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Embryo transfer under ultrasound guidance improves pregnancy rates after in-vitro fertilization

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Between October 1998 and January 1999, we examined the influence of ultrasound guidance in embryo transfer on pregnancy rate in 362 patients from our in-vitro fertilization (IVF)–embryo transfer programme. These patients were prospectively randomized into two groups: 182 had ultrasound-guided embryo replacement, and 180 had clinical touch embryo transfer. There were no statistically significant differences between the two groups with respect to age, cause of infertility and in the characteristics of the IVF cycle. The pregnancy rate was significantly higher among the ultrasound-guided embryo transfer group (50%) compared with the clinical touch group (33.7%) ($P < 0.002$). Furthermore, there was also a significant increase in the implantation rate: 25.3% in the ultrasound group compared with 18.1% in the clinical touch group ($P < 0.05$). In conclusion, ultrasound assistance in embryo transfer significantly improved pregnancy and implantation rates in IVF.

Key words: clinical touch/implantation rate/IVF/pregnancy rate/ultrasound-guided embryo transfer

Introduction

Embryo transfer is the last step on the way to pregnancy in the in-vitro fertilization (IVF) cycle. Since the first pregnancy was achieved by such means (Steptoe and Edwards, 1978), certain aspects of the technique have remained largely unchanged. IVF technique however, has continued to evolve, the most significant developments being in ovulation induction (Diedrich *et al*., 1988), the use of intracytoplasmic sperm injection (ICSI) (Palermo *et al*., 1992) and culture media (Gardner *et al*., 1998). Despite these improvements, the majority of transferred embryos fail to implant. This failure may be due to poor embryo quality, lack of uterine receptivity, or the technique of embryo transfer itself.

The use of ultrasound guidance to facilitate embryo transfer has been described by various authors (Strickler *et al*., 1985; Woolcott and Stanger, 1997). However, a significantly higher rate of pregnancy following ultrasound guidance has not been consistently demonstrated. Moreover, a recent study found no significant difference between ultrasound-guided and clinical touch uterine embryo transfers (Kan *et al*., 1999).

The aim of this prospective randomized study was to investigate further the use of transabdominal ultrasound scan guidance during embryo transfer, to compare it with the alternative practice of clinical touch, and to assess its effect on rates of pregnancy and implantation.

Materials and methods

Patients

Between October 1998 and January 1999, 362 patients from our IVF–embryo transfer treatment programme underwent embryo transfer. These patients were prospectively and randomly assigned to two groups: 182 had ultrasound-guided and 180 had clinical touch embryo transfer. The mean age of the women in the ultrasound group was 34.6 ± 4.0 years, compared with 34.5 ± 4.1 years in the clinical touch group.

Routine ovarian stimulation (Neofertinorm®; Serono, Madrid, Spain) with down-regulation (leuprolide acetate 0.5 mg/day s.c.; Procrin®; Abbot, Madrid, Spain) was used. Ovarian response was monitored using vaginal ultrasound scan and serial serum oestradiol determinations as described previously (Barri *et al*., 1988). Human chorionic gonadotrophin (HCG; Profasi®, Serono), 10 000 IU i.m. or s.c., was administered 36 h before ultrasound-directed oocyte recovery (Carreras *et al*., 1987). Embryo transfer was performed on day 2 or 3 with cleaved embryos, and on day 5 or 6 with blastocyst stage following oocyte retrieval. The luteal phase was supported either by vaginal administration of micronized progesterone 600 mg/day (Utrogestan®; Seid, Barcelona, Spain) or HCG 2500 IU (Profasi®; Serono) on days 2, 4 and 6 after oocyte retrieval, according to ovarian response.

The details of laboratory procedures for IVF, including ICSI, have been described previously (Calderón *et al*., 1995; Boada *et al*., 1997).

Technique of embryo transfer

Embryos were selected for transfer according to morphology and cleavage criteria. When regular embryos with 4–8 cells, but without cytoplasmic fragments, were available, they were selected for transfer on day 2 or 3 after oocyte retrieval. Embryonic score was established (Plachot *et al.*, 1990) depending on the number of cells and the percentage of cytoplasmic fragments. Up to two (exceptionally three) expanded blastocysts with distinctive trophectoderm and inner cell mass obtained using sequential media (Chouteau *et al.*, 1998) were replaced on day 5 or 6 (Veiga *et al.*, 1999).

The number of embryos replaced to reduce multiple pregnancies depended upon the age of the patient, the number of embryos available for replacement, and the number of previous IVF attempts (Tur, 1994).

The preparation for embryo transfer was the same for both experimental groups. Patients were positioned supine (lithotomy position) and the cervix was exposed using a bivalve speculum. The external cervical os was then cleaned with a phosphate-buffered saline (PBS) solution (Dulbecco's PBS solution; Irvine Scientific, Santa Anna, CA, USA). Finally, the mucus in the cervical canal was

removed by means of a sterile Teflon catheter (Malleable Stylet Wallace, SIMCARE, Lancing, West Sussex, UK) connected to a syringe.

The Edwards–Wallace embryo replacement catheter (SIMS Portex Ltd, Kent, UK) connected to an insulin syringe was used for all transfers. The catheter was first loaded with transfer medium [50% synthetic serum substitute (Irvine Scientific) and IVF-50 medium for early cleavage or G2.2 medium (Scandinavian IVF Science, Göthenburg, Sweden) for blastocysts], taking care to avoid air bubbles. The embryos were then loaded in the catheter.

The catheter was handed to the clinician, who inserted it through the cervical canal. It was at this point that a difference in technique was introduced between the two groups. In the ultrasound-guided group both the insertion and the positioning of the catheter were facilitated by transabdominal ultrasound [Toshiba, Barcelona, Spain; TOSBEE (SSA-240A) convex 3.75 MHz]. The embryo(s) were slowly released when the ultrasound scan showed the catheter to be within 1.5 cm of the fundus of the uterine cavity. Ultrasound also allowed the visualization of the 'transfer bubble' after the embryos had been expelled (Figure 1).

In the clinical touch group, the embryo(s) were released according to the clinician's feeling as to the position of the catheter (i.e. as close to the fundus as possible without touching it).

In all transfers, only 30 µl of transfer medium, containing the embryo(s) were expelled into the uterine cavity. After transfer, the catheter was checked under a stereomicroscope to ensure that all embryos had been transferred.

The difficulty of the replacement was determined by the following criteria: very easy, when the catheter passed smoothly through the cervix; easy, when the catheter required a rigid outer Teflon introducer; and difficult, when the use of a tenaculum was required in addition to the above.

Pregnancy was assessed by serum β-HCG determination 14 days after embryo transfer. Clinical pregnancy was confirmed by ultrasonography at 6–8 weeks of amenorrhoea, and further monitored at 12–16 weeks.

Data analysis

SPSS for Windows (Chicago, IL, USA) was used for statistical analysis. The chi square test was used to compare qualitative variables, and Student's *t*-test to compare quantitative variables. The significance level was set at $P = 0.05$.

Results

There were no significant differences between the two groups with respect to age (ultrasound group: 34.6 ± 4.0 years; clinical touch group 34.5 ± 4.1 years) or cause of infertility, the male factor being the most frequent cause in both groups (ultrasound group 35.3%; clinical touch group 37.2%) (Table I). Neither were there any significant differences in the characteristics of the IVF cycle (oestradiol concentration on the day of HCG administration, number of oocytes retrieved and transferred (ultrasound group 2.9 ± 0.8 embryos; clinical touch group 2.8 ± 0.8 embryos), and quality of embryos transferred (Table II), day of embryo transfer and luteal phase support treatment (Table III).

In contrast, a significant difference was found between the two groups with respect to both pregnancy and implantation rates (Table IV). In the ultrasound group, 91 out of 182 (50%) patients became pregnant compared with 61 out of 180 patients (33.9%) in the clinical group ($P < 0.002$). Furthermore, 25.3% of the embryos transferred implanted successfully using ultrasound, whereas an implantation rate of 18.1% was achieved in the clinical group ($P < 0.01$). In addition, 93.4% of the pregnancies achieved following ultrasound-guided transfer had a successful outcome (ongoing pregnancies) compared

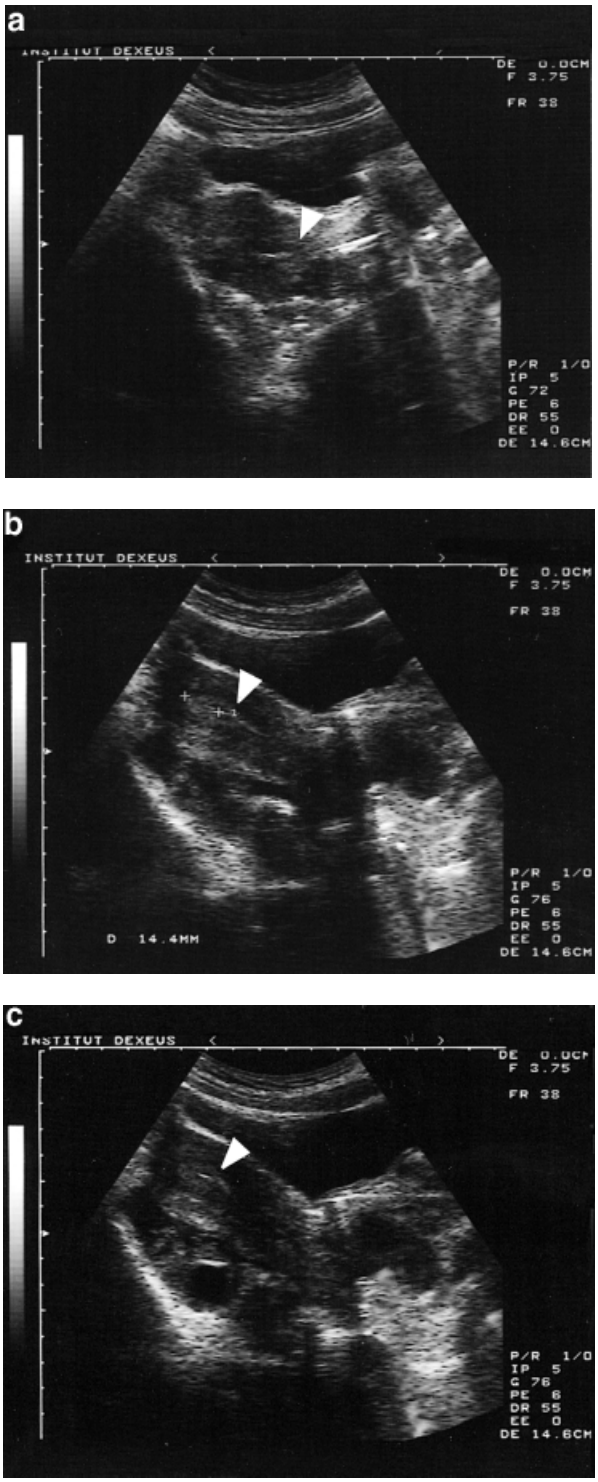

Figure 1. Ultrasound-monitored embryo transfer. (**a**) The arrowhead indicates the tip of the catheter inserted into the uterine cavity. (**b**) The catheter tip (arrowhead) is visualized at a distance of 1.5 cm from the fundus of the uterine cavity. (**c**) The 'transfer bubble' can be seen after the embryo transfer.

Table I. Patients' clinical history

	Ultrasound group (n = 182)	Clinical group (n = 180)
Mean (± SD) age (years)	34.6 ± 4.0	34.5 ± 4.1
Cause of infertility		
Tubal	52 (28.5)	47 (26.2)
Male	64 (35.3)	67 (37.2)
Unexplained	20 (10.9)	13 (7.2)
Endometriosis	12 (6.5)	11 (6.1)
More than one diagnosis	34 (18.8)	42 (23.3)

There were no significant differences.
Values in parentheses are percentages.

Table II. Characteristics of IVF cycle in both groups

	Ultrasound group (n = 182)	Clinical group (n = 180)
Oestradiol concentration on HCG day (pg/ml)	1934.8 ± 865.6	1920.3 ± 932.4
No. of oocytes retrieved	13.8 ± 7.5	13.6 ± 8.2
No. of embryos	7.5 ± 4.9	7.6 ± 4.8
No. of embryos transferred	2.9 ± 0.8	2.8 ± 0.8
Embryonic 'score'	24.3 ± 7.8	23.8 ± 7.4

Values are means ± SD.
There were no significant differences.

Table III. Comparison of day of embryo transfer and type of luteal phase support in IVF cycle in both groups

	Ultrasound group (n = 182)	Clinical group (n = 180)
Day of embryo transfer (n)		
+ 2	149	154
+ 3	21	10
+ 5	7	11
+ 6	5	5
Luteal phase support (n)		
Progesterone	114	120
HCG	68	60

There were no significant differences.
HCG = human chorionic gonadotrophin.

Table IV. Pregnancy rate, implantation rate and evolution of pregnancy

	Ultrasound group (n = 182)	Clinical group (n = 180)
Pregnancy rate[a]	91/182 (50.0)	61/180 (33.9)
Implantation rate[b]	134/529 (25.3)	92/509 (18.1)
Ongoing pregnancies[c]	85/91 (93.4)	52/61 (85.2)

Values in parentheses are percentages
[a,b,c]Groups were significantly different, $P < 0.002$, 0.01 and 0.05 respectively.

with 85.2% in the clinical group ($P < 0.05$) (Table IV). There was one case of ectopic pregnancy in each patient group: 1.09% (1/91) of the ultrasound group, and 1.6% (1/61) of the clinical touch group.

Table V. Pregnancy rate by number of embryos transferred

No. of embryos transferred	Ultrasound group (n = 182)	Clinical group (n = 180)
1	3/13 (23.1)	4/15 (26.7)
2[a]	13/28 (46.4)	3/27 (11.1)
3[b]	57/104 (57)	45/112 (44.1)
⩾4	18/37 (66.7)	9/26 (33.3)

Values in parentheses are percentages
[a,b]Groups were significantly different, $P < 0.004$ and < 0.05 respectively.

When comparing the difficulty of embryo transfer in the two groups, no significant difference was observed in the difficulty of the procedure between ultrasound-guided transfers and clinical touch ones (very easy + easy transfers: 98.3% versus 97.2%).

When pregnancy rates were compared in relation to the number of embryos transferred in both groups, the difference between the two groups was only significant in the transfers of two or three embryos (Table V).

Discussion

Embryo transfer is a critical part of any IVF cycle, and many aspects of the process have been studied, including the type of catheter used (Wisanto *et al*., 1989; Gonen *et al*., 1991), the presence of blood on the catheter (Goudas *et al*., 1998), the importance of 'mock embryo transfer' (Knutzen *et al*., 1992), and the necessity of bed rest following embryo transfer (Sharif *et al*., 1998). With respect to the latter, a recent study (Woolcott and Stanger, 1998) demonstrated that standing shortly after embryo transfer did not change the position of the 'transfer air bubble', and therefore is unlikely to be a significant factor in terms of pregnancy rates. What seems clear however, is that the long treatment process will only result in successful pregnancy if good quality embryos are accurately placed within the uterus.

In a recent paper (Kovacs, 1999), the clinicians' attitude with respect to factors related to embryo transfer were analysed. The most important factor was considered to be the removal of hydrosalpinges before IVF treatment, and surprisingly, the use of ultrasonic monitoring of transfer was considered by the clinicians to be in the position of lowest priority.

Following studies first conducted during the mid-1980s (Strickler *et al*., 1985; Leong *et al*., 1986), the possibility of improving pregnancy rate by using ultrasound guidance has also been considered. These researchers argued that the use of ultrasound facilitated both the positioning of the catheter tip in the fundus of the uterine cavity and visualization of the 'transfer bubble' ejection.

A subsequent study (Hurley *et al*., 1991) investigated the use of transvaginal ultrasound guidance in embryo transfer, but did not identify a significant difference between this and the usual clinical-feel method. However, it must be taken into account that the ultrasound guidance was only used after clinical insertion of the catheter.

This prospective randomized study is, to our knowledge,

the largest to date. High pregnancy and implantation rates were found in the ultrasound-guided group (50% and 25.3% respectively) compared with the clinical touch group (33.9% and 18.07% respectively). The advantage of the use of ultrasound in guiding the embryo replacement is that it assures the correct positioning of the catheter.

In a prospective study using abdominal ultrasound (Al-Shawaf *et al*., 1993), it was found that there was no significant affect of ultrasound on pregnancy outcome. More recently, in what was until then the largest prospective study comparing clinical and ultrasound-guided embryo transfer (Kan *et al*., 1999), there was still no significant improvement seen in either pregnancy or implantation rates. A possible key factor in both of these studies is that the catheter tip was positioned 1 cm from the fundus of the uterine cavity. In our study, the tip of the catheter was positioned with the use of ultrasound at 1.5 cm from the fundus of the uterine cavity. Although some studies have found no relationship between the site of embryo deposition in the uterus and pregnancy outcome (Rosenlund *et al*., 1996), our recommendation is confirmed in two other studies (Waterstone *et al*., 1991; Naaktgeboren *et al*., 1997), in which a significant increase in pregnancy rate was reported by changing the position of the catheter so as to avoid placement of embryos close to the uterine fundus. Furthermore, it was demonstrated (Lesny *et al*., 1998) that contact between the catheter and the uterine fundus stimulates junctional zone contractions which can reduce the likelihood of pregnancy. It was also noted (Fanchin *et al*., 1998) that more uterine contractions at the time of embryo transfer were associated with a lower clinical and ongoing pregnancy rate, and also that the difficulty of embryo transfer was associated with uterine contractions. Recently (Lesny *et al*., 1999), the difficulty of embryo transfer was related to the possibility of an increased incidence of ectopic pregnancy. In our study, the incidence of ectopic pregnancy was higher in the clinical touch group, although this difference was not statistically significant. Therefore, the use of ultrasound assistance appears to be beneficial not only in knowing the position of the catheter in the uterine cavity, but also in avoiding uterine contractions that may increase the rate of ectopic pregnancies.

Clinical judgement during embryo transfer has been shown to be often unreliable (Woolcott and Stanger, 1997): in 17.4% of clinical touch transfers, the outer guiding catheter inadvertently abutted the fundal endometrium, while in 24.8% the outer guiding cannula indented the endometrium and in 33.1% the transfer catheter became embedded in the endometrium. Such data indicate the various ways in which embryos can be inadvertently lost during a transfer procedure.

According to our results, the use of ultrasound resulted in higher rates of pregnancy and implantation when compared with clinical touch replacements. Multiple pregnancy rate may be reduced in certain patients by the appropriate replacement with ultrasound of only two good quality embryos.

Due to the simplicity and good outcomes described above, ultrasound guidance would appear to be an essential factor in improving the results of embryo transfer and, moreover, is one which offers our patients the maximum possibility of a successful pregnancy.

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