved in the recruitment of subjects. The same group of doctors and nurses were responsible for conducting embryo transfer in the two groups.

Ovarian Stimulation Protocol

The following protocols for ovarian stimulation were applied according to age, ovarian function, serum concentration of follicle stimulating hormone (FSH) on days 3–5 of the menstrual cycle, the thickness of endometrium and the clinical history of the patients.

- 1) *Regular regimen*: Patients were given an intramuscular injection of 1.875–3.75 mg of Gonadotrophin Releasing Hormone Agonist (GnRH-a; Enantone, Abbott laboratories, Japan) on day 21 of the menstrual cycle. On days 3–5 of the next cycle, FSH (Gonal-F, Serono, Switzerland) was administered at a concentration of 150–300 IU/day. When the mean diameter of the leading follicles was more than 18 mm, 10,000 IU of human Chorionic Gonadotrophin (hCG; Profasi, Serono, Switzerland) was injected.
- 2) *Mini-dose regimen*: Patients were treated with oral contraceptives for at least 21 days. Oral contraceptives were stopped when ovarian function was inhibited. After 3 days, GnRH-a (Decapeptide) was given daily in a 0.1 mg dose. On days 2–3 of the menstrual cycle, FSH was given at 150–300 IU/day. When the mean diameter of the leading follicles was more than 18 mm, 10,000 IU of hCG was injected.
- 3) *Oral contraceptives plus GnRH-a*: Patients had been treated with oral contraceptives for 21–28 days, and in the latter 7 days, 0.1 mg GnRH-a (Decapeptide) was given

daily. On days 2–3 of the menstrual cycle, FSH was given in a 75–150 IU/day dose. Then, 10,000 IU of hCG was injected when the mean diameter of the leading follicles was more than 8 mm.

IVF/ICSI Procedure

Transvaginal ultrasound-guided oocyte retrieval was scheduled after 36 hours of hCG injection. IVF or ICSI was performed about 5 h after oocyte retrieval. Oocytes were inseminated with a calculated volume of sperm suspension treated by Percoll, which resulted in a concentration of about 50,000/mL progressively moving spermatozoa. After incubation for 18–20 h, corona cells that remained attached to the oocyte were removed using drawn Pasteur pipettes. For ICSI, after oocyte retrieval, cells of the cumulus and corona were removed by rinsing for 30–60 seconds in HEPES-buffered human tubal fluid medium (HTF; Irvine, CA) supplemented with a 10% pasteurized human plasma protein solution and with 40–80 IU hyaluronidase/mL, followed by aspiration of the oocytes in and out of hand-drawn glass pipettes. Oocytes were rinsed several times in culture medium and incubated until the injection procedure. Just before the ICSI procedure, 0.5 μ L of a seminal plasma-free sperm suspension was pipetted into a 5 μ L droplet of a 10% polyvinylpyrrolidone solution. Injection was performed in 15 μ L droplets of HEPES-buffered HTF solution. After incubation for 18–20 h, oocytes were checked for the presence of pronuclei, then washed twice and transferred to fresh medium. Embryos were cultured in 20 μ L droplets under mineral oil. The embryo score was established by reference to the number of cells and the morphology.

Embryo Transfer Procedure

One to three embryos with the highest scores were selected for transfer 2–3 days after the retrieval. For abdominal ultrasound-guided embryo transfer, the patients were placed in a lithotomic position and then transabdominal ultrasound (Aloka SSD-1400 3.5 MHz Aloka Co. Ltd., Japan) was applied to examine the pelvis, uterus, and cervix. A bivalve speculum was used to expose the cervix. After the cervix was cleaned with saline, a Wallace embryo transfer catheter (SIMS Portex. Ltd., Kent, UK) was used for embryo transfer. These catheters can be seen clearly with the ultrasound. The outer cannula of the

Fig. 1. Abdominal ultrasound-guided embryo transfer. (A) A strong echo was visualized when the head of the inner catheter was inserted into the uterine cavity; (B) Embryos releasing: A strong echo near the uterine fundus was seen when the embryos were released from the internal catheter. After the catheter withdrew, the echo still remained in the uterine cavity.

catheter was bent and inserted into the cervical canal 4–5 cm from the external os to arrive at the internal os, with caution taken to avoid the cannula advancing into the uterine cavity. The inner catheter was rinsed and loaded with HTF medium. The embryos were then loaded in the catheter. Under the guidance of ultrasound, a strong echo was visualized on the connection between the body of the uterus and the inner os of the cervix (Fig. 1A), then the inner transfer catheter with the loaded embryos was inserted into the uterine cavity approximately 6 cm. The embryos were slowly injected when the ultrasound scanner showed the catheter to be within 1.5–2.0 cm of the fundus of the uterine cavity. The visualization of a strong ultrasound echo between the head of the inner catheter and the uterine cavity was judged as a successful embryo transfer (Fig. 1B).

For the controls, the inner catheter with the loaded embryos was inserted into the uterine cavity about 6 cm. The embryos were slowly released according to the clinician's judgment of the position of the catheter. A malleable obturator was used if difficulty was encountered in the process.

After transfer, the catheter was checked under a microscope to make sure that all embryos had been transferred. All the patients were kept in bed for a minimum of 6 h after their operations.

According to the serum concentration of Estradiol (E_2), the patients were treated with 2,000 IU of Profasi once every 3 days when $E_2 < 10,000$ pmol/mL, or with progesterone at 20–60 mg/day when $E_2 > 10,000$ pmol/mL. Pregnancy was determined by urine β -hCG testing

2 weeks after embryo transfer. Clinical pregnancy was confirmed by ultrasound at 6–7 weeks of gestation. Clinical pregnancy and implantation rates were used to evaluate the efficiency of the two embryo transfer methods.

Statistical Analysis

A *t*-test was used to compare the clinical histories and characteristics of IVF cycles between the cases and the controls. The chi-squared test was used to compare the clinical pregnancy and implantation rates. Values were considered significant when $p \leq 0.05$.

RESULTS

In total, there were 178 cases and 152 controls. There were no significant differences between the two groups with respect to age, causes of infertility, duration of infertility, and the ovarian stimulation protocols (Table I). In addition, no significant differences were observed between the two groups in terms of their IVF cycles (Table II). In contrast, the ultrasound-guided implantation group had a significantly higher rate of clinical pregnancy and implantation (Table III).

DISCUSSION

With the rapid development of assisted reproductive techniques (ART), ovulation stimulation

Table I. Clinical History of the Patients

	Ultrasound-guided group	Regular group	<i>p</i> -value
Cycles	178	152	
Age (years)	32.2 ± 3.9	32.5 ± 4.2	0.504
Cause of infertility (%)			
1. Tubal	65 (36.5)	58 (38.2)	0.759
2. Male	68 (38.2)	57 (37.5)	0.896
3. Endometriosis	24 (13.5)	18 (11.8)	0.656
4. Unexplained	12 (6.7)	12 (7.9)	0.688
5. Mixed	9 (5.1)	7 (4.6)	0.849
Duration of infertility (years)	5.8 ± 2.1	5.6 ± 1.6	0.328
Ovarian stimulation protocol			
1. Regular regimen	114 (64.0)	106 (69.7)	0.157
2. Mini-dose regimen	20 (11.2)	17 (11.2)	0.157
3. Oral contraceptives plus GnRH-a	44 (24.8)	29 (19.1)	0.157

protocols, ICSI techniques and the embryo culture mediums have been modified greatly (10–13). Methods to improve the implantation rate have become a key area of research in the field of ART. The embryo implantation rate in the process of IVF-ET depends on uterine receptivity, quality of embryos and techniques of embryo transfer (14). Uterine receptivity and the quality of the embryos can be improved by optimization of both the ovarian stimulation protocol and the in vitro culture. Since the establishment of IVF, embryo transfer has been performed mainly depending on doctors' personal experience and judgment. Experience-based methods have many negative factors that may result in a total failure of the whole IVF procedure. These potentially negative elements include: 1) initiation of uterine contractility that may lead to immediate or delayed expulsion of the embryos, especially for patients who experience difficult transfers; 2) the presence of cervical mucus that may plug the tip of the catheter or entangle the embryo and drag it out during withdrawal of the catheter; and 3) for patients with acute utero-cervical angulations, cervical stenosis or anatomical distortion of the cervical canal, the regular method without ultrasound guidance cannot put the embryos in the correct location in the uterine

cavity due to failure to pass the catheter through the internal os (15).

The application of abdominal ultrasound has led to major improvements in embryo transfer (16,17). Our observations have demonstrated that the use of ultrasound significantly increases the rate of implantation and clinical pregnancy. Compared with the traditional method, the abdominal ultrasound-guided embryo transfer has a number of benefits. Firstly, doctors can clearly see the position of the uterus, the angle between the body of the cervix and the uterus, the length and direction of the uterine cavity and the cervical canal. This helps the gynecologist to choose a suitable catheter for the embryo transfer. It has been reported that the main cause for embryos to fail to arrive at the internal os is that the catheter is not level with the axis of the uterine body and cervical canal (15,18). With the guidance of ultrasound, the catheter can be bent to follow the axis and can easily pass through the cervical canal, which also avoids over-stimulation and injury of the cervix and uterus. Secondly, the whole process of catheterization and releasing the embryos can be visualized on the ultrasound image, which makes it easier to place the embryos on the correct position. Clinical judgment during

Table II. Characteristics of IVF Cycles in the Two Groups

	Ultrasound-guided group	Regular group	<i>p</i> -value
Cycles	178	152	
FSH concentration on day 3–5 (IU/L)	2.8 ± 0.8	3.0 ± 1.1	0.064
E_2 concentration on HCG day (pmol/L)	7,839.1 ± 3,871.4	7,541.5 ± 3,624.6	0.472
Number of oocytes retrieved	8.6 ± 3.4	9.4 ± 4.2	0.061
Fertilization rate (%)	81.1 ± 19.3	83.7 ± 16.2	0.184
The number of embryos transferred	2.3 ± 0.53	2.2 ± 0.46	0.067
Embryo scores	11.7 ± 0.53	11.6 ± 0.49	0.076
Days of embryo culture in vitro	2.45 ± 0.4	2.42 ± 0.2	0.380

Table III. Clinical Pregnancy and Implantation Rates in the Two Groups

	Ultrasound-guided group	Regular group	<i>p</i> -value
Cycles	178	152	
Clinical pregnancy (%)	37.1 (66/178)	25.0 (38/152)	0.019
Embryo implantation (%)	19.6 (80/409)	12.6 (42/334)	0.011

embryo transfer has often been shown to be unreliable. With transvaginal ultrasound, Woolcott *et al.* observed that among 121 cases undergoing traditional embryo transfer, the outer catheter touched the uterine fundus in 17.4% of cases, indented the endometrium in 24.8%, embedded in the endometrium in 20.8% and inserted into the fallopian tube in 7.4% (19). Keeping an appropriate distance between the tip of the internal catheter and the fundus of the uterus is an important point for a successful embryo transfer. Kojima *et al.* reported that 1.5 cm was a more suitable distance than 1 cm, and significantly improved the clinical pregnancy rate; distances of less than 1 cm also decreased the success of embryo implantation. In our study, a strong ultrasound echo was visualized at the time the embryos were released and 1.5–2 cm was the optimal distance, similar to the reported data (20). The visualization of embryo releasing also increases the cooperation between doctors and patients. Thirdly, ultrasound-guided embryo transfer significantly decreases the opportunity for stimulation of the uterine cervix and fundus, thereby reducing the chance of the uterus contracting. Excessive uterine contractions at the time of embryo transfer have been associated with a lower clinical pregnancy rate. Stimulation of the cervix causes the release of oxytocin, thus increasing uterine contractility (21). When the transfer catheter touches the uterine fundus, it induces strong contractions of cervix and uterine fundus (22). Additionally, avoiding unnecessary operation and removing cervical mucus with soft catheters are of vital importance for the success of embryo transfer.

In summary, ultrasound-guided embryo transfer appears to significantly increase the rate of embryo implantation and clinical pregnancy. To date, 330 patients have been recruited into our study: 178 cases and 152 controls. The study will be continued to increase the number of patients in each of the two groups. Further evaluation of the larger cohort will strengthen the statistical validity of our results. However, it seems likely that, in the future, ultrasound-

guided embryo transfer will be recommended as a regular procedure in IVF-ET.

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REFERENCES

1. Edwards RG: Clinical approaches to increasing uterine receptivity during human implantation. *Hum Reprod* 1995;10(Suppl. 2):60–66
2. Al-Shawaf T, Dave R, Harper J, *et al.*: Transfer of embryos into the uterus: How much do technical factors affect pregnancy rates? *J Assist Reprod Genet* 1993;10(1):31–36
3. Cohen J: Embryo replacement technology. San Francisco 31 Annual postgraduate course 1998; ASRM
4. Coroleu B, Carreras O, Veiga A, *et al.*: Embryo transfer under ultrasound guidance improves pregnancy rates in in-vitro fertilization. *Hum Reprod* 2000;15(3):616–620
5. Coroleu B, Barri PN, Carreras O, *et al.*: The influence of the depth of embryo replacement into the uterine cavity on implantation rates after IVF: A controlled, ultrasound-guided study. *Hum Reprod* 2002;17(2):341–346
6. Coroleu B, Barri PN, Carreras O, *et al.*: The usefulness of ultrasound guidance in frozen-thawed embryo transfer: A prospective randomized clinical trial. *Hum Reprod* 2002;17(11):2885–2890
7. Matorras R, Urquijo E, Mendoza, *et al.*: Ultrasound-guided embryo transfer improved pregnancy rates and increases the frequency of easy transfer. *Hum Reprod* 2002;17(7):1762–1766
8. Wood EG, Batzer FR, Go KJ, *et al.*: Ultrasound-guided soft catheter embryo transfers will improve pregnancy rates in in-vitro fertilization. *Hum Reprod* 2000;15(1):107–112
9. Tang OS, Ng EH, So WW, *et al.*: Ultrasound guided embryo transfer: A prospective randomized controlled trial. *Hum Reprod* 2001;16(11):2310–2315
10. Van Wely M, Westergaard LG, Bossuyt PM, *et al.*: Effectiveness of human menopausal gonadotropin versus recombinant follicle-stimulating hormone for controlled ovarian hyperstimulation in assisted reproductive cycles: A meta-analysis. *Fertil Steril* 2003;80(5):1086–1093
11. Weissman A, Farhi J, Royburt M, *et al.*: Prospective evaluation of two stimulation protocols for low responders who were undergoing in vitro fertilization-embryo transfer. *Fertil Steril* 2003;79(4):886–892
12. Pool TB: Recent advances in the production of viable human embryos in vitro. *Reprod Biomed Online* 2002;4(3):294–302
13. Cooke S, Quinn P, Kime L, *et al.*: Improvement in early human embryo development using new formulation sequential stage-specific culture media. *Fertil Steril* 2002;78(6):1254–1260

14. Chien LW, Au HK, Chen PL, *et al.*: Assessment of uterine receptivity by the endometrial-subendometrial blood flow distribution pattern in women undergoing in vitro fertilization-embryo transfer. *Fertil Steril* 2002;78(2):245–251
15. Mansour RT, Aboulhar MA: Optimizing the embryo transfer. *Hum Reprod* 2002;17(5):1149–1153
16. Sallam HN, Sadek SS: Ultrasound-guided embryo transfer: A meta-analysis of randomized controlled trials. *Fertil Steril* 2003;80(4):1042–1046
17. Buckett WM: A meta-analysis of ultrasound-guided versus clinical touch embryo transfer. *Fertil Steril* 2003;80(4):1037–1041
18. Sallam HN, Agameya AF, Rahman AF, *et al.*: Ultrasound measurement of the uterocervical angle before embryo transfer: A prospective controlled study. *Hum Reprod* 2002;17(7):1767–1772
19. Woolcott R, Stanger J: Ultrasound tracking of the movement of embryo-associated air bubbles on standing after transfer. *Hum Reprod* 1998;13(8):2107–2109
20. Kojima K, Nomiya M, Kumamoto T, *et al.*: Transvaginal ultrasound-guided embryo transfer improves pregnancy and implantation rates after IVF. *Hum Reprod* 2001;16(12):2578–2582
21. Dorn C, Reinsberg J, Schlebusch H, *et al.*: Serum oxytocin concentration during embryo transfer procedure. *Eur J Obstet Gynecol Reprod Biol* 1999;87(1):77–80
22. Lesny P, Killick SR, Robinson J, *et al.*: Junctional zone contractions and embryo transfer: Is it safe to use a tenaculum? *Hum Reprod* 1999;14(9):2367–2370