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Ultrasound measurement of the uterocervical angle before embryo transfer: a prospective controlled study

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BACKGROUND: The study aim was to determine whether moulding the embryo transfer catheter according to the uterocervical angle measured by ultrasound could improve pregnancy and implantation rates. METHODS: Patients were alternately allocated to one of two groups. In the ultrasound-guided group (n = 320), the catheter was moulded according to the uterocervical angle measured by abdominal ultrasound. In controls (n = 320), embryo transfer was performed using the 'clinical feel' method. RESULTS: Moulding the embryo transfer catheter according to the uterocervical angle significantly increased clinical pregnancy [(OR = 1.57, 95% CI (1.08–2.27)] and implantation rates [(OR = 1.47, 95% CI (1.10–1.96)] compared with the 'clinical feel' method. It also significantly reduced difficult transfers [(OR = 0.25, 95% CI (0.16–0.40)] and blood during transfers [OR = 0.71, 95% CI (0.50–0.99)]. Patients with large angles (>60°) had significantly lower pregnancy rates compared with those with no angle [OR = 0.36, 95% CI (0.16–0.52)]. CONCLUSIONS: Moulding the embryo transfer catheter according to the uterocervical angle measured by ultrasound increases clinical pregnancy and implantation rates and diminishes the incidence of difficult and bloody transfers.

Key words: embryo transfer/IVF/pregnancy rate/ultrasound/uterocervical angle

Introduction

Embryo transfer is an essential step in IVF and ICSI, but is also the least successful, as few embryos replaced inside the uterine cavity result in clinical pregnancies. Indeed, it has been estimated that 85% of the replaced embryos fail to implant (Edwards, 1995).

Numerous attempts to improve the technique of embryo transfer have been described, including the need for a gentle and atraumatic technique (Nabi *et al*., 1997; Goudas *et al*., 1998), performing a trial transfer before the actual procedure (Mansour *et al*., 1990; Sharif *et al*., 1995), removing the cervical mucus prior to embryo transfer (Mansour *et al*., 1994; Nabi *et al*., 1997), avoiding the use of a tenaculum (Lesny *et al*., 1999a), using specific catheters (Ghazzawi *et al*., 1999; Wood *et al*., 2000), depositing the embryos near the uterine fundus (Egbase *et al*., 2000), using a fibrin sealant (Feichtinger *et al*., 1992; Ben-Rafael *et al*., 1995), routine administration of antibiotics following embryo transfer (Fanchin *et al*., 1998a; Egbase *et al*., 1999), as well as performing the procedure under ultrasound guidance (Strickler *et al*., 1985; Hurley *et al*, 1991; Prapas *et al*., 1995, 2001; Woolcott and Stanger, 1997; Kan *et al*., 1999; Lindheim *et al*., 1999; Coroleu *et al*., 2000; Wood *et al*., 2000; Tang *et al*., 2001).

Abdominal ultrasound was used mainly in ultrasound-guided transfers (Strickler *et al*., 1985; Prapas *et al*., 1995, 2001; Kan *et al*., 1999; Lindheim *et al*., 1999; Coroleu *et al*., 2000; Wood *et al*., 2000; Tang *et al*., 2001), but transvaginal ultrasound-guided embryo transfer has also been described (Hurley *et al*., 1991; Woolcott and Stanger, 1997). However, in all these studies, although ultrasound was used to confirm that the embryos have been properly deposited inside the uterine cavity and to follow the embryo-associated air bubble afterwards, no attempt was made to measure the uterocervical angle and mould the catheter accordingly.

The aim of this study was to measure the uterocervical angle immediately before embryo transfer by use of abdominal ultrasound, and to mould the transfer catheter according to the degree of angulation. It was hypothesized that this modified technique would result in improved clinical pregnancy and implantation rates in patients undergoing IVF and ICSI.

Materials and methods

Patients

Between February 1, 1999 and the end of August 2001, 640 consecutive cycles in 539 patients undergoing IVF and ICSI in the authors' unit were studied prospectively. Patients were alternately allocated to one of two groups. In the first group (n = 320), embryos were transferred under ultrasound guidance, whilst in the control group (n = 320) embryos were transferred without ultrasound

Table I. Criteria of studied patients

Criterion	Ultrasound-guided (n = 320)	No ultrasound (n = 320)	P
Age (years)	32.4 ± 5.5	33.2 ± 5.9	NS
Duration of infertility (years)	7.7 ± 4.8	8.6 ± 5.8	NS
No. of HMG ampoules	31.0 ± 8.2	29.8 ± 7.0	NS
No. of oocytes	9.5 ± 7.4	8.9 ± 6.4	NS
No. of embryos	5.2 ± 5.8	5.7 ± 4.7	NS
No. of transferred embryos	2.9 ± 1.4	2.8 ± 1.4	NS
Midluteal progesterone (ng/ml)	45.0 ± 31.6	42.5 ± 16.3	NS

Values are mean ± SD.
NS = not significant.

guidance, but using the 'clinical feel' method. There were no statistically significant differences between the two groups with regard to patient age, duration of infertility, mean number of HMG ampoules administered, mean number of oocytes retrieved, or mean number of embryos transferred (Table I). Cycles from patients who received cryopreserved embryos or who requested general anaesthesia were excluded from the study.

Ovarian stimulation protocol

Controlled ovarian stimulation was achieved using a triptorelin (Ferring, Kiel, Germany) short down-regulation protocol followed by HMG stimulation (Pergonal®, Serono, Switzerland or Humegon®, Organon, The Netherlands). Monitoring was effected by serial ultrasound scanning, and HCG (Profasi®, Serono, Switzerland; 10 000 IU) was administered by i.m. injection when the leading follicle reached 18 mm in diameter, as described elsewhere (Sallam *et al.*, 1982). Oocyte retrieval was performed 36 h later through the transvaginal, ultrasound-guided route (Combison 310; Kretztechnik AG, Zipf, Austria). Embryos were transferred 48 h after retrieval, and only class I and II embryos were replaced. Luteal phase support was effected by oral administration of 600 mg/day of micronized progesterone (Utrogestan; Laboratoires Bessins-Iscovesco, Paris, France) in three divided doses. The serum β-HCG concentration was measured 2 weeks after embryo transfer in order to determine the occurrence of pregnancy. Clinical pregnancy was defined as the observation of a fetus with a pulsating heart at 6–8 weeks after embryo transfer. The implantation rate was defined as the number of sacs seen on ultrasound in relation to the number of embryos replaced.

Measurement of the uterocervical angle

Measurement of the uterocervical angle was performed using abdominal ultrasound (Scanner 100 LC; Pie Medical, Maastricht, the Netherlands) immediately before embryo transfer, and with the patient in the lithotomy position. Patients were asked to maintain a full urinary bladder before the procedure was carried out. A trans-abdominal ultrasound scan was then performed and a mid-plane longitudinal section obtained. The uterocervical angle is the angle between a line joining the external cervical os and internal cervical os, and a line joining the internal cervical os and uterine fundus. Patients were divided into four categories depending on the degree of angulation: (i) no angle, Figure 1A; (ii) small angle ($<30°$, Figure 1B); (iii) moderate angle (30–60°, Figure 1C); and (iv) large angle ($>60°$, Figure 1D).

Embryo transfer procedure

In all patients, embryo transfer was performed 48 h after oocyte retrieval with the patient in the lithotomy position. The procedure was performed under sterile conditions; the patient was draped, a sterile speculum was inserted into the vagina and the cervix exposed. The cervical mucus was aspirated using a mucus aspirator and the cervix was then cleansed with a swab soaked with culture medium (BM9 medium; CCD, Paris, France).

A Frydman TDT catheter (CCD) was used in all cycles. This catheter consists of an outer rigid sheath with an obturator and an inner soft catheter. The outer sheath with its obturator was passed first through the cervix until located 1 cm above the internal cervical os. In ultrasound-guided transfer patients, this outer sheath (with its obturator) was moulded while still in its sterile sleeve, according to the uterocervical angle measured. In control patients, no ultrasound was used and the outer sheath was passed through the cervix using the 'clinical feel' method. All transfers were performed by the same clinician (H.N.S.).

Once the outer sheath was in place, the embryologist (A.F.R.) was informed. The inner soft catheter was then fitted with a 1 ml tuberculin-type syringe and flushed with culture medium (BM9). The embryos were then loaded in the distal end of the inner catheter in a volume of 15–25 µl of culture medium (BM9) preceded and followed by 1 cm of air. The inner catheter containing the embryos was then advanced inside the outer catheter to 1 cm below the uterine fundus, according to the uterine length obtained by sounding at the initial visit of the patient. The embryos were then gently deposited into the uterine cavity. The catheter was left *in situ* for 30–60 s and then withdrawn gently. In the ultrasound-guided group the position of the catheter tip and the release of the air bubble containing the embryos in the uterine fundus were also confirmed by ultrasound, by a third clinician (A.F.A.) holding the abdominal transducer throughout the procedure. In all cases, the catheter was then checked under a dissecting microscope for retained embryos. If these were found, they were reloaded and transferred again. The patients were asked to remain in bed for 15–30 min after the procedure.

Data analysis

Statistical analysis was performed using the Microstat software package. The χ^2-test was used to compare qualitative variables, and Student's t-test to compare quantitative variables. The significance level was set at $P = 0.05$. The analysis was carried out on an intention-to-treat basis.

Results

Difficulties encountered during embryo transfer

Difficulties were encountered during embryo transfer in 27 instances in ultrasound-guided patients (8.4%) compared with 86 (26.8%) in controls (Tables II and III). This difference was statistically significant ($\chi^2 = 36.941$; $P < 0.00001$). The odds ratios (OR) for diminishing the incidence of difficulties were 0.25 (95% CI 0.16–0.40). Changing the catheter to a more rigid catheter with a ball-pointed tip (Labotect; Labor-Technik, Göttingen, Germany) was necessary in three instances (0.9%) in ultrasound-guided patients and five (1.6%) in controls [$\chi^2 = 0.506$, $P = 0.4767$ (not significant)]. Negotiation of the cervical canal was necessary in 16 instances (5%) in ultrasound-guided cases compared with 45 (14.1%) in controls ($\chi^2 = 15.239$, $P < 0.0001$). Volsellum use was required in five (1.6%) ultrasound-guided patients, and in 31 (9.7%) controls ($\chi^2 = 19.897$, $P < 0.00001$), while embryos were retained inside the catheter in three (0.9%) and four instances (1.3%) respectively (P = NS). Transmyometrial transfer was performed in one

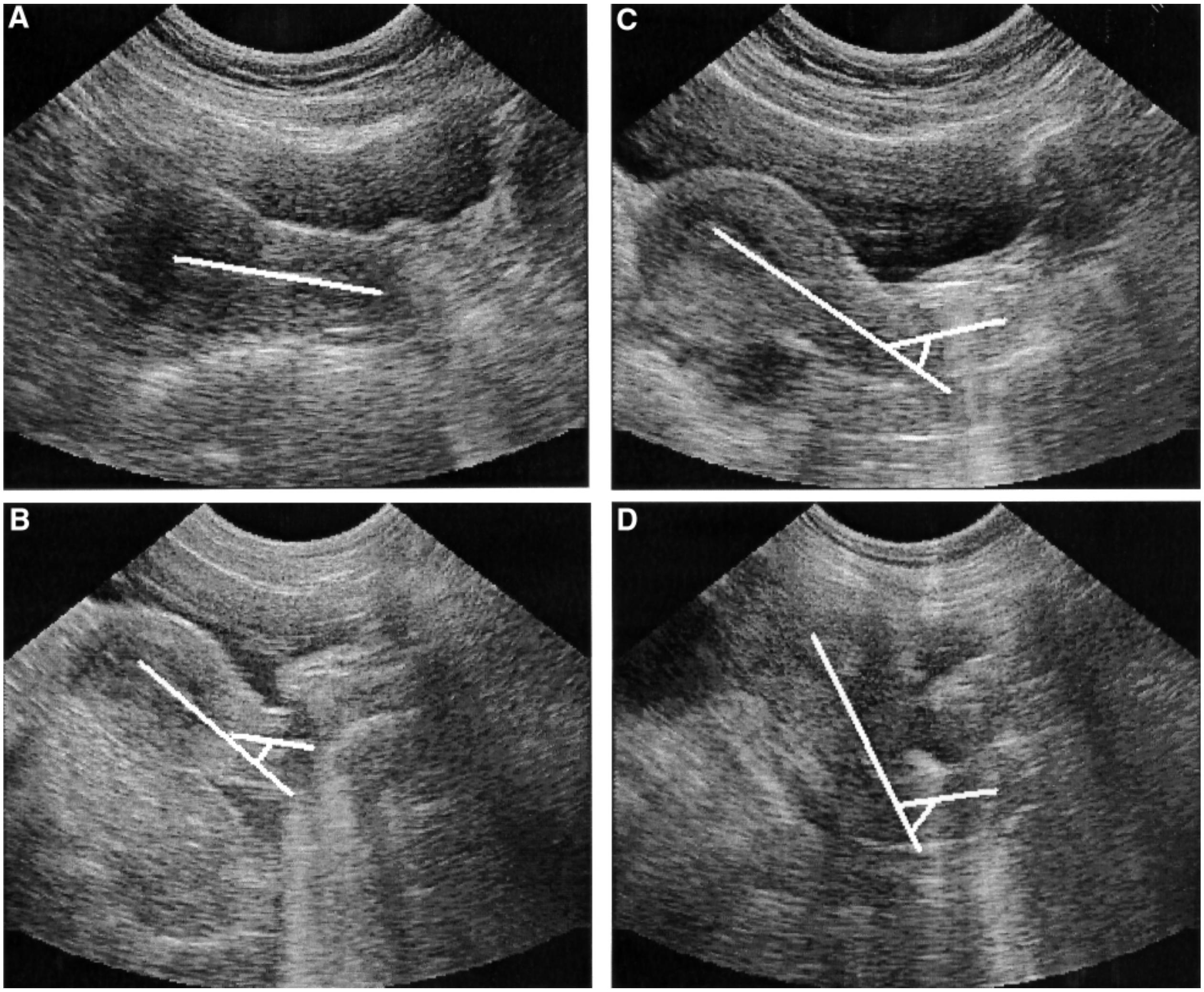

Figure 1. Abdominal ultrasound showing (**A**) absence of the uterocervical angle; (**B**) a small uterocervical angle (<30°); (**C**) a moderate uterocervical angle (30–60°); and (**D**) a large uterocervical angle (>60°).

Table II. Difficulties encountered during embryo transfer, presence of blood during embryo transfer, clinical pregnancy rate and implantation rate in both studied groups

Type of difficulty	Ultrasound-guided (n = 320)	No ultrasound (n =320)	P
Change of catheter	3 (0.9)	5 (1.6)	NS
Negotiation of cervix	16 (5)	45 (14.1)	<0.0001
Use of volsellum	5 (1.6)	31 (9.7)	<0.00001
Retained embryos	3 (0.9)	4 (1.3)	NS
Transmyometrial transfer	0 (0)	1 (0.3)	<0.00001
All difficulties	27 (8.4)	86 (26.8)	<0.00001
Blood on catheter wall	65 (20.3)	52 (16.3)	NS
Blood on catheter tip	16 (5.0)	43 (13.4)	<0.005
Blood from cervix	3 (0.9)	12 (3.8)	<0.05
All bloody transfers	84 (26.3)	107 (33.4)	<0.05
Clinical pregnancy rate (%)	26.25	18.43	<0.02
Implantation rate (%)	13.79	9.82	<0.01
No. of ectopic pregnancies	1	3	NS

Values in parentheses are percentages.
NS = not significant.

control subject due to difficulty in bypassing the internal cervical os, even after ultrasound use.

Presence of blood during embryo transfer

Blood was present during embryo transfer in a total of 84 patients of the ultrasound-guided group (26.3%) and 107 (33.4%) of the controls (Tables II and III); this difference was statistically significant (χ^2 = 3.948; $P < 0.05$). The OR for diminishing the incidence of blood during transfer were 0.71 (95% CI 0.50–0.99). Blood was seen on the catheter outer wall in 65 instances (20.3%) in the ultrasound-guided group, and in 52 instances (16.3%) in controls [χ^2 = 1.768, P = 0.1837 (not significant)], while blood from the cervix was seen in three (0.9%) and 12 (3.8%) cases respectively (χ^2 = 5.530, $P < 0.05$). Blood on the catheter tip was seen in 16 cases (5.0%) after ultrasound guidance, and in 43 controls (13.4%) (χ^2 = 13.611, $P < 0.005$).

Clinical pregnancy and implantation rates

A total of 143 clinical pregnancies (22.3%) resulted (Tables II and III). Of these pregnancies, 84 occurred in the ultrasound-

Table III. Odds ratio (OR) and 95% confidence intervals (CI) for measuring the uterocervical angle by ultrasound before embryo transfer versus not using ultrasound

Procedure/difficulty	OR (95% CI)
Negotiation of cervix	0.32 (0.18–0.58) [e]
Use of volsellum	0.15 (0.06–0.39) [f]
Retention of embryos in catheter	0.75 (0.17–3.40)
Changing the catheter	0.60 (0.14–2.53)
All difficult transfers	0.25 (0.16–0.40) [g]
Blood on catheter wall	1.31 (0.88–1.95)
Blood on catheter tip	0.34 (0.19–0.62) [d]
Blood coming from cervix	0.24 (0.07–0.85) [a]
All bloody transfers	0.71 (0.50–0.99) [a]
Clinical pregnancy rate	1.57 (1.08–2.27) [b]
Implantation rate	1.47 (1.10–1.96) [c]
Ectopic pregnancies	0.33 (0.03–3.19)

Superscripts denote statistical significance: [a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.01$; [d]$P < 0.005$; [e]$P < 0.0001$; [f]$P < 0.00001$; [g]$P < 0.000001$.

Table IV. Distribution of uterocervical angle and clinical pregnancy rate (CPR) in the ultrasound-guided embryo transfer group ($n = 320$)

Angle size (°)	*n* (%)	Pregnancies (*n*)	CPR (%)
No angle	39 (12.2)	14	35.9
Small (<30)	32 (10)	11	34.4
Moderate (30–60)	119 (37.2)	37	31.1
Large (>60)	130 (40.6)	22	16.9[a]
Total	320 (100)	84	26.3

[a]Statistically significantly different from patients with no angle ($P < 0.02$).

directed group (26.25%) and 59 in controls (18.43%); this difference was statistically significant ($\chi^2 = 5.628$, $P < 0.02$). The OR for increasing the clinical pregnancy rate were 1.57 (95% CI 1.08–2.27). A total of 1824 embryos was replaced; of these, 928 were in the ultrasound-guided group (mean 2.9 embryos per transfer) and 896 in the control group (mean 2.8 embryos per transfer). A total of 128 embryos was implanted in the ultrasound-guided group (implantation rate 13.79%) compared with 88 in controls (implantation rate 9.82%); this difference was also statistically significant ($\chi^2 = 6.888$, $P < 0.01$). The ORs for increasing the implantation rate were 1.47 (95% CI 1.10–1.96). One ectopic pregnancy occurred in the ultrasound-guided group (0.3%) and three in the control group (0.9%), but this difference was not statistically significant ($\chi^2 = 1.006$, $P = 0.3158$).

Relationship between uterocervical angle and clinical pregnancy rate

In the ultrasound-guided group, no uterocervical angle was seen in 39 (12.2%) of the 320 cycles studied, a small angle (<30°) was found in 32 cycles (10%), a moderate angle (30–60°) in 119 cycles (37.2%), and a large angle (>60°) in 130 cycles (40.6%). The clinical pregnancy rates were 35.9, 34.4, 31.1 and 16.9% respectively in these four subgroups (Table IV). When compared with patients with no uterocervical angle, the clinical pregnancy rate was significantly diminished in patients with large angles ($\chi^2 = 6.443$, $P < 0.02$), but not with patients with either small angles [$\chi^2 = 0.018$, $P = 0.8937$ (not significant)] or moderate angles [$\chi^2 = 0.30$, $P = 0.5775$ (not significant)]. The OR for diminished pregnancy rates for patients with large angles compared with those with no angles were 0.36 (95% CI 0.16–0.52).

Relationship between difficult transfers and clinical pregnancy rate

Among 143 clinical pregnancies that occurred in 640 cycles studied, 124 were in 527 cycles with easy transfers (23.5%), compared with 19 in 113 cycles with difficult transfers (16.8%). This difference was not statistically significant ($\chi^2 = 2.361$; $P = 0.1244$), but in order to reach statistical significance, the least number needed to treat would be 610 cycles in each arm of the study (taking 0.05 as the level of significance and accepting an 80% probability of detecting a true difference).

Relationship between presence of blood during embryo transfer and clinical pregnancy rate

Among 143 pregnancies that occurred in 640 cycles studied, 113 were in 447 cycles with no blood found during transfer (25.3%), compared with 30 in 193 cycles with blood found during transfer (15.5%). This difference was statistically significant ($\chi^2 = 7.363$; $P < 0.01$). The OR for diminished pregnancy rates in cycles with blood during embryo transfer compared with those with bloodless transfers were 0.54 (95% CI 0.35–0.84).

Discussion

The present study showed that ultrasound measurement of the uterocervical angle before embryo transfer improves clinical pregnancy and implantation rates in patients undergoing IVF and ICSI. Ultrasound-guided embryo transfer was first suggested in 1985 (Strickler *et al*., 1985), when 16 such transfers were performed and compared with 12 transfers guided by 'clinical feel'. This small study was followed by further investigations of abdominal (Prapas *et al*., 1995, 2001; Kan *et al*., 1999; Lindheim *et al*., 1999; Coroleu *et al*., 2000; Wood *et al*., 2000; Tang *et al*., 2001) as well as vaginal ultrasound-guided embryo transfers (Hurley *et al*., 1991; Woolcott and Stanger, 1997). Most of these studies reported improvements in the pregnancy and/or implantation rates, though the results were statistically significant only in the three larger studies (Prapas *et al*., 2001; Coroleu, 2000; Tang *et al*., 2001). In particular, one prospective study reported a statistically significant improvement in clinical pregnancy and implantation rates over blind (non-ultrasound-guided) embryo transfers (Coroleu *et al*., 2000). Another study reported a significant improvement in implantation rates but not in clinical pregnancy rates (Tang *et al*., 2001), whilst a third study reported improvement in overall pregnancy and implantation rates, when embryos were replaced on days 3 and 4 but not on day 5 after oocyte retrieval (Prapas *et al*., 2001).

In all these studies, however, ultrasound was mainly used to confirm that the tip of the transfer catheter was correctly positioned in the uterine fundus before embryos were actually deposited, and to follow the embryo-associated air bubble afterwards. In the present study, ultrasound was mainly used to measure the uterocervical angle immediately before embryo transfer, and the outer sheath of the TDT catheter was moulded according to the angle before its introduction into the uterine cervix. Ultrasound was also used to confirm the correct deposition of embryos into the uterine fundus. The results showed that this new technique improves both clinical pregnancy and implantation rates when embryo transfer is performed 48 hours after oocyte retrieval.

It was necessary to measure the angle immediately before the transfer, as it was found during preliminary studies that the uterocervical angle changes with the state of bladder fullness. Although, as had been suggested previously (Lewin *et al*., 1997), patients were asked to maintain a full bladder in order to straighten the uterus, only 12.2% of them had no uterocervical angle during transfer, and 40.6% had an angle >60°. The clinical pregnancy rate was found to be significantly diminished for patients with larger angles (>60°) compared with those with no angle, and it is suggested that these patients should have a very full bladder during transfer. Whether the routine use of a volsellum in these patients would improve the pregnancy rate remains to be seen (Johnson and Bromham, 1991), as recent studies have shown that this practice leads to the initiation of uterine contractions (Dorn *et al*., 1999; Lesny *et al*., 1999a).

The incidence of ectopic pregnancies was also lower in the ultrasound-guided group than in the 'clinical feel' group, but the difference was not statistically significant; however, this may have been due to the infrequent nature of extrauterine pregnancies. Previous studies have shown that correct positioning of the catheter in relation to uterine size diminishes the incidence of ectopic pregnancies (Egbase *et al*., 2000), and that difficult embryo transfers were associated with an increased incidence of ectopic pregnancies (Lesny *et al*., 1999b).

The present study also showed that ultrasound measurement of the uterocervical angle before embryo transfer significantly diminished the incidence of difficult transfers. In particular, the incidences of negotiating the cervical canal and using a volsellum were significantly reduced in the ultrasound-guided group compared with the 'clinical feel' group. The use of a volsellum has been shown previously to release oxytocin (Dorn *et al*., 1999) and to initiate junctional zone uterine contractions (Lesny *et al*., 1999a), the presence of which was also found to diminish pregnancy and implantation rates (Fanchin *et al*., 1998b).

Changing the embryo transfer catheter to a more rigid one with a ball point was necessary in three cases in the ultrasound-guided group compared with five cases in the clinical feel group. Embryos were also retained inside the catheter in three ultrasound-guided patients compared with four in controls, whilst a repeat transfer was carried out in seven instances. These differences were not statistically significant, and previous studies have shown that these practices (changing the catheter and repeated transfers) do not adversely affect either IVF pregnancy rates or outcome (Nabi *et al*., 1997; Tur-Kaspa *et al*., 1998).

It was found that difficult transfers were associated with lower clinical pregnancy rates compared with easy transfers, though the difference was not statistically significant. The results of the present study agree with those of others (Tur-Kaspa *et al*., 1998; Burke *et al*., 2000), who found no difference in pregnancy rates between easy and difficult transfers. On the contrary, our technique was found to reduce significantly the incidence of blood appearance during transfers, thereby confirming the opinion of many IVF experts in Australia and the UK who reported that avoidance of blood during embryo transfer was the second most important prerequisite for a successful transfer (Kovacs, 1999; Salha *et al*., 2001). The present findings also agree with other reports which showed that blood outside, but not inside, the catheter after embryo transfer was associated with reduced rates of embryo implantation and clinical pregnancy (Goudas *et al*., 1998). These findings therefore suggest that measuring the uterocervical angle with ultrasound before embryo transfer increases the clinical pregnancy and implantation rates mainly by diminishing bloody rather than difficult transfers. The presence of blood during embryo transfer occurred more often in the present study than in others, perhaps due to the fact that a catheter with a rigid outer sheath was used throughout. However, this incidence could most likely be reduced with the use of softer catheters, but this must be confirmed by a comparative study (Wood *et al*., 2000).

In the present study, transvaginal transmyometrial embryo transfer was performed on one occasion in a control patient due to difficulty in bypassing the internal cervical os even after ultrasound use, though the patient did not become pregnant. This technique has been described previously and resulted in a clinical pregnancy rate of 36.5% per attempt (Kato *et al.*, 1993), whilst others reported three clinical pregnancies out of 13 attempts (Sharif *et al*., 1996).

Although the present study was not randomized controlled in design, it was felt that strict adherence to the policy of alternate allocation and the large number of patients studied were the next best alternatives. It has been calculated that the least number needed to treat in order to improve the pregnancy rate from 18 to 28% was 296 patients in each arm of the study, taking 5% as the significance level and accepting an 80% probability of finding a true difference. Nevertheless, a randomized controlled study is needed to confirm the present findings.

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